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A MOLECULAR PHYLOGENETIC PERSPECTIVE ON THE EVOLUTIONARY HISTORY OF TERRARANAN FROGS, A VERTEBRATE MEGA-RADIATION

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ABSTRACT

Terraranans are one of the largest vertebrate radiations, with over 900 species (13% of all amphibians) distributed in the West Indies, Central America, and South America. Unlike most frogs, they lay eggs on land which hatch into froglets rather than tadpoles (direct development). In order to better understand the evolutionary pattern of this group, I have gathered DNA sequence data for over 300 terraranan species. Several main results have emerged. First, most terraranan species fall into four main groups (genera), each predominating in a different geographic region (Central America, the West Indies, northern and western South America, and southeast Brazil). Molecular clock analyses show that these genera are relatively young (between 20 and 50 million years old). Because land connections among Central America, the West Indies, and South America were broken by the beginning of the Cenozoic, these times of divergence suggest parallel origins by overwater dispersal for the West Indian and Central American clades. By using broad taxonomic sampling, and performing analyses with multiple nuclear and mitochondrial genes, a well-supported phylogeny was produced which is the basis of a new classification of terraranan frogs. An enigmatic undescribed frog was placed in a 17-gene molecular phylogeny. This phylogeny demonstrated that it represents the most basal species in Terrarana. Furthermore, this same analysis identified the marsupial frogs as the closest relatives of Terraranans. Together they form an enlarged clade of direct-developing species, suggesting this life-history trait evolved earlier than previously thought. Phylogenies and divergence time analyses of several non-terraranan vertebrate clades (West Indian *Leptodactylus*, lungfishes, and cartilaginous fishes) are also presented, which provide contrasts in diversification and biogeographic history to the terraranans.

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CHAPTER 1
General Introduction

1.1 Overview

The relationships and evolutionary history of the 6500+ living species of amphibians have traditionally been difficult to resolve. This difficulty has arisen from many causes, including the relatively poor fossil record of amphibians, the parallel morphological effects in unrelated groups of miniaturization and paedomorphosis (Yeh 2002; Wiens et al. 2005), and a relatively higher degree of morphological conservatism (and hence lack of useful characters) (Wiens 2008) as compared to some other vertebrate groups. However, in the past several years, employing molecular sequence data has revolutionized the understanding of amphibian relationships.

After a somewhat slow start, molecular phylogenetic studies of amphibian relationships have accumulated at an increasing rate (Hedges and Maxson 1993; Feller and Hedges 1998; Zardoya and Meyer 2001; Zhang et al. 2003; San Mauro et al. 2005). Most notably, Frost et al. (2006) and Roelants et al. (2007) both assembled data sets containing hundreds of species and multiple genetic markers representing the breadth of amphibian diversity. Frost et al., in their analysis, combined a patchwork of mitochondrial and nuclear sequence data (mostly mitochondrial) with coded morphological data in a parsimony framework; their explicit goal was to infer the evolutionary relationships of all amphibians and to use these data to present a new taxonomy of the class. Roelants et al., on the other hand, combined data from multiple nuclear loci and one mitochondrial gene and analyzed their data using likelihood and Bayesian methodology. Their explicit goal was to produce a timescale of amphibian evolution in order to identify times of increased cladogenesis; producing a fully-resolved phylogeny was secondary. Neither of these analyses included enough data to robustly infer some interfamilial relationships, especially among frogs, or enough species to fully understand within-family relationships. In addition, the study by Frost et al. has been severely criticized on methodological grounds by workers who prefer model-based analyses (e. g. Wiens 2008). Notwithstanding these limitations and criticisms, together these two studies have provided a general phylogenetic framework against which to

gauge subsequent studies, as well as a wealth of publicly available nucleotide sequence data.

Of the three living amphibian orders, the relationships among families of salamanders and caecilians (even to species level in salamanders) have now largely been resolved through the use of both mitochondrial and nuclear sequence data (Weisrock et al. 2005; Wiens et al. 2005; Roelants et al. 2007; Hedges et al. 1993; Wilkinson et al. 2002; San Mauro et al. 2004). However, salamanders and caecilians represent only about 10% of amphibian species; there are about 6000 known living or recently extinct species of frogs (AmphibiaWeb 2009). A number of recent studies have used large sequence data sets to infer the evolutionary relationships of frogs as a whole, as well as genus- and species-level relationships in many families (Bossuyt and Milinkovitch 2000; Biju and Bossuyt 2003; Hoegg et al. 2004; Darst and Cannatella 2004; Faivovich et al. 2005; Wiens et al. 2005; Bossuyt et al. 2006; Grant et al. 2006; Frost et al. 2006; Roelants et al. 2007; Guayasamin et al. 2008; Santos et al. 2009). The major goal of the work described in this dissertation has been to better understand the evolutionary history of one relatively poorly-known subgroup of frogs – the New World direct-developing frogs – from a molecular phylogenetic perspective.

As a necessary consequence of this work, the taxonomy of the New World direct-developing frogs has been extensively modified. The species formerly placed in tribe Eleutherodactylini of the family Leptodactylidae (mainly in *Eleutherodactylus* – formerly the largest vertebrate genus) have been transferred to five families united in the unranked taxon Terrarana, many in new or resurrected genera. Thus, the entities referred in the text of this dissertation alternately as "eleutherodactylines" and "terraranans" should be taken as equivalent in content, with the understanding that the term terraranan post-dates most of the earlier studies upon which my dissertation work builds.

The terraranan frogs are a species-rich (900+ species) group of frogs, distributed from the southern United States to Argentina and the West Indies, which are characterized by a derived developmental mode whereby the tadpole stage has been lost; eggs are laid on land and hatch into froglets. Prior to the work presented below, evolutionary relationships within the group, and between terraranans and other frogs, were not well resolved. The vast majority of species (over 700) were lumped in the

enormous genus *Eleutheordactylus*, including all species in Central America and the West Indies. Anatomical evidence supported the presence of a clade (subgenus *Craugastor*) uniting most Central American species (Lynch 1986). Hedges (1989a) recognized three additional subgenera (*Euhyas*, *Pelorius*, *and Syrrhophus*) based on allozyme electrophoretic data, which were additionally supported by some morphological similarities. However, the majority of species, including a handful in Central America, about two-fifths of the West Indian species, and all of the South American species, were left in the paraphyletic subgenus *Eleutherodactylus*, and the relationships of the other South American terraranans (e. g. *Phrynopus*, *Ischnocnema*, *Holoaden*, etc.) to the five subgenera of *Eleutherodactylus* were not resolved. Nor were the relationships of terraranans to other frogs well understood. Terraranans have traditionally been treated as one component of the ill-defined family Leptodactylidae (i.e., as the tribe Eleutherodactylini within the subfamily Telmatobiinae). Leptodactylidae was a prime example of a paraphyletic "wastebasket taxon", and for the most part included all the advanced Neotropical frogs (nobleobatrachians, formerly bufonoids or hyloids) with the exception of those that had obvious morphological synapomorphies, such as the true toads (Bufonidae), the treefrogs (Hylidae), and the dartfrogs (Dendrobatidae).

The earliest molecular phylogenetic analyses of DNA sequence data which included or focused on terraranans did not include enough species to infer any strong conclusions regarding relationships within terraranans, or between terraranans and other frogs (Darst and Cannatella 2004; Crawford and Smith 2005; Lehr et al. 2005; Frost et al. 2006; Roelants et al. 2007). Those studies focusing on terraranans did so at a low taxonomic level: Crawford and Smith used nuclear and mitochondrial sequence data to infer relationships within the subgenus *Craugastor*, a group already supported by morphological and electrophoretic data (Lynch 1986; Hedges 1989a), while Lehr et al. used mitochondrial data to demonstrate the paraphyly of the terraranan genus *Phrynopus*. Above the genus level, the most notable discovery was the linking of the enigmatic, miniaturized brachycephalid frogs of southeast Brazil (also a direct-developing group) with the terraranans (Darst and Cannatella 2004; Frost et. al 2006). These phylogenetic analyses also generally suggested that terraranans had no close relatives, often placing them at or near the base of a radiation of advanced Neotropical frogs. However, support

for the placement of terraranans was not significant in any analysis. Without a robust, well resolved phylogeny, any potential study of terraranan evolutionary history (including biogeography, character evolution, and developmental evolution, among others) has been difficult.

In Chapter 2, molecular phylogenetic data are used to explore the biogeographic history of the terraranan frogs. Like a number of other frog groups, such as the more familiar toads, treefrogs, and dartfrogs, the terraranans are widely distributed and highly diverse in both Central and South America. However, unlike all other frog groups, the terraranans are also highly diverse in the West Indies, with 163 species (no other group of frogs has more than 13 West Indian species). It has not been clear if terraranans arrived in Central America and the West Indies via dispersal events, or if they instead originated through ancient (Mesozoic) vicariance, when the proto-Antilles comprised a land bridge between North and South America. Most of the West Indian vertebrate biota is thought to have arrived through over-water dispersal (Hedges 1996b), while that in Central America has been thought to represent a mixture of ancient vicariant elements with more recent dispersals (Savage 1982). The diversity of the West Indian terraranan fauna, coupled with relatively old times of divergence obtained from immunological clocks (Hass and Hedges 1991; Hedges 1996a), has suggested the possibility of a vicariant origin for terraranans. Likelihood, Bayesian, and neighbor-joining molecular phylogenetic analyses of mitochondrial and nuclear gene data for 276 terraranan species are presented in Chapter 2. These analyses demonstrate that most species fall into three clades, distributed in Central America, South America, and the West Indies, respectively. While the Central American clade was already known (see above), it had been previously thought that the West Indian species comprised multiple groups, some more closely related to South American species (Lynch and Duellman 1997). A Bayesian relaxed molecular clock analysis, calibrated with fossil and geologic data, results in Cenozoic dates for the origins of both the Central American and West Indian clades, suggesting origins for both by oceanic dispersal events (land connections had been severed by the end of the Mesozoic).

The work presented in Chapter 3 is more specifically systematic in nature. While taxon sampling in Heinicke et al. (2007) was adequate to understand the biogeographic history of the major terraranan groups, that study did not include a number of poorly-known, terraranan genera, taxon sampling was relatively sparse in some parts of South America, and relationships among the major clades were not significantly resolved. Thus, sequence data were obtained for an additional 68 terraranan species, including exemplars of most genera. Phylogenetic analyses resulted in a more robust estimate of terraranan relationships, with the inter-relationships of many genera being resolved. Most notably, the closest relatives of the large West Indian and Central American clades are identified as small groups (of only nine and two species) distributed in northwestern South America and southeast Brazil, respectively. Based on this new phylogeny, a new taxonomy of terraranan frogs was presented (see Appendix).

Chapter 4, instead of having a single focus, includes three separate aspects: describing a new terraranan frog so distinct that it represents a new, basal family, determining the interrelationships of the terraranan families, and determining the relationships among the nobleobatrachian frogs, specifically identifying how terraranans are related to other species in this radiation. The study presented in Chapter 3 included an undescribed species, that while externally similar to terraranans of the genus *Pristimantis*, was so distinct genetically that it fell outside Terrarana and was instead intermixed among the outgroups. Subsequently, two additional examples of this species were obtained by a collaborator. Phylogenetic analyses of 17 genes, including this new species as well as exemplars of all major nobleobatrachian groups, show that this species does indeed represent a new family: the basal family of terraranans. Furthermore, the analyses show that the terraranan families Craugastoridae and Strabomantidae (described in the research presented in Chapter 3) are closest relatives, and that Terrarana as a whole is not the basal group within Nobleobatrachia, but is instead related to the marsupial frogs (Hemiphractidae). Notably, the hemiphractids, like terraranans, are direct developers; nearly all other nobleobatrachians demonstrate normal larval development. Thus, the evolution of this life history trait appears to have evolved earlier, and less frequently, than previously believed.

Chapters 5, 6, and 7 present molecular phylogenies (estimated in chapter 5, taken from the literature in Chapters 6 and 7) and divergence times (estimated in all chapters) of three non-terraranan groups of vertebrates: West Indian frogs of the genus *Leptodactylus*, lungfishes, and cartilaginous fishes. While these may seemingly be unrelated to the evolutionary history of terraranan frogs, each has different comparative value. Chapter 5 is the most directly comparable, as it pertains to another group of frogs that has successfully colonized the West Indies. However, unlike in *Eleutherodactylus*, a large radiation stemming from a single species, there are only three species of *Leptodactylus* endemic to the West Indies. The data presented in Chapter 5 show that these three species descend from two separate ancestors, and that one of the species is actually not distinct at the molecular level. Times of divergence between these species and their mainland relatives are more recent, but not especially so, as compared to the divergence between West Indian *Eleutherodactylus* and its mainland relatives, thus demonstrating a striking contrast in post-colonization cladogenesis between *Eleutherodactylus* and *Leptodactylus*. The lungfish data provide another opportunity to examine the relative importance of vicariance and dispersal in historical biogeography. Like frogs, lungfish are generally intolerant of saltwater, and thus can be assumed to have similar limitations in overwater dispersal ability. Results of molecular clock analyses, presented in Chapter 6, show that Cretaceous Gondwanan vicariance is possible explanation the divergence between African and South American lungfish. Thus, even though molecular clock analyses suggest ancient vicariance is unlikely for terraranans, the same analyses suggest that ancient vicariance can not be discounted for other groups. Chapter 7 presents a timetree of cartilaginous fishes. While the extant diversity of this group is similar to that of terraranans (~1,200 species vs. ~900 terraranans), times of divergence are far older, with the earliest divergences estimated to have occurred over 400 million years ago. This suggests that terraranans have diversified at a much faster rate, perhaps because allopatric divergence is more likely in land than marine environments. Recent analyses of vertebrate diversification rates suggests that neither group has speciated at a particularly remarkable rate, although the diversification of lungfishes, in contrast, appears to have been remarkably slow (Alfaro et al. 2009).

In the remainder of this introduction, I briefly review some of the themes not expanded upon above with which my dissertation work has dealt.

1.2 Molecular clocks and amphibian evolution

Ideally, a complete fossil record would allow for a comprehensive understanding of the evolutionary history of a group. Unfortunately, the fossil record has many gaps, and is substantially incomplete in the case of amphibians. For example, although there are over 900 species of living terraranan frog, the only known pre-Quarternary fossil is of an amber-preserved *Eleutherodactylus* from Hispaniola (Poinar and Cannatella 1985). Thus, molecular timescales are critical for an understanding of diversification or historical biogeography of extant amphibians. In the past several years, a number of studies have appeared which provide molecular timescales of evolution in amphibians (reviewed in Cannatella et al. 2009; Bossuyt and Roelants 2009; Vieites et al. 2009; Gower and Wilkinson 2009). These studies have proven critical in broadening the understanding of amphibian evolutionary history. In addition to the analysis presented in Chapter 2 of this dissertation, several other studies have used molecular clocks to infer biogeographic history. Bossuyt and Milinkovitch (2001) showed that several groups of Old World frogs likely spent millions of years rafting on India prior to its collision with Asia. Links between frogs in India and the Seychelles appear to represent a case of ancient vicariance (Biju and Bossuyt 2003). Overseas dispersal of amphibians is not only a phenomenon in the West Indies; molecular clock data also support oceanic dispersals among frogs of the Indian Ocean islands (Vences et al. 2003). In addition to biogeographic hypotheses, timetrees have also been assembled to test other aspects of amphibian biology. Analysis of diversification rates suggests several pulses of increased cladogenesis in amphibian history, including one directly postdating the K-T extinction event, suggesting that amphibians diversified to fill newly vacated niches (Roelants et al. 2007). Molecular times of divergence have also been used as evidence to identify fossil relatives of living amphibians (Zhang et al. 2005). The general trend from the molecular clock data is that the earliest divergences among living amphibians are relatively old, predating the Mesozoic (Cannatella et al. 2009). However, the nobleobatrachian frogs, which include half of all living amphibian species, are much younger, having diversified

near the K-T boundary (San Mauro et al. 2005; Roelants et al. 2007). Thus, the Cenozoic dates of divergence obtained for terraranan frogs in this thesis are not surprising.

1.3 Dispersal, vicariance, and molecular clocks

In the preceding section, several examples were given of molecular timetrees being used to infer biogeographic history. Because putative vicariant events can generally be constrained to a particular time frame (e.g. vicariance between Africa and South America must have occurred prior to the opening of the Atlantic ~100 Ma), it is possible to falsify them by demonstrating that dates of divergence obtained from molecular clocks (assuming proper calibrations and phylogeny reconstruction) do not overlap with these timeframes. Conversely, dates of divergence overlapping with the time frames in question may be taken to suggest the viability of vicariance as an explanation for currently-observed distributional patterns.

Based on these principles, an increasing number of studies (including that presented in Chapter 2) have demonstrated that many divergences formerly suggested to result from vicariance actually represent cases of oceanic dispersal, often over long distances. Most, notably, a number of clades with "Gondwanan" distributions are much too young, based on timetrees, to have originated by vicariance. Among these are the southern beech, *Nothofagus* (Knapp et al. 2005; Cook and Crisp 2005) and the galaxiid fishes (Waters et al. 2000). However, ancient vicariance can explain other range disjuntions, including in lungfishes (see Chapter 6) as well as in other clades such as cichlid fishes (Azuma et al. 2008) and geckos (Gamble et al. 2008). These examples illustrate that neither mechanism can be assumed. Therefore, use of putative vicariant events as geologic calibrations of molecular clocks must be done with caution.

1.4 Systematics in the molecular era.

There is a perception that, while there may still be much undiscovered diversity among invertebrates, plants, fungi, protists, and prokaryotes, work in both branches of vertebrate systematics (phylogenetics and taxonomy) is largely complete. However, recent advances in these fields, aided by the use of genetic data, show that not to be the case. Often, these advances based on molecular data spur re-evaluations of other types of

data (e.g., morphology) and allow the evolution of a group to be seen in a new light. One of the more notable recent advances in vertebrate phylogenetics is the recognition that whales are highly nested in the even-toed hoofed mammal radiation (Graur and Higgins 1994; Gatesy et al. 1996; Shimamura et al. 1997; Gatesy et al. 1999; Nikaido et al. 1999), which has subsequently received support from paleontological data (Thewissen et al. 2001; Boisserie et al. 2005). The discovery of the eutherian superordinal clades, which apparently evolved on separate landmasses, demonstrates widespread morphological convergence (i.e. among insectivores, ungulates) in the living placental mammals (Springer et al. 1997; Stanhope et al. 1998). Likewise, molecular data show widespread morphological convergence in birds, with forms such as waders, divers, and raptors having evolved multiple times (Van Tuinen et al. 2001; Hackett et al. 2008). In reptiles, the iguanians, which were thought to be the most basal squamates (lizards and snakes), are instead closely related to snakes and anguimorph lizards (monitor lizards and relatives) (Vidal and Hedges 2004, 2005; Townsend et al. 2004). This seemingly unnatural group has been shown to share the ability to produce venom, including in many species ("harmless" snakes, all iguanians, and most anguimorphs) not previously believed to be venomous (Fry et al. 2006). Within amphibians, molecular phlylogenetic data suggest major life-history reversals have occurred, with several groups of frogs and salamanders which evolved direct-development subsequently having species revere back to normal (larval) development (Chippendale et al. 2004; Wiens et al. 2007). In fish, molecular phylogenetic data have shown that enigmatic deep-sea fishes thought to belong to three distinct families instead represent larval, adult male, and adult female forms of the same species (Johnson et al. 2009).

In vertebrate taxonomy, new species are being described at a surprisingly rapid rate. Since the start of my graduate work in 2004, for example, the focal group of this thesis (Terraranan frogs) has grown from 780 to 923 described species, an increase of 17% in a span of five years. This growth is not unique, but mirrors the increase in known species numbers seen in amphibians as a whole, and molecular data suggest that the actual number of species is vastly higher (Vieites et al. 2009). The growth in numbers of described vertebrate species is not simply the result of taxonomic revisions of known entities. Many recent discoveries instead represent new genera and families. These

include several genera of large mammals (hoofed mammals and primates) from the Old World tropics (Dung et al. 1993; Jones et al. 2005; Davenport et al. 2006); a new genus of rodent from a family thought extinct for millions of years (Jenkins et al. 2005; Dawson et al. 2006; Huchon et al. 2007); a new genus of salamander distributed thousands of miles and a continent away from all other species in its family (Min et al. 2005); a new, relict, family of frog in India with ancient links to Gondwana (Biju and Bossuyt 2003); a new family of frog from the ancient tepuis of the Guiana Shield, which is the subject of Chapter 4 of this thesis (Heinicke et al. 2009); and, perhaps most surprisingly, a new genus of salamander from the eastern United States (Camp et al. 2009). Clearly, much is still to be learned of the systematics of vertebrates, including amphibians, and the work described in this thesis represents a small contribution to filling these gaps in knowledge.

1.5 Mitochondrial data in molecular phylogenies. Phylogenies based on sequence data obtained from mitochondrial genes have received criticism because strongly-supported conflicts with nuclear-gene data have been observed on numerous occasions (e.g. Leache and McGuire 2006; Spinks and Shaffer 2007). Taken to the extreme, it has been suggested that mitochondrial sequence data should not be used as direct evidence of species' relationships (Taggart et al. 2001). However, many of these conflicts can be explained as resulting from one of several causes. Long-branch attraction is the likely cause of conflict in studies using mitochondrial data where species diverged so long ago (often hundreds of millions of years) that substitution saturation has occurred (e.g. Inoue et al. 2003; Arnason et al. 2004). Conflicts in phylogenies of more recently diverged species may result from introgressive hybridization (e.g. Verkaar et al. 2004; Spinks and Shaffer 2009) or incomplete lineage sorting (e.g. McGuire et al. 2007).

Often lost in criticisms of mitochondrial data are its advantages as a phylogenetic marker. These include the single-copy nature of the mitochondrial genome (resulting in no heterozygous sequences except in rare cases of heteroplasmy), the relatively faster rate of evolution (resulting in the ability to resolve branches too short for data from single nuclear markers to resolve) (Avise 2000), relative ease of amplification due to high copy numbers of the mitochondrion in the cell, the lack of length-variable introns in the mitochondrial genome, and the presence of "ready-made" priming sites in conserved

stem regions of structural RNA. Because of these advantages, the phylogenetic analyses in this thesis have employed mitochondrial data from several genes (especially 12S and 16S rRNA), in concert with sequence data from multiple nuclear loci. The divergences among terraranans that are the focus of this thesis are probably too recent for any strong effects of long branch attraction; conversely, hybridization and incomplete lineage sorting are of more concern in phylogeographic studies of recent population divergences. No strongly-supported discordance was observed among nuclear and mitochondrial data, supporting the use of these data in phylogeny inference.

CHAPTER 2

Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal

Note: Modified from Heinicke, M. P., Duellman, W. E., and Hedges, S. B. 2007. *Proceedings of the National Academy of Sciences (USA)* **104:** 10092-10097. MPH carried out laboratory and computational research, and co-drafted manuscript. WED supplied tissue samples and contributed to manuscript. SBH directed research, collected tissue samples, and co-drafted manuscript.

2.1 Abstract

Approximately one-half of all species of amphibians occur in the New World tropics, which includes South America, Middle America, and the West Indies. Of those, 27% (801 species) belong to a large assemblage, the eleutherodactyline frogs, which breed out of water and lay eggs that undergo direct development on land. Their wide distribution and mode of reproduction offer potential for resolving questions in evolution, ecology, and conservation. However, progress in all of these fields has been hindered by a poor understanding of their evolutionary relationships. As a result, most of the species have been placed in a single genus, *Eleutherodactylus*, which is the largest among vertebrates. Our DNA sequence analysis of a major fraction of eleutherodactyline diversity revealed three large radiations of species with unexpected geographic isolation: a South American Clade (393 sp.), a Caribbean Clade (171 sp.), and a Middle American Clade (111 sp.). Molecular clock analyses reject the prevailing hypothesis that these frogs arose from land connections with North and South America and their subsequent fragmentation in the Late Cretaceous (80–70 Mya). Origin by dispersal, probably over water from South America in the early Cenozoic (47–29 million years ago, Mya), is more likely.

2.2 Introduction

The evolutionary tree of amphibians is now being revealed at a rapid pace, largely from DNA sequence analyses (Bossuyt et al. 2006; Frost et al. 2006; Wiens et al. 2005;

Parra-Olea et al. 2004; Zhang et al. 2006). However, the evolutionary history of a major assemblage of frogs is not well understood. These are the eleutherodactylines and the related genus *Brachycephalus*, which comprise 13% (812 sp.) of all known species of amphibians and 27% of those occurring in the New World tropics (AmphibiaWeb 2007). Unlike most temperate species, these frogs reproduce on land and undergo direct development, bypassing the tadpole stage (Lynch and Duellman 1997). Most are relatively small, typically 20–50 mm in length. A majority of the species has been placed in *Eleutherodactylus* and, together with several other genera, assigned to the tribe Eleutherodactylini of the neobatrachian family Leptodactylidae (Lynch 1971), superfamily Hyloidea (Frost 2007). However, molecular phylogenies of small sets of representative species over the last two decades have suggested that both the family-level and genus-level classification is in need of revision (Frost et al. 2006; Hass and Hedges 1991; Hedges 1989a; Crawford and Smith 2005; Darst and Cannatella 2004; Ruvinsky and Maxson 1996).

Terrestrial breeding and direct development have allowed eleutherodactyline frogs to occupy a diversity of ecological niches and have facilitated their wide distribution (Figure 2-1). Eleutherodactylines occur on almost every island in the Caribbean and display near total endemicity to single-island banks. Their elevational range also is broad, with some species occurring up to 4,400 m in the Andes of South America. Thus, they are a model group for studying Neotropical biogeography and evolution. With this in mind, we assembled samples and available sequences of 276 species of eleutherodactylines and *Brachycephalus* for several mitochondrial and nuclear genes. Our goal was to identify the major groups of species and their times of divergence, to better understand the historical biogeography of eleutherodactyline frogs and the region in general. Our results revealed several major and, for the most part, geographically isolated, clades of eleutherodactyline frogs and showed that the Middle American and West Indian eleutherodactylines owe their origin to Cenozoic over-water dispersal, not from land connections in the Mesozoic.

Figure 2-1. Composite distribution of eleutherodactyline frogs and *Brachycephalus* (812 sp.). "Middle America" refers to Central America and Mexico. No evolutionary groupings are implied.

2.3 Materials and Methods

Taxon Sampling. Our data set encompasses ≈34% of known eleutherodactyline diversity with 276 species in 12 of 18 genera, including at least one representative of every genus with more than five described species. Included species were concentrated in the largest genera, with 140 species of *Eleutherodactylus*, 87 of *Pristimantis*, 14 of *Craugastor*, 17 of *Phrynopus*, and four of the Southeast Brazil Clade. Two hylid species, *Agalychnis callidryas* (South America) and *Litoria caerulea* (Australia), were included for calibration of divergence times. Seven additional hyloid species and a more distant ranoid species (*Rana catesbeiana*) were included as out groups. In addition to broadly sampling the eleutherodactyline genera, our data set also spans a broad geographic range. Included were seven eleutherodactyline species from Southeast Brazil (plus one species

of *Brachycephalus*), 116 from other parts of South America, 19 Middle American species, and 140 West Indian species. These regions respectively contain totals of 47, 477, 154, and 149 described species.

Data Collection. Our study included data from three mitochondrial genes: 12S ribosomal RNA (12S), 16S ribosomal RNA (16S), and intervening tRNA-Valine. In addition, fragments from two nuclear protein-coding genes were sequenced: recombination-activating gene 1 (*Rag-1*) and the tyrosinase gene (*Tyr*). Approximately 90% of the sequences used are previously uncharacterized. Data were collected as overlapping sets (Table 2-1) of 280 species (two genes), 146 species (three genes), and 65 species (five genes).

We chose the *12S* and *16S* genes because of their slower rate of evolution, as compared to other mitochondrial genes, in an attempt to avoid saturation problems (multiple nucleotide substitutions at the same site). The fragment of nuclear gene *Tyr* was chosen because it has proven informative in other anuran studies (Frost et al. 2006; Bossuyt and Milinkovitch 2000). The fragment of nuclear gene *Rag-1* is from the relatively faster-evolving first half of the gene.

For the 280-species data set, partial 12S and 16S sequences were assembled for 277 in-group and three out-group species and used to define major clades (here recognized as genera and subgenera). This data set consists of a ≈350-bp fragment of 12S concatenated with a ≈800-bp fragment of 16S. For the 146-species data set, complete 12S and 16S sequences (≈2.5 kb), including the intervening tRNA and fragments of the flanking tRNA sequences, were assembled for 136 species representing all major groups as defined by the partial data set, the same three out-group species, and seven additional hyloid out-group species. This data set was used to test groups found with the 280-species data set, confirm rooting within eleutherodactylines by using additional out groups, and define subgroups within the largest clades. For the 65-species data set, we also included sequences from a 493-bp region of *Tyr* and a 639-bp region of *Rag-1*. This sample included representatives of most major clades and subclades, except where specimen availability or quality were limiting.

Tissue samples were hand-collected by using approved methods (Pennsylvania State University Institutional Animal Care and Use Committee approval 17632, for those

collected by S.B.H.). They were frozen in liquid nitrogen or preserved in ethanol and kept cold during transport. Additional ethanol-preserved or frozen tissue samples were obtained from museum sources or other researchers. In the laboratory, samples were maintained at -80°C. In addition to sequences generated from these tissues, some sequences were obtained from GenBank. Table 2-1 lists all individual specimens used in this study, including source, tissue collection number (if applicable), museum voucher number (if available), genes sampled, and corresponding GenBank accession numbers for each sequence.

Genomic DNA was extracted using the Qiagen DNeasy tissue extraction kit under the manufacturer's protocol. PCR amplification of samples was performed in 50-ml reactions using AmpliTaq DNA polymerase and ThermoPol buffer (New England Biolabs). For amplification of mitochondrial genes, each reaction contained ThermoPol buffer at 1×, dNTPs at 4 mM, forward and reverse primers at 1 mM, one unit of polymerase, and 1 ml of extracted DNA (more for low-quality tissue). For amplification of nuclear genes, dNTP was increased to 6.6 mM, polymerase to 2.5 units, and extracted DNA to 5 ml. Standard reaction conditions were an initial hold for 5 min at 94°C, followed by 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s. After 40 cycles, a final hold of 72°C for 7 min was performed before terminating the reaction at 4°C. For low- or nonyielding samples, annealing temperature was dropped from 50°C to 46°C. Primers used in PCR reactions were obtained from the literature or designed in the lab (see Table 2-2). Amplified PCR products were isolated by running on agarose gels and filtering with Millipore Ultrafree-DA gel filters or by vacuum filtration using Millipore Multiscreen filters.

Cycle sequencing was performed by using either the Amersham Pharmacia DYEnamic ET terminator cycle sequencing kit or ABI BigDye terminator cycle sequencing kit under manufacturers' guidelines. DNA sequencing was performed with an ABI 3100-Avant or 3730 genetic analyzer. Cycle sequencing and analysis were performed by the authors or by the Pennsylvania State University Nucleic Acid Facility. All fragments were sequenced in both forward and reverse directions.

Before analyses involving any sequence, all chromatograms were fully inspected, and all sequences were compared against their reverse complement to detect any call

errors. Embedded primer sequences were deleted from all sequence fragments before assembly or alignment. Alignments of *12S* and *16S* sequences were created by using CLUSTAL X under default parameters. Resulting alignments were inspected for errors and compared against secondary structure models available from the European ribosomal RNA database. Regions of uncertain homology were excluded from analysis. Sequences for *Rag-1* and *Tyr* were aligned by eye.

Phylogenetic Analyses. Reconstructions of phylogenies for all data sets were performed by using ME, ML, and Bayesian methods. For ML and Bayesian analyses, the 65-species data set was divided into three partitions: 12S and 16S, *Rag-1*, and *Tyr*. ME analyses were implemented in MEGA 3.1 (Kumar et al. 2004) by using the TN + Γ model of evolution. PAUP 4b10 (Swofford 2003) was used to estimate the γ-parameter, and branch support was assessed with 2,000 bootstrap replicates. ML analyses used RAxML-VI-HPC v.2.0 (Stamatakis 2006), accessed at the San Diego Supercomputing Center. For each data set, 100 alternative runs were performed under the GTRMIX model of evolution. Other parameters were maintained at default settings. Nonparametric bootstrap analysis (1,000 replicates) was used to provide branch support values for the most likely tree of 100 found in each data set. MrBayes 3.1 (Ronquist and Huelsenbeck 2003) was used to perform Bayesian analyses. Bayesian analyses used the GTR + I + Γ model of evolution, with all parameters unlinked in partitioned analyses. For the 65-species data set, all phylogenetic analyzes were performed by using only the two nuclear genes in addition to analyses employing both the mitochondrial nuclear data, to ensure that mitochondrial and nuclear data produced results that were not significantly divergent.

In certain cases, species deemed important to the study were missing large parts of data for one gene. This was due in part to the lack of available tissues or GenBank sequences and in part to the inability to amplify or sequence certain regions. In these instances, the available sequences were used, and unknown regions were coded as missing data. Additionally, 7 species in the 65-species data set were missing data for one of the two nuclear genes (Table 2-1). In the case of *Limnophys anomalus*, the sequence of the closely related species *L. bufoniformis* was substituted for the *Tyr* fragment because *L. anomalus* was the only species of *Limnophys* in the 65 species data set and *Tyr* is the least variable gene in our study.

Model choice for likelihood and minimum evolution analyses were based partly on the limitations of the software packages used. Our preferred model for all analyses was GTR + I + G. However, neither RAxML nor MEGA can employ this model. Therefore, substitute models available in each package close to the GTR + I + G model were chosen for likelihood and minimum evolution analyses.

Bayesian analyses were run for 20,000,000 (280-species), 10,000,000 (146-species), or 2,000,000 (65-species) generations with three heated and one cold chain. Chains were sampled every 1,000, 500, or 100 generations, respectively. The first 25% of samples were discarded as burn-in. To ensure that this was an adequate number of samples discarded for each analysis, plots of log likelihood vs. generation were produced for every Bayesian analysis. In all cases, the region of increasing log likelihood values was encompassed in the first 25% of samples. Convergence for each Bayesian analysis was assessed by using the program Tracer 1.3 (Rambaut and Drummond 2005) to obtain estimated sample sizes for each model parameter (six substitution frequency categories, four nucleotide frequency categories, g-parameter, proportion of invariant sites, tree length, and log likelihood). Estimated sample sizes of each parameter were >100 for both independent runs of nearly all analyses, except for a substitution frequency (ESS = 99) and a nucleotide frequency parameter (ESS = 90) for one of the 146-species runs, the tree length parameter (ESS = 98) for the other 146-species run, and the tree length parameter (ESS = 94) for one run of the 65-species nuclear + mitochondrial data set. All ESS values were >200 when the two independent runs of each analysis were combined.
For the 65-species dataset, ME, ML, and Bayesian analyses were run with the nuclear gene data set separately (data not shown). The same major clades appeared in all of these analyses.

Divergence Time Estimation. Times of divergence were estimated for the 65-species data set by using the T3 version of Multidivtime (Thorne and Kishino 2002; Yang and Yoder 2003). The assumed topology was from the five-gene ML analysis. The data were divided into three partitions, as in the phylogenetic analyses. In addition to estimating times by using all available data, timing analyses were also performed by using mitochondrial and nuclear data separately. A total of five calibrations, including

both upper and lower bounds within and outside the eleutherodactylines, were used based on geologic and fossil evidence.

Geologic times and boundaries of periods used here are from a recent update (Gradstein et al. 2005). The five chosen calibrations were based on several independent lines of evidence. Jamaica did not become permanently emergent until 10 Mya (Mitchell 2004; Donovan 2002), setting a maximum time for basal divergences in the Jamaican clade (including representative species *E. gossei* and *E. luteolus*). The Hispaniolan South Island has a similar geologic history (Iturralde-Vinent and MacPhee 1999), setting a maximum time of 10 Mya for the basal divergence in the South Island clade (e.g., between representative species *E. thorectes* and *E. caribe*). An *Eleutherodactylus* fossil in amber from northern Hispaniola (Poinar and Cannatella 1987) is dated 15-20 Mya (Iturralde-Vinent and MacPhee 1996). The fossil is assumed to be in the subgenus *Eleutherodactylus* based on location (North Island of Hispaniola), age (older than uplift of South Island), and normal head width as compared with members of the subgenus *Pelorius* (which are wide-headed) (Hedges 1989a). This establishes a minimum date for the origin of the lineage leading to the subgenus *Eleutherodactylus* on Hispaniola (divergence of the subgenera *Pelorius* and *Eleutherodactylus*). Australian hylids (pelodryadines) are most closely related to South American hylids (phyllomedusines), and probably arrived by overland dispersal via Antarctica (Maxson et al. 1975; Duellman and Trueb 1986). The time window for this dispersal was 35-70 Mya (Sanmartin and Ronquist 2004; Woodburne and Case 1996; Li and Powell 2001; Springer et al. 1998), providing constraints for the divergence of representatives *Litoria caerulea* and *Agalychnis callidryas*.

Multidivtime requires prior estimates for rttm, rtsd, rtrate, rtratesd, brownmean, brownsd, and bigtime. The prior for the rttm (in-group root) parameter was set at 65 Mya based on recent data (Roelants et al. 2007) that place hyloid family divergences near the K-T boundary. Rttmsd, the standard deviation of rttm, was set at 25 Mya. This is a conservative estimate, considering that the true date of the ingroup root is almost certainly less than one standard deviation from rttm in either direction. Higher values of rttm (80 Mya) and rttmsd (40 Mya) were also used to test their results on timing analyses. Changing these priors had little effect on resulting dates, which differed by <3% when

both rttm and rttmsd are increased, and by even less if only one of the priors was increased. The prior for rtrate was set at 0.0075 for the mt analysis, 0.0015 for the nuclear analysis, and 0.0035 for the combined analysis. These values were obtained by first dividing the typical root-to-tip branch length for each gene (as determined by estbranches in the Multidivtime package) by rttm, and then taking the average of these values for the genes used in each respective analysis. As a conservative measure, the prior for rtratesd was set equal to rtrate in each analysis. The prior for brownmean was set at 0.0125, arrived at by dividing one by rttm. The prior for brownsd was set equal to brownmean because of the large measure of uncertainty in the prior for brownmean. Bigtime was set at 150 Mya. All other parameters (minab, newk, othk, thek) were maintained at default values. Analyses were run for 1,100,000 generations, with a sample frequency of 100 after a burn-in of 100,000 generations.

2.4 Results

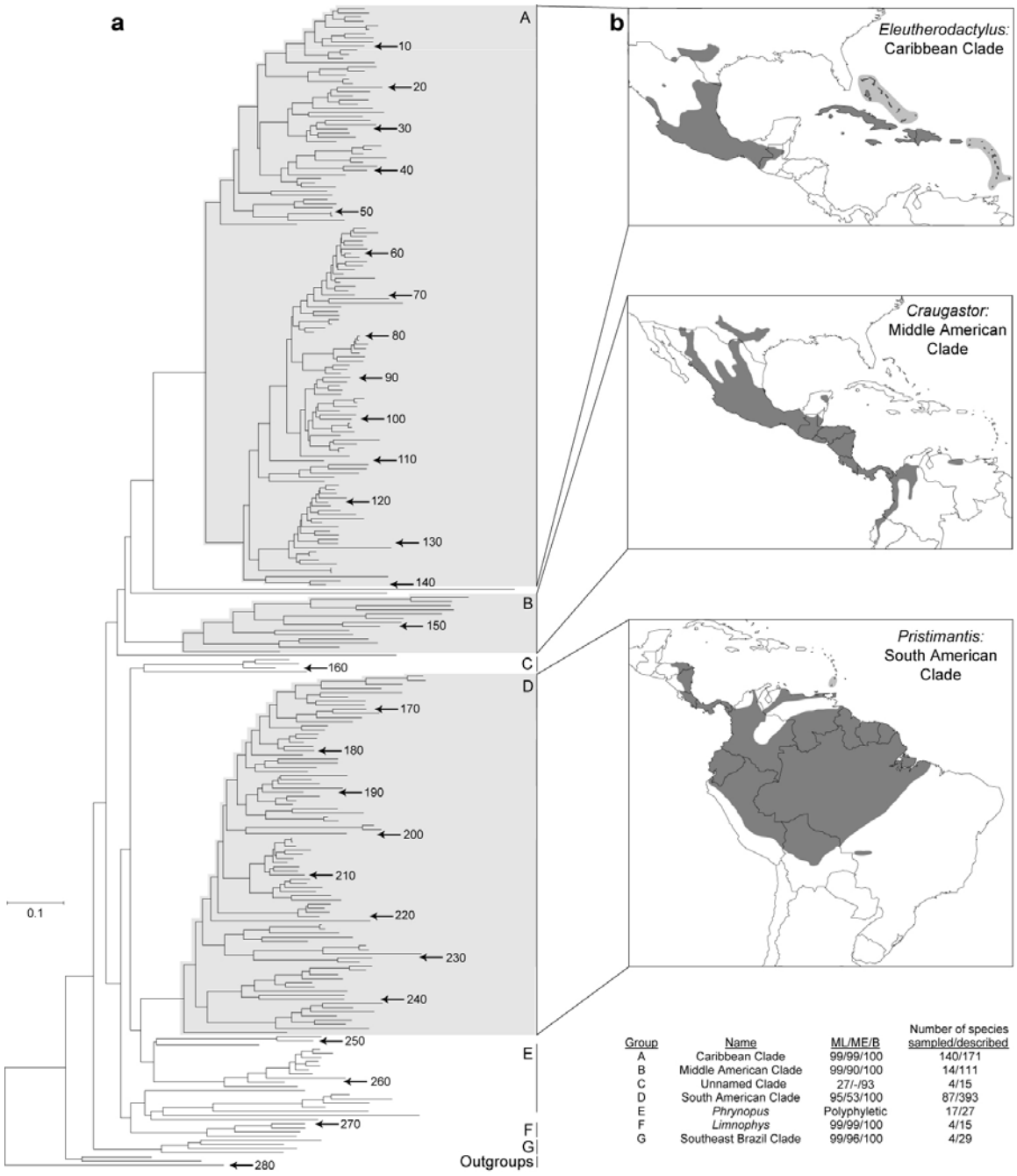
Major Clades of Eleutherodactylines. After alignment and removal of ambiguous regions, the 280-species data set encompassed 1,206 sites. The 146- and 65-species data sets included 2,578 and 3,709 sites, respectively. Maximum likelihood (ML), minimum evolution (ME), and Bayesian methods defined the same major clades for all data sets [Figs.2-2 and 2-3]. Support values for these three groups were variable in the 280-species data set, but were uniformly significant for all methods when the data sets encompassing more nucleotide sites were used.

The three largest and most diverse groups of species are largely defined by geography, with one dominant group each in the Caribbean region, Middle America, and northern South America. A smaller fourth group is found in southeast Brazil. By using past species–group affiliations, it was possible to assign species not included in this study to these major genetically defined clades. The first major group, which we call the Caribbean Clade (*Eleutherodactylus*), consists of the West Indian members of the subgenus *Eleutherodactylus* (47 sp.), the subgenus *Pelorius* of Hispaniola (6 sp.), the West Indian subgenus *Euhyas* (91 sp.), and the subgenus *Syrrhophus* (26 sp.) of southern North America, Middle America, and Cuba.

A second large group (111 sp.) of eleutherodactyline frogs occurs in Middle America, and already has been recognized as the subgenus or genus *Craugastor* (Frost et al. 2006; Hedges 1989a; Crawford and Smith 2005; Lynch 1986). Our analyses indicate a slightly different composition of this Middle American Clade. Previous definitions included some primarily South American species (Lynch 2000), which we find to form a separate clade that is, instead, most closely related to other South American eleutherodactylines (see below). The single remaining South American endemic, the distinctive *C. biporcatus*, warrants further study with DNA sequences to verify its placement in *Craugastor* (Savage and Myers 2002).

The third and largest group defined in our analyses includes nearly 400 species centered in the Andes but with species also occurring elsewhere in northern South America. A few species in this group extend into Central America, including nine endemic to southern portions of that region. Also, two species occur in the southernmost islands of the Lesser Antilles (*Pristimantis euphronides* and *P. shrevei*). This South American Clade includes species formerly placed in the *Eleutherodactylus unistrigatus*, *conspicillatus*, and 13 other species groups (Lynch and Duellman 1997). We use the available name *Pristimantis* Jiménez de la Espada, 1870 for this previously undefined clade.

Besides these three major clades, our analyses suggest that most of the 31 species in southeastern Brazil formerly placed in *Eleutherodactylus* form a separate, smaller clade (Figs. 2-2, 2-3). Our sparse taxonomic sampling from this region makes it difficult to determine the composition of this group, but the joining of four diverse species (*E. guentheri*, *E. hoehnei*, *E. parvus*, and *E. juipoca*) in a well supported group, suggests that other species from the region believed to be closely related to them also are part of that group, which takes the available name *Ischnocnema* Reinhardt and Lütken, 1862. Four southeast Brazilian species in our analysis that are not part of that clade are *E. binotatus*, which has an unusual karyotype (Siqueira et al. 2004), *Holoaden bradei*, *Barycholos ternetzi*, and *Brachycephalus ephippium*. These species also branch basally among eleutherodactylines but are not closely related to other species or groups.



Group	Name	ML/ME/B	Number of species sampled/described
A	Caribbean Clade	99/99/100	140/171
B	Middle American Clade	99/90/100	14/111
C	Unnamed Clade	27/-/93	4/15
D	South American Clade	95/53/100	87/393
E	*Phrynopus*	Polyphyletic	17/27
F	*Limnophys*	99/99/100	4/15
G	Southeast Brazil Clade	99/96/100	4/29

Figure 2-2. Major clades of eleutherodactyline frogs. (*a*) ML phylogeny of 280 species of frogs including eleutherodactylines, *Brachycephalus*, and three out-group species. Species are numbered according to Table 2-1. Major groups with support values (ML bootstrap/ME bootstrap/Bayesian posterior probability), number of species sampled, and total number of described species per clade are indicated. ML, ME, and Bayesian trees including taxon names and all confidence values are available (http://www.pnas.org/content/104/24/10092/suppl/DC1). (*b*) Distribution of Caribbean, Middle American, and South American clades.

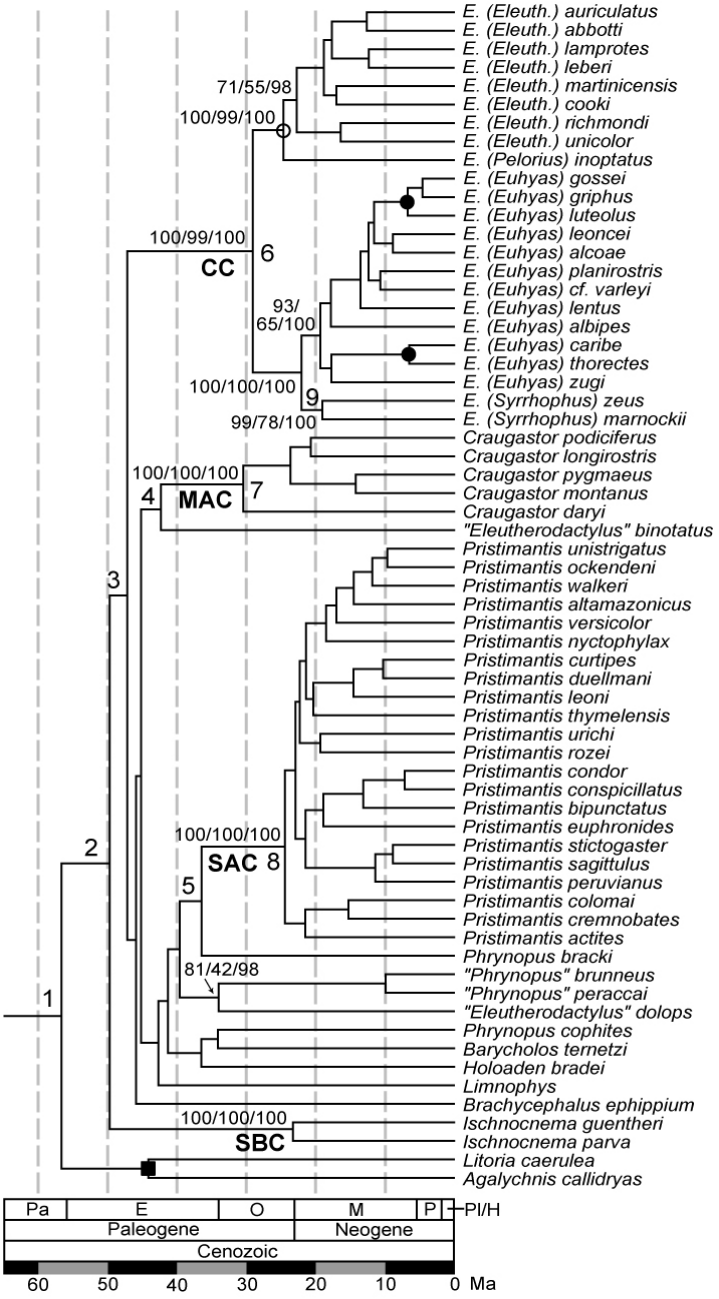


Figure 2.3. A time tree of eleutherodactyline frogs. The tree topology is derived from a ML analysis of 61 eleutherodactylines, *Brachycephalus*, and three out-group species. Support values for groups mentioned in the text are indicated at nodes (ML/ME/Bayesian posterior probability). Calibration nodes are indicated by open circle (minimum constraint), filled circles (maximum constraint), or filled square (minimum and maximum constraints). The two proposed oceanic dispersal events are on the branches leading to the Caribbean Clade (CC) and the Middle American Clade (MAC). [The South American Clade (SAC) and Southeast Brazil Clade (SBC) are indicated.] Times and credibility intervals for numbered nodes are shown in Table 2-3. Geologic epochs are abbreviated as follows: Paleocene (Pa), Eocene (E), Oligocene (O), Miocene (M), Pliocene (P), Pleistocene (Pl), Holocene (H).

These major clades of species with definitive geographic patterns account for 87% of the 812 species of eleutherodactyline frogs and *Brachycephalus*. The remaining 106 species are all native to South America, mostly Andean, and are best characterized by their basal position in the phylogenetic trees (Figs. 2-2, 2-3), suggesting that they represent an early stage of evolution of the group. Their relationships and those of the three major clades remain unresolved. Among these are the representatives of the (formerly *Craugastor*) *anomalus* and *bufoniformis* groups, which cluster strongly with a species in the *E. sulcatus* group. For this clade, we apply the available name *Limnophys* Jiménez de la Espada, 1871. The genus *Phrynopus* is polyphyletic, with species forming several independent groups, as was found elsewhere (Lehr et al. 2005). Two species of *Phrynopus* cluster with species of the *E. nigrovittatus* and *E. dolops* groups. Other genera in this category of deeply branching lineages include *Oreobates* and *Phyllonastes*.

Times of Divergence. Dates of divergence obtained by using nuclear data, mitochondrial data, or all data are similar for most nodes; reported dates refer to the combined data (Table 2-3). The eleutherodactyline lineage diverged from other hyloid frogs near the Mesozoic–Cenozoic boundary (57 Mya, C.I. = 78–44 Mya), as found elsewhere (Roelants et al. 2007), with initial divergences occurring among eleutherodactylines ≈50 Mya (Fig. 2-3). The Caribbean Clade (*Eleutherodactylus*) diverged from its extant mainland relatives ≈47 Mya and began diversification ≈29 Mya, setting upper and lower bounds for the date that the West Indies was colonized. Assuming no extinction of the mainland source lineage, the dispersal most likely occurred early in that time interval rather than later. Similarly, the Middle American Clade (*Craugastor*) diverged 42 Mya and began diversification 31 Mya. Middle American and Cuban *Syrrhophus* split ≈19 Mya. The Southeast Brazil Clade diverged from other eleutherodactylines ≈50 Mya. The South American Clade (*Pristimantis*) diverged from other eleutherodactylines 37 Mya and began an explosive diversification 24 Mya.

2.5 Discussion

Major Clades of Tropical Frogs. The discovery of three major and geographically defined groups of these tropical amphibians was unexpected. Previous

studies on eleutherodactylines had been hampered by too few useful morphological characters and too few samples for molecular analysis. Although the Middle American Clade was known (Crawford and Smith 2005; Lynch 1986), it had included species in South America shown here to be misclassified based on our sequence analyses. The Caribbean and South American clades, on the other hand, were unpredicted. Previous studies had assumed a close relationship between West Indian members of the subgenus *Eleutherodactylus* and the species-rich *unistrigatus* group (now in *Pristimantis*) in South America (Lynch and Duellman 1997; Hass and Hedges 1991; Hedges 1989a; Joglar 1989). In part, this was based on shared morphological characters that may be associated with climbing habits (Hedges 1989a). Our results show, however, that diverse morphologies and habits have evolved independently in the Caribbean and South American Clades. The geographical separation of these large clades highlights a general pattern, the greater importance of geography, revealed in many molecular phylogenetic studies (e.g., Zhang et al. 2006; Bossuyt et al. 2004).

Middle America and the Caribbean. The origin of the Middle American and West Indian terrestrial vertebrates has focused on two competing models in the context of current geologic models for the region (Hedges 2001; Iturralde-Vinent and MacPhee 1999; Pindell 1994). The vicariance model suggests that they arose in the Late Cretaceous (80–70 Mya) by fragmentation of a continuous land mass (proto-Antilles) and its biota located between North and South America (Rosen 1975, 1985; Savage 1982). This occurred as the Caribbean tectonic plate moved eastward, carrying the West Indian fauna and isolating the Middle American fauna from its South American counterparts. This is in contrast to an origin of these faunas by dispersal, on flotsam from continental source areas. One difficulty for the vicariance model has been the great age (Cretaceous) of the groups required for this model, which is largely unsupported by the fossil record (Pregill 1981). Also, the fauna of the West Indies is peculiar in missing many higher-level groups, indicative of dispersal (Williams 1989). Geologic evidence does not rule out the possibility of a proto-Antillean island chain or corridor, but does not favor the substantial emergence of land in the Antilles before the mid-Eocene (37–49 Mya) (Iturralde-Vinent and MacPhee 1999).

Molecular clock analyses have yielded mixed results, although most groups have shown Cenozoic divergences with their closest relatives on the mainland (Hedges 2001; Hedges et al. 1992b; Hedges 1996a). Estimates of Cretaceous divergence between West Indian and mainland representatives of insectivores (Roca et al. 2004), xantusiid lizards (Roca et al. 2004; Hedges 2006b), and (in past studies) eleutherodactyline frogs (Hass and Hedges 1991; Hedges 1996a), suggested that those groups may be vicariant relicts of the proto-Antilles even if most others are not. However, the relictual nature of the distribution of xantusiid lizards and West Indian insectivores raises the possibility of Cenozoic dispersal to the West Indies and subsequent extinction of those mainland source populations (Hedges 2006b). Studies indicating Cretaceous ages for Middle American and West Indian eleutherodactylines either assumed proto-Antillean vicariance (Crawford and Smith 2005) or used geologic calibrations that have since been revised (Hass and Hedges 1991).

Based on our results, the Middle American and Caribbean clades of eleutherodactylines originated through dispersal from South America during the Cenozoic. For these clades to have originated through proto-Antillean vicariance, Mesozoic ages (e.g., 80–70 Mya) are required for divergences between these groups and their South American relatives. Instead, our data (Table 2-3) indicate that a single event 42–31 Mya established eleutherodactylines in Middle America, and another 47–29 Mya established eleutherodactylines in the West Indies (Fig. 2-4*a*). Early speciation in the Caribbean Clade was confined to Hispaniola and Cuba. The paleogeography of the West Indies in the mid-Cenozoic was substantially different from that today (Iturralde-Vinent and MacPhee 1999). Land connections between Cuba, northern Hispaniola, and Puerto Rico probably existed in the Late Eocene (≈35 Mya), facilitating dispersal among the islands. A proposed dry-land connection to South America at this time (Iturralde-Vinent and MacPhee 1999) lacks geologic support and remains controversial (Hedges 2001, 2006b). After subsidence in the Oligocene (23–34 Mya), land connections were broken, probably isolating the western Caribbean lineage (subgenera *Euhyas* plus *Syrrhopus*) in Cuba from the eastern Caribbean lineage (subgenera *Eleutherodactylus* plus *Pelorius*) in northern Hispaniola and Puerto Rico (Fig. 2-4*b*).

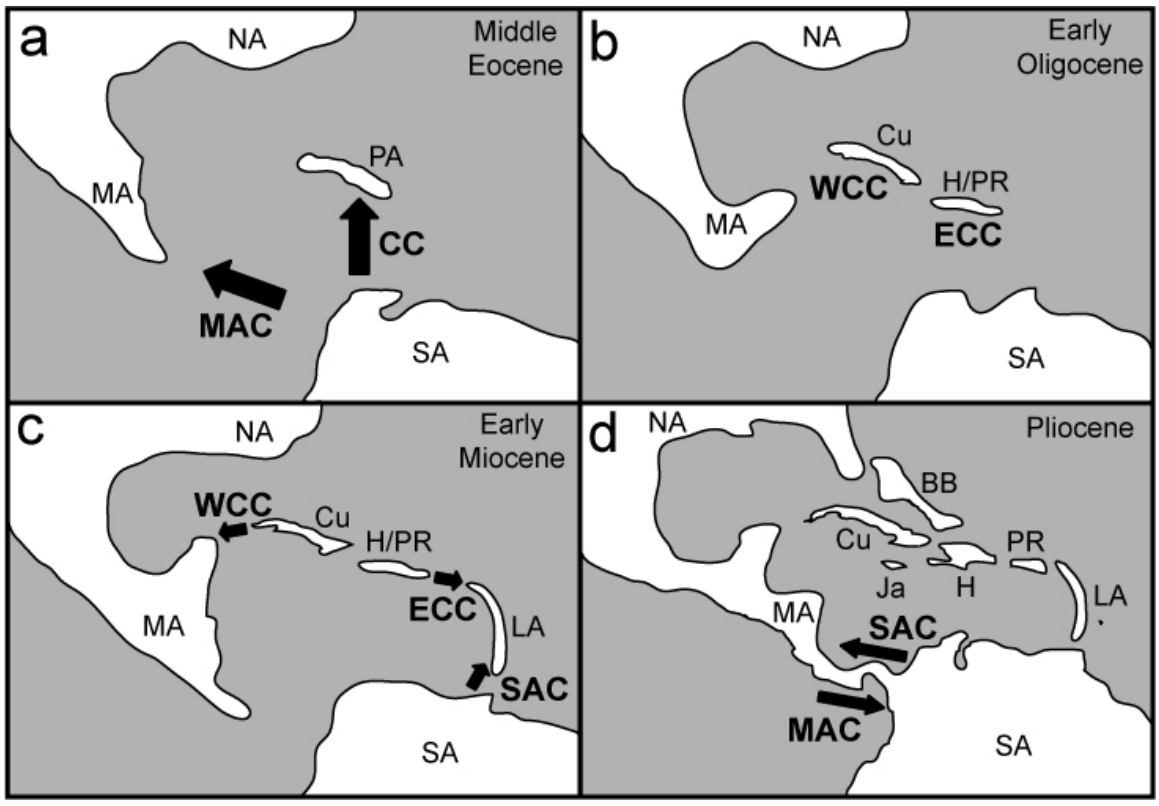


Figure 2-4. Biogeographic model showing the origin of the Middle American and Caribbean clades of eleutherodactyline frogs. Location of exposed land is conjectural and based on a synthesis of models (Iturralde-Vinent and MacPhee 1999; Pindell 1994; Pindell and Kennan 2002). Landmasses are abbreviated as follows: North America (NA), Middle America (MA), South America (SA), Proto-Antilles (PA), Cuba (Cu), Hispaniola (H), Puerto Rico (PR), Lesser Antilles (LA), Jamaica (Ja), Bahama Bank (BB). (*a)* Middle Eocene (49–37 Mya), when dispersal over water from South America probably occurred, leading to the origin of the Middle American Clade (MAC) and Caribbean Clade (CC). (*b*) Early Oligocene (≈30 Mya), when land subsidence and higher sea levels led to isolation of a western Caribbean (WCC) lineage on Cuba and an eastern Caribbean (ECC) lineage on Hispaniola and Puerto Rico. (*c*) Early Miocene (≈20 Mya), when dispersal from Cuba to the mainland led to a radiation of the subgenus *Syrrhopus* in southern North America and northern Middle America and when the Lesser Antilles were colonized by members of the ECC and South American Clade (SAC). (*d*) Pliocene (≈3 Mya), when closing of the Isthmus of Panama allowed overland dispersal of species of the MAC to South America and species of the SAC to Middle America.

In the Early Miocene (19 Mya), an over-water dispersal occurred from western Cuba to southern North America within the subgenus *Syrrhophus* (Fig. 2-4*c*), as indicated by some earlier molecular studies (Hass and Hedges 1991; Hedges 1989a). It is possible that this lineage initially evolved in isolation to the north of the Middle American Clade, although the distributions of these two groups currently overlap. Dispersal from the

Greater Antilles to the mainland has been found in other vertebrate groups, including turtles (Seidel 1988; 1996) and anoline lizards (Nicholson et al. 2005). Other Miocene dispersals of eleutherodactylines, most probably over water, occurred among islands in the West Indies (Fig. 2-3). The direction of some of these dispersal events would have been against the present-day water currents, which flow primarily from southeast to northwest. However, current flow within the Caribbean may have been different in the past, before the emergence of the Isthmus of Panama (Droxler et al. 1998).

A striking pattern in these results is the absence of subsequent successful colonizations of eleutherodactyline frogs in Middle America and the West Indies from South America after their origin in the early Cenozoic. Of the few exceptions, two species of the South American clade now occupy the southernmost Lesser Antilles (St. Vincent and Grenada) and 18 species of the South American Clade now occur in Middle America (including nine endemics). In the latter case, the presence of some or all of those species may be explained by dispersal over land after the emergence of the Isthmus of Panama (≈3 Mya). However, the possibility of over-water dispersal for *C. biporcatus* or endemic Central American *Pristimantis* cannot be excluded. Our data set includes none of these endemics, so no determination based on times of divergence can be made. Whether or not there were failed colonizations to Middle America and the West Indies as a result of competition (Williams 1969) is unknown. Also, if the Middle American Clade and Caribbean Clade are later found to be closest relatives, the possibility that there was a stepwise dispersal (South America to one clade and from that clade to the other) should be considered.

South America. Most of the basal branches of eleutherodactylines, with some dating to the early Cenozoic, occur in South America (Fig. 2-3). This indicates that South America was the place of origin for the group, as it was for hyloid frogs in general (Darst and Cannatella 2004; Ruvinsky and Maxson 1996). However, the great diversity of species, including the South American Clade of 393 species, is associated with Andes. The Andean uplift is relatively recent, occurring mostly in the last 10–20 million years (Gregory-Wodzicki 2002; MacFadden 2006). Rapid diversification within the South American Clade, which began 24 Mya and has continued to the present, was probably linked with this uplift. Mountain-building and associated climatic changes resulted in

repeating patterns of habitat isolation, which, in turn, probably resulted in genetic isolation and speciation in these amphibians (Lynch and Duellman 1997).

Despite the large number of South American species included in this analysis (123 sp.), we are missing a majority of species including many from southeastern Brazil. Our results indicate that the eleutherodactyline fauna of southeastern Brazil is distinct and includes several basal clades. This region is an isolated area of montane rainforest and is a region of endemism for other amphibians (Duellman 1999).

2.6 Acknowledgments

Lori Fritz, Jennifer R. Grubb, Genna M. Lutz, Molly E. Means, Rebecca Montrose, Michael R. Tracy, and Cecelia Youngblood contributed to the initial generation of data for this project. We thank Jonathan A. Campbell and Eric N. Smith (University of Texas, Arlington, TX), David Cannatella (University of Texas, Austin, TX), Luis A. Coloma (Catholic University of Ecuador, Quito, Ecuador), Andrew J. Crawford (Smithsonian Tropical Research Institute, Balboa, Republic of Panama), Luis Diaz (Museum of Natural History, Havana, Cuba), Ronald Heyer and Addison Wynn (Smithsonian Institution, Washington, DC), Edgar Lehr (University of Kansas), John D. Lynch (National University of Colombia, Bogata, Colombia), Linda R. Maxson (University of Iowa, Iowa City, IA), Lily Rodriguez (University of San Marcos, Lima, Peru), Michael Schmid (University of Würzburg, Würzburg, Germany), and Steven Werman (Mesa State College, Grand Junction, CO), for providing some samples and sequences; Paul Mann for discussion of geological history; and Bernard Moret for providing computer time on the NSF-funded CIPRES cluster at the San Diego Supercomputer Center. Richard Thomas (University of Puerto Rico, San Juan, Puerto Rico) contributed significantly to field work in the West Indies. This research was supported by National Aeronautics and Space Administration Grant NNA04CC06A (to S.B.H.) and by National Science Foundation (NSF) Grants 8906325, 9123556, 9525775, and 9615643 (to S.B.H.), 8307115 (to Richard Highton, University of Maryland, College Park, MD), 8805920 (to W.E.D.), and especially by NSF-Assembling the Tree of Life (AToL) Amphibia-Tree Project Grant 0334952 (to David Cannatella and David Hills, University of Texas, Austin, TX).

Table 2-1. Specimens used in this study. GenBank accession number, datasets in which species appear, and museum voucher numbers (for newly-sequenced specimens) are reported. Museum abbreviations are AMNH (American Museum of Natural History), BWMC (Bobby Witcher Memorial Collection, Avila University), KU (University of Kansas Museum of Natural History), MNHNCu (National Museum of Natural History, Havana, Cuba), MVZ (Museum of Vertebrate Zoology, University of California, Berkeley), QCAZ (Catholic University of Ecuador, Museum of Zoology), UPRRP (University of Puerto Rico, at Rio Piedras, Museum of Natural History), USNM (United States National Museum of Natural History, Smithsonian Institution), UTA (University of Texas at Arlington, Museum of Natural History), MHNSM (Universidad Nacional Mayor de San Marcos, Lima, Peru). Specimens are numbered as they appear in Fig. 2-2.

			Museum	Genbank Accession Number				Dataset		
Number	Genus	Species	Voucher	12S	16S	Rag1	Tyr	280	146	65
1	*Eleutherodactylus*	*portoricensis*	USNM326885	EF493720	EF493548			x		
2	*Eleutherodactylus*	*wightmanae*	USNM326905	EF493721	EF493549			x		
3	*Eleutherodactylus*	*coqui*	USNM305421	EF493722	EF493550			x		
4	*Eleutherodactylus*	*schwartzi*	No voucher	EF493723	EF493551			x		
5	*Eleutherodactylus*	*gryllus*	USNM269304	EF493724	EF493552			x		
6	*Eleutherodactylus*	*sp. 1*	UPRRP6361	EF493538	EF493365			x		
7	*Eleutherodactylus*	*cochranae*	USNM326775	EF493725	EF493555			x		
8	*Eleutherodactylus*	*hedricki*	USNM564995	EF493726	EF493553			x		
9	*Eleutherodactylus*	*brittoni*	USNM326765	EF493727	EF493554			x		
10	*Eleutherodactylus*	*antillensis*	USNM326747	EF493728	EF493556			x		
11	*Eleutherodactylus*	*eneidae*	USNM326857	EF493729	EF493557			x		
12	*Eleutherodactylus*	*locustus*	USNM326861	EF493730	EF493558			x		
13	*Eleutherodactylus*	*cooki*	USNM326784	EF493539		EF493413	EF493455	x	x	x
14	*Eleutherodactylus*	*flavescens*	USNM331662	EF493731	EF493559			x		
15	*Eleutherodactylus*	*martinicensis*	USNM565001	EF493343		EF493419	EF493456	x	x	x
16	*Eleutherodactylus*	*amplinympha*	USNM564978	EF493732	EF493560			x		
17	*Eleutherodactylus*	*johnstonei*	USNM336018	EF493733	EF493561			x		
18	*Eleutherodactylus*	*pinchoni*	USNM565006	EF493734	EF493562			x		
19	*Eleutherodactylus*	*barlagnei*	USNM564982	EF493735	EF493563			x		
20	*Eleutherodactylus*	*auriculatus*	USNM564980	EF493344		EF493417	EF493458	x	x	x
21	*Eleutherodactylus*	*bartonsmithi*	USNM309753	EF493736	EF493576			x		
22	*Eleutherodactylus*	*glamyrus*	USNM564987	EF493737	EF493575			x		
23	*Eleutherodactylus*	*mariposa*	MNHNCu591	EF493738	EF493573			x		
24	*Eleutherodactylus*	*ronaldi*	USNM309760	EF493739	EF493574			x		
25	*Eleutherodactylus*	*eileenae*	No voucher	EF493740	EF493577			x		
26	*Eleutherodactylus*	*minutus*	USNM331987	EF493741	EF493578			x		
27	*Eleutherodactylus*	*poolei*	USNM332236	EF493742	EF493579			x		
28	*Eleutherodactylus*	*haitianus*	No voucher	EF493743	EF493583			x		
29	*Eleutherodactylus*	*abbotti*	USNM564974	EF493540		EF493412	EF493457	x	x	x
30	*Eleutherodactylus*	*audanti*	USNM331514	EF493744	EF493584			x		
31	*Eleutherodactylus*	*sp. 2*	USNM337773	EF493745	EF493580			x		
32	*Eleutherodactylus*	*parabates*	USNM332136	EF493746	EF493581			x		
33	*Eleutherodactylus*	*pituinus*	USNM332229	EF493747	EF493582			x		
34	*Eleutherodactylus*	*ionthus*	USNM309757	EF493748	EF493564			x		
35	*Eleutherodactylus*	*guantanamera*	MNHNCu590	EF493749	EF493565			x		
36	*Eleutherodactylus*	*varians*	USNM309763	EF493750	EF493566			x		
37	*Eleutherodactylus*	*leberi*	USNM309758	EF493342		EF493403	EF493459	x	x	x
38	*Eleutherodactylus*	*melacara*	USNM309733	EF493751	EF493567			x		
39	*Eleutherodactylus*	*lamprotes*	USNM564997	EF493379			EF493460	x	x	x
40	*Eleutherodactylus*	*fowleri*	USNM269266	EF493752	EF493568			x		
41	*Eleutherodactylus*	*wetmorei*	USNM332369	EF493753	EF493569			x		
42	*Eleutherodactylus*	*auriculatoides*	USNM331627	EF493754	EF493572			x		
43	*Eleutherodactylus*	*patriciae*	No voucher	EF493755	EF493570			x		
44	*Eleutherodactylus*	*montanus*	USNM332069	EF493756	EF493571			x		
45	*Eleutherodactylus*	*richmondi*	USNM326894	EF493541			EF493461	x	x	x

Number	Genus	Species	Museum Voucher	Genbank Accession Number				Dataset		
				12S	16S	Rag1	Tyr	280	146	65
46	*Eleutherodactylus*	*unicolor*	USNM326897	EF493542		EF493398	EF493462	x	x	x
47	*Eleutherodactylus*	*hypostenor*	USNM257731	EF493757	EF493585			x		
48	*Eleutherodactylus*	*parapelates*	USNM257726	EF493758	EF493587			x		
49	*Eleutherodactylus*	*ruthae*	USNM257752	EF493759	EF493586			x		
50	*Eleutherodactylus*	*nortoni*	USNM257744	EF493760	EF493588			x		
51	*Eleutherodactylus*	*chlorophenax*	USNM257730	EF493543	EF493589			x		
52	*Eleutherodactylus*	*inoptatus*	USNM331931	EF493380		EF493405	EF493463	x	x	x
53	*Eleutherodactylus*	*counouspeus*	USNM329989	EF493719				x	x	
54	*Eleutherodactylus*	*cundalli*	USNM266364	EF493761	EF493612			x		
55	*Eleutherodactylus*	*glaucoreius*	USNM305366	EF493762	EF493613			x		
56	*Eleutherodactylus*	*cavernicola*	USNM266357	EF493763	EF493614			x		
57	*Eleutherodactylus*	*gossei*	USNM327419	EF493716		EF493410	EF493466	x	x	x
58	*Eleutherodactylus*	*junori*	USNM269239	EF493764	EF493617			x		
59	*Eleutherodactylus*	*griphus*	USNM564992	EF493381		EF493415	EF493465	x	x	x
60	*Eleutherodactylus*	*pentasyringos*	USNM266455	EF493765	EF493615			x		
61	*Eleutherodactylus*	*pantoni*	USNM327822	EF493766	EF493616			x		
62	*Eleutherodactylus*	*orcutti*	USNM327808	EF493767	EF493619			x		
63	*Eleutherodactylus*	*alticola*	USNM266340	EF493768	EF493620			x		
64	*Eleutherodactylus*	*fuscus*	USNM266380	EF493769	EF493618			x		
65	*Eleutherodactylus*	*jamaicensis*	USNM327594	EF493770	EF493621			x		
66	*Eleutherodactylus*	*andrewsi*	USNM327267 USNM327274	EF493544	EF493623			x		
67	*Eleutherodactylus*	*nubicola*	USNM327777	EF493771	EF493622			x		
68	*Eleutherodactylus*	*grabhami*	USNM327565	EF493772	EF493624			x		
69	*Eleutherodactylus*	*sisyphodemus*	USNM266467	EF493773	EF493625			x		
70	*Eleutherodactylus*	*luteolus*	USNM327744	EF493545			EF493464	x	x	x
71	*Eleutherodactylus*	*riparius*	n/a	Y10944				x	x	
72	*Eleutherodactylus*	*rivularis*	USNM565009	EF493376	EF493626			x		
73	*Eleutherodactylus*	*toa*	USNM306544	EF493774	EF493627			x		
74	*Eleutherodactylus*	*cuneatus*	USNM564985	EF493775	EF493608			x		
75	*Eleutherodactylus*	*turquinensis*	USNM348803	EF493776	EF493609			x		
76	*Eleutherodactylus*	*darlingtoni*	USNM307236	EF493777	EF493610			x		
77	*Eleutherodactylus*	*leoncei*	USNM564999	EF493375	EF493715	EF493404	EF493468	x	x	x
78	*Eleutherodactylus*	*alcoae*	USNM564977	EF493382		EF493406	EF493469	x	x	x
79	*Eleutherodactylus*	*armstrongi*	USNM329962	EF493778	EF493611			x		
80	*Eleutherodactylus*	*rhodesi*	USNM332259	EF493779	EF493629			x		
81	*Eleutherodactylus*	*weinlandi*	USNM332332	EF493780	EF493630			x		
82	*Eleutherodactylus*	*grahami*	USNM564990	EF493781	EF493632			x		
83	*Eleutherodactylus*	*pictissimus*	USNM266310	EF493782	EF493631			x		
84	*Eleutherodactylus*	*lentus*	USNM564998	EF493717		EF493418	EF493471	x	x	x
85	*Eleutherodactylus*	*monensis*	USNM565002	EF493783	EF493633			x		
86	*Eleutherodactylus*	*probolaeus*	USNM322252	EF493784	EF493634			x		
87	*Eleutherodactylus*	*bresslerae*	USNM564983	EF493785	EF493635			x		
88	*Eleutherodactylus*	*ricordii*	USNM565008	EF493786	EF493636			x		
89	*Eleutherodactylus*	*acmonis*	USNM564975	EF493787	EF493637			x		
90	*Eleutherodactylus*	*orientalis*	USNM565003	EF493373	EF493592			x		
91	*Eleutherodactylus*	*etheridgei*	USNM335715	EF493794	EF493593			x		
92	*Eleutherodactylus*	*limbatus*	USNM565000	EF493795	EF493590			x		
93	*Eleutherodactylus*	*iberia*	MNHNCu1001	EF493374	EF493591			x		
94	*Eleutherodactylus*	*cubanus*	No voucher	EF493796	EF493594			x		
95	*Eleutherodactylus*	*casparii*	USNM564984	EF493788	EF493599			x		
96	*Eleutherodactylus*	*planirostris*	USNM565007	EF493346		EF493396	EF493470	x	x	x
97	*Eleutherodactylus*	*guanahacabibes*	USNM564993	EF493789	EF493600			x		
98	*Eleutherodactylus*	*tonyi*	No voucher	EF493790	EF493602			x		
99	*Eleutherodactylus*	*rogersi*	USNM565010	EF493372	EF493603			x		
100	*Eleutherodactylus*	*goini*	USNM335725	EF493791	EF493604			x		
101	*Eleutherodactylus*	*thomasi*	No voucher	EF493370	EF493605			x		
102	*Eleutherodactylus*	*blairhedgesi*	No voucher	EF493371	EF493606			x		
103	*Eleutherodactylus*	*pinarensis*	USNM565005	EF493792	EF493607			x		
104	*Eleutherodactylus*	*atkinsi*	USNM335686	EF493797	EF493598			x		
105	*Eleutherodactylus*	*gundlachi*	USNM564994	EF493798	EF493597			x		
106	*Eleutherodactylus*	*cf. varleyi*	MNHNCu1002	EF493345		EF493408	EF493467	x	x	x
107	*Eleutherodactylus*	*intermedius*	USNM564996	EF493799	EF493595			x		
108	*Eleutherodactylus*	*varleyi*	USNM335732	EF493800	EF493596			x		

Number	Genus	Species	Museum Voucher	Genbank Accession Number				Dataset		
				12S	16S	Rag1	Tyr	280	146	65
109	*Eleutherodactylus*	*pezopetrus*	USNM565004	EF493793	EF493601			x		
110	*Eleutherodactylus*	*greyi*	USNM564991	EF493801	EF493628			x		
111	*Eleutherodactylus*	*emiliae*	No voucher	EF493368	EF493638			x		
112	*Eleutherodactylus*	*dimidiatus*	USNM564986	EF493802	EF493640			x		
113	*Eleutherodactylus*	*maestrensis*	MNHNCu1003	EF493369	EF493639			x		
114	*Eleutherodactylus*	*albipes*	USNM564976	EF493386		EF493409	EF493475	x	x	x
115	*Eleutherodactylus*	*schmidti*	USNM332313	EF493803	EF493641			x		
116	*Eleutherodactylus*	*eunaster*	No voucher	EF493804	EF493646			x		
117	*Eleutherodactylus*	*caribe*	USNM314179	EF493385		EF493411	EF493472	x	x	x
118	*Eleutherodactylus*	*corona*	KU218431	EF493807	EF493645			x		
119	*Eleutherodactylus*	*heminota*	USNM331829	EF493806	EF493649			x		
120	*Eleutherodactylus*	*amadeus*	USNM329866	EF493805	EF493644			x		
121	*Eleutherodactylus*	*bakeri*	USNM564981	EF493808	EF493647			x		
122	*Eleutherodactylus*	*glaphycompus*	USNM292259	EF493383				x	x	
123	*Eleutherodactylus*	*dolomedes*	KU218434	EF493809	EF493648			x		
124	*Eleutherodactylus*	*glanduliferoides*	USNM564989	EF493546	EF493364			x		
125	*Eleutherodactylus*	*thorectes*	USNM565011	EF493384		EF493416	EF493473	x	x	x
126	*Eleutherodactylus*	*jugans*	USNM331952	EF493810	EF493652			x		
127	*Eleutherodactylus*	*apostates*	USNM564979	EF493811	EF493650			x		
128	*Eleutherodactylus*	*oxyrhynchus*	USNM332073	EF493812	EF493651			x		
129	*Eleutherodactylus*	*rufifemoralis*	No voucher	EF493813	EF493653			x		
130	*Eleutherodactylus*	*furcyensis*	USNM331673	EF493814	EF493654			x		
131	*Eleutherodactylus*	*paulsoni*	USNM310833	EF493815	EF493659			x		
132	*Eleutherodactylus*	*glandulifer*	USNM564988	EF493816	EF493655			x		
133	*Eleutherodactylus*	*sciagraphus*	USNM332316	EF493817	EF493656			x		
134	*Eleutherodactylus*	*ventrilineatus*	USNM332320	EF493818	EF493658			x		
135	*Eleutherodactylus*	*brevirostris*	USNM329968	EF493819	EF493657			x		
136	*Eleutherodactylus*	*zugi*	USNM335744	EF493347		EF493401	EF493474	x	x	x
137	*Eleutherodactylus*	*klinikowskii*	MNHNCu1004	EF493547	EF493363			x		
138	*Eleutherodactylus (Syrrhophus)*	*marnockii*	No voucher n/a	EF493820 DQ283102	EF493642 DQ283101	EF493399	EF493476	x	x	x
139	*Eleutherodactylus (Syrrhophus)*	*symingtoni*	No voucher	EF493821	EF493643			x		
140	*Eleutherodactylus (Syrrhophus)*	*zeus*	USNM335740	EF493718		EF493402	EF493477	x	x	x
141	*Barycholos*	*ternetzi*	n/a		DQ283094		DQ284144	x	x	x
142	*"Eleutherodactylus"*	*binotatus*	USNM303077 n/a	EF493361		EF493397	DQ282918	x	x	x
143	*Craugastor*	*rhodopis*	n/a	DQ283317				x	x	
144	*Craugastor*	*mexicanus*	n/a	AY326006				x	x	
145	*Craugastor*	*podiciferus*	MVZ12020	EF493360		EF493450	EF493481	x	x	x
146	*Craugastor*	*bransfordii*	AMNH-A124398	EF493822	EF493661			x		
147	*Craugastor*	*longirostris*	KU177803	EF493395		EF493454	EF493482	x	x	x
148	*Craugastor*	*fitzingeri*	n/a	AY326001				x	x	
149	*Craugastor*	*sandersoni*	UTA-A49803	EF493712				x	x	
150	*Craugastor*	*punctariolus*	n/a	DQ283168				x	x	
151	*Craugastor*	*pygmaeus*	UTA-A55241	EF493711		EF493451	EF493479	x	x	x
152	*Craugastor*	*montanus*	UTA-A51105	EF493530		EF493453	EF493478	x	x	x
153	*Craugastor*	*augusti*	n/a	DQ283271				x	x	
154	*Craugastor*	*bocourti*	UTA-A55235	EF493713				x	x	
155	*Craugastor*	*alfredi*	n/a	DQ283318				x	x	
156	*Craugastor*	*daryi*	UTA-A57940	EF493531		EF493452	EF493480	x	x	x
157	*Brachycephalus*	*ephippium*	n/a n/a	AY326008 DQ283091			DQ282917	x	x	x
158	*"Phrynopus"*	*peraccai*	KU178266	EF493710		EF493420	EF493485	x	x	x
159	*"Phrynopus"*	*brunneus*	KU178258	EF493357		EF493422	EF493484	x	x	x
160	*"Eleutherodactylus"*	*elassodiscus*	KU177282	EF493358				x	x	
161	*"Eleutherodactylus"*	*dolops*	No voucher	EF493394		EF493414	EF493483	x	x	x
162	*Pristimantis*	*walkeri*	KU218116	EF493518		EF493428	EF493490	x	x	x
163	*Pristimantis*	*luteolateralis*	KU177807	EF493517				x	x	
164	*Pristimantis*	*parvillus*	KU177821	EF493351				x	x	
165	*Pristimantis*	*chalceus*	KU177638	EF493675				x	x	
166	*Pristimantis*	*ockendeni*	KU222023	EF493519		EF493434	EF493496	x	x	x
167	*Pristimantis*	*unistrigatus*	KU218057	EF493387		EF493444	EF493505	x	x	x
168	*Pristimantis*	*cajamarcensis*	KU217845	EF493823	EF493663			x		

Number	Genus	Species	Museum Voucher	Genbank Accession Number				Dataset		
				12S	16S	Rag1	Tyr	280	146	65
169	*Pristimantis*	*ceuthospilus*	KU 212216		EF493520			x	x	
170	*Pristimantis*	*lirellus*	KU212226		EF493521			x	x	
171	*Pristimantis*	*imitatrix*	KU215476	EF493824	EF493667			x		
172	*Pristimantis*	*croceoinguinis*	KU217862	EF493669	EF493665			x		
173	*Pristimantis*	*altamazonicus*	KU215460		EF493670	EF493441		x	x	x
174	*Pristimantis*	*orestes*	KU218257		EF493388			x	x	
175	*Pristimantis*	*simonbolivari*	KU218254		EF493671			x	x	
176	*Pristimantis*	*riveti*	KU218035		EF493348			x	x	
177	*Pristimantis*	*versicolor*	KU218096		EF493389	EF493431	EF493493	x	x	x
178	*Pristimantis*	*phoxocephalus*	KU218025		EF493349			x	x	
179	*Pristimantis*	*spinosus*	KU218052		EF493673			x	x	
180	*Pristimantis*	*cryophilius*	KU217863		EF493672			x	x	
181	*Pristimantis*	*rhodoplichus*	KU219788		EF493674			x	x	
182	*Pristimantis*	*wiensi*	KU219796	EF493377	EF493668			x		
183	*Pristimantis*	*petrobardus*	KU212293	EF493825	EF493367			x		
184	*Pristimantis*	*melanogaster*	MHNSM-WED56846	EF493826	EF493664			x		
185	*Pristimantis*	*simonsii*	n/a	AM039709	AM039641			x		
186	*Pristimantis*	*appendiculatus*	KU177637		EF493524			x	x	
187	*Pristimantis*	*pycnodermis*	KU218028		EF493680			x	x	
188	*Pristimantis*	*dissimulatus*	KU179090		EF493522			x	x	
189	*Pristimantis*	*calcarulatus*	KU177658		EF493523			x	x	
190	*Pristimantis*	*orcesi*	KU218021		EF493679			x	x	
191	*Pristimantis*	*glandulosus*	KU218002		EF493676			x	x	
192	*Pristimantis*	*inusitatus*	KU218015		EF493677			x	x	
193	*Pristimantis*	*acerus*	KU217786		EF493678			x	x	
194	*Pristimantis*	*schultei*	KU212220		EF493681			x	x	
195	*Pristimantis*	*bromeliaceus*	KU291702		EF493351			x	x	
196	*Pristimantis*	*subsigillatus*	KU218147		EF493525			x	x	
197	*Pristimantis*	*nyctophylax*	KU177812		EF493526	EF493425	EF493487	x	x	x
198	*Pristimantis*	*shrevei*	No voucher		EF493692			x	x	
199	*Pristimantis*	*euphronides*	BWMC6918		EF493527	EF493427	EF493489	x	x	x
200	*Pristimantis*	*rozei*	No voucher		EF493691	EF493429	EF493491	x	x	x
201	*Pristimantis*	*gentryi*	KU218109		EF493511			x	x	
202	*Pristimantis*	*truebae*	KU218013		EF493512			x	x	
203	*Pristimantis*	*curtipes*	KU217871		EF493513	EF493435	EF493497	x	x	x
204	*Pristimantis*	*vertebralis*	KU177972		EF493689			x	x	
205	*Pristimantis*	*buckleyi*	KU217836		EF493350			x	x	
206	*Pristimantis*	*devillei*	KU217991		EF493688			x	x	
207	*Pristimantis*	*surdus*	KU177847		EF493687			x		
208	*Pristimantis*	*quinquagesimus*	KU179374		EF493690			x	x	
209	*Pristimantis*	*duellmani*	n/a KU217998		AY326003	EF493438	EF493500	x	x	x
210	*Pristimantis*	*thymalopsoides*	KU177861		EF493514			x	x	
211	*Pristimantis*	*ocreatus*	KU208508		EF493682			x	x	
212	*Pristimantis*	*pyrrhomerus*	KU218030		EF493683			x	x	
213	*Pristimantis*	*festae*	KU218234		EF493515			x	x	
214	*Pristimantis*	*leoni*	KU218227		EF493684	EF493433	EF493495	x	x	x
215	*Pristimantis*	*verecundus*	QCAZ12410		EF493686			x	x	
216	*Pristimantis*	*celator*	KU177684		EF493685			x	x	
217	*Pristimantis*	*chloronotus*	n/a		AY326007			x	x	
218	*Pristimantis*	*thymelensis*	QCAZ16428		EF493516	EF493442	EF493503	x	x	x
219	*Pristimantis*	*supernatis*	n/a		AY326005			x	x	
220	*Pristimantis*	*sp. 1*	n/a		AY326002			x	x	
221	*Pristimantis*	*urichi*	USNM336098		EF493699	EF493426	EF493488	x	x	x
222	*Pristimantis*	*latidiscus*	KU218016		EF493698			x	x	
223	*Pristimantis*	*colomai*	QCAZ17101		EF493354	EF493440	EF493502	x	x	x
224	*Pristimantis*	*cruentus*	AMNH-A12444-448		EF493697			x	x	
225	*Pristimantis*	*ridens*	AMNH-A124551		EF493355			x	x	
226	*Pristimantis*	*cremnobates*	KU177252		EF493528	EF493424	EF493486	x	x	x
227	*Pristimantis*	*w-nigrum*	n/a		AY326004			x	x	
228	*Pristimantis*	*actites*	KU217830		EF493696	EF493432	EF493494	x	x	x
229	*Pristimantis*	*lanthanites*	KU222001		EF493695			x	x	
230	*Pristimantis*	*crenunguis*	KU177730	EF493693	EF493666			x		

Number	Genus	Species	Museum Voucher	Genbank Accession Number				Dataset		
				12S	16S	Rag1	Tyr	280	146	65
231	*Pristimantis*	*labiosus*	QCAZ19771		EF493694			x	x	
232	*Pristimantis*	*sp. 2*	MHNSM-LR4341		EF493356			x	x	
233	*Pristimantis*	*conspicillatus*	QCAZ28448		EF493529	EF493437	EF493499	x	x	x
234	*Pristimantis*	*condor*	KU217857		EF493701	EF493443	EF493504	x	x	x
235	*Pristimantis*	*citriogaster*	KU212278		EF493700			x	x	
236	*Pristimantis*	*achatinus*	KU217809	EF493827	EF493660			x		
237	*Pristimantis*	*lymani*	KU218019		EF493392			x	x	
238	*Pristimantis*	*fenestratus*	MHNSM9298		EF493703			x	x	
239	*Pristimantis*	*bipunctatus*	KU291638		EF493702	EF493430	EF493492	x	x	x
240	*Pristimantis*	*skydmainos*	MHNSM10071		EF493393			x	x	
241	*Pristimantis*	*toftae*	KU215493		EF493353			x	x	
242	*Pristimantis*	*rhabdolaemus*	KU173492		EF493706			x	x	
243	*Pristimantis*	*pluvicanorus*	n/a		AY843586			x	x	
244	*Pristimantis*	*sagittulus*	KU291635		EF493705	EF493439	EF493501	x	x	x
245	*Pristimantis*	*stictogaster*	KU291659		EF493704	EF493445	EF493506	x	x	x
246	*Pristimantis*	*aniptopalmatus*	KU291627		EF493390			x	x	
247	*Pristimantis*	*peruvianus*	MHNSM9267		EF493707	EF493436	EF493498	x	x	x
248	*Pristimantis*	*caprifer*	KU177680		EF493391			x	x	
249	*Oreobates*	*quixensis*	KU178249-250 n/a	EF493828 AY819344	EF493662 AY819474			x	x	
250	*Oreobates*	*sp.*	n/a	DQ283060	DQ283061			x		
251	*Phrynopus*	*parkeri*	n/a	AM039707	AM039639			x		
252	*Phrynopus*	*juninensis*	n/a	AM039725	AM039657			x		
253	*Phrynopus*	*kauneorum*	n/a	AM039718	AM039650			x		
254	*Phrynopus*	*tautzorum*	n/a	AM039720	AM039652			x		
255	*Phrynopus*	*barthlenae*	n/a	AM039717	AM039649			x		
256	*Phrynopus*	*horstpauli*	n/a	AM039715	AM039647			x		
257	*Phrynopus*	*pesantesi*	n/a	AM039724	AM039656			x		
258	*Phrynopus*	*bufoides*	n/a	AM039713	AM039645			x		
259	*Phrynopus*	*heimorum*	n/a	AM039703	AM039635			x		
260	*Phrynopus*	*bracki*	USNM286919		EF493709	EF493421	EF493507	x	x	x
261	*Phrynopus*	*sp. 1*	KU291634		EF493708			x	x	
262	*Phrynopus*	*cophites*	KU173497		EF493537	EF493423	EF493508	x	x	x
263	*Phrynopus*	*iatamasi*	n/a	AM039712	AM039644			x		
264	*Phrynopus*	*wettsteini*	n/a	AM039711	AM039643			x		
265	*Phrynopus*	*sp. 2*	n/a	AM039710	AM039642			x		
266	*Phrynopus*	*sp. 3*	n/a		AY843720			x		
267	*Phrynopus*	*peruvianus*	KU173495		EF493714			x	x	
268	*Phyllonastes*	*sp.*	n/a	AM039714	AM039646			x		
269	*Holoaden*	*bradei*	USNM207945	EF493378	EF493366	EF493449		x	x	
270	*Limnophys*	*anomalus*	KU177627		EF493534	EF493447		x	x	x/2
271	*Limnophys*	*bufoniformis*	n/a		DQ283165		DQ282942	x	x	x/2
272	*Limnophys*	*necerus*	KU179076		EF493535			x	x	
273	*Limnophys*	*sulcatus*	KU218055		EF493536			x	x	
274	*Ischnocnema*	*guentheri*	No voucher		EF493533	EF493407	EF493510	x	x	x
275	*Ischnocnema*	*hoehnei*	No voucher		EF493359			x		
276	*Ischnocnema*	*parvus*	No voucher		EF493532	EF493400	EF493509	x	x	x
277	*Ischnocnema*	*juipoca*	n/a		DQ283093			x	x	
278	*Litoria*	*caerulea*	n/a No voucher		AY843692		AY844131 EF493446	x	x	x
279	*Agalychnis*	*callidryas*	n/a n/a		DQ283423		DQ283018 EF493362	x	x	x
280	*Rana*	*catesbeiana*	n/a No voucher n/a		M57527 DQ283257		EF493448 DQ282959	x	x	x
n/a	*Bufo*	*melanostictus*	n/a		AY458592				x	
n/a	*Centrolene*	*prosoblepon*	n/a		AY843574				x	
n/a	*Ceratophrys*	*cornuta*	n/a		AY326014				x	
n/a	*Cryptobatrachus*	*sp.*	n/a		AY326050				x	
n/a	*Dendrobates*	*sylvaticus*	n/a		AY364569				x	
n/a	*Hyla*	*chinensis*	n/a		AY458593				x	
n/a	*Leptodactylus*	*pentadactylus*	n/a		AY326017				x	

Table 2-2. Primers used in this study.

Primer	Sequence[a]	Dir.	Location[b]	Source
	12s/tRNA-Val/16s			
12L9	AAAGCAHRRCACTGAARATGYYDAGA	F	229-254	this study
12L29E	AAAGCRTAGCACTGAAAATGCTAAGA	F	229-254	this study
12.1L4E	TACACATGCAAGTYTCCGC	F	322-340	this study
12L12E	CAAACTGGGATTAGATACCCCACTATG	F	697-723	this study
12L15	CAAACTGGGATTAGATACCCCACTAT	F	697-722	this study
12.2L4E	GCTTAAAACCYAARGGAYTTGACG	F	775-798	this study
12H42	GCTGCACCTTGACCTGACGTATTG	R	939-961	this study
12L27	ACGTCAGGTCAARGTGCAGC	F	943-962	this study
12H46E	GCTGCACYTTGACCTGACGT	R	943-962	this study
12L30E	GTACAMACCGCCCGTCACCCTC	F	1097-1118	this study
12.2H1E	TCCGGTATACTTACCATGTTAC	R	1175-1196	this study
12L34	GTAACATGGTAAGYRTACCGGA	F	1175-1196	this study
12H10	CACYTTCCRGTRCRYTTACCRTGTTACGACTT	R	1170-1201	this study
16L43E	CTYGTACCTTTTGCATCATGGTTTA	F	1462-1486	this study
16H50	TARACCATRATGCAAAAGGTAC	R	1465-1486	this study
16L19	AATACCTAACGAACTTAGCGATAGCTGGTT	F	1614-1644	this study
16H49E	AACCAGCTATMRCTAAGTTCGSTAGG	R	1618-1644	this study
16L33E	AAGTWGGCCTAARAGCAGCCAYCTTT	F	1792-1817	this study
16H48E	AAAGRTGGCTGCTYTYAGGCC	R	1797-1817	this study
16L28E	AAGTRGGCCTAARAGCAGCCA	F	1792-1812	this study
16L42	GGCCTRATAGCAGCCAYCT	F	1797-1815	this study
16H46	TCWTGTTACTAGTTYTARCAT	R	1919-1939	this study
16L37	GATTAYAAGAAAAAGAAGGAACTCGGCA	F	2082-2109	this study
16H41	GAGGCGATGTTTTTGGTAAACAGGC	R	2122-2144	this study
16L34	TTTAACGGCCGCGGTATCCTAACCG	F	2186-2210	this study
16H24	TACCTTCGCACGGTTAGKRTACCGCGGCCGTT	R	2190-2220	this study
16L29E	TATCCTAACCGTGCRAAGCTAGC	F	2200-2222	this study
16L1	CTGACCGTGCAAAGGTAGCGTAATCACT	F	2204-2231	this study
16H36E	AAGCTCCAWAGGGTCTTCTCGTC	R	2341-2363	this study
16H37	TTACTCCGGTCTGAACTCAGATC	R	2710-2732	this study
16H25	GACCTGGATTACTCCGGTCTGAACTCAGAT	R	2711-2740	this study
16H1	CTCCGGTCTGAACTCAGATCACGTAGG	R	2703-2729	this study
16H47	AAAGRGCTTAGRTCTTTYGCA	R	2903-2923	this study
	Rag-1			
R182	GCCATAACTGCTGGAGCATYAT	F	1391-1412	D. Cannatella, pers. comm
Rag1FF2	ATGCATCRAAAATTCARCAAT	F	1411-1431	this study
Rag1FR2	CCYCCTTTRTTGATAKGGWCATA	R	2029-2051	this study
R270	AGYAGATGTTGCCTGGGTCTTC	R	2051-2072	D. Cannatella, pers. comm
	Tyr			
Tyr1C	GGCAGAGGAWCRTGCCAAGATGT	F	101-123	Bossuyt and Milinkovitch (2000)
TyrFE	GTTGTYGTATCTACCTCRCC	F	122-141	this study
TyrRE	GMAGGGAATGGTGAARTTCTC	R	635-655	this study
Tyr1G	TGCTGGGCRTCTCTCCARTCCCA	R	656-678	Bossuyt and Milinkovitch (2000)

[a]written 5' to 3'.

[b]Location on reference sequences: AY458592 (mitochondrial), L19324 (Rag-1), AY333967 (Tyr).

Table 2.3. Times of divergence for major nodes in Figure 2-3.

Node	Divergence	Time	95% CI[a]
1	Eleutherodactylines + *Brachycephalus*/hylid frogs	56.79	(43.52, 78.13)
2	Southeast Brazil Clade (SBC)/other species	49.79	(37.18, 68.67)
3	Caribbean Clade (CC)/other eleutherodactylines	47.28	(35.09, 65.26)
4	Middle American Clade (MAC)/other eleutherodactylines	42.39	(30.99, 58.99)
5	South American Clade (SAC)/other eleutherodactylines	36.52	(26.56, 50.81)
6	Last common ancestor of Caribbean Clade	29.09	(20.95, 40.35)
7	Last common ancestor of Middle American Clade	30.51	(21.67, 43.17)
8	Last common ancestor of South American Clade	24.45	(17.30, 34.82)
9	Middle American *Syrrhophus*/Cuban *Syrrhophus*	19.05	(13.06, 26.92)

[a]Bayesian credibility interval.

CHAPTER 3

New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation

Note: Modified from Hedges, S. B., Duellman, W. E., Heinicke, M. P. 2008. *Zootaxa* **1737**: 1-182. MPH carried out laboratory and computational research. SBH, WED, and MPH drafted paper, with MPH focusing on molecular phylogenetic aspects. Systematic accounts based on the molecular phylogeny were largely composed by SBH and WED, and are provided as an appendix.

3.1 Abstract

New World frogs recently placed in a single, enormous family (Brachycephalidae) have direct development and reproduce on land, often far away from water. DNA sequences from mitochondrial and nuclear genes of 344 species were analyzed to estimate their relationships. The molecular phylogeny in turn was used as the basis for a revised classification of the group. The 882 described species are placed in a new taxon, Terrarana, and allocated to four families, four subfamilies, 24 genera, 11 subgenera, 33 species series, 56 species groups, and 11 species subgroups. Systematic accounts are provided for all taxa above the species level. Two families (Craugastoridae and Strabomantidae), three subfamilies (Holoadeninae, Phyzelaphryninae, and Strabomantinae), six genera (*Bryophryne, Diasporus, Haddadus, Isodactylus, Lynchius*, and *Psychrophrynella*), and two subgenera (*Campbellius* and *Schwartzius*) are proposed and named as new taxa, 13 subspecies are considered to be distinct species, and 613 new combinations are formed. Most of the 100 informal groups (species series, species groups, and species subgroups) are new or newly defined. *Brachycephalus* and *Ischnocnema* are placed in Brachycephalidae, a relatively small clade restricted primarily to southeastern Brazil. Eleutherodactylidae includes two subfamilies, four genera, and five subgenera and is centered in the Caribbean region. Craugastoridae contains two genera and three subgenera and is distributed mainly in Middle America. Strabomantidae is distributed primarily in the Andes of northwestern South America and includes two

subfamilies, 16 genera, and three subgenera. Aspects of the evolution, biogeography, and conservation of Terrarana are discussed.

3.2 Introduction

The twenty-first Century has witnessed a renaissance in systematic biology with respect to theory, methodology, and taxonomy, and perhaps most significantly the application of systematics to such diverse fields as ecology, behavior, and conservation, among others. This resurgence has occurred principally with the sequencing of DNA and use of newly developed methods of analysis. Thus, systematists have discovered a new array of tools and characters for the inference of phylogenetic relationships. These innovative approaches are being used from the levels of local phylogeography to ascertaining the relationships among prokaryotes and eukaryotes. The only work that has attempted to determine the phylogenetic relationships among all living taxa of amphibians is that by Frost et al. (2006), in which DNA sequences of 522 species were used to create a phylogenetic tree representing nearly 6000 species. One small twig in their molecular tree was a newly classified "Brachycephalidae" represented by 16 species. The phylogeny and classification presented here for that same group are based on DNA sequences from 344 species. Thus, that small twig has grown into a major branch of the amphibian evolutionary tree.

This monograph concerns a large evolutionary radiation (882 species) of New World frogs that breed on land and have direct development. Except for one species known to be ovoviviparous (Drewry and Jones 1976), all of these species are known (or presumed) to lay eggs in terrestrial situations where they hatch into froglets, thereby bypassing the tadpole stage. These frogs range from the southern United States to northern Argentina, although they are most diverse in mountains of Central America, the West Indies, and South America. They represent about 13% of all known amphibian species and 28% of the amphibian taxa in the New World tropics (AmphibiaWeb 2007; Frost 2007; IUCN 2006). Almost all of the included species have been called "eleutherodactylines" at some point in time. A notable exception is the small genus *Brachycephalus* (11 species) which in recent years has become affiliated with this clade in molecular phylogenies.

The "eleutherodactylines" have been considered to be a subunit (subfamily Eleutherodactylinae or tribe Eleutherodactylini of Telmatobiinae) of Leptodactylidae (Lynch 1971) whereas *Brachycephalus* has been placed in its own family, Brachycephalidae (e.g., Noble 1931) or in Atelopodidae (Griffiths 1959). Based on life-history data, Duellman and Lynch (1969) suggested that *Brachycephalus* was not an atelopodid. Analyses of molecular data over the last decade (Ruvinsky and Maxson 1996; Darst and Cannatella 2004; Frost et al. 2006; Roelants et al. 2007) have indicated that Leptodactylidae is not monophyletic and that the "eleutherodactylines" and *Brachycephalus* belong to a separate lineage of neobatrachian anurans. However, the paraphyly of "eleutherodactylines," with respect to *Brachycephalus*, implied by some of these analyses, cannot be taken as strong evidence given the small representation (1–5%) of "eleutherodactylines" in those studies. Even in our recent study with expanded coverage (Heinicke et al. 2007), and in this study (see below), the position of *Brachycephalus* with respect to "eleutherodactylines" has been difficult to resolve. While other lineages have been difficult to resolve as well, the importance of *Brachycephalus* concerns its taxonomic priority in determining the family name.

From an evolutionary and taxonomic standpoint, this clade of frogs is one of the most poorly known major groups of vertebrates. Although new species are readily recognized and described, taxonomists have been unable to agree, for the most part, on how to organize those species into genera, subgenera, and species groups to make this complex more manageable (e.g., Lynch 1976; Savage 1987; Hedges 1989a; Lynch and Duellman 1997; Frost et al. 2006). As a result, most species have been placed in *Eleutherodactylus*, which for years has remained the largest vertebrate genus. Species continue to be described at an increasing rate (Figure 3-1). This rate of species discovery was approximately 1–2 species per year during the 19th Century, but then rose sharply during the latter half of the 20th Century to 15 species per year, and continues to increase (Figure 3-1).

The taxonomic confusion in part reflects the paucity of characters available for study and the plasticity of the few "useful" characters. Characters such as skin texture, relative length of digits, and size of digital discs have been used extensively, but such characters are of functional importance and subject to evolutionary convergence. For

example, almost all arboreal species have large digital tips, and most ground-dwelling species have small digital tips. The larger digital tips of the arboreal species, of course, aid them in climbing. Most major clades of "eleutherodactylines," such as the Middle American Clade and Caribbean Clade, have arboreal species with large digital tips. Even the states of the single morphological character believed to be the most reliable, the orientation of the trigeminal nerve relative to jaw musculature (Lynch 1986), have been shown to have evolved independently in different lineages in molecular phylogenies (Frost et al. 2006; Heinicke et al. 2007). Despite their plasticity, these characters are often useful in defining some clades, as evidenced by agreement with other data sets such as DNA sequences, and they remain major aspects of the organism and cannot be overlooked.

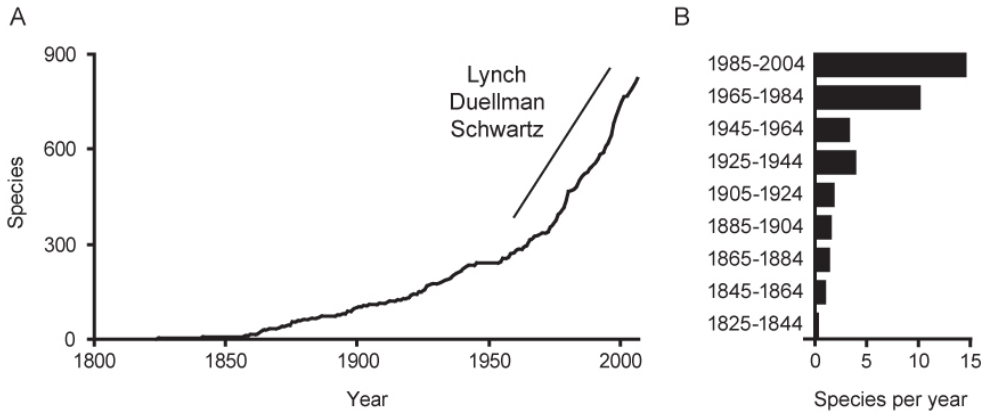


Figure 3-1. The history of discovery of terraranan frogs. (A) Discovery curve showing cumulative number of valid species through time, illustrating an increase in rate in recent decades. The names of the three herpetologists mostly responsible for the rate increase are noted. (B) Average number of valid species described each year, calculated over intervals of 20 years.

Chromosome analyses of "eleutherodactylines" have revealed a surprisingly rapid rate of change for amphibians (including closely related families Hylidae and Bufonidae), comparable to that in mammals (Bogart 1981; Bogart and Hedges 1995). However, chromosome variation, by itself, generally has not proven useful in the classification of "eleutherodactyline" frogs (e.g., Savage 1987; Bogart and Hedges 1995). As data from more species become available, chromosome data will likely be more phylogenetically informative.

Initial molecular studies of *Eleutherodactylus* and relatives using allozymes and albumin immunology (Miyamoto 1983, 1984, 1986; Hedges 1989a, 1989b; Hass and Hedges 1991; Kaiser et al. 1994) showed promise for resolving relationships, but those studies were hampered by limited taxonomic sampling and limitations of the methods themselves. Until recently, only a few comparative studies using DNA sequences have been undertaken with these frogs, and they too have had limited taxon sampling, including less than 5% of "eleutherodactylines." One study included 12 species (Darst and Cannatella 2004), another 39 species (Crawford and Smith 2005) a third used 16 species (Frost et al. 2006), a fourth 21 taxa, and a fifth 24 taxa (Padial et al. 2007a) (Padial et al. 2007b).

Recently, we completed a DNA sequence analysis of 277 species of "eleutherodactylines" and *Brachycephalus* using mitochondrial and nuclear genes (Heinicke et al. 2007). The resulting tree resolved three major groups: a Caribbean Clade of 140 species (representing 185 species), a Middle American Clade of 14 species (representing 111 species) and a South American Clade of 87 species (representing 397 species). A smaller Southeast Brazil Clade was also defined that we recognized as *Ischnocnema*. The Middle American Clade had been recognized previously as the subgenus or genus *Craugastor* (Lynch 1986; Crawford and Smith 2005; Frost et al. 2006), although the analysis by Heinicke et al. (2007) redefined the group by showing that members of two South American species groups were not part of the clade. The Caribbean and South American clades were both new and unpredicted, because previous analyses had assumed a close relationship between a South American group (the "*Eleutherodactylus*" *unistrigatus* Species Group) and a large assemblage of species in the West Indies of the subgenus *Eleutherodactylus*.

Although our study included approximately one-third of the species in this large clade, the taxonomic coverage was best in the West Indies and comparatively weak in South America. A small sample of *Phrynopus,* a genus of terrestrial species lacking T-shaped terminal phalanges and digit discs, suggested that the genus is polyphyletic, as had been noted previously (Duellman and Hedges 2005; Lehr et al. 2005). The Southeast Brazil Clade included representatives of several species groups not thought to be closely related, suggesting that many or most species from that region also belong to that clade.

Not unexpectedly, we found that the deepest branches in the tree were of lineages in South America, including *Brachycephalus*, and these nodes were, for the most part, unresolved.

Herein, we have added new sequences to that data set and use the results to reorganize the classification of this large clade of frogs. In general, we have taken a conservative approach, and have minimized changes at the generic level so as to cause the least disruption for users of the taxonomy while still reflecting the new evolutionary information. Where appropriate, we use the subgenus category to organize major groups within large genera. This approach provides systematists and evolutionary biologists with information they need for hypothesis testing yet retains the binomens used frequently by non-systematists in field guides and checklists. For example, most of the species in South America are placed in the genus *Pristimantis*, most in Central America are placed in *Craugastor*, and nearly all in the West Indies are retained in *Eleutherodactylus*. Because of the large number of species involved, we also make use of the informal categories of species series, species group, and species subgroup. We include distribution maps of the higher taxa.

3.3 Materials and Methods

Molecular Analyses. All methods used for the collection and analysis of the new DNA sequences presented here follow those in Heinicke et al. (2007). The sequences have been deposited in GenBank and are EU186650–780. Localities, tissue numbers, and museum numbers for the new sequences are in Table 3-1. Included among the new sequences are samples from specimens that are being described elsewhere as new species or specimens for which an accurate identification has not yet been made. Although each sequence is treated as a separate species here, several may turn out to be species already included, as suggested by some close relationships seen in the trees in the case of several pairs of sequences. These unidentified sequences will be referred to by their GenBank accession numbers or museum catalog numbers. For this study, we have added a total of 72 ingroup and eight outgroup species to the data set of Heinicke et al. (2007), as well as new nuclear gene sequences for eight previously-sampled taxa (Table 3-1).

As in Heinicke et al. (2007), sequence data consisted of three overlapping sets. Partial sequences for the mitochondrial 12S (~350 bp) and 16S (~800 bp) rRNA genes were included for all 362 (344 ingroup) species. A dataset of 216 species (198 ingroup) includes the full 12S and 16S sequences, along with the intervening tRNA-Valine (~2,500 bp total). For 80 of these species (77 ingroup), data were also obtained for two nuclear exon gene regions of RAG-1 (639 bp) and Tyrosinase precursor (Tyr; 493 bp). Substantial amounts of 12S or 16S data were missing for some of the included species, due to either their not being available on GenBank or the inability to amplify these regions. In these cases the unsequenced regions were coded as missing data. In addition, one species in the 80-species set (*Eleutherodactylus counouspeus*) is missing data for the Tyr gene, and another (*Strabomantis anomalus*) could not be amplified for that gene so instead we used the Tyr sequence of a related species (*S. bufoniformis*).

Genomic DNA was extracted from frozen or ethanol-preserved tissue samples using a Qiagen DNeasy tissue extraction kit under the manufacturer's protocol. PCR amplification of samples was performed in 50-µl reactions using AmpliTaq DNA polymerase and ThermoPol buffer (New England Biolabs). Each polymerase chain reaction for mitochondrial products contained ThermoPol buffer at 1×, dNTPs at 4 µM, forward and reverse primers at 1 µM, one unit of polymerase, and 1 µl of extracted DNA (more for low-quality tissue). For amplification of nuclear genes, dNTP was increased to 6.6 µM, Taq polymerase to 2.5 units, and extracted DNA to 5 µl. Standard reaction conditions were an initial hold for 5 min at 94°C, followed by 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s. After 40 cycles, a final hold of 72°C for 7 min was performed before terminating the reaction at 4°C. For low- or nonyielding samples, annealing temperature was dropped to 46°C. In some cases, a second round of PCR was performed using primer pairs inside the initially generated fragment. Primers used in PCR reactions were identical to those of Heinicke et al. (2007). Amplified PCR products were isolated by running on agarose gels and filtering with Millipore Ultrafree-DA gel filters or by vacuum filtration using Millipore Multiscreen filters. Cycle sequencing was performed by the Pennsylvania State University Nucleic Acid Facility. All fragments were sequenced in both forward and reverse directions.

Before analyses, all chromatograms were fully inspected, and all sequences were compared against their reverse complement to detect any call errors. Embedded primer sequences were deleted from all sequence fragments before assembly or alignment. Alignments of 12S and 16S sequences were created using CLUSTAL W (Thompson et al. 1994) using the following scoring parameters: match (3), transition (1), transversion (0), gap opening penalty (10), gap extension penalty (5), delay divergent sequences (40%). Resulting alignments were inspected for obvious errors and compared against frog secondary structure models (including *Eleutherodactylus*) available from the European ribosomal RNA database, and modified accordingly. Regions deemed poorly aligned were excluded from analysis. In general, these are loop regions of variable sequence length greater than ~6 bases having low sequence identity (<50%), but no strict algorithm was used. Sequences for RAG-1 and Tyrosinase precursor were aligned by eye; Tyr contained no alignment gaps and all gaps in RAG-1 consisted of codon deletions with the open reading frame preserved. Final alignments for each gene are available from TreeBASE (accession number S2061).

For all datasets, maximum likelihood analyses were performed. In addition, Bayesian and distance (NJ) methods were employed for the dataset of 80 species. For the 80-species set, three partitions (12S and 16S, RAG-1, Tyr) were introduced for the Bayesian and likelihood analyses, with model parameters unlinked across the partitions. Distance analysis was performed using MEGA 3.1 (Kumar et al. 2004), while Bayesian analysis used MrBayes 3.1 (Ronquist and Huelsenbeck 2003) and likelihood analyses used RAxML-VI-HPC v2.0 (Stamatankis 2006). Bayesian analysis used the GTR + I + Γ model of evolution. Neither MEGA nor RAxML implement this model; instead, TN + Γ (MEGA) and GTRMIX (RAxML) were used. The Γ-parameter for the distance analysis was estimated in PAUP 4b10 (Swofford 2003). All parameters for ML and Bayesian analyses were estimated by the programs during the runs. Gaps were treated as missing data.

For each ML analysis, 100 alternative runs were performed. Model parameters in RAxML are estimated by the program and do not require input. Nonparametric bootstrap analysis (1,000 replicates) was performed to provide branch support to the most likely tree of the 100 runs for each data set. Bootstrap analysis (1,000 replicates) was also used

to provide branch support for the NJ analysis. Posterior probabilities were used to determine branch support for the Bayesian analysis. The Bayesian analysis of 80 species was performed as two independent runs for 2,000,000 generations with three heated and one cold chain. Chains were sampled every 1,000 generations. The first 25% of samples were discarded as burn-in. To ensure that this was an adequate number of samples discarded, a plot of log likelihood vs. generation was produced and showed that the region of increasing log likelihood values was encompassed in the first 25% of samples. Convergence was assessed by using the program Tracer 1.3 (Rambaut and Drummond 2005) to obtain estimated sample sizes for each model parameter (six substitution frequency categories, four nucleotide frequency categories, Γ-parameter, proportion of invariant sites, tree length, and log likelihood). Estimated sample sizes of each parameter were substantially greater than 100 for the sum of both independent runs, although values for a small number of parameters were less than 100 when the independent runs were treated separately. However, the convergence metric employed by MrBayes (average standard deviation of split frequencies) was less than 0.01 (=0.006) at the conclusion of the run.

Systematic Accounts. A major goal of this work is a taxonomic revision where new families, genera, and other taxa are described. Here, we mention some of the procedures that we used in the systematic accounts. In all cases, taxa are listed in alphabetical order. Accounts of all taxa are given in the Appendix.

A classification provides a way to organize species to facilitate communication and further study. At the same time, a new classification of one group, such as this one, must integrate with that of other groups, and should consider the history of the classification as it relates to stability. Above all it should reflect the evolutionary history of the organisms. For this last consideration, we relied mostly on the molecular phylogenetic analyses (Figures 3-2, 3-3, 3-4). The species-rich analyses (Figures 3-2, 3-3) provided guidance for taxonomic decisions at lower levels (e.g., species groups and series) whereas the gene-rich analyses (Figure 3-4) provided guidance for decisions at higher levels, although all three analyses were consulted in many cases. If we were to define taxa solely on relationships based on molecular analyses, we would not be able to

classify the majority of species in the group, because so many have not yet been sequenced. Instead, we use the molecular phylogenies as primary guidance in establishing a conventional morphology-based classification that is useful for all species.

There is no biological meaning associated with any taxonomic rank or level above the species level, or at least none intended here. However, higher taxa (e.g., families) that contain too many lower taxa (e.g., species) can hinder further research simply because of their large size. Because the group in question here, with more than 850 species, is currently considered a single family, Brachycephalidae (Frost et al. 2006) that is larger than nearly any other family of tetrapods, our first decision was to make it more manageable by splitting the group into four families. This necessitated the creation of a higher-level taxon to contain those four families. We chose an unranked taxon to avoid putting in place yet another formal name (superfamily rank) given the volatility of anuran taxonomy in recent years (Frost et al. 2006) and the potential problems it might raise in dealing with existing superfamily names (e.g., Hyloidea) that may apply to this group.

Definitions of families, subfamilies, genera, and subgenera follow standardized format (Lynch 1971; Lynch and Duellman 1997). That format is essentially a numerical list of characters deemed to be important or useful in classification, although not all are necessarily diagnostic except when considered in combination. In the past, characters used in the definitions of genera and subgenera have varied because of different characters emphasized by different researchers (e.g., Savage 1987; Hedges 1989a; Lynch and Duellman 1997). Nonetheless we have attempted to standardize those definitions, at least within a genus or subgenus, for comparative purposes. Although rare, individual morphological characters that appear to have diagnostic value on their own are noted in the Remarks section of each account. This major taxonomic revision would not have been necessary if large numbers of shared derived morphological characters were already recognized in these frogs.

In cases where clades could be defined within formal taxa, we recognize the following informal taxa: series, species groups, and species subgroups. Of course, any informal taxon—including those above the family group level—does not fall under the formal rules of the code. Nonetheless, we consider all taxa in this classification as evolutionary units (monophyletic groups) except as indicated (some previously defined

species groups of the genus *Pristimantis* are retained here pending further study). For consistency, we name each species series, species group, and species subgroup based on the earliest described species contained in the taxon. As more species are discovered and described and these informal taxa become larger and unmanageable, it is likely that some will be replaced with formal names (e.g., subgenera and genera) and other informal names will be created to accommodate new lower-level clades. Such is the normal evolution of a classification.

Among these informal categories, decisions on which taxonomic level (species series, species group, or species subgroup) to use, within a genus or subgenus, were based largely on convenience in classifying the species. For example, the category of species series was generally used for groups containing a large number of species requiring further subdivision (into species groups) and/or which represent deep divergences within a genus or subgenus. The category of species subgroup was used for relatively small groups of species not requiring further subdivision at present. Within the large subgenus *Pristimantis* (*Pristimantis*), which will require more sequence data to establish a stable classification, we have continued to recognize previously defined species groups until those much needed data become available.

Definitions of species series, species groups, and species subgroups describe the potentially diagnostic aspects of the morphology of the taxon as well as some information on ecology and habits. The characters usually include body size and proportions, skin texture, coloration, and vocalization. As one would expect with such nested low-level clades, diagnostic characters for species groups in one genus may not be the same as those for another genus, and therefore this section does not have a numbered list. Because this study is based largely on a molecular phylogeny, we make no claim that any of these taxa, formal or informal, are fully diagnosable now based on morphology, but we anticipate that further study of these clades will reveal such characters in the future.

For terminology specific to "eleutherodactyline" frogs, we follow Lynch and Duellman (1997). Body size is reported as maximum snout-vent length (SVL) in adult females of each species, taken from the literature. Except in rare cases, females are larger than males. For head width (HW), we list ranges of proportions for adult females (HW/SVL x 100), taken from the literature. Numbering of digits in the hand follows

conventional standards of Fingers I through IV, although we are aware that the first digit in the hand has been lost in anurans (Fabrezi and Alberch 1996). The relationship of the trigeminal nerve and adductor musculature was obtained from the literature and from dissections accomplished with the aid of Luchol's solution; terminology follows Lynch (1986). Nine species were dissected; all had the "S" condition of the adductor musculature. The specimens are: *Bryophryne cophites*, KU 138907; *Diasporus diademata,* KU 37467; *Dischidodactylus duidensis,* AMNH 23192; *Lynchius parkeri,* KU 181354; *Niceforonia nana,* KU 169122; *Noblella lochites,* KU 177356; *Noblella* "*peruviana,*" KU 173329; *Phrynopus montium,* KU 138880; *Psychrophrynella laplacai,* KU 154556. In the case of vocalizations of species from the West Indies, some data are based on personal observations (SBH). Distribution maps of genera and subgenera are based on maps of distributions of species by the Global Amphibian Assessment (IUCN 2006), with some modification to improve accuracy. Although we often point out geographic patterns, we do not use geography as a defining character of a taxon.

3.4 Results

After alignment and removal of ambiguous regions, the 362-species dataset encompassed 1207 sites of the 12S and 16S rRNA genes. The 216-species dataset included 2578 sites of the complete 12S and 16S rRNA genes. The 80-species dataset included 3709 sites of the complete 12S and 16S rRNA genes and portions of the nuclear genes for RAG-1 and Tyrosinase. We present Maximum likelihood (ML) trees for all three datasets, respectively (Figures. 3-2, 3-3, 3-4), and include bootstrap confidence values on nodes. For the 80-species tree we also included neighbor-joining bootstrap values and Bayesian confidence values (posterior probabilities).

The same four major geographic clades included in our earlier study (Heinicke et al. 2007) were obtained here: A Caribbean Clade (*Eleutherodactylus*), a Middle American Clade (*Craugastor*), a South American Clade (*Pristimantis*), and a Southeast Brazil Clade (*Ischnocnema*). However, the additional species and sequences clarified relationships of many poorly known taxa, identified other taxa allied to these genera, and added resolution of some deeper branches in the tree that were previously unresolved.

Based on the molecular phylogeny, we recognize four families, four subfamilies, 24 genera, 11 subgenera, 33 species series, 56 species groups, and 11 species subgroups within Terrarana. Two families (Craugastoridae and Strabomantidae), three subfamilies (Holoadeninae, Phyzelaphryninae, and Strabomantinae), six genera (*Bryophryne, Diasporus, Haddadus, Isodactylus, Lynchius*, and *Psychrophrynella*), and two subgenera (*Campbellius* and *Schwartzius*) are newly described (see Appendix). Relationships among the four families of Terraranan are not resolved; however, all but Strabomantidae receive significant support for monophyly in the character-rich dataset (Fig. 3-4), and the closest relatives of many genera are also identified. The molecular phylogeny (Fig. 3-4) shows that *Brachycephalus* is closest to the genus *Ischnocnema*, together forming an expanded southeast Brazil clade. The closest relatives of both the Central American clade (*Craugastor*) and the West Indian clade (*Eleutherodactylus*) are identified with significant support as the South American genera *Haddadus* and *Diasporus*, respectively. Relationships among and within other clades are discussed more fully in relevant the systematic accounts (Appendix).

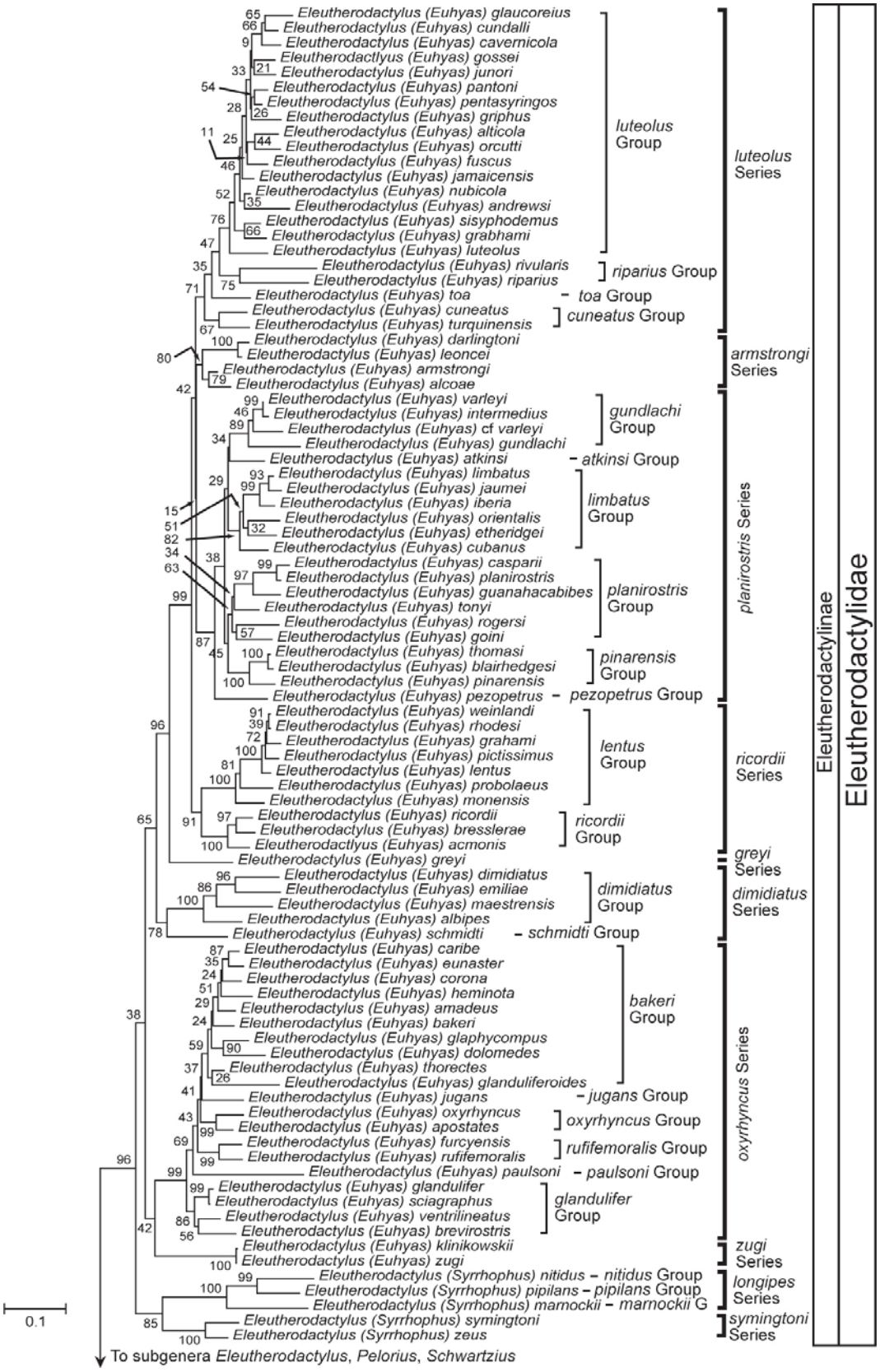


Fig. 3-2 A. (continues on next page)

Figure 3-2 B. (continues on next page)

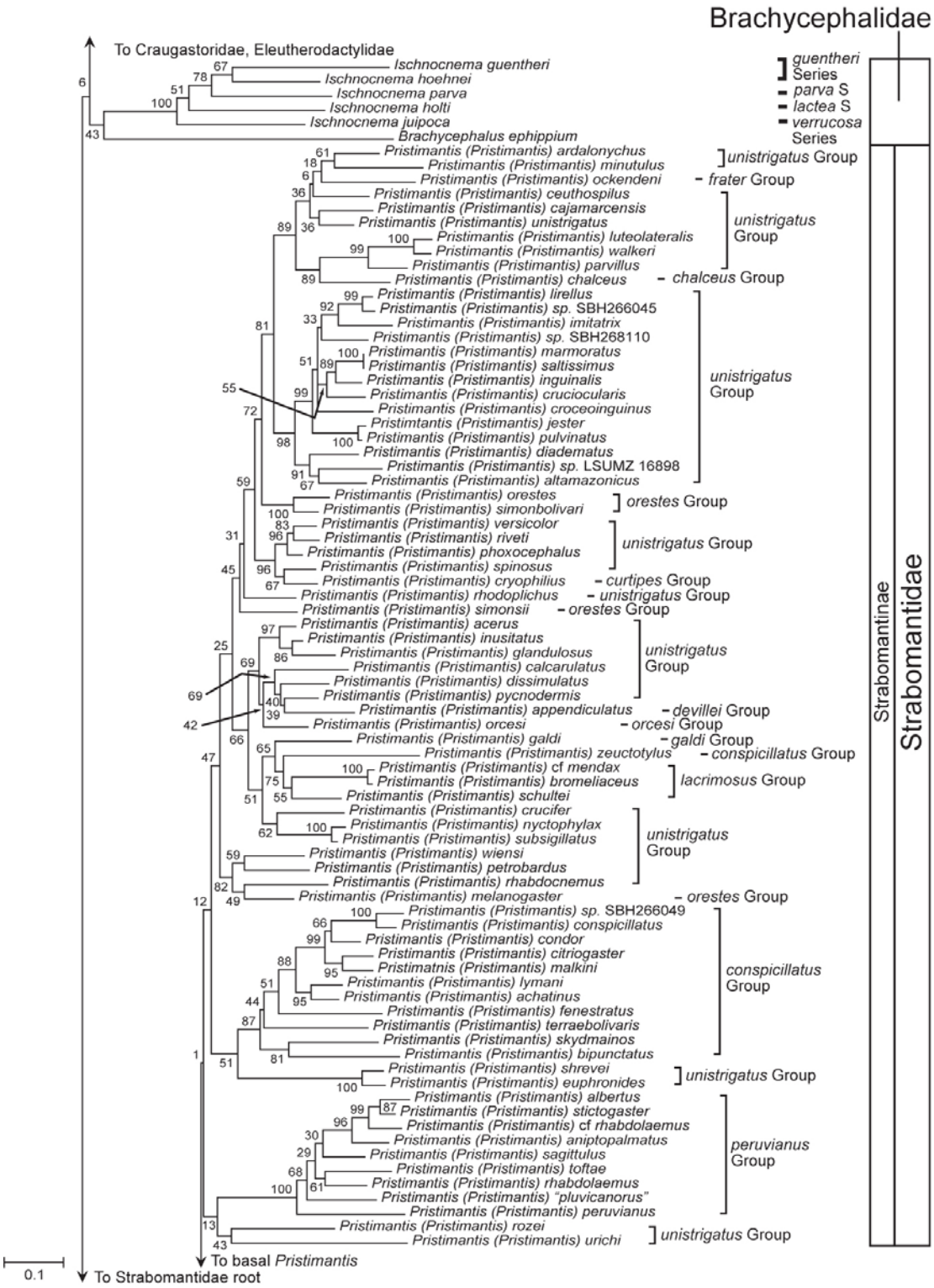


Figure 3-2 C. (continues on next page)

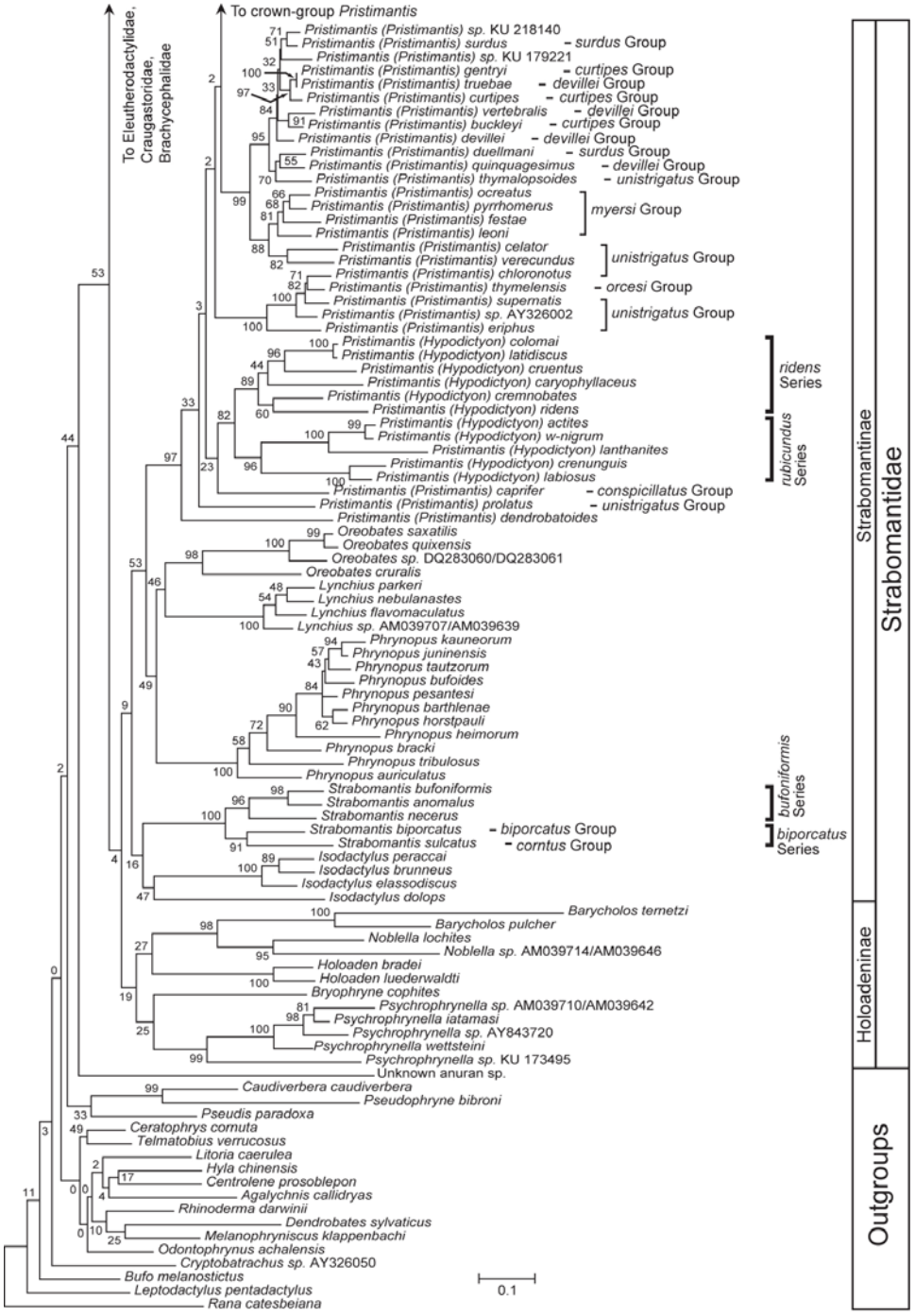


Figure 3-2 D. Maximum likelihood phylogeny of 362 species of frogs. The data set consists of 1,207 base pairs of aligned mitochondrial DNA sequences of the 12S rRNA and 16S rRNA genes. Bootstrap support values are shown on nodes. Classification of the species is indicated. Where species identification is not known, either the sequence accession number (continuous series of letters and numbers) or museum voucher number (letters separated from numbers) is given. (A) First segment (top) of tree. (B) Second segment of tree. (C) Third segment of tree. (D) Fourth segment (bottom) of tree.

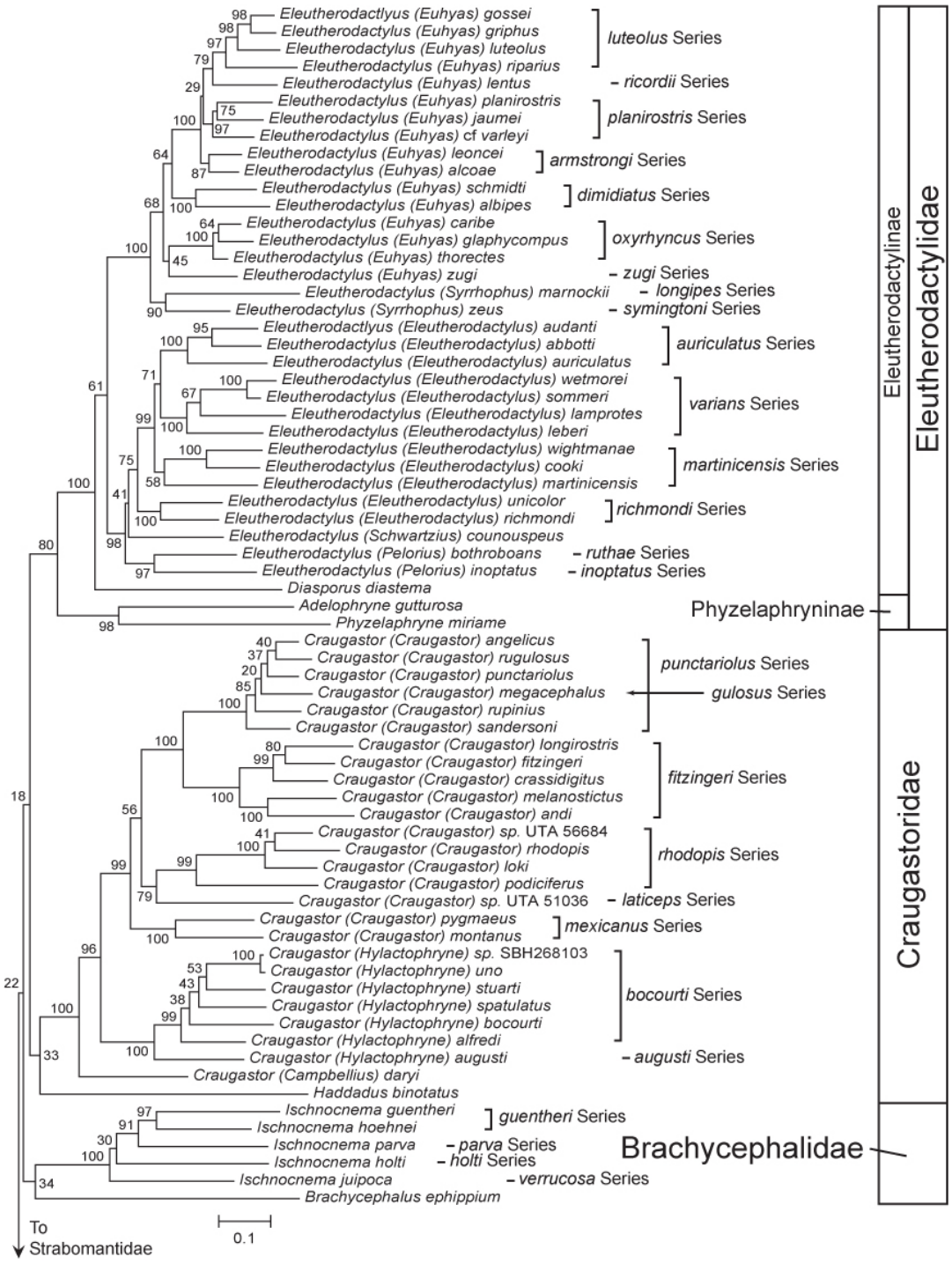


Figure 3-3 A. (continues on next page)

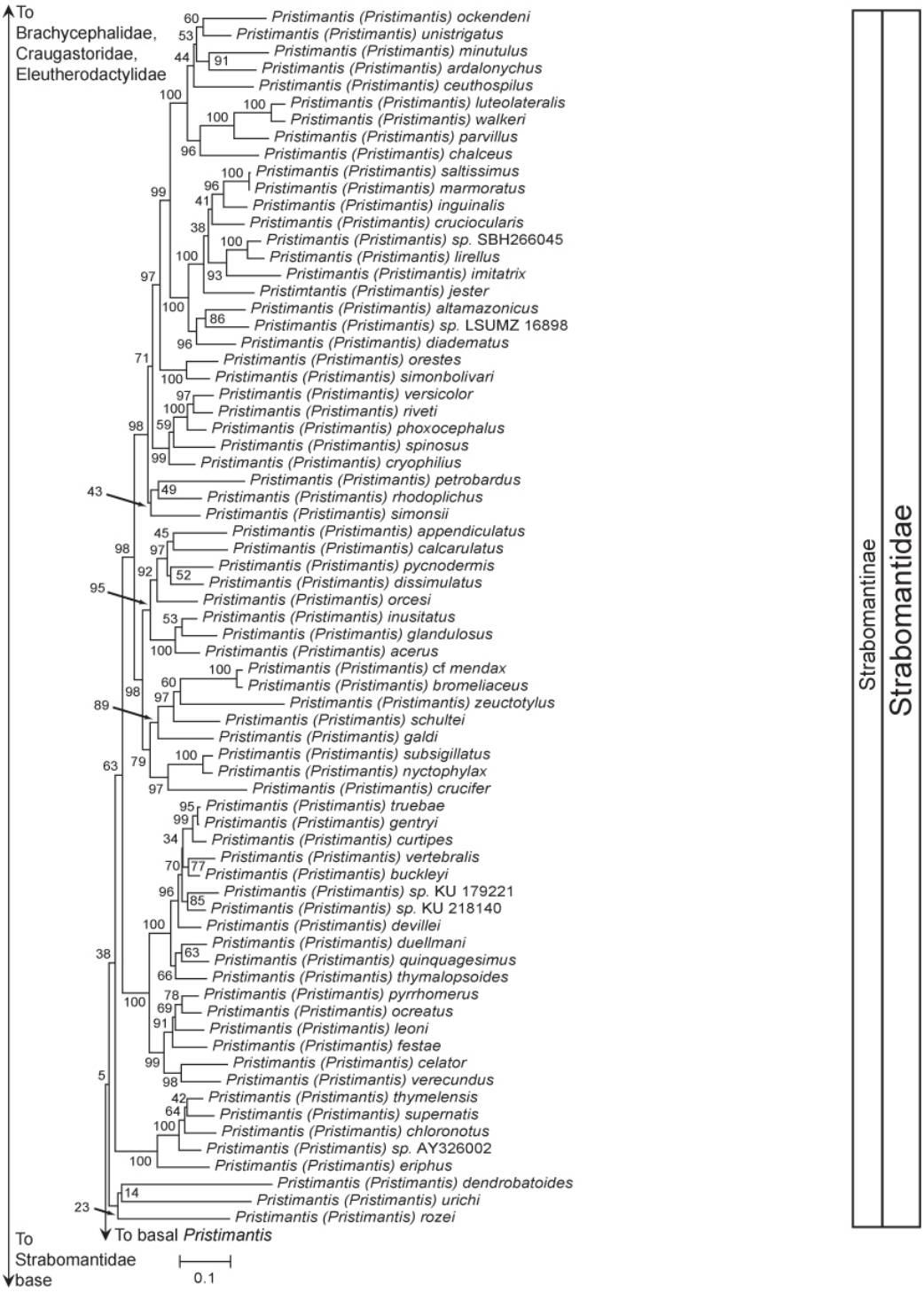


Figure 3-3 B. (continues on next page)

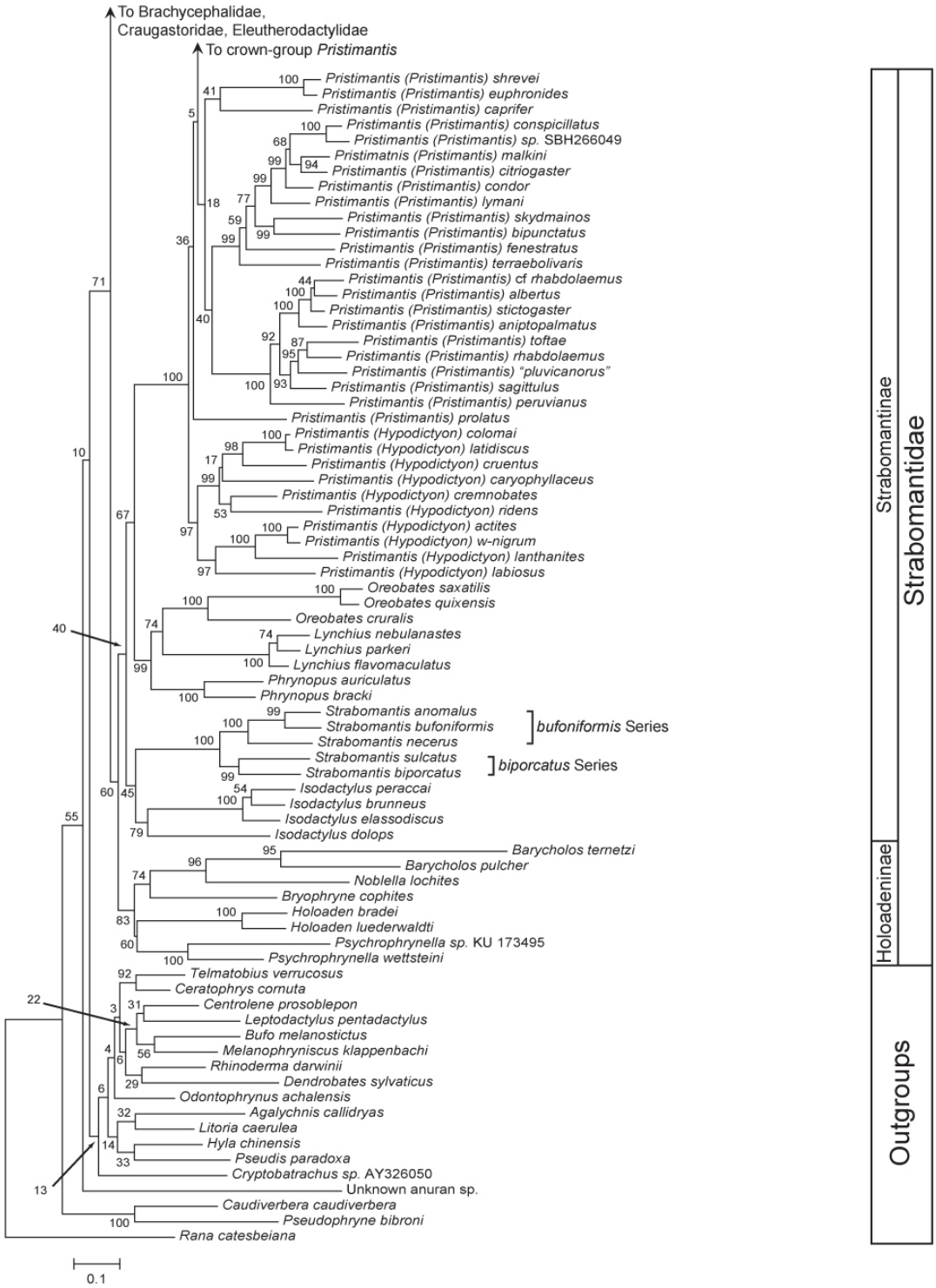


Figure 3-3 C. Maximum likelihood phylogeny of 216 species of frogs. The data set consists of 2578 base pairs of aligned mitochondrial DNA sequences of the 12S rRNA and 16S rRNA genes, including the intervening transfer RNA Valine. Bootstrap support values are shown on nodes. Classification of the species is indicated. (A) First segment (top) of tree. (B) Second segment (middle) of tree. (C) Third segment (bottom) of tree.

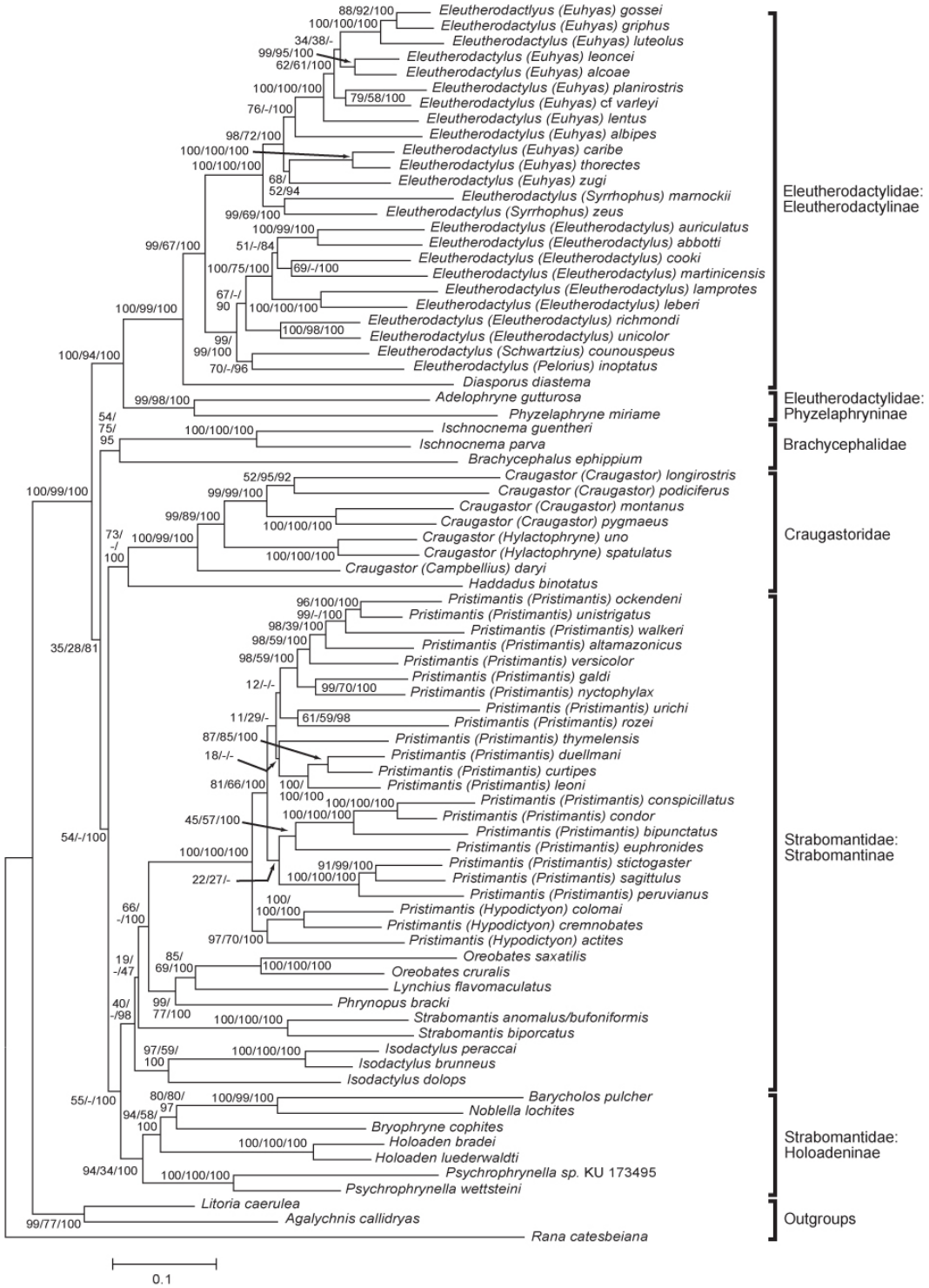


Figure 3-4. Maximum likelihood phylogeny of 80 species of frogs. The data set consists of 3709 base pairs of aligned DNA sequences, including mitochondrial DNA (12S rRNA, tRNA-Valine, and 16S rRNA) and nuclear DNA (RAG-1 and Tyr genes). Bootstrap support values and posterior probabilities (ML/NJ/Bayesian) are shown on nodes. Classification of the species is indicated.

3.5 Discussion

Classification. The new names and rearrangements that we introduce herein represent a major change in the classification of this large group of frogs. We have proposed these changes so that the classification better reflects phylogeny, as inferred from DNA sequence data (Figures 3-2, 3-3, 3-4) and have increased the number of families so that it is more manageable for future research. We have also identified morphological characters that support the classification, where they are known, but further research will be needed to determine shared derived morphological characters for many of the taxa. Our definitions of the four families, while based on the molecular phylogeny, largely correspond to geography (Figure 3-5). Brachycephalidae now corresponds to the small Southeast Brazil Clade. Craugastoridae consists of the Middle American Clade (*Craugastor*) and its closest relatives in South America (*Haddadus*). Eleutherodactylidae consists of the Caribbean Clade (*Eleutherodactylus*) and its closest mainland relatives (*Diasporus*, *Adelophryne*, and *Phyzelaphryne*). Strabomantidae includes a Northwest South America Clade (*Pristimantis*) and 15 small genera that are distributed almost entirely in South America. Considering the average number of species in a family of anurans (~100), the allocation of 882 species to only four families is still conservative.

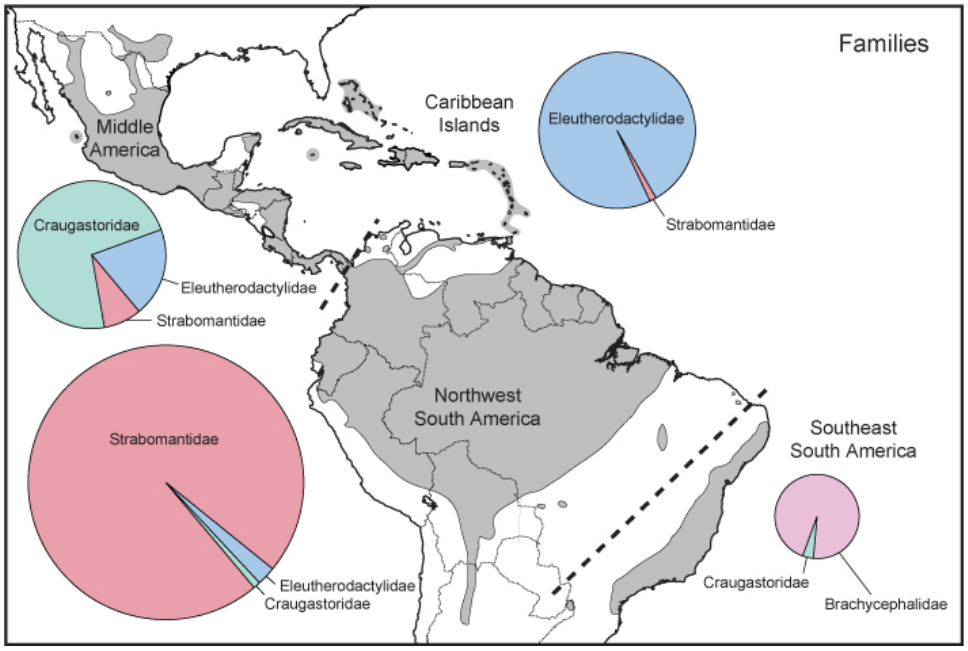


Figure 3-5. Proportion of species in each family of Terrarana, by geographic region. The area of each circle also is proportional to the total number of species in region.

The allocation of generic names was guided by two criteria (1) phylogenetic relationships and (2) binomial stability. Because binomens are the most widely used components of the classification by non-systematists, it is important to minimize unnecessary generic changes, especially those based only on molecular evidence (field identification requires morphological or geographic evidence). We have accomplished this by making wide use of the category of subgenus and several informal categories (species series, species group, and species subgroup). As a result, all but two species occurring on Caribbean Islands are placed in a single genus, *Eleutherodactylus*, 73% of the species occurring in Middle America are placed in *Craugastor*, 77% of the species in northwestern South America are placed in *Pristimantis*, and 82% of species in southeastern South America are placed in either *Ischnocnema* or *Brachycephalus* (Figure 3-6). In Middle America, the three additional terraranan genera occurring in that region can be distinguished based on morphological characters. In South America, the chief difficulty for field identification (to genus) is among the non-*Pristimantis* species of the Andes, where a variety of morphologically similar genera (*Phrynopus, Noblella, Oreobates, Lynchius, Isodactylus, Niceforonia, Bryophryne,* and *Psychrophrynella*) occur, albeit in much smaller numbers than *Pristimantis*.

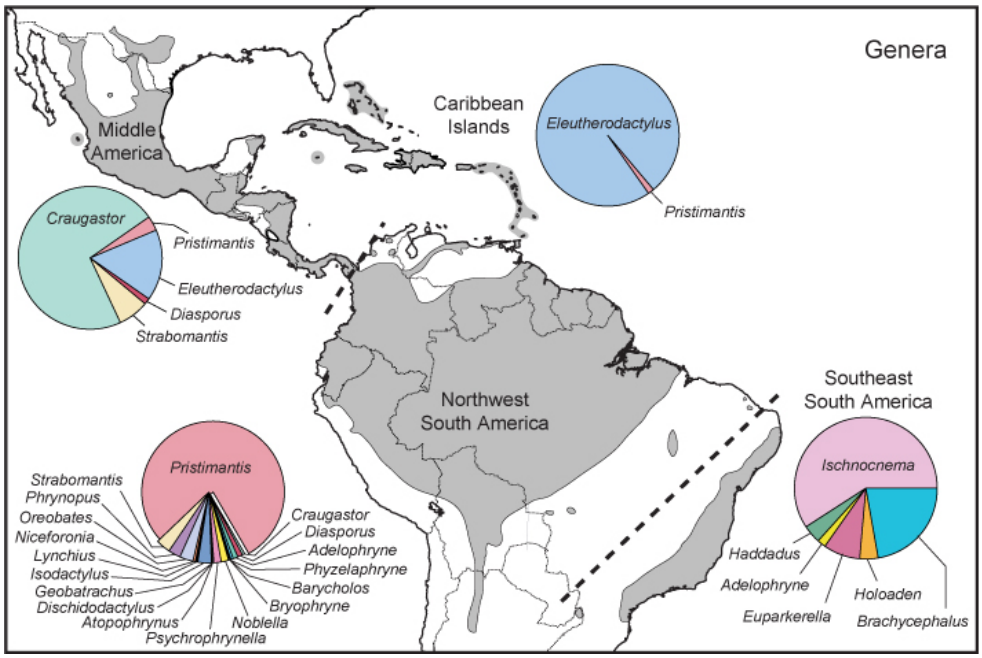


Figure 3-6. Proportion of species in each genus of Terrarana, by geographic region.

The relatively large number of taxa used for West Indian (Caribbean) species reflects the dense taxon-sampling for that region in our molecular phylogenies. Although longer sequences are needed and species will continue to be discovered, we do not anticipate major changes in the classification of West Indian eleutherodactylids. Our taxon sampling is sparser in Middle America. The subgenus *Syrrhophus* of *Eleutherodactylus* remains poorly sampled in that region and the species group definitions are based on morphology. More than one-third of the genus *Craugastor* has been sampled in molecular studies, and the phylogeny obtained here is well-supported at most nodes (Figures 3-2, 3-3, 3-4). Nearly all of the species not sampled can be assigned to taxa based on their affinities with sampled species. Additional species will be discovered, but we do not anticipate major changes in classification of *Craugastor* in the future, except for the recognition of additional lower divisions (groups and subgroups). South America remains the most poorly sampled geographic region. Of the 23 genera occurring in South America, five have yet to be sampled in any molecular study. We have no doubt that some of the species and genera not yet sampled will require refinement of the generic classification when they become sampled. As we noted above in the account for *Pristimantis*, inclusion of more than 100 species in our molecular phylogeny was insufficient to revise the classification for most of the genus. Therefore, much more systematic work remains ahead in Terrarana.

Evolution and Biogeography. As in most organisms, speciation in terraranans is not well-understood. However, their evolutionary history occurred almost entirely in the Cenozoic Era (65–0 million years ago, Ma) (Heinicke et al. 2007), thereby implying a relatively high rate of speciation. This evolutionary success probably can be attributed to the reproductive mode of these anurans. The innovation of direct development allowed them to disperse more widely over the land surface and exploit more terrestrial habitats, including some far from running or standing water. Thus rivers and other bodies of water have become barriers rather than conduits for gene flow. It has also been suggested that the small, terrestrial clutches of direct developing anurans are more suseptable to complete mortality (death of the entire clutch) compared with aquatic breeders, possibly increasing the rate of fixation of alleles (Dubois 2005). This and other hypotheses to

explain different rates of speciation among amphibians are reviewed elsewhere (Vences and Wake 2007).

Terraranans have an unusually rapid rate of chromosome change (Bogart 1981; Kaiser et al. 1994; Bogart and Hedges 1995), but it is not yet clear how such change bears on the mechanism or rate of speciation in these frogs. They definitely are most diverse (species rich) in upland areas where their distributional and elevational ranges tend to be relatively small. The observation in molecular phylogenies (e.g., Figure 3-2) that closely related species are often allopatric, especially on Caribbean islands where sampling is dense, suggests that allopatric speciation is the predominant mode of speciation in Terrarana. Geologic uplift and climate change during the Cenozoic would have frequently isolated populations of these forest-dwelling species, leading to speciation. However the details of this process and the subsequent changes leading to adaptive differences among species are unclear.

Terraranans have encountered and adapted to similar environments throughout the range of the group. In the process they have undergone evolutionary convergence in ecological habits and morphology. Several "ecomorphs" have been described for West Indian species of *Eleutherodactylus* (Hedges 1989a, 1989b); these probably are broadly applicable in Terrarana. For example, the aquatic (or stream) ecomorph includes species that occur in aquatic habitats, usually rocky streams, and have a streamlined body form, interdigital webbing, and large, round digital tips. The bromeliad ecomorph includes species adapted to bromeliads and have a dorsoventrally flattened body, large and rounded digital tips, and eyes oriented more forward on the head (Hedges et al. 1992a). Other ecomorphs have been named, but a more comprehensive survey is needed to determine the occurrence of ecomorphs more generally among terraranans and whether or not they form discrete categories in adaptive space.

The new data here do not alter the major aspects of the biogeographic scenario that we presented recently elsewhere, based on the molecular phylogeny and estimated times of divergence (Heinicke et al. 2007). Terraranans arose in South America and dispersed, probably across marine waters, to colonize Middle America (*Craugastor*) and the Antilles (*Eleutherodactylus*) in the Mid-Cenozoic (47–29 Ma). However, the new data provide greater resolution of relationships and have allowed us to identify the closest

relatives of the Middle American and Caribbean clades. *Haddadus* appears to be the closest relative of *Craugastor* whereas *Diasporus* is the closest relative of *Eleutherodactylus*. In the case of the Caribbean Clade, the phylogenetic tree (Figure 3-4) shows that *Diasporus* breaks up what was previously a long branch leading to *Eleutherodactylus* (Heinicke et al. 2007) and indicates that the dispersal probably occurred late in the interval 47–29 Ma.

By including additional representatives of the subgenus *Syrrhophus* we found that the mainland and Cuban members are reciprocally monophyletic. This is consistent with the distribution of characters such as the presence of dentigerous processes of the vomers in the Cuban species and their absence in mainland *Syrrhophus*. It also further supports the origin of the mainland members of *Syrrhophus* by dispersal from Cuba. Nuptial pads in males are common in mainland terraranans but are absent in all species of *Eleutherodactylus* and the closely related genera *Diasporus*, *Adelophryne* and *Phyzelaphryne*, establishing this character as diagnostic for the family. Also in common among *Eleutherodactylus* and those three related genera is small body size. The average maximum SVL of species of *Eleutherodactylus* is only 33.6 mm and the smallest species of tetrapod (tied with *Brachycephalus didactylus*) is a Cuban member of that genus (Estrada and Hedges 1996a). The average size of species in the three closely related genera is 18.1 mm. This contrasts with body size in *Craugastor*, in which the average maximum SVL is 52.9 mm, or 57% larger. The two species of *Haddadus*, the closest relative of *Craugastor*, have maximum SVLs of 17 and 64 mm. Although this suggests an inheritance of ancestral body size in these large adaptive radiations, each genus contains species near the lower and upper limits of size for Terrarana indicating that size has been not been constrained. Nonetheless, miniaturization in vertebrates often is associated with loss of characters and fusion of bones (Trueb and Alberch 1985; Hanken and Wake 1993), and therefore some defining characteristics of *Eleutherodactylus* (e.g., absence of nuptial pads) may be the consequence of having a diminutive ancestor.

The relationships of the subgenera and species series of the Middle American Clade (*Craugastor*) (Figures 3-2, 3-3), considered along with their distributions, allow a reconstruction of the biogeographic history of that clade. As was also noted by Crawford and Smith (2005) the two most basal lineages (here designated as the subgenera

Campbellius and *Hylactophryne*) are restricted to southern North America and northern Central America, indicating an initial colonization of the *Craugastor* lineage in that region. No species within those subgenera occurs further south than Honduras. In the mid-Cenozoic (31–42 Ma) when this initial colonization was estimated to have occurred (Heinicke et al. 2007), there may have been emergent land on the either the Chortis Block (now southern Guatemala, Honduras, El Salvador, and northern Nicaragua) or the southern portion of the North American continent (e.g., southern Mexico and Guatemala) or both (Pindell 1994; Iturralde-Vinent and MacPhee 1999; Hedges 2006b). Crawford and Smith (2005) found that the next most basal lineage was the "*C. gollmeri* group" (our *C. laticeps* Species Series), which includes taxa whose ranges extend southward into Panama. However, our molecular phylogeny (Figures 3-2, 3-3) differs in showing the next most basal lineage to be the *C. mexicanus* Species Series, which is restricted to Mexico and Guatemala. In our phylogeny, evidence of a migration south of Honduras is not seen until the next more derived node in the tree. All five of the remaining species series of the subgenus *Craugastor* form a monophyletic group that contains species that range at least as far south as Panama. Of that clade, two species series (*C. fitzingeri* and the *C. gulosus* species series) contain species that range further south, into South America. The southern portion of present-day Central America (Costa Rica and Panama) became emergent relatively late in the Cenozoic and therefore would have been unavailable for initial colonization, explaining this stepwise southward migration. Only four species of *Craugastor* occur in South America, and the implication is that their distributions have extended southwards only recently, after the emergence of the Isthmus of Panama in the Pliocene.

In South America, the Southeast Brazil Clade (Brachycephalidae) has existed as long as the clade represented by the family Strabomantidae, approximately 30–50 million years, yet the former led to only 40 described species whereas the latter has led to more than ten times that number (518 species). Strabomantids are distributed over a wider area, but they are most diverse in the Andes of western South America. As has been noted elsewhere, mountain building (in addition to associated climatic change) probably resulted in habitat isolation and increased rates of speciation in these frogs (Lynch and Duellman 1997).

Within Strabomantidae the divergence of Holoadeninae and Strabomantinae occurred approximately 40–45 Ma (Heinicke et al. 2007). The greatly disjunct distributions of the two species of *Barycholos* in the relatively dry lowlands of Pacific Ecuador and Colombia and eastern Amazonian Brazil suggest a widespread distribution of an ancestor prior to the major uplift of the Andes in the Miocene and Pliocene and an earlier differentiation of *Noblella,* which now inhabits Amazonian lowlands and the eastern cordilleras of the Andes. That part of the holoadenine ancestral stock that gave rise to *Bryophryne* and *Psychrophrynella* was associated later with the uplift of the Andes in Peru and Bolivia that occurred primarily since the early Miocene, 23 Ma (Gregory-Wodzicki 2002; MacFadden 2006).

Early evolution of strabomantine frogs involved the differentiation of at least four major clades approximately 30–40 Ma (Heinicke et al. 2007): *Isodactylus*, *Pristimantis*, *Strabomantis*, and the clade consisting of *Lynchius*, *Oreobates*, and *Phrynopus*. At least the differentiation of *Strabomantis* must have occurred before the major uplift of the northern Andes during the Pliocene (5.3–1.8 Ma), inasmuch as members of that genus are on both sides of the Andes. *Isodactylus* has one species in the Amazon Basin and several in the Andes, and apparently most of the speciation took place during the major Andean orogeny, since the early Miocene. Padial et al. (2007a) proposed that the early differentiation of the subgenus *Yunganastes* was coincident with the Andean orogeny. Our molecular phylogeny identifies several previously recognized species groups of *Pristimantis* that were based solely on morphological features. These include the *Pristimantis myersi, orestes,* and *conspicillatus* species groups. Within the latter, as defined by Lynch and Duellman (1997), two groups are apparent—the *P. conspicillatus* group of larger and mostly Amazonian frogs and the *P. peruvianus* group of smaller and mostly Andean frogs. On the other hand, all members of the *P. curtipes, devillei,* and *surdus* species groups, as defined by Lynch and Duellman (1997) are nested together and intermixed in a clade that also contains one species in the *P. unistrigatus* Group (*P. thymalopsoides*). Members of the "catch-all" *P. unistrigatus* Species Group, as defined by Lynch and Duellman (1997) appear in 10 different clades in the species-rich tree (Figure 3-2). Obviously molecular data are needed for many more species of *Pristimantis* before a reasonably clear picture of phylogenetic relationships will be visible. It also is

apparent that careful re-examination of morphological characters is required to accurately define species groups within *Pristimantis.*

As we noted for some groups of *Eleutherodactylus* in the West Indies and some of *Craugastor* in Middle America, allopatric speciation also accounts for the great diversity of upland strabomantids in the Andes. Allopatric distributions within elevational belts were emphasized for groups of *Pristimantis* in Andean Colombia and Ecuador (Lynch 1997; Lynch and Duellman 1997). Such patterns are especially evident in the *Pristimantis* (*Pristimantis*) *curtipes, galdi,* and *orcesi* groups (Lynch et al. 1997), *myersi* group (Lynch and Duellman 1997), and *orestes* group (Duellman and Pramuk 1999). Lynch and Duellman (1997) also pointed out latitudinal displacement of closely related species of *Strabomantis* on the Pacific lowlands of Colombia and Ecuador and of *Pristimantis* on the Pacific slopes of the Andes in Colombia and Ecuador.

Several genera or groups of species include species in the lowlands and others in the Andes. An analysis of sequence data from six species of *Oreobates* by Padial et al. (2008) shows the Amazonian species to be basal to the five Andean species. In our analyses, the otherwise upland (Andean) *Pristimantis peruvianus* group has the lowland *P. peruvianus* as the basal taxon. Based on morphology, Lynch (1997) postulated that *Strabomantis sulcatus* was basal to other members of the genus that occur in the Andes. All of these suggest that Andean taxa evolved from basal stocks in the lowlands, which existed before the uplift of the Andes. However, this apparent generalization may not hold true for all lineages. Based on morphological data, Lynch et al. (1997) postulated that in the "*Eleutherodactylus nigrovittatus*" Group a lowland species was imbedded in a clade of highland species; we are unable to refute this hypothesis because we lack molecular data for that lowland species that together with its Andean relatives is placed here in the genus *Isodactylus.*

In South America, the highlands of the great Andean mountain chain contain the greatest diversity of anurans on the continent (Duellman 1999), and a major component of that diversity is the family Strabomantidae. Of the 16 genera in Strabomantidae, only four (*Barycholos, Dischidodactylus, Euparkerella,* and *Holoaden*), with a total of 10 species, do not occur in the Andes. Eight genera (*Atopophrynus, Bryophryne, Geobatrachus, Lynchius, Niceforonia, Noblella, Phrynopus,* and *Psychrophrynella*), with

a total of 58 species, are endemic to the Andes. The remaining genera (*Isodactylus, Oreobates, Pristimantis,* and *Strabomantis*) have representatives in the lowlands, but the vast majority of the 450 species inhabit the Andes, the northern, tropical part of which obviously is the center of strabomantid diversity (Figure 3-6).

Conservation. The recent Global Amphibian Assessment (Stuart et al. 2004; IUCN 2006) found that 38% of terraranans are threatened and that 15% of terraranans are in the highest threat categories (critically endangered or extinct). Another 20% may also be threatened but there are insufficient data to determine their status. On Caribbean islands, the proportions are the highest of any region, for terraranans or for amphibians as a whole: 76% of the species are threatened and 40% are critically endangered. The threats are complex and still not well understood, although all potential causes involve the action of humans. Habitat loss is considered to be the overall major threat to amphibians in the Neotropics, affecting nearly 90% of the threatened species. Pollution and disease are the two other most commonly recorded threats (Stuart et al. 2004).

The fungal disease cytridiomycosis, caused by the species *Batrachochytrium dendrobatidis*, is central to many discussions of amphibian decline. Mass mortality of amphibians associated with the appearance of the fungus at localities in Panama provides compelling evidence that the fungus is the proximal cause of declines (Lips et al. 2006). On the other hand, the fungus is known to occur in other areas where it has not affected the resident amphibians (Daszak et al. 2005). Moreover, some frog populations have declined at the same time and to the same degree as co-occurring lizard populations suggesting that the proximal cause was not the fungus (Whitfield et al. 2007). In the latter case, those authors suggested that the declines were tied to a reduction in leaf litter as a result of global warming. Human-induced climate change already had been implicated in a previous study, although it was suggested in that case that warmer temperatures favored spread of the fungal disease (Pounds et al. 2006a).

Considering that lissamphibians and chytrid fungi probably have coexisted for at least 300 million years, it is unlikely that a disease would emerge naturally (without human influence), at this point in time, and potentially eradicate many species and clades of species that have evolved for tens of millions of years. Thus, if cytridiomycosis is the

major proximal cause of amphibian decline, humans in some way must be affecting its distribution or enhancing conditions for its growth through climate change (Pounds et al. 2006a). In this respect, it is important to identify the place of origin of this disease, if possible (Morgan et al. 2007).

While the importance of fungal disease as a major cause of amphibian declines continues to be debated (Mendelson et al. 2006; Pounds et al. 2006b; Wake 2007), the importance of habitat destruction is well-established. Almost all species of terraranans are forest dwelling, and thus deforestation proportionately decreases numbers of individuals. Deforestation obviously can lead to species extinctions as well. As a rough guide, the species-area relationship (MacArthur and Wilson 1967) predicts that the destruction of 90% of forest habitat is expected to lead to a 50% reduction in the number of species. In the West Indies, where human population density is at its highest in the New World, approximately 90% of original forests have been destroyed (Hedges and Woods 1993; Smith et al. 2005; Hedges 2006a). However, there is no evidence yet that 50% of the species have disappeared in the West Indies, and no expectation that they should do so immediately. Patches of forest often remain in the midst of broadly deforested areas, maintaining populations and species, if only temporarily. In Haiti, where forests have all but disappeared (Smith et al. 2005), much of the frog fauna—including 33 species of *Eleutherodactylus* endemic to Haiti and not found in the Dominican Republic—has survived in these precarious forest patches which also will soon disappear. For these Haitian frogs, habitat destruction is a more obvious threat than fungal disease. One of the better forested islands is Cuba, but even there only 15% of original forests remain intact (Smith et al. 2005).

Streamside species of terraranans seem to have suffered the most declines. In Central America, most of the species in *Craugastor* (*Campbellius*) and the *Craugastor* (*Craugastor*) *punctariolus* Species Group apparently have disappeared (Campbell 1999; McCranie and Wilson 2002; Savage 2002). In the West Indies, aquatic species on Jamaica (*Eleutherodactylus orcutti*), Hispaniola (*E. semipalmatus*), and Puerto Rico (*E. karlschmidti*) have not been seen in decades and may be extinct (Hedges 1993, 1999). In contrast, close relatives of those species, living in the same areas, appear to be unaffected. *Batrachochytrium dendrobatidis* is known to be in the West Indies, on at least Cuba

(Díaz et al. 2007) and Puerto Rico (Burrowes et al. 2004). However, the Cuban terraranan fauna—including aquatic species—has not shown any obvious declines at present (Hedges 1993, 1999; Hedges and Díaz In press). Continued monitoring of these species will be important in the future (Díaz et al. 2007).

Finally, the outcome of this reclassification has some important conservation implications. Saving the majority of the world's biodiversity would be prohibitively expensive, and therefore conservation practices invariably involve prioritization. The selection process of protected areas in individual countries is an example of prioritization at a local scale, whereas the concept of biodiversity "hot spots" (Myers et al. 2000) is an example of this on a global scale. Such systems of prioritization often rely on classifications and the maximizing of taxonomic diversity. We believe that this new classification better reflects the evolutionary history of these species, as well as their diversity, and therefore will better serve the conservation community as it faces difficult decisions ahead.

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Table 3-1. Specimens and sequences used in this study. Locality information for all samples is associated, and retrievable, with the GenBank accession numbers. Museum vouchers and tissue catalog numbers are given for all specimens sequenced for this study and for Heinicke et al. (2007). New sequences for this study have GenBank accession numbers EU186650–780; species with newly-generated sequences are denoted with an asterisk; n/a = not applicable. Museum abbreviations are AMNH (American Museum of Natural History), BWMC (Bobby Witcher Memorial Collection, Avila University), CVULA (Collection of Vertebrates, University of the Andes, Mérida, Venezuela), KU (University of Kansas Natural History Museum), LSUMZ (Louisiana State University, Museum of Zoology), MNHNCu (National Museum of Natural History, Havana, Cuba), MHNSM (Universidad Nacional Mayor de San Marcos, Lima, Peru), MVZ (Museum of Vertebrate Zoology, University of California, Berkeley), MZUSP (Museum of Zoology, University of São Paulo), QCAZ (Catholic University of Ecuador, Museum of Zoology), ROM (Royal Ontario Museum, Toronto, Canada), UPRRP (University of Puerto Rico, at Rio Piedras, Museum of Natural History), USNM (United States National Museum of Natural History, Smithsonian Institution), UTA (University of Texas at Arlington, Museum of Natural History).

Species	Museum Voucher	GenBank Accession Number 12S	16S	Rag1	Tyr
*Brachycephalus ephippium**	n/a	AY326008		n/a	n/a
	n/a	DQ283091		n/a	DQ282917
	USNM207716	n/a	n/a	EU186761	n/a
*Ischnocnema guentheri**	No voucher	EF493533		EF493407	EF493510
Ischnocnema hoehnei	No voucher	EF493359		n/a	n/a
*Ischnocnema holti**	USNM 318165	EU186740	EU186722	n/a	n/a
Ischnocnema parva	No voucher	EF493532		EF493400	EF493509
Ischnocnema juipoca	n/a	DQ283093		n/a	n/a
Craugastor daryi	UTA 57940	EF493531		EF493452	EF493480
*Craugastor crassidigitus**	MVZ 207248	EU186733	EU186715	n/a	n/a
Craugastor fitzingeri	n/a	AY326001		n/a	n/a
Craugastor longirostris	KU177803	EF493395		EF493454	EF493482
*Craugastor andi**	MVZ 207254	EU186687		n/a	n/a
*Craugastor emcelae**	AMNH 124468	EU186738	EU186720	n/a	n/a
*Craugastor melanostictus**	MVZ 203856	EU186683		n/a	n/a

*Craugastor megacephalus**	MVZ 207243	EU186688		n/a	n/a
*Craugastor laticeps**	MVZ 143299	EU186731	EU186713	n/a	n/a
*Craugastor lineatus**	MVZ 143301	EU186732	EU186714	n/a	n/a
*Craugastor sp.**	UTA 51036	EU186697		n/a	n/a
Craugastor montanus	UTA 51105	EF493530		EF493453	EF493478
Craugastor pygmaeus	UTA 55241	EF493711		EF493451	EF493479
*Craugastor angelicus**	MVZ 149762	EU186681		n/a	n/a
*Craugastor obesus**	AMNH 124540	EU186737	EU186719	n/a	n/a
Craugastor punctariolus	n/a	DQ283168		n/a	n/a
*Craugastor rugulosus**	MVZ 207279	EU186680		n/a	n/a
*Craugastor rupinius**	KU 289861	EU186669		n/a	n/a
Craugastor sandersoni	UTA-A49803	EF493712		n/a	n/a
Craugastor bransfordii	AMNH 124398	EF493822	EF493661	n/a	n/a
Craugastor podiciferus	MVZ12020	EF493360		EF493450	EF493481
*Craugastor loki**	MVZ 137064	EU186685		n/a	n/a
Craugastor rhodopis	n/a	DQ283317		n/a	n/a
*Craugastor sp.**	UTA 56684	EU186675		n/a	n/a
Craugastor augusti	n/a	DQ283271		n/a	n/a
*Craugastor tarahumarensis**	No voucher	EU186702		n/a	n/a
Craugastor alfredi	n/a	DQ283318		n/a	n/a
Craugastor bocourti	UTA-A55235	EF493713		n/a	n/a
*Craugastor sp.**	No voucher	EU186698		n/a	n/a
*Craugastor sp.**	No voucher	EU186703		n/a	n/a
*Craugastor spatulatus**	AMCC 118375	EU186674		EU186749	EU186770
*Craugastor stuarti**	MVZ 143310	EU186684		n/a	n/a
*Craugastor uno**	AMCC 118080	EU186673		EU186748	EU186769
	n/a	n/a	n/a	n/a	DQ282918
Haddadus binotatus	USNM303077	EF493361		EF493397	n/a
*Diasporus diastema**	MVZ 203844	EU186682		EU186752	EU186773
Eleutherodactylus abbotti	USNM564974	EF493540		EF493412	EF493457
*Eleutherodactylus audanti**	No voucher	EU186662		n/a	n/a
Eleutherodactylus haitianus	No voucher	EF493743	EF493583	n/a	n/a
Eleutherodactylus notidodes	USNM331514	EF493744	EF493584	n/a	n/a
Eleutherodactylus parabates	USNM332136	EF493746	EF493581	n/a	n/a
Eleutherodactylus pituinus	USNM332229	EF493747	EF493582	n/a	n/a
Eleutherodactylus sp.	USNM337773	EF493745	EF493580	n/a	n/a
Eleutherodactylus auriculatus	USNM564980	EF493344		EF493417	EF493458
Eleutherodactylus bartonsmithi	USNM309753	EF493736	EF493576	n/a	n/a
Eleutherodactylus eileenae	No voucher	EF493740	EF493577	n/a	n/a
Eleutherodactylus glamyrus	USNM564987	EF493737	EF493575	n/a	n/a
Eleutherodactylus mariposa	MNHNCu591	EF493738	EF493573	n/a	n/a
Eleutherodactylus ronaldi	USNM309760	EF493739	EF493574	n/a	n/a
Eleutherodactylus minutus	USNM331987	EF493741	EF493578	n/a	n/a
Eleutherodactylus poolei	USNM332236	EF493742	EF493579	n/a	n/a
Eleutherodactylus antillensis	USNM326747	EF493728	EF493556	n/a	n/a
Eleutherodactylus brittoni	USNM326765	EF493727	EF493554	n/a	n/a
Eleutherodactylus cochranae	USNM326775	EF493725	EF493555	n/a	n/a
Eleutherodactylus hedricki	USNM564995	EF493726	EF493553	n/a	n/a
Eleutherodactylus gryllus	USNM269304	EF493724	EF493552	n/a	n/a

Eleutherodactylus juanariveroi	UPRRP6361	EF493538	EF493365	n/a	n/a
Eleutherodactylus cooki	USNM326784	EF493539		EF493413	EF493455
Eleutherodactylus eneidae	USNM326857	EF493729	EF493557	n/a	n/a
Eleutherodactylus locustus	USNM326861	EF493730	EF493558	n/a	n/a
Eleutherodactylus coqui	USNM305421	EF493722	EF493550	n/a	n/a
Eleutherodactylus portoricensis	USNM326885	EF493720	EF493548	n/a	n/a
Eleutherodactylus schwartzi	No voucher	EF493723	EF493551	n/a	n/a
*Eleutherodactylus wightmanae**	USNM 326915	EU186651		n/a	n/a
Eleutherodactylus flavescens	USNM331662	EF493731	EF493559	n/a	n/a
Eleutherodactylus amplinympha	USNM564978	EF493732	EF493560	n/a	n/a
Eleutherodactylus barlagnei	USNM564982	EF493735	EF493563	n/a	n/a
Eleutherodactylus johnstonei	USNM336018	EF493733	EF493561	n/a	n/a
Eleutherodactylus martinicensis	USNM565001	EF493343		EF493419	EF493456
Eleutherodactylus pinchoni	USNM565006	EF493734	EF493562	n/a	n/a
*Eleutherodactylus richmondi**	USNM326894	EF493541		EU186758	EF493461
Eleutherodactylus unicolor	USNM326897	EF493542		EF493398	EF493462
Eleutherodactylus fowleri	USNM269266	EF493752	EF493568	n/a	n/a
*Eleutherodactylus lamprotes**	USNM564997	EF493379		EU186759	EF493460
Eleutherodactylus auriculatoides	USNM331627	EF493754	EF493572	n/a	n/a
Eleutherodactylus montanus	USNM332069	EF493756	EF493571	n/a	n/a
Eleutherodactylus patriciae	No voucher	EF493755	EF493570	n/a	n/a
Eleutherodactylus leberi	USNM309758	EF493342		EF493403	EF493459
Eleutherodactylus melacara	USNM309733	EF493751	EF493567	n/a	n/a
Eleutherodactylus guantanamera	MNHNCu590	EF493749	EF493565	n/a	n/a
Eleutherodactylus ionthus	USNM309757	EF493748	EF493564	n/a	n/a
Eleutherodactylus olibrus	USNM309763	EF493750	EF493566	n/a	n/a
Eleutherodactylus diplasius	USNM332369	EF493753	EF493569	n/a	n/a
*Eleutherodactylus sommeri**	USNM 332341	EU186654		n/a	n/a
*Eleutherodactylus wetmorei**	No voucher	EU186652		n/a	n/a
Eleutherodactylus alcoae	USNM564977	EF493382		EF493406	EF493469
Eleutherodactylus armstrongi	USNM329962	EF493778	EF493611	n/a	n/a
Eleutherodactylus darlingtoni	USNM307236	EF493777	EF493610	n/a	n/a
Eleutherodactylus leoncei	USNM564999	EF493375	EF493715	EF493404	EF493468
Eleutherodactylus albipes	USNM564976	EF493386		EF493409	EF493475
Eleutherodactylus dimidiatus	USNM564986	EF493802	EF493640	n/a	n/a
Eleutherodactylus emiliae	No voucher	EF493368	EF493638	n/a	n/a
Eleutherodactylus maestrensis	MNHNCu1003	EF493369	EF493639	n/a	n/a
Eleutherodactylus rucillensis	USNM332313	EF493803	EF493641	n/a	n/a
*Eleutherodactylus schmidti**	No voucher	EU186653		n/a	n/a
Eleutherodactylus greyi	USNM564991	EF493801	EF493628	n/a	n/a
Eleutherodactylus cuneatus	USNM564985	EF493775	EF493608	n/a	n/a
Eleutherodactylus turquinensis	USNM348803	EF493776	EF493609	n/a	n/a
Eleutherodactylus cavernicola	USNM266357	EF493763	EF493614	n/a	n/a
Eleutherodactylus cundalli	USNM266364	EF493761	EF493612	n/a	n/a
Eleutherodactylus glaucoreius	USNM305366	EF493762	EF493613	n/a	n/a
Eleutherodactylus fuscus	USNM266380	EF493769	EF493618	n/a	n/a
Eleutherodactylus gossei	USNM327419	EF493716		EF493410	EF493466
Eleutherodactylus junori	USNM269239	EF493764	EF493617	n/a	n/a
Eleutherodactylus pantoni	USNM327822	EF493766	EF493616	n/a	n/a
Eleutherodactylus pentasyringos	USNM266455	EF493765	EF493615	n/a	n/a

Eleutherodactylus jamaicensis	USNM327594	EF493770	EF493621	n/a	n/a
Eleutherodactylus grabhami	USNM327565	EF493772	EF493624	n/a	n/a
*Eleutherodactylus luteolus**	USNM327744	EF493545		EU186757	EF493464
Eleutherodactylus sisyphodemus	USNM266467	EF493773	EF493625	n/a	n/a
Eleutherodactylus alticola	USNM266340	EF493768	EF493620	n/a	n/a
	USNM327267	EF493544	n/a	n/a	n/a
Eleutherodactylus andrewsi	USNM327274	n/a	EF493623	n/a	n/a
Eleutherodactylus griphus	USNM564992	EF493381		EF493415	EF493465
Eleutherodactylus nubicola	USNM327777	EF493771	EF493622	n/a	n/a
Eleutherodactylus orcutti	USNM327808	EF493767	EF493619	n/a	n/a
Eleutherodactylus riparius	n/a	Y10944		n/a	n/a
Eleutherodactylus rivularis	USNM565009	EF493376	EF493626	n/a	n/a
Eleutherodactylus toa	USNM306544	EF493774	EF493627	n/a	n/a
Eleutherodactylus amadeus	USNM329866	EF493805	EF493644	n/a	n/a
Eleutherodactylus bakeri	USNM564981	EF493808	EF493647	n/a	n/a
Eleutherodactylus caribe	USNM314179	EF493385		EF493411	EF493472
Eleutherodactylus corona	KU218431	EF493807	EF493645	n/a	n/a
Eleutherodactylus dolomedes	KU218434	EF493809	EF493648	n/a	n/a
Eleutherodactylus eunaster	No voucher	EF493804	EF493646	n/a	n/a
Eleutherodactylus glanduliferoides	USNM564989	EF493546	EF493364	n/a	n/a
Eleutherodactylus glaphycompus	USNM292259	EF493383		n/a	n/a
Eleutherodactylus heminota	USNM331829	EF493806	EF493649	n/a	n/a
Eleutherodactylus thorectes	USNM565011	EF493384		EF493416	EF493473
Eleutherodactylus brevirostris	USNM329968	EF493819	EF493657	n/a	n/a
Eleutherodactylus glandulifer	USNM564988	EF493816	EF493655	n/a	n/a
Eleutherodactylus sciagraphus	USNM332316	EF493817	EF493656	n/a	n/a
Eleutherodactylus ventrilineatus	USNM332320	EF493818	EF493658	n/a	n/a
Eleutherodactylus jugans	USNM331952	EF493810	EF493652	n/a	n/a
Eleutherodactylus apostates	USNM564979	EF493811	EF493650	n/a	n/a
Eleutherodactylus oxyrhynchus	USNM332073	EF493812	EF493651	n/a	n/a
Eleutherodactylus paulsoni	USNM310833	EF493815	EF493659	n/a	n/a
Eleutherodactylus furcyensis	USNM331673	EF493814	EF493654	n/a	n/a
Eleutherodactylus rufifemoralis	No voucher	EF493813	EF493653	n/a	n/a
Eleutherodactylus atkinsi	USNM335686	EF493797	EF493598	n/a	n/a
Eleutherodactylus gundlachi	USNM564994	EF493798	EF493597	n/a	n/a
Eleutherodactylus intermedius	USNM564996	EF493799	EF493595	n/a	n/a
Eleutherodactylus varleyi	USNM335732	EF493800	EF493596	n/a	n/a
Eleutherodactylus cf. varleyi	MNHNCu1002	EF493345		EF493408	EF493467
Eleutherodactylus cubanus	No voucher	EF493796	EF493594	n/a	n/a
Eleutherodactylus etheridgei	USNM335715	EF493794	EF493593	n/a	n/a
Eleutherodactylus iberia	MNHNCu1001	EF493374	EF493591	n/a	n/a
*Eleutherodactylus jaumei**	MNHNCu 1002	EU186672		n/a	n/a
Eleutherodactylus limbatus	USNM565000	EF493795	EF493590	n/a	n/a
Eleutherodactylus orientalis	USNM565003	EF493373	EF493592	n/a	n/a
Eleutherodactylus pezopetrus	USNM565004	EF493793	EF493601	n/a	n/a
Eleutherodactylus blairhedgesi	No voucher	EF493371	EF493606	n/a	n/a
Eleutherodactylus pinarensis	USNM565005	EF493792	EF493607	n/a	n/a
Eleutherodactylus thomasi	No voucher	EF493370	EF493605	n/a	n/a
Eleutherodactylus casparii	USNM564984	EF493788	EF493599	n/a	n/a
Eleutherodactylus goini	USNM335725	EF493791	EF493604	n/a	n/a

Eleutherodactylus guanahacabibes	USNM564993	EF493789	EF493600	n/a	n/a
Eleutherodactylus planirostris	USNM565007	EF493346		EF493396	EF493470
Eleutherodactylus rogersi	USNM565010	EF493372	EF493603	n/a	n/a
Eleutherodactylus tonyi	No voucher	EF493790	EF493602	n/a	n/a
Eleutherodactylus grahami	USNM564990	EF493781	EF493632	n/a	n/a
Eleutherodactylus lentus	USNM564998	EF493717		EF493418	EF493471
Eleutherodactylus monensis	USNM565002	EF493783	EF493633	n/a	n/a
Eleutherodactylus pictissimus	USNM266310	EF493782	EF493631	n/a	n/a
Eleutherodactylus probolaeus	USNM322252	EF493784	EF493634	n/a	n/a
Eleutherodactylus rhodesi	USNM332259	EF493779	EF493629	n/a	n/a
Eleutherodactylus weinlandi	USNM332332	EF493780	EF493630	n/a	n/a
Eleutherodactylus acmonis	USNM564975	EF493787	EF493637	n/a	n/a
Eleutherodactylus bresslerae	USNM564983	EF493785	EF493635	n/a	n/a
Eleutherodactylus ricordii	USNM565008	EF493786	EF493636	n/a	n/a
Eleutherodactylus klinikowskii	MNHNCu1004	EF493547	EF493363	n/a	n/a
Eleutherodactylus zugi	USNM335744	EF493347		EF493401	EF493474
Eleutherodactylus chlorophenax	USNM257730	EF493543	EF493589	n/a	n/a
Eleutherodactylus inoptatus	USNM331931	EF493380		EF493405	EF493463
Eleutherodactylus nortoni	USNM257744	EF493760	EF493588	n/a	n/a
Eleutherodactylus aporostegus	USNM257752	EF493759	EF493586	n/a	n/a
*Eleutherodactylus bothroboans**	USNM 332278	EU186655		n/a	n/a
Eleutherodactylus hypostenor	USNM257731	EF493757	EF493585	n/a	n/a
Eleutherodactylus parapelates	USNM257726	EF493758	EF493587	n/a	n/a
*Eleutherodactylus counouspeus**	USNM329989	EF493719		EU186760	n/a
	n/a	DQ283102	DQ283101	n/a	n/a
Eleutherodactylus marnockii	No voucher	EF493820	EF493642	EF493399	EF493476
*Eleutherodactylus pipilans**	AMCC 118110	EU186729	EU186711	n/a	n/a
*Eleutherodactylus nitidus**	AMCC 118239	EU186730	EU186712	n/a	n/a
Eleutherodactylus symingtoni	No voucher	EF493821	EF493643	n/a	n/a
Eleutherodactylus zeus	USNM335740	EF493718		EF493402	EF493477
*Adelophryne gutturosa**	ROM 39578	EU186679		EU186751	EU186772
*Phyzelaphryne miriamae**	LSUMZ 16935	EU186689		EU186753	EU186774
*Barycholos pulcher**	KU 217781	EU186727	EU186709	EU186744	EU186765
Barycholos ternetzi	n/a	n/a	DQ283094	n/a	DQ284144
Bryophryne cophites	KU173497	EF493537		EF493423	EF493508
*Holoaden bradei**	USNM207945	EF493378	EF493366	EF493449	EU186779
*Holoaden luederwaldi**	MZUSP 131872	EU186728	EU186710	EU186747	EU186768
*Noblella lochites**	KU 177356	EU186699		EU186756	EU186777
Noblella sp.	n/a	AM039714	AM039646	n/a	n/a
Psychrophrynella iatamasi	n/a	AM039712	AM039644	n/a	n/a
*Psychrophrynella sp.**	KU173495	EF493714		EU186762	EU186780
Psychrophrynella sp.	n/a	AM039710	AM039642	n/a	n/a
Psychrophrynella sp.	n/a	AY843720		n/a	n/a
*Psychrophrynella wettsteini**	KU 183049	EU186696		EU186755	EU186776
Isodactylus brunneus	KU178258	EF493357		EF493422	EF493484
Isodactylus dolops	No voucher	EF493394		EF493414	EF493483
Isodactylus elassodiscus	KU177282	EF493358		n/a	n/a
Isodactylus peraccai	KU178266	EF493710		EF493420	EF493485
*Lynchius flavomaculatus**	KU 218210	EU186667		EU186745	EU186766
*Lynchius nebulanastes**	KU 181408	EU186704		n/a	n/a

*Lynchius parkeri**	KU 181307	EU186705		n/a	n/a
Lynchius sp.	n/a	AM039707	AM039639	n/a	n/a
*Oreobates cruralis**	KU 215462	EU186666		EU186743	EU186764
	KU178249-250	EF493828	EF493662	n/a	n/a
Oreobates quixensis	n/a	AY819344	AY819474	n/a	n/a
*Oreobates saxatilis**	KU 212327	EU186726	EU186708	EU186742	EU186763
Oreobates sp.	n/a	DQ283060	DQ283061	n/a	n/a
Phrynopus auriculatus	KU291634	EF493708		n/a	n/a
Phrynopus barthlenae	n/a	AM039717	AM039649	n/a	n/a
Phrynopus bracki	USNM286919	EF493709		EF493421	EF493507
Phrynopus bufoides	n/a	AM039713	AM039645	n/a	n/a
Phrynopus heimorum	n/a	AM039703	AM039635	n/a	n/a
Phrynopus horstpauli	n/a	AM039715	AM039647	n/a	n/a
Phrynopus juninensis	n/a	AM039725	AM039657	n/a	n/a
Phrynopus kauneorum	n/a	AM039718	AM039650	n/a	n/a
Phrynopus pesantesi	n/a	AM039724	AM039656	n/a	n/a
Phrynopus tautzorum	n/a	AM039720	AM039652	n/a	n/a
*Phrynopus tribulosus**	KU 291630	EU186725	EU186707	n/a	n/a
*Pristimantis dendrobatoides**	ROM 43318	EU186735	EU186717	n/a	n/a
Pristimantis colomai	QCAZ17101	EF493354		EF493440	EF493502
Pristimantis cremnobates	KU177252	EF493528		EF493424	EF493486
Pristimantis cruentus	AMNH12444-448	EF493697		n/a	n/a
Pristimantis latidiscus	KU218016	EF493698		n/a	n/a
Pristimantis ridens	AMNH-A124551	EF493355		n/a	n/a
Pristimantis achatinus	KU217809	EF493827	EF493660	n/a	n/a
Pristimantis actites	KU217830	EF493696		EF493432	EF493494
Pristimantis crenunguis	KU177730	EF493693	EF493666	n/a	n/a
Pristimantis labiosus	QCAZ19771	EF493694		n/a	n/a
Pristimantis lanthanites	KU222001	EF493695		n/a	n/a
Pristimantis w-nigrum	n/a	AY326004		n/a	n/a
Pristimantis bipunctatus	KU291638	EF493702		EF493430	EF493492
Pristimantis caprifer	KU177680	EF493391		n/a	n/a
Pristimantis citriogaster	KU212278	EF493700		n/a	n/a
Pristimantis condor	KU217857	EF493701		EF493443	EF493504
Pristimantis conspicillatus	QCAZ28448	EF493529		EF493437	EF493499
Pristimantis fenestratus	MHNSM9298	EF493703		n/a	n/a
Pristimantis lymani	KU218019	EF493392		n/a	n/a
*Pristimantis malkini**	QCAZ 28296	EU186663		n/a	n/a
Pristimantis skydmainos	MHNSM10071	EF493393		n/a	n/a
Pristimantis sp.	MHNSM-LR4341	EF493356		n/a	n/a
*Pristimantis terraebolivaris**	No voucher	EU186650		n/a	n/a
*Pristimantis zeuctotylus**	ROM 43978	EU186678		n/a	n/a
Pristimantis chalceus	KU177638	EF493675		n/a	n/a
Pristimantis buckleyi	KU217836	EF493350		n/a	n/a
Pristimantis cryophilius	KU217863	EF493672		n/a	n/a
Pristimantis curtipes	KU217871	EF493513		EF493435	EF493497
Pristimantis gentryi	KU218109	EF493511		n/a	n/a
Pristimantis appendiculatus	KU177637	EF493524		n/a	n/a
Pristimantis devillei	KU217991	EF493688		n/a	n/a

Pristimantis quinquagesimus	KU179374	EF493690		n/a	n/a
Pristimantis truebae	KU218013	EF493512		n/a	n/a
Pristimantis vertebralis	KU177972	EF493689		n/a	n/a
Pristimantis ockendeni	KU222023	EF493519		EF493434	EF493496
*Pristimantis galdi**	QCAZ 32368	EU186670		EU186746	EU186767
Pristimantis bromeliaceus	KU291702	EF493351		n/a	n/a
*Pristimantis cf mendax**	MTD 45080	EU186659		n/a	n/a
Pristimantis schultei	KU212220	EF493681		n/a	n/a
Pristimantis festae	KU218234	EF493515		n/a	n/a
Pristimantis leoni	KU218227	EF493684		EF493433	EF493495
Pristimantis ocreatus	KU208508	EF493682		n/a	n/a
Pristimantis pyrrhomerus	KU218030	EF493683		n/a	n/a
Pristimantis orcesi	KU218021	EF493679		n/a	n/a
Pristimantis thymelensis	QCAZ16428	EF493516		EF493442	EF493503
Pristimantis melanogaster	MHNSM-WED56846	EF493826	EF493664	n/a	n/a
Pristimantis orestes	KU218257	EF493388		n/a	n/a
*Pristimantis simonsii**	KU 212350	EU186665		n/a	n/a
Pristimantis simonbolivari	KU218254	EF493671		n/a	n/a
*Pristimantis albertus**	KU 291675	EU186695		n/a	n/a
Pristimantis aniptopalmatus	KU291627	EF493390		n/a	n/a
Pristimantis peruvianus	MHNSM9267	EF493707		EF493436	EF493498
Pristimantis "pluvicanorus"	n/a	AY843586		n/a	n/a
Pristimantis rhabdolaemus	KU173492	EF493706		n/a	n/a
*Pristimantis cf rhabdolaemus**	MTD 45073	EU186660		n/a	n/a
Pristimantis sagittulus	KU291635	EF493705		EF493439	EF493501
Pristimantis stictogaster	KU291659	EF493704		EF493445	EF493506
Pristimantis toftae	KU215493	EF493353		n/a	n/a
	KU217998	n/a	n/a	EF493438	EF493500
Pristimantis duellmani	n/a	AY326003		n/a	n/a
Pristimantis surdus	KU177847	EF493687		n/a	n/a
*Pristimantis sp.**	KU 179221	EU186700		n/a	n/a
*Pristimantis sp.**	KU 218140	EU186661		n/a	n/a
Pristimantis acerus	KU217786	EF493678		n/a	n/a
*Pristimantis altamazonicus**	KU215460	EF493670		EF493441	EU186778
*Pristimantis ardalonychus**	KU 212301	EU186664		n/a	n/a
Pristimantis cajamarcensis	KU217845	EF493823	EF493663	n/a	n/a
Pristimantis calcarulatus	KU177658	EF493523		n/a	n/a
*Pristimantis caryophyllaceus**	MVZ 203810	EU186686		n/a	n/a
Pristimantis celator	KU177684	EF493685		n/a	n/a
Pristimantis ceuthospilus	KU 212216	EF493520		n/a	n/a
Pristimantis chloronotus	n/a	AY326007		n/a	n/a
Pristimantis croceoinguinis	KU217862	EF493669	EF493665	n/a	n/a
*Pristimantis crucifer**	KU 177733	EU186736	EU186718	n/a	n/a
*Pristimantis cruciocularis**	KU 291673	EU186656		n/a	n/a
*Pristimantis diadematus**	KU 221999	EU186668		n/a	n/a
Pristimantis dissimulatus	KU179090	EF493522		n/a	n/a
*Pristimantis eriphus**	QCAZ 32705	EU186671		n/a	n/a
Pristimantis euphronides	BWMC6918	EF493527		EF493427	EF493489
Pristimantis glandulosus	KU218002	EF493676		n/a	n/a
Pristimantis imitatrix	KU215476	EF493824	EF493667	n/a	n/a

*Pristimantis inguinalis**	ROM 40164	EU186676		n/a	n/a
Pristimantis inusitatus	KU218015	EF493677		n/a	n/a
*Pristimantis jester**	ROM 43302	EU186734	EU186716	n/a	n/a
Pristimantis lirellus	KU212226	EF493521		n/a	n/a
Pristimantis luteolateralis	KU177807	EF493517		n/a	n/a
*Pristimantis marmoratus**	ROM 43913	EU186692		n/a	n/a
*Pristimantis minutulus**	KU 291677	EU186657		n/a	n/a
Pristimantis nyctophylax	KU177812	EF493526		EF493425	EF493487
Pristimantis parvillus	KU177821	EF493351		n/a	n/a
Pristimantis petrobardus	KU212293	EF493825	EF493367	n/a	n/a
Pristimantis phoxocephalus	KU218025	EF493349		n/a	n/a
*Pristimantis prolatus**	KU 177433	EU186701		n/a	n/a
Pristimantis pycnodermis	KU218028	EF493680		n/a	n/a
*Pristimantis rhabdocnemus**	KU 291651	EU186724	EU186706	n/a	n/a
Pristimantis rhodoplichus	KU219788	EF493674		n/a	n/a
Pristimantis riveti	KU218035	EF493348		n/a	n/a
Pristimantis rozei	No voucher	EF493691		EF493429	EF493491
*Pristimantis saltissimus**	ROM 43310	EU186693		n/a	n/a
Pristimantis shrevei	No voucher	EF493692		n/a	n/a
*Pristimantis sp.**	No voucher	EU186658		n/a	n/a
*Pristimantis sp.**	LSUMZ 16898	EU186690		n/a	n/a
*Pristimantis sp.**	No voucher	EU186739	EU186721	n/a	n/a
Pristimantis sp.	n/a	AY326002		n/a	n/a
Pristimantis spinosus	KU218052	EF493673		n/a	n/a
Pristimantis subsigillatus	KU218147	EF493525		n/a	n/a
Pristimantis supernatis	n/a	AY326005		n/a	n/a
Pristimantis thymalopsoides	KU177861	EF493514		n/a	n/a
Pristimantis unistrigatus	KU218057	EF493387		EF493444	EF493505
Pristimantis urichi	USNM336098	EF493699		EF493426	EF493488
Pristimantis verecundus	QCAZ12410	EF493686		n/a	n/a
Pristimantis versicolor	KU218096	EF493389		EF493431	EF493493
Pristimantis walkeri	KU218116	EF493518		EF493428	EF493490
Pristimantis wiensi	KU219796	EF493377	EF493668	n/a	n/a
*Pristimantis pulvinatus**	KU 181015	EU186741	EU186723	n/a	n/a
*Strabomantis biporcatus**	CVULA 7073	EU186691		EU186754	EU186775
Strabomantis sulcatus	KU218055	EF493536		n/a	n/a
Strabomantis anomalus	KU177627	EF493534		EF493447	n/a
Strabomantis bufoniformis	n/a	DQ283165		n/a	DQ282942
Strabomantis necerus	KU179076	EF493535		n/a	n/a
	n/a	DQ283423		n/a	DQ283018
Agalychnis callidryas	n/a	n/a	n/a	EF493362	n/a
Bufo melanostictus	n/a	AY458592		n/a	n/a
Caudiverbera caudiverbera	n/a	DQ283439		n/a	n/a
Centrolene prosoblepon	n/a	AY843574		n/a	n/a
Ceratophrys cornuta	n/a	AY326014		n/a	n/a
Cryptobatrachus sp.	n/a	AY326050		n/a	n/a
Dendrobates sylvaticus	n/a	AY364569		n/a	n/a
Hyla chinensis	n/a	AY458593		n/a	n/a
Leptodactylus pentadactylus	n/a	AY326017		n/a	n/a
Litoria caerulea	n/a	AY843692		n/a	AY844131

	No voucher	n/a	n/a	EF493446	n/a
Melanophryniscus klappenbachi	n/a	AY843699		n/a	n/a
Odontophrynus achalensis	n/a	DQ283248		n/a	n/a
Pseudis paradoxa	n/a	AY843740		n/a	n/a
Pseudophryne bibroni	n/a	AY843742		n/a	n/a
Rhinoderma darwinii	n/a	DQ283324		n/a	n/a
Telmatobius verrucosus	n/a	DQ283040		n/a	n/a
*Unidentified hyloid sp.**	ROM 40161	EU186677		EU186750	EU186771
Rana catesbeiana	n/a	M57527		n/a	n/a
	n/a	DQ283257		n/a	DQ282959
	No voucher	n/a	n/a	EF493448	n/a

CHAPTER 4

A new frog family (Anura: Terrarana) and an expanded direct-developing clade revealed by molecular phylogeny.

Note: Modified from Heinicke, M. P., Duellman, W. E., Trueb, L., Means, D. B., MacCulloch, R. D., and Hedges, S. B. 2009. *Zootaxa* **2211**: 1-35. MPH carried out laboratory and computational research. SBH and WED examined external morphology. LT examined osteology. DBM and RDM collected the new species, and provided natural history data. MPH and SBH drafted paper, with other authors contributing text in their sections. Systematic accounts appearing in this paper were largely composed by SBH, WED, and LT and are provided in the Appendix.

4.1 Abstract.

Three frogs of a new species found in cloud forests on two nearby mountains in Guyana were included in a molecular phylogeny of 17 nuclear and mitochondrial genes (10,739 aligned sites) that revealed that their closest relative is Terrarana (Brachycephalidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae) and their next-closest relative is Hemiphractidae (marsupial frogs). We place these frogs in a new family, genus, and species which is strongly supported as the basal clade within Terrarana: Ceuthomantidae n. fam., *Ceuthomantis smaragdinus* n. gen, n. sp. Morphological evidence supports the placement of two other species from the Guiana Highlands, *Pristimantis aracamuni* (Barrio-Amorós and Molina) and *P. cavernibardus* (Myers and Donnelly), in the new family and genus. This close phylogenetic relationship of terraranans and marsupial frogs, nearly all of which have direct development, supports an hypothesis that direct development evolved early in the evolution of this huge clade (~1000 species), for which we propose the unranked taxonomic epithet Orthobatrachia.

4.2 Introduction

During the past five years, phylogenetic studies of frogs based on molecular data have resulted in many taxonomic changes at the familial and generic levels—Darst and Cannatella 2004, Faivovich et al. 2005, Wiens et al. 2005, Frost et al. 2006, Grant et al. 2006, Crawford and Smith 2005, and Guayasamin et al. 2008. Frogs formerly placed in

the immense, diverse genus *Eleutherodactylus* were subjected to phylogenetic analyses of both mitochondrial and nuclear genes by Hedges et al. (2008). The analysis of 344 species resulted in the recognition of four families (Brachycephalidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae) placed in the unranked taxon Terrarana, with one "unknown Anuran sp." (Hedges et al., 2008, fig. 2) lying between Terrarana and the outgroups. This unidentified juvenile frog resembled *Pristimantis,* by far the largest genus in the Strabomantidae with 426 species (AmphibiaWeb 2009); this small frog was found on Mt. Ayanganna, Guyana, by A. Lathrop and C. Cox in October 2000. In July 2007 one of us (D.B.M) collected several species of *Pristimantis* on nearby Mt. Kopinang, Guyana. Genetic sequences obtained from tissues of these specimens revealed that two individuals were essentially the same as the "unknown anuran." Morphologically, the frogs resembled some species of *Pristimantis*. However, the phylogenetic analyses of sequences from 17 genes revealed that these specimens are not only distinct from Strabomantidae but represent an evolutionary lineage so distant that its closest relative is the clade containing all terraranan frogs (i.e., 4 families and ~900 species). Unique morphological traits further supported their position in the molecular tree and showed that they necessitate placement in a new family, which is described herein.

4.3 Materials and methods

General. In the field, specimens were handled and euthanized according to approved animal care protocols. After tissues were removed and placed in 95% ethanol, specimens were fixed in formalin and subsequently stored in 70% ethanol. We use the classification proposed by Hedges et al. (2008). Museum abbreviations are: AMNH = American Museum of Natural History, New York, USA; KU = Herpetological collection in the Biodiversity Institute (formerly Natural History Museum), University of Kansas, Lawrence, USA; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley, USA; ROM = Royal Ontario Museum, Toronto, Canada.

Morphology. External observations and measurements were taken under a Leica stereo-zoom microscope. Measurements were taken to the nearest 0.1 mm with dial

calipers. Measurements and external morphological features are those defined by Lynch and Duellman (1997), except that the term dentigerous processes of vomers is used instead of vomerine odontophores. Snout–vent length is abbreviated SVL. In order to maintain consistency, the numbered arrangement in the diagnosis also follows that in Lynch and Duellman (1997). Sex was determined by examination of the gonads. The nature of the adductor musculature and of the glandlike protrusions on the dorsum was determined by dissection of KU 315000.

We follow Myers and Donnelly's (1997) terminology for emarginate conditions of digital tips: An indented margin is defined as a broad, shallow concavity—e.g., *Pristimantis crenunguis* (Lynch) (Lynch and Duellman 1997, fig. 15C). A notched margin is defined as a distinct, narrow concavity—e.g., *Pristimantis aracamuni* (Barrio-Amorós and Molina 2006, fig. 2); *P. cavernibardus* (Myers and Donnelly 1997, fig. 37A). In *Dischidodactylus duidensis* (Rivero), the ungual flap is indented and longitudinally divided (Lynch 1979, fig. 3); the same condition exists in *D. colonnelloi* (Ayarzagüena 1985, fig. 3).

The osteological description is based on high-resolution tomographs of the skeleton of KU 315000, and comparisons are made with tomographs of other Terrarana. These were scanned on the OMNI-X high-resolution x-ray CT scanner at the Center for Quantitative Imaging at Pennsylvania State University at voxel dimensions of 0.03–0.05 mm. CT images and animations of the specimens presented here are available at DigiMorph (http://digimorph.org/). Terminology for the cranial osteology follows Trueb (1993). Proportions are based on measurements that were made from the tomograph with the measuring tool in Adobe Photoshop® Version 10.0. Except where noted, osteological measurements are those defined by Trueb (1977).

There is a notable discrepancy in the numbering of the digits in the hand. The description of external features of the hand follows the standard practice of the median (preaxial) digit ("thumb") being designated Finger I. Alberch and Gale (1985) and Fabrezi and Alberch (1996) showed that during development the first (preaxial) digit is lost, so that the first digit ("thumb") of anurans actually is Digit II. This arrangement is becoming standard in osteological studies. Consequently, in the description of external

features, the digits on the hand are referred to as Fingers I, II, III, and IV; the same digits in the osteological description are designated Digits II, III, IV, and V.

Molecular analyses. We sequenced or obtained from GenBank data from 11 nuclear and six mitochondrial genes totaling 10,739 bases, for exemplars of 39 nobleobatrachian and four outgroup taxa, as well as three samples of the new family (Table 4-1). The nuclear genes were 28S ribosomal RNA (28S), cellular myelocytomatosis (c-myc), chemokine receptor 4 (CXCR4), Histone H3 (HH3), sodium-calcium exchanger 1 (NCX1), proopiomelanocortin A (POMC), recombination activating protein 1 (RAG-1), Rhodopsin (Rho), seventh in absentia (SIA), solute carrier family 8 member 3 (SLC8a3), and Tyrosinase precursor (Tyr). The mitochondrial genes were 12S ribosomal RNA (12S), tRNA-Valine (tRNAV), 16S ribosomal RNA (16S), tRNA-Leucine (tRNAL), NADH dehydrogenase 1 (ND1), and cytochrome b (CytB). Most taxa are chimeric, consisting of sequences from several species within a genus or, in cases where sequences of congeners were not available, between closely related genera. Species composition of the chimeric sequences was guided by the results of previous molecular phylogenetic analyses (Frost et al. 2006; Wiens et al. 2005; Faivovich et al. 2005; Grant et al. 2007; Darst and Cannatella 2004; Guayasamin et al. 2008; Roelants et al. 2007). All nobleobatrachian families (sensu Frost 2009: Aromobatidae, Brachycephalidae, Bufonidae, Centrolenidae, Ceratophryidae, Craugastoridae, Cycloramphidae, Dendrobatidae, Eleutherodactylidae, Hemiphractidae, Hylidae, Hylodidae, Leiuperidae, Leptodactylidae, Strabomantidae) were represented, as well as multiple taxa of the most diverse families, or those families rendered polyphyletic in previous molecular phylogenetic studies of nobleobatrachians (Darst and Cannatella 2004; Faivovich et al. 2005; Frost et al. 2006; Grant et al. 2006; Roelants et al. 2007). The four outgroups included ranid, limnodynastid, myobatrachid, and *Calyptocephallela* sequences, representing the closest families outside Nobleobatrachia.

For specimens sequenced in this study (Table 4-1), genomic DNA was extracted from frozen or ethanol-preserved tissue samples using a Qiagen DNeasy Blood and Tissue kit. Polymerase chain reactions were performed at 50 μL volume using AmpliTaq

DNA polymerase and ThermoPol buffer (NEB). Primer sequences were obtained from the literature (Biju and Bossuyt 2003; Bossuyt and Milinkovitch 2000; Faivovich et al. 2005; Frost et al. 2006; Heinicke et al. 2007; Roelants and Bossuyt 2005; Roelants et al. 2007; Wiens et al. 2005). Standard reaction conditions were initial denaturation at 94° C (5 m), followed by 40 cycles of 94° C (30 s), 55° C (30 s), 72° C (60 s), and a final extension at 72° C (7 m). For some poor-yielding samples, annealing temperature was dropped from 55° C to 50° C or 46° C, and the duration of the annealing step was increased to 45 s. Amplified PCR products were purified via gel filtration or vacuum filtration (Millipore). Cycle sequencing was performed in forward and reverse directions for all samples, at the Pennsylvania State University Nucleic Acids Facility.

Newly generated sequences (GenBank accession numbers GQ345132–GQ345340) were combined with those obtained from GenBank (Table 4-2) and aligned using MUSCLE 3.6 under default parameters (Edgar 2004). Protein coding sequences were adjusted manually so that gaps corresponded with codon insertions or deletions. No premature stop codons were detected. 12S, 16S, and 28S ribosomal RNA alignments were refined based on structure models of *Eleutherodactylus riparius* Estrada and Hedges (Y10944) and *Xenopus laevis* (Daudin) (X02995) from the European ribosomal RNA database, using RNAsalsa 0.7.4 (Stocsits 2009) under default parameters. Poorly conserved loop regions of the ribosomal gene alignments were identified and excluded using Gblocks 0.91b (Castresana 2000) and the following parameters: maximum number of contiguous nonconserved regions (4), minimum length of a block (6), allowed gap positions (with half), and other parameters at default values. Third positions within codons of the mitochondrial ND1 and cytochrome b genes showed strong evidence of saturation when plots of transitions and transversions vs. genetic distance were made in DAMBE 5.0.25 (Xia and Xie 2001) and were excluded from the alignment to avoid biasing the non-model based analyses. For some taxa and genes, data were not available or could not be sequenced and were coded as missing data (Table 4-2). Single-gene neighbor-joining trees were produced to verify the presence of no strongly conflicting gene trees before concatenation of the genes into the final alignment. The final alignment includes 2,379 bases of mitochondrial structural RNA genes, 798 bases of mitochondrial

protein-coding genes, 662 bases of nuclear structural RNA genes, and 6,900 bases of nuclear protein-coding genes.

In addition to this complete dataset, a shorter alignment was constructed without chimeric taxa, except one terminal that includes sequences of the former conspecifics *Thoropa miliaris* (Spix) and *T. taophora* (Miranda-Ribeiro). This reduced dataset includes sequences of the mitochondrial 12S, 16S, tRNAV, tRNAL, and ND1 genes, and the nuclear CXCR4, NCX1, RAG-1, and SLC8a3 genes, totaling 2,379 bases of mitochondrial structural RNA genes, 542 bases of mitochondrial protein-coding genes, and 3,631 bases of nuclear protein-coding genes. Both alignments have been deposited in TreeBASE, with accession number SN4553.

Molecular phylogenetic analyses were performed on both alignments using maximum likelihood (ML), Bayesian, and maximum parsimony (MP) methods, implemented in RAxML-VI-HPC 2.2.1, MrBayes 3.1.2, and MEGA 4.0, respectively (Stamatakis 2006; Huelsenbeck and Ronquist 2001; Tamura et al. 2007). For ML and Bayesian analyses, the nucleotide sequence data were divided into four partitions based on gene location (nuclear or mitochondrial genome) and type (structural RNA or protein-coding genes), with all parameters unlinked across these partitions. Alignment gaps were treated as missing data. In both cases, the best-fitting evolutionary model was identified as GTR + I + Γ under the Akaike information criterion using the program Modeltest 3.7 (Posada and Crandall 1998; Posada and Buckley 2004). For Bayesian analyses, this model was chosen. Because RAxML does not implement models with invariant sites, the GTR + gamma model was used for ML analyses. For the ML analyses, 100 independent searches were performed on the original dataset, and branch support was assessed for the most likely tree of these 100 runs with nonparametric bootstrapping (2,000 replicates). The Bayesian analyses were performed as two parallel runs for 15,000,000 or 20,000,000 generations, sampled every 500 generations. Each run employed three heated and one cold chain, with a temperature parameter of 0.25. The first 25% of samples were discarded as burnin. Convergence was assessed by the standard deviation of split frequencies (< 0.01 in all cases), potential scale reduction factors (approaching 1 for all parameters), and estimated sample sizes of parameters, using Tracer 1.3 (Rambaut and Drummond 2005) (> 100 for all parameters in each independent run across all partitions).

Branch support was assessed with posterior probabilities. For the MP analyses, close neighbor interchange searches were implemented, and 2,000 bootstrap replicates were run to provide branch support values.

A timescale of nobleobatrachian evolution was estimated using the topology from the ML analysis of the full dataset, but with the reduced alignment to avoid timing with chimeric taxa, and a Bayesian relaxed-clock model implemented in the T3 version of Multidivtime (Thorne and Kishino 2002; Yang and Yoder 2003). For comparative purposes, analyses were also performed on the same topology with the full alignment, and using the ML topology obtained using the reduced dataset (with the reduced alignment). The same partitions employed in phylogenetic analyses were also used in timetree estimation.

A total of five minimum and one maximum constraint were used as calibrations. The minimum divergence time between *Eleutherodactylus* and *Diasporus* was set at 15 million years ago (Ma), based on an amber-preserved *Eleutherodactylus* from Hispaniola (Iturralde-Vinent and MacPhee 1996; Poinar and Cannatella 1987). The minimum divergence time between the two members of *Bufo* sensu lato (*Rhinella* and *Duttaphrynus*) and *Melanophryniscus* was set at 24 Ma, based on fossil remains of "*Bufo*" from the Salla Beds of Bolivia (Báez and Nicoli 2004). Remains of *Hyla* from the Miocene of Austria set the minimum divergence time between *Hyla* and *Acris* at 16 Ma (Sanchiz 1998). Fossil evidence of *Calyptocephalella* dates to 61 Ma, setting the minimum divergence between it and myobatrachids (Báez 2000). The divergence time between *Litoria* and *Phyllomedusa* was constrained between 35 and 70 Ma, based on the timeframe when Australian hylids (represented by *Litoria*) could disperse from South America through Antarctica (Li and Powell 2001; Sanmartin and Ronquist 2004; Springer et al. 1998; Woodburne and Case 1996). Analyses were also performed with single calibrations removed, to gauge the relative effects of each calibration on the obtained divergence times.

For the analyses, priors of several other parameters are required, with some settings recommended by the creators of the software. The prior for root-to-tip age, rttm, was set at 145 (with 1 time unit equaling 1 million years), and its standard deviation at 40, based on recent molecular estimates of the divergence times between

nobleobatrachians and myobatrachids (Roelants et al. 2007; Wiens et al. 2007). The rate prior, rtrate, was set at 0.0017, which is approximately the value of a root-to-tip branch length divided by the rttm. The standard deviation for rtrate was also set to 0.0017. The parameters brownmean and brownsd were set at 0.007, based on the recommendation that these values should be approximately 1 or 2 divided by rttm. Bigtime was set at 300. All other parameters (minab, newk, othk, thek) were maintained at default values. The analyses were run for 1,100,000 generations, with sampling every 100 generations and a burnin of 100,000 generations.

4.4 Results.

Based on the distinctivness of these frogs and the results of the phylogenetic analysis, we describe a new family, Ceuthomantidae, to contain the new genus and species *Ceuthomantis smaragdinus* (Fig. 4-1). The description is given in the Appendix. We refer *Pristimantis aracamuni* and *P. cavernibardus* to *Ceuthomantis* based on their sharing, with *Ceuthomantis smaragdinus*, a unique combination of five characters (cited in Appendix). However, we consider this arrangement to be tentative because genetic data are unavailable for either species and both lack the paired dorsal gland-like structures of *C. smaragdinus*. An unusual behavioral trait (for terraranans) — diurnal calling — may be shared by these three species. *Ceuthomantis aracamuni* were found during the day on moss and rocks in a small creek (Barrio-Amorós and Molina 2006), and *C. cavernibardus* were calling during the day in caves formed by granite boulders or on roots and moss (Myers and Donnelly 1997). At the type locality of *C. smaragdinus,* frogs of an unknown species (perhaps *C. smaragdinus*) were calling vociferously during the day from a site where a small stream emerged amongst large boulders. Barrio-Amoros and Brewer- Carias (2008) reported hearing *P.* cf. *cavernibardus* calling during rainy or cloudy days on Sarisariñama.

Barrio-Amorós and Brewer-Carías (2008) reported "*Pristimantis*" cf. *cavernibardus* from elevations of 1100–1375 m of Sarisariñama Tepui, which is about 380 km NNE of Cerro Aracamuni and Sierra Tapirapecó. Their color photograph (Fig. 13) shows a narrow nearly phosphorescent interorbital bar like that in *Ceuthomantis smaragdinus.* The tepuis in extreme southern Venezuela and in Guyana seem to harbor a

biota that is distinct from the tepuis on the northern part of the Guiana Highlands in Venezuela (McDiarmid and Donnelly 2005).

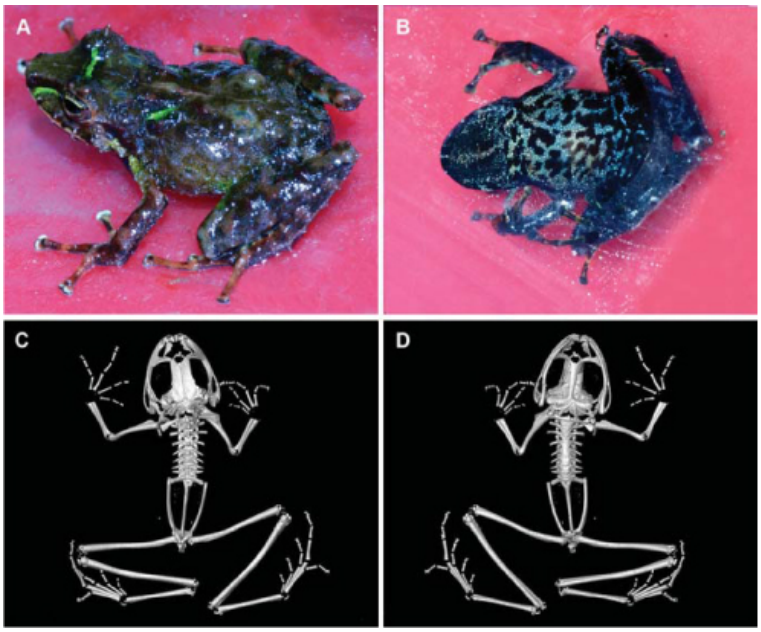

Figure 4-1. Dorsal (A) and ventral (B) views of *Ceuthomantis smaragdinus* in life; and high-resolution tomographs of *Ceuthomantis smaragdinus*, dorsal (C) and ventral (D).

Ceuthomantis cavernibardus has large, unpigmented eggs (Myers and Donnelly 1997); these are typical of direct-developing species of terraranans. The only female of *C. smaragdinus* is a subadult with small, unpigmented eggs in the oviducts. Consequently, direct development of terrestrial eggs on *C. smaragdinus* can only be assumed. Large, unpigmented eggs also are associated with frogs that have nonfeeding tadpoles, including hemiphractids (Duellman 2007; Wells 2007); consequently, additional data are needed to confirm the reproductive mode of *Ceuthomantis*.

Inasmuch as the osteological data for *Ceuthomantis smaragdinus* were obtained from a tomograph, the only direct comparisons are made with representatives of the other four families of Terrarana for which tomographs exist. These are *Ischnocnema guentheri* (Steindachner) of the Brachycephalidae, *Haddadus binotatus* (Spix) of the Craugastoridae, *Eleutherodactylus gossei* Dunn of the Eleutherodactylidae, and *Pristimantis pulvinatus* (Rivero) of the Strabomantidae (Figs. 4-1, 4-2, and 4-3).

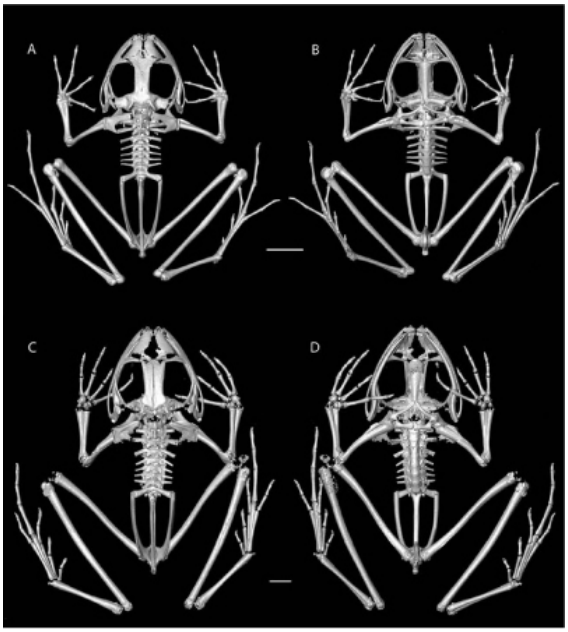

Figure 4-2. High-resolution tomographs of terraranan frogs representing two families (left, dorsal view; right, ventral view). (A–B) Brachycephalidae, *Ischnocnema guentheri* (KU92816); (C–D) Craugastoridae, *Haddadus binotatus* (KU92808). Scale bars = 5 mm.

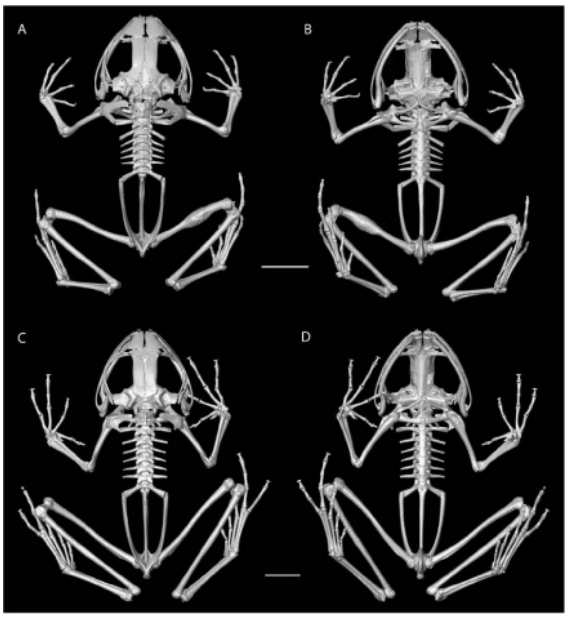

Figure 4-3. High-resolution tomographs of terraranan frogs representing two families (left, dorsal view; right, ventral view). (A–B) Eleutherodactylidae, *Eleutherodactylus gossei* (SBH266440); (C–D) Strabomantidae, *Pristimantis pulvinatus* (KU166368). Scale bars = 5 mm.

Comparison of the taxa reveals several rather striking differences. *Ischnocnema, Eleutherodactylus,* and *Pristimantis* have rather well-ossified skeletons in contrast to that of *C. smaragdinus*. As a result, note that the anterolateral part of the braincase is complete, although the sphenethmoid may be marginally ossified dorsally (*E. gossei*); likewise, the exooccipitals are synostotically united to one another and to the prootics so as to produce well-developed otic regions. The nasals are large; ventrally, vomers and robust pterygoids are present. The neural arches of Presacrals I and II are fused. The epiphyses of the long bones are uniformly mineralized and ossification of the carpal and tarsal elements is clearly evident.

The shape of the head (dorsal/ventral profiles) of *Ceuthomantis* is distinctly different from that of *Pristimantis, Ischnocnema,* and *Eleutherodactylus,* which one could reasonably interpret as being more "typical" of terraranans, with their broadly arced jaws and almost triangular heads. In contrast, the head of *Ceuthomantis,* with its narrow otic region and wide preorbital region, has an overall shape somewhat reminiscent of a quadrangular caudate skull. Note the disproportionately large rostrum in contrast to that of *Pristimantis,* and the shape of the mandible in ventral view; it is strongly sigmoid in *Ischnocnema, Eleutherodactylus,* and *Pristimantis,* and only weakly so in *Ceuthomantis.* The transverse processes of the presacral vertebrae of *Ceuthomantis* are much shorter than those of *Ischnocnema, Eleutherodactylus,* and *Pristimantis,* and the sacral diapophyses are less robustly developed. *Ceuthomantis* lacks well-developed preacetabular ilium, whereas *Ischnocnema, Eleutherodactylus, Haddadus,* and *Pristimantis* possesses distinct, well-developed preacetabular ilia. The terminal phalanges are small, knobby expansions in *Ischnocnema, Eleutherodactylus,* and *Haddadus,* whereas they are larger and have a distinctive hourglass shape in *Ceuthomantis* and a broadly expanded, gracile T-shape in *Pristimantis.*

Of the four genera and families available for comparison with *Ceuthomantis,* it bears a few features in common with the craugastorid, *Haddadus.* In *Haddadus binotatus,* the anterior braincase (sphenethmoid) is scarcely ossified and the otic capsule is very poorly developed. The neural arches of Presacrals I and II are not fused, and the transverse processes of the presacrals are short, resembling those of *Ceuthomantis.*

Despite the reduced ossification of *Haddadus*, the carpal and tarsal elements are mineralized in contrast to those in *Ceuthomantis.*

There is a brief osteological description for one other species included in *Ceuthomantis*, *C. cavernibardus* (Myers and Donnelly 1997). The authors noted that in this species the skull is a "little wider than long," and that the nasals are "moderate, not medial contact, well separated from frontoparietals by sphenethmoid." These comments suggest that *C. cavernibardus* has larger nasals and that the sphenethmoid is ossified dorsally, in contrast to *C. smaragdinus.* Likewise, *C. cavernibardus* has vomers, whereas *C. smaragdinus* lacks them. Both taxa have widely separated occipital condyles on short stalks, similar parasphenoids, squamosals, and pterygoids. Likewise, as described by Myers and Donnelly (1997), the configurations of the axial column, and pectoral and pelvic girdles seem to resemble one another; however, based on their comments about the tarsal elements and the skeleton in general, it is evident that the skeleton of *C. cavernibardus* is more completely ossified than is that of *C. smaragdinus.*

Phylogenetic Relationships of *Ceuthomantis.* In order to determine the relationships of Ceuthomantidae, we estimated a molecular phylogeny with sequences from 17 nuclear and mitochondrial genes and exemplars of all nobleobatrachian families. For both the full and limited alignments, all analyses support the position of the new family as the basal family of Terrarana, and support marsupial frogs (Hemiphractidae) as the closest relatives of Ceuthomantidae + Terrarana (Figs. 4-4, 4-5, 4-6). Support values are significant (≥ 95%) for placement of the new family as the closest relative of, but outside the four terraranan families with both Bayesian analyses and the ML analysis of the complete dataset. The Terrarana + Ceuthomantidae + Hemiphractidae clade received significant support only from the Bayesian analysis of the full dataset, and moderate support from the ML analysis of the full dataset.

Individual gene trees (not shown) revealed no strongly conflicting phylogenetic signal. In general, the gene trees did not include enough data to resolve relationships among families, and only relationships within families received moderate (bootstrap > 70%) support. However, for most genes, the new family is recovered either as the closest relative of Terrarana or embedded in Terrarana.

No evidence was found for the polyphyly of marsupial frogs (Faivovich et al. 2005; Frost et al. 2006; Wiens et al. 2005), consistent with some other recent analyses (Wiens et al. 2007; Guayasamin et al. 2008). Previous molecular phylogenetic analyses have identified various close relatives of Terrarana, including some or all hemiphractids (Faivovich et al. 2005; Wiens et al. 2005), and phyllomedusine + pelodryadine hylids (Roelants et al. 2007), or placed Terrarana outside most other nobleobatrachians (Darst and Cannatella 2004; Frost et al. 2006), but none of those proposed relationships had significant support. For example, Guayasamin et al. (2008) included four genera of hemiphractid frogs. They recovered a clade with significant support that included all marsupial frogs in the nuclear and complete phylogenies but not in the mitochondrial analysis; in all analyses terraranans were in a polytomy within Nobleobatrachia and not significantly linked to hemiphractids.

Within Terrarana, ML and Bayesian analyses strongly support Craugastoridae and Strabomantidae as closest relatives. Eleutherodactylidae is recovered as basal to Brachycephalidae, Craugastoridae, and Strabomantidae in all analyses, but with low support. Most relationships among other nobleobatrachian families remain unresolved. The major exception is the significantly supported close relationship between Leptodactylidae and Leiuperidae (removed from Leptodactylidae by Grant et al., 2006), which has been recovered with non-significant support in other studies (Darst and Cannatella 2004; Faivovich et al. 2005; Frost et al. 2006; Roelants et al. 2007). Conversely, two other former components of Leptodactylidae, Ceratophryidae and Cycloramphidae, are rendered polyphyletic in all analyses.

The results of the molecular phylogenetic analyses (Figs. 4-4, 4-5, 4-6) are largely compatible with recent hypotheses regarding overall terraranan relationships and evolution (Heinicke et al. 2007; Hedges et al. 2008). The four previously-named families—Brachycephalidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae—were each found to be monophyletic, with all but Strabomantidae receiving significant support. However, the lone representative of the strabomantid subfamily Holoadeninae (*Psychrophrynella*) is embedded among the four exemplars of Strabomantinae, a subfamily that received only poor support previously (Hedges et al. 2008). Considering the limited sampling of strabomantids in this study, any revision of the content of the

strabomantid subfamilies must await future analyses with more taxa. Previous studies have suggested that West Indian *Eleutherodactylus* and Middle American *Craugastor* originated via dispersal from South America (Lynch 1971; Hedges et al. 1989a; Crawford and Smith 2005; Heinicke et al. 2007). The discovery of the basal terraranan, *Ceuthomantis*, reinforces a South American origin for Terrarana as a whole, whereas a strabomantid + craugastorid clade supports separate origins of terraranans in Middle America and the West Indies.

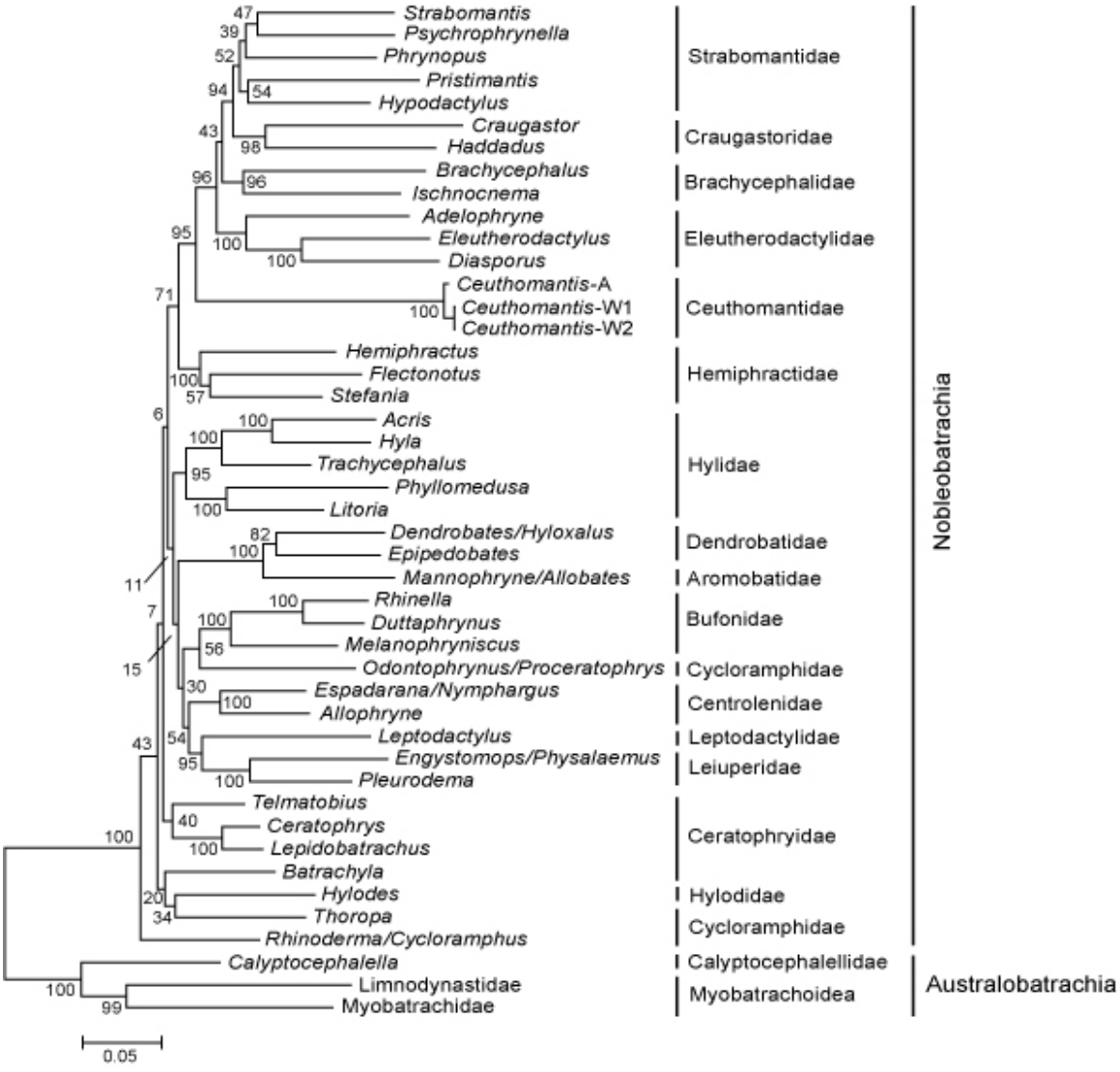

Figure 4-4. Maximum likelihood phylogeny of nobleobatrachian frogs represented by selected genera and constructed using sequences from 17 genes. The tree is rooted with Ranidae (not shown). Bootstrap support values are indicated at nodes. Higher classification is indicated to the right.

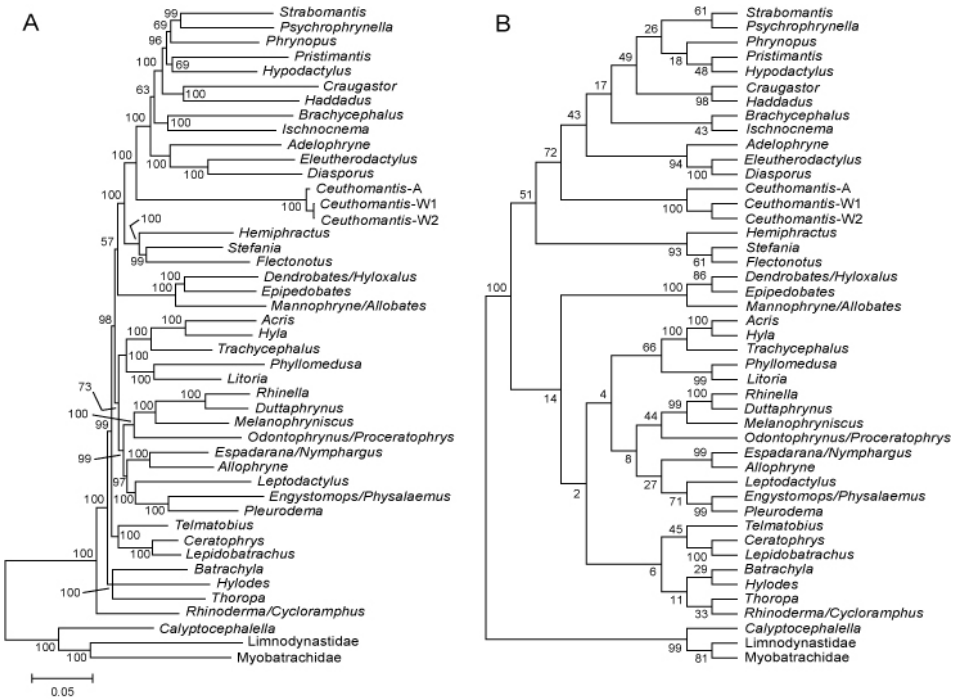

Figure 4-5. (A) Bayesian and (B) maximum parsimony phylogenies of nobleobatrachian frogs represented by selected genera and constructed using sequences from 17 genes. The trees are rooted with Ranidae (not shown). Support values (Bayesian posterior probabilities or MP bootstrap values) are indicated at nodes.

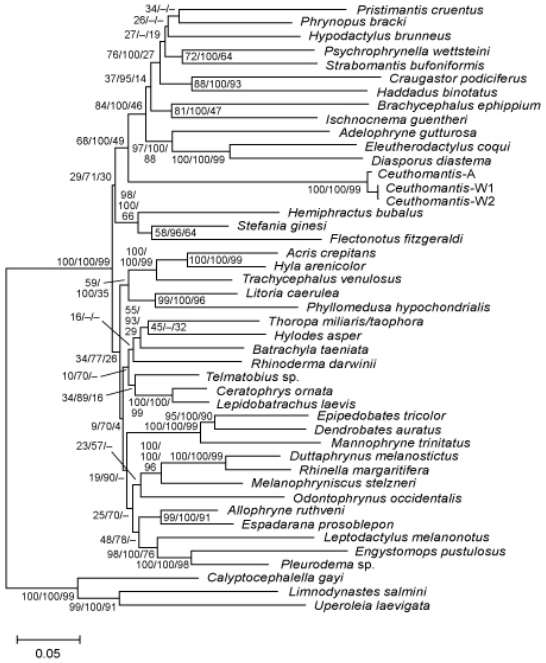

Figure 4-6. Phylogeny of nobleobatrachian frogs represented by selected species and constructed using sequences from 9 genes. The tree is rooted with *Rana temporaria* (not shown). Support values (ML bootstrap/Bayesian posterior probability/MP bootstrap) are indicated at nodes. Bayesian and MP support values are not given in cases where those phylogenies conflicted with the ML phylogeny.

Times of divergence within Terrarana (Fig. 4-7, Table 4-3) are similar among all analyses. Multiple repeated runs of the initial analysis, using the same alignment, parameters and tree topology, resulted in times no more than 0.5% different from the initial analysis at each node. No single calibration had undue effects on the resulting times. Removal of individual minimum constraints resulted in times differing by less than two percent at any node. Removal of the single maximum constraint had slightly greater effects, resulting in times five percent older on average. Employing the complete sequence alignment (with chimeric sequences) also had little effect, resulting in times differing by an average of one percent. Using the topology from the ML analysis of the reduced dataset had greater effects, resulting in times seven percent older on average. Recent molecular clock analyses of terraranans (Heinicke et al. 2007; Roelants et al. 2007) produced somewhat younger divergence times (ten percent on average) than this analysis, although results are more similar for some key nodes. The older dates obtained in this study may result from one of several potential causes, including differences in taxon sampling, phylogeny, and sequences used. These older inferred times do not affect previous hypotheses of terraranan biogeographic history (Heinicke et al. 2007; Hedges et al. 2008). The timetree indicates that *Craugastor* and *Haddadus* diverged in the early Cenozoic, about 42 (58–29) Ma, nearly identical to the previous estimate of 42 (59–31) Ma (Heinicke et al. 2007). *Eleutherodactylus* and *Diasporus* (not timed in Heinicke et al. 2007) diverged in the mid-Cenozoic, 32 (46–21) Ma. In contrast, previous studies based on immunological distances (Hedges et al. 1992b; Hedges 1996b) and DNA sequence data (Crawford and Smith 2005), and calibrated differently, obtained older time estimates indicating origins in the Late Cretaceous or early Cenozoic. The radiation leading to most other nobleobatrachian families occurred rapidly across the K-T boundary, similar to, but slightly older than, a previously-inferred explosive post-Cretaceous diversification (Roelants et al. 2007).

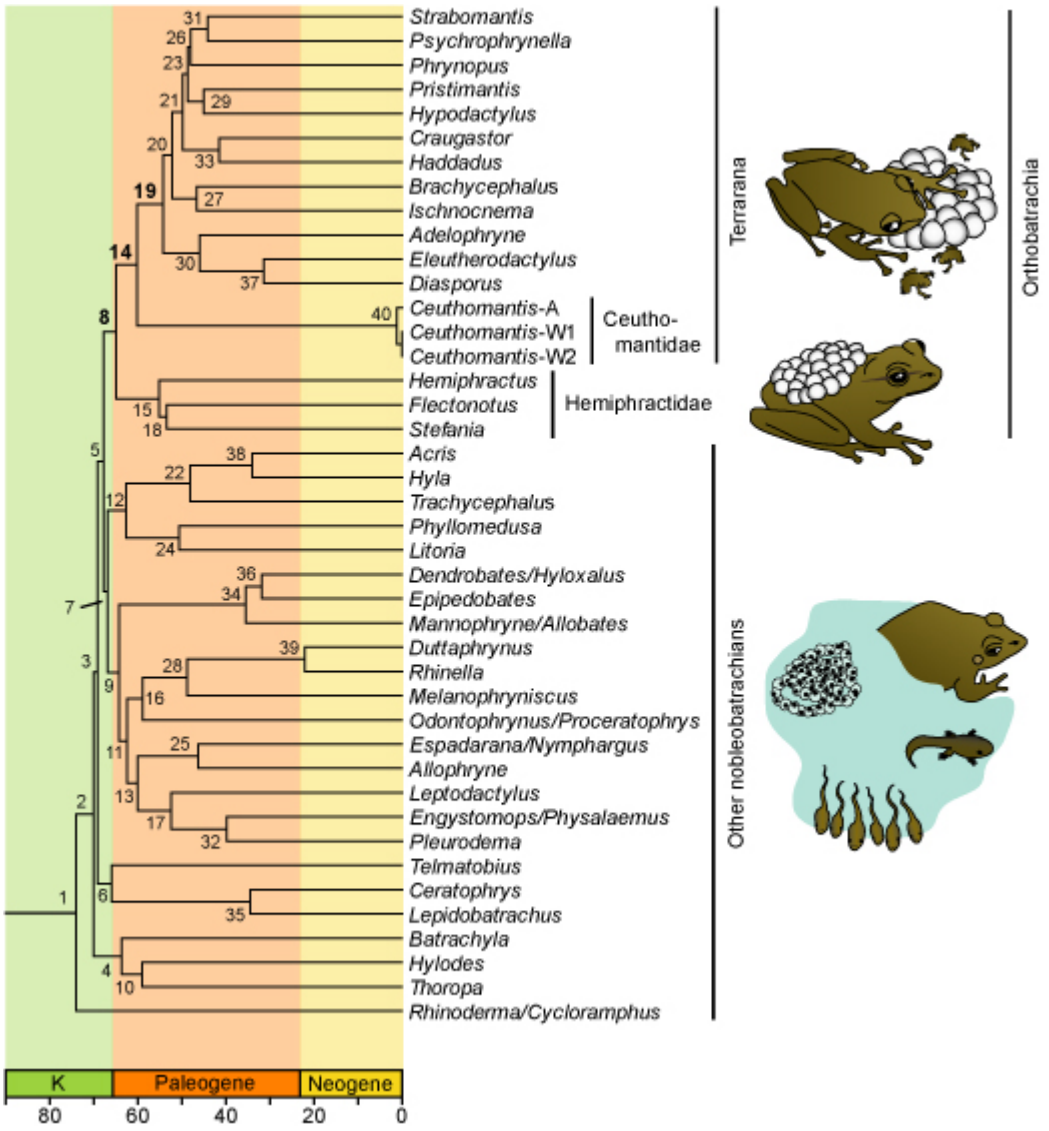

Figure 4-7. Timetree of nobleobatrachian frogs represented by selected genera and estimated with a Bayesian analysis of sequences from 9 genes, based on a topology obtained from the ML analysis of 17 genes (Figure 4-4). Numbers on nodes refer to time estimates and credibility intervals of time estimates (Table 4-3); those in bold are nodes discussed in the text. Illustrations portray the major reproductive modes of the genera and families, with most direct-developing species (i.e., no aquatic larvae) contained in Terrarana (Ceuthomantidae and four other families) that nearly always lay eggs on substrate, and Hemiphractidae that carry their eggs on their backs. Nearly all other nobleobatrachians have aquatic larvae (e.g., tadpoles).

Outside Terrarana, even > 10 kb of sequence data are not able to resolve interfamilial relationships. Resolution of a major exception, the Terrarana + Hemiphractidae clade, is probably facilitated by the early-diverging position of

Ceuthomantidae. This helps to break up the long phylogenetic branch leading to Terrarana, an action in general known to improve the accuracy of phylogenetic analysis (Heath et al. 2008). Most of the other basal branches in Nobleobatrachia are characterized by very short internodes (Fig. 4-4), which may confound efforts to resolve these early divergences even with increased gene sampling (Rokas and Carroll 2006; Wiens et al. 2008). Some recent analyses suggest that such short internodes are resolvable, however, and additional support may be provided through the discovery of shared rare genomic changes (e. g. Janecka et al. 2007). Resolution of these branches in future studies is critical to place into context the emergence of the most successful nobleobatrachian groups (bufonids, dendrobatoids, and hylids, in addition to terraranans) from the "leptodactylids" at the base of the tree.

Evolution of Direct Development in Noblebatrachia. Terrarana, including this new family of frogs, is part of an even larger radiation of frogs— Nobleobatrachia— distributed primarily in the New World and containing nearly half (3,224 species in 17 families) of all living amphibians (AmphibiaWeb 2009; Frost 2009). We propose here an unranked taxon within Noblebatrachia that includes Terrarana and Hemiphractidae based on their close phylogenetic relationship (Fig. 4-4) and sharing direct development among most species: Orthobatrachia (Greek: *ortho*, direct; and *batrachos*, frog). By linking together groups sharing the same, advanced, reproductive mode, the discovery of Ceuthomantidae and recognition of Orthobatrachia provides a better understanding of the evolution of direct development in anurans (Hanken et al. 1997; Callery et al. 2001), at least within the major group of direct-developing frogs in the Noblebatrachia.

Except for a single live-bearing (ovoviviparous) species, *Eleutherodactylus jasperi* Drewry and Jones (Wake, 1978), all terraranans presumably undergo direct development and lay large, terrestrial, unpigmented eggs that bypass the tadpole stage and hatch into froglets. Development has not been observed for most terraranan species, but direct development has been confirmed for at least some species in all families (excluding Ceuthomantidae), including one or more species in the genera *Brachycephalus*, *Ischnocnema*, *Craugastor*, *Eleutherodactylus*, *Diasporus*, *Barycholos*, *Bryophryne*, *Holoaden*, *Pristimantis*, *Psychrophrynella*, *Strabomantis* and *Yunganastes*

(e. g. Pombal et al. 1994; Lynn and Lutz 1946; Valett and Jameson 1961; Schwartz and Henderson 1991; Ovaska and Rand 1991; Caramaschi and Pombal 2001; Catenazzi 2006; Lutz 1958; Hödl 1990; De la Riva 2007; Heatwole 1962; De la Riva and Lynch, 1997). Hemiphractids are unique in that the embryos of different species reflect various stages of development (Wassersug and Duellman 1984. Of the five genera of hemiphractids, all species in three (*Cryptobatrachus*, *Hemiphractus*, *Stefania*) have direct development; in *Flectonotus* eggs hatch as nonfeeding larvae with well-developed hind limbs and forelimbs. Most species of *Gastrotheca* have direct development but in some others the eggs hatch at a range of developmental states (Duellman 2007). The Brazilian microhylid *Myersiella microps* Duméril and Bibron, along with several bufonids of the genera *Oreophrynella*, *Osornophryne* (presumed), and *Rhinella* (presumed) are the only non-orthobatrachians in the New World that have direct development of terrestrial eggs (Izecksohn et al. 1971; McDiarmid and Gorzula 1989; Gluesenkamp and Acosta 2001; Duellman and Trueb 1986).

Direct development also is characteristic *Arthroleptis* in Africa (Blackburn, 2008) and of two major clades in Southeast Asia and the Australo-Papuan Region (Ceratobatrachidae and asterophryine microhylids, respectively), as well as a few other frogs (Bossuyt and Milinkovitch 2000; Duellman 2007; Meegaskumbura et al. 2002; Pikacha et al. 2008). Together, the 975 species of orthobatrachian frogs with direct development include 73% of all direct-developing frog species in the World and 96% of those in the New World. A Bayesian molecular clock analysis shows the divergence between Terrarana and Hemiphractidae to be approximately 65 (48–89) Ma (Fig. 4-7, Table 4-3). This suggests that at least terrestrial reproduction, if not direct development, had evolved among South American frogs by that time.

Although terraranans and hemiphractids are both direct-developing groups, the degree of specialization and developmental attributes differ between them. The development of terraranans has been characterized as the most ontogenetically advanced of all frogs, such that most traces of the tadpole stage in the embryo have been lost (Thibaudeau and Altig 1999). Embryonic respiratory structures consist of an expanded, vascularized tail or small external gills derived from Branchial Arch III; these are reabsorbed prior to hatching (Duellman and Trueb 1986). In contrast, hemiphractid

embryonic respiratory structures consist of large bell-shaped external gills derived from Branchial Arches I and II (Duellman and Trueb 1986). Direct-developing species of hemiphractids have retained enough larval characteristics such that the tadpole stage has been able to re-evolve one or more times in *Gastrotheca* and possibly *Flectonotus* (Duellman and Hillis 1987; Wassersug and Duellman 1984; Wiens et al. 2007). Unlike most aquatic frogs that have small, pigmented eggs, *Ceuthomantis cavernibardus* has large, unpigmented eggs typical of direct-developing species of terraranans. However, such eggs also are associated with frogs that have nonfeeding tadpoles, including hemiphractids (Duellman 2007; Wells 2007); therefore additional data are needed to confirm the reproductive mode of *Ceuthomantis*. Determination of the reproductive mode in Ceuthomantidae may provide insight into the origin of developmental differences between Terrarana and Hemiphractidae.

4.5 Discussion

The ancient highlands of the Guiana Shield represent a unique biogeographic region in South America where endemism is high, especially among certain groups of plants (Steyermark 1986), birds (Mayr and Phelps 1967), and amphibians (Señaris and MacCulloch 2005; McDiarmid and Donnelly 2005). Eight genera of anurans with 45 recognized species are endemic to the Guiana Highlands. In addition to *Ceuthomantis* with three species, there are two genera of bufonids—*Oreophrynella* (8 species) and the monotypic *Metaphryniscus*—two genera of hylids—*Myersiohyla* (4 species) and *Tepuihyla* (8 species)—the strabomantid genus *Dischidodactlyus* with two species (Ayarzagüena 1986; Lynch 1979); also there are the hemiphractid genus *Stefania* with 18 species (MacCulloch et al. 2006) and the monotypic dendrobatid genus *Minyobates.* In addition there are many endemic species including 12 *Pristimantis* (Strabomantidae) (Myers and Donnelly 2008), one clade of four species of *Hyalinobatrachium* and one species of *Vitreorana* (Centrolenidae) (Guayasamin et al. 2009), as well as eight species of *Anomaloglossus* (Dendrobatidae) and at least six species of *Hypsiboas* and one of *Osteocephalus* (Hylidae) (MacCulloch and Lathrop 2005). With exploration of many other tepuis or granitic mountains the number of endemic taxa certainly will increase significantly.

The discovery of *Ceuthomantis* highlights the importance of geologically old regions of continents, such as the Guiana Shield, for harboring relict biodiversity of evolutionary importance, as was emphasized in the discovery of Nasikabatrachidae in India (Biju and Bossuyt 2003; Hedges 2003). In addition to *Ceuthomantis*, early-branching lineages of several frog families occur in this region, as shown by three other molecular phylogenies. The endemic treefrogs of the genus *Myersiella* are the closest relatives of the clade containing all of the other South American hylines (Faivovich et al., 2005). *Minyobates* seems to be the closest relative of all other dendrobatine frogs (Grant et al. 2006). The hemiphractid genus *Stefania* is basal to the genus *Gastrotheca* (Wiens et al. 2007). Whereas geologically active areas, such as the Andes, may have higher rates of speciation, the older, more stable regions may act as evolutionary refugia for "living fossils." These early-branching lineages can provide a wealth of biological information far beyond that which can be gleaned from fossils alone.

4.6 Acknowledgments

We thank Alan Walker and Timothy Ryan (Pennsylvania State University) for making available highresolution computed tomography facilities; Charles Myers (AMNH) for unpublished data on *Ceuthomantis cavernibardus* and loan of specimens; César Barrio-Amorós, Andrew Crawford, Ronald Crombie, Mary Davies, Stefan Gorzula, Ronald Heyer, Marinus Hoogmoed, Peter Houde, John Lynch, Linda Maxson, John Murphy, Charles Myers, and the Museum of Vertebrate Zoology (U. California, Berkeley) for collecting or providing samples used in the study; the AmphibiaTree Project (David Cannatella and David Hillis) for partial financial support; César Barrio-Amorós for comments and discussion; Andrew Campbell for macrophotography; J. Soberon for translating the resumen; Lainey Lee and Madelyn Owens for assistance with graphics; and Andrew Crawford, Juan Guayasamin, and an anonymous reviewer for many helpful suggestions that greatly improved the manuscript. S.B.H. and L.T. were supported by grants from the National Science Foundation and S.B.H. was supported by grants from the National Aeronautics and Space Administration. Fieldwork by R.D.M. was funded by Royal Ontario Museum (ROM) Governors, the ROM Centre for Biodiversity and Conservation Biology and a Smithsonian BDG Fellowship. D.B.M. was

supported by the National Institutes of Health (to Jerrold Meinwald and Valerie C. Clark, Cornell University).

Table 4-1. Specimens newly sequenced for molecular analyses.

Species	Lab tissue number	Museum voucher	Locality
Rhinella margaritifera	268430	MSH 5249-50	French Guiana: Petit-Saut, Sinnamary River
Phyllomedusa hypochondrialis	268431	USNM-FS 46785	Brazil: Rio Tapajos
Flectonotus fitzgeraldi	268432	KU 192399–400	Trinidad and Tobago: Trinidad, 6.4 km N Arima
Stefania ginesi	268433	LM 1056	Venezuela: Amazonas, Abacapa Tepui
Hemiphractus bubalus	268434	KU 178588	Ecuador: Pastaza, Mera
Mannophryne trinitatus	171009	n/a	Trinidad and Tobago: Trinidad, Paria River (near Brasso Seco)
Mannophryne trinitatus	268435	UIMNH 94439–441	Trinidad and Tobago: Trinidad, Arima-Blanchisseuse Road
Brachycephalus ephippium	268117	USNM 207716	Brazil: São Paulo, Eugenio Lefevre
Ischnocnema guentheri	267345	USNM-FS 053312	Brazil: São Paulo, ***Estação*** Biológica de Jureia
Ischnocnema parva	267328	USNM-FS 053232	Brazil: São Paulo, ***Estação*** Biológica de Boracéia
Craugastor fitzingeri	194926	FMNH 257745	Costa Rica: Puntarenas, Wilson Botanical Garden
Craugastor podiciferus	266082	MVZ 12020	Costa Rica: Heredia, Chompipe, vicinity of Volcán Barba
Haddadus binotatus	267339	USNM 303077	Brazil: São Paulo, ***Estação*** Biológica de Boracéia
Eleutherodactylus cooki	160048	USNM 326784	USA: Puerto Rico, El Yunque
Eleutherodactylus planirostris	267470	n/a	USA: Florida, Monroe Co., Key West
Diasporus diastema	268025	MVZ 203844	Costa Rica: Cartago, 1.9 km S Tapanti Bridge over Río Grande de

			Orosi
Adelophryne gutturosa	268015	ROM 39578	Guyana: District 7, Mount Ayanganna
Pristimantis cruentus	267876	AMNH 12444–448	Panama: Ratibor, Finca Ojo de Agua
Phrynopus bracki	171045	USNM 286919	Peru: Pasco, 2.9 km N, 5.5 km E Oxapampa
Hypodactylus brunneus	267860	KU 178258	Ecuador: Carchí, 14.6 km NW Carchí
Hypodactylus dolops	267862	JDL 17574	Colombia
Strabomantis biporcatus	268087	CVULA 7073	Venezuela: Sucre, Parque Nacional de Paria, Las Melenas, Peninsula de París
Strabomantis necerus	267885	KU 179076	Ecuador: Carchí, Maldonado
Psychrophrynella wettsteini	268101	KU 183049	Bolivia: La Paz, 2.3 km S Unduavi
Psychrophrynella usurpator	267889	KU 173495	Peru: Cusco, Abra Acanacu, 25 km NNE Paucartambo
Ceuthomantis smaragdinus	268011	ROM 40161	Guyana: District 7, Mount Ayanganna
Ceuthomantis smaragdinus	268267	KU 315000	Guyana: Potaro-Siparuni, Wokomung Massif, Mt. Kopinang
Ceuthomantis smaragdinus	268268	KU 300000	Guyana: Potaro-Siparuni, Wokomung Massif, Mt. Kopinang
Proceratophrys melanopogon	268436	USNM 208125	Brazil: São Paulo; São José de Barreiro, Fazada de Vendo
Thoropa taophora	268437	USNM 209318	Brazil: Salesopolis, near ***Estação*** Biológica de Boracéia
Hylodes nasus	268438	USNM 245925	Brazil: Rio de Janiero, near Parque Nacional de Tijuca
Pleurodema marmoratum	268439	KU 173341	Peru: Cuzco, 36 km NW Ollantaytambo, Abra Málaga
Limnodynastes tasmaniensis	268440	n/a	Australia: Tasmania, Hamilton, Greenwich House

Table 4-2. GenBank accession numbers. Numbers with "GQ" are new to this study. Aligned sequence length is given for each gene. For ND1 anc CytB, length after removal of third codon positions is given in parentheses.

Taxon	12S/tRNAV/16S	tRNAL/ND1	CytB	28S	c-myc A
Melanophryniscus	AY325999	AY819463 AY948744	DQ502444	AY844306	AY819167
Rhinella	AY843573 AY819331/AF375514	AY819461	AY843795	AY844205	AY819165
Duttaphrynus	AY458592	AY458592	AY458592	DQ283658	
Espadarana/Nymphargus	AY843574	AY819466	AY843796	AY844206	AY819170
Allophryne	AY843564	AY819458	AY843786		AY819162
Trachycephalus	AY326048	AY819514	EU034077	AY844322	AY819217
Hyla	EF566960	AY819494	AY843824	AY844241	AY819197
Acris	EF566970	AY819491	AY843782	AY844194	AY819194
Litoria	AY326038	AY819531	AY843938	AY844304	AY819234
Phyllomedusa	AY843724	AY819535 AY948748	AY843969	AY844329	AY819239
Flectonotus	AY843589 AY819355/DQ679381	AY819486	AY843809	AY844215	AY819189
Stefania	AY843768 DQ679266/DQ679417	AY819490 DQ679373	AY844013	AY844354	AY819193
Hemiphractus	AY843594 DQ679263/DQ679412	AY819489 DQ679370	AY843813	**AA000000**	AY819192
Mannophryne/Allobates	DQ502131	AY819469	DQ502562	DQ503024	AY819173
Dendrobates/Hyloxalus	AY364565	AY819470	DQ502491	AY844211	AY819174
Epipedobates	AY364577		DQ502584	DQ283461	
Brachycephalus	AY326008	**AA000000**	**AA000000**	DQ282494	**AA000000**
Ischnocnema	EF493533	**AA000000**	**AA000000**	DQ283495	EU025679
Craugastor	EF493360	**AA000000**	**AA000000**	DQ283648	**AA000000**
Haddadus	EF493361		**AA000000**	DQ283493	**AA000000**
Eleutherodactylus	EF493539 **AA000000**	**AA000000**	**AA000000**	DQ283629	AY211282
Diasporus	EU186682		**AA000000**	**AA000000**	**AA000000**
Adelophryne	EU186679	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Pristimantis	EF493697	AY948758	EU368884	AY844213	AY819177
Phrynopus	EF493709		**AA000000**	**AA000000**	**AA000000**
Hypodactylus	EF493357	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Strabomantis	EU186691	**AA000000**	**AA000000**	DQ283555	**AA000000**
Psychrophrynella	EU186696	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Ceuthomantis-W1	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Ceuthomantis-W2	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Ceuthomantis-A	EU186677	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Batrachyla	AY843572 AY389157	AY948759	AY843794	AY844204	
Ceratophrys	AY326013	AY523774	AY843797	AY844207	AY819176
Lepidobatrachus	DQ283152	AY819475		DQ283543	AY819179
Telmatobius	DQ283040 DQ347049/DQ347333	AY819478	DQ502448	AY844355	AY819182
Odontophrynus/Proceratophrys	AY843704	AY948757	AY843949	AY844309	**AA000000**
Thoropa	DQ283331	**AA000000**	DQ502607	**AA000000**	**AA000000**
Rhinoderma/Cycloramphus	DQ283324	AY523783	DQ502589	DQ283654	
Hylodes	DQ502171	**AA000000**	DQ502606	DQ503009	**AA000000**
Pleurodema	AY843733	AY948753	AY843979	**AA000000**	**AA000000**
Physalaemus/Engystomops	AY843729 DQ337249	AY819477	AY843795	AY844330	AY819181

Leptodactylus	AY843688 AY364359/DQ347060	AY948760	AY843934	AY844302	AY337266
Calyptocephalella	DQ283439	AY819471		DQ283748	AY819175
Myobatrachidae	DQ283221	AY948768	AY843988	DQ283644	AY819185
Limnodynastidae	AY326071	AY523775	**AA000000**	DQ283643	**AA000000**
Ranidae	AY326063	M57527 AF314018	AY522428	DQ283522	AY819188
Sequence Length (bp)	835 / 73 / 1399	73 / 813 (542)	385 (256)	662	420

Table 4-2 (continued)

Taxon	c-myc B	CXCR4	NCX1	POMC	RAG-1 A
Melanophryniscus	AY819247	AY948784	AY948822	AY819082 DQ158263	AY948927
Rhinella	AY819244	DQ306529	**AA000000**	AY819080	DQ158354
Duttaphrynus		AY364167	AY948805	DQ158317	AY364197
Espadarana/Nymphargus	AY819250	AY364193	AY948834	AY819085	AY364223
Allophryne	AY819242			AY819077	
Trachycephalus	AY819291	AY364185	AY948824	AY819132	AY364215
Hyla	AY819271	AY364190	EF107241	AY819112	AY364220
Acris	AY819268	EF107468	EF107244	AY819109	EF107304
Litoria	AY819308	AY948783	AY948821	AY819149	AY948926
Phyllomedusa	AY819313	AY948786	AY948826	AY819153	AY948929
Flectonotus	AY819265	**AA000000**	**AA000000**	AY819104	DQ679274
Stefania	AY819267	**AA000000**	**AA000000**	AY819108 DQ679338	DQ679308
Hemiphractus	AY819266	**AA000000**	**AA000000**	DQ679335	DQ679303
Mannophryne/Allobates	AY819253		**AA000000**	AY819088	**AA000000**
Dendrobates/Hyloxalus	AY819254	AY364184	AY948823	AY819089	AY364214
Epipedobates		EF107458	EF107233		EF107295
Brachycephalus	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Ischnocnema	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Craugastor	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Haddadus	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Eleutherodactylus	**AA000000**	EF107500	EF107282	**AA000000**	EF107341
Diasporus		**AA000000**	**AA000000**	**AA000000**	**AA000000**
Adelophryne	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Pristimantis	AY819256	AY948792	AY948836	DQ158260	AY948935
Phrynopus	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Hypodactylus		**AA000000**	**AA000000**	**AA000000**	**AA000000**
Strabomantis		**AA000000**	**AA000000**	**AA000000**	**AA000000**
Psychrophrynella		**AA000000**	**AA000000**	**AA000000**	**AA000000**
Ceuthomantis-W1	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Ceuthomantis-W2		**AA000000**	**AA000000**	**AA000000**	**AA000000**
Ceuthomantis-A	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Batrachyla		AY948793	AY948837		AY948936
Ceratophrys	AY819255	AY364188	AY523718	AY819091	AY364218
Lepidobatrachus	AY819258	EF107461	EF107236	AY819094	EF107298
Telmatobius	AY819260	EF107464	EF107239	AY819097	DQ347275

Odontophrynus/Proceratophrys	**AA000000**	AY948791	AY948835	**AA000000**	AY948934
Thoropa	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Rhinoderma/Cycloramphus		AY364192	AY523733		AY364222
Hylodes	**AA000000**	**AA000000**	**AA000000**	**AA000000**	**AA000000**
Pleurodema	**AA000000**	AY948789	AY948831	**AA000000**	AY948932
Physalaemus/Engystomops		EF107462	EF107237	AY819096	EF107299
Leptodactylus	AY337266	AY364194	AY948838	DQ158259	AY364224
Calyptocephalella		EF107495	EF107275	AY819090	EF107334
Myobatrachidae	AY819262	EF107474	EF107251	AY819100	EF107310
Limnodynastidae	**AA000000**	AY364189	AY523719	AY819099	AY364219
Ranidae		EF017988	EF018012	AY819103	DQ347231
Sequence Length (bp)	317	682	1282	531	556

Table 4-2 (continued)

Taxon	RAG-1 B	Rho	SIA	SLC8a3	Tyr
Melanophryniscus	AY844478	DQ283765	AY844899	AY948878	
Rhinella	AY844370	AY844547	AY844775	**AA000000**	EF364358
Duttaphrynus	DQ158394	AF249097	DQ282815	AY948851	
Espadarana/Nymphargus	AY844371	AY844548	AY844776	AY948896	AY844029
Allophryne	AY844361	AY844538	AY844766		
Trachycephalus	AY844493	AY844707	AY844912	AY948880	AY844149
Hyla	AY844391	AY844577	AY844802	EF107393	AY844048
Acris	AY844358	AY844533	AY844762	EF107403	AY844019
Litoria	AY323767	AY844685	AY844893	AY948877	AY844131
Phyllomedusa	AY844496	AY844711	AY844916	AY948882	AY844153
Flectonotus	AY844379	AY844562	AY844788	**AA000000**	AY844038
Stefania	AY844528	AY844756	AY844951	**AA000000**	AY844353
Hemiphractus	AY844382	AY844566	AY844792	**AA000000**	
Mannophryne/Allobates	DQ503345	DQ503236	DQ503097	**AA000000**	DQ503136
Dendrobates/Hyloxalus	DQ503304	AY364395	AY844781	AY948879	DQ347160
Epipedobates	DQ503354	DQ283768	DQ503104	EF107381	DQ282902
Brachycephalus	**AA000000**	DQ283808	DQ282673	**AA000000**	DQ282919
Ischnocnema	**AA000000**	DQ283809	**AA000000**	**AA000000**	EF493510
Craugastor	**AA000000**	DQ283960	DQ282808	**AA000000**	EF493481
Haddadus	**AA000000**	DQ283807	**AA000000**	**AA000000**	DQ282918
Eleutherodactylus	**AA000000**	DQ283937	**AA000000**	EF107445	EF493455
Diasporus	**AA000000**		**AA000000**	**AA000000**	EU186773
Adelophryne	**AA000000**	**AA000000**	**AA000000**	**AA000000**	EU186772
Pristimantis	DQ679272	AY844559	**AA000000**	AY948898	EF493502
Phrynopus	**AA000000**	**AA000000**	**AA000000**	**AA000000**	EF493507
Hypodactylus	**AA000000**	**AA000000**	**AA000000**	**AA000000**	EF493484
Strabomantis	**AA000000**		DQ282718	**AA000000**	EU186775
Psychrophrynella	**AA000000**		**AA000000**	**AA000000**	EU186776

Ceuthomantis-W1		**AA000000**	**AA000000**	**AA000000**	
Ceuthomantis-W2			**AA000000**	**AA000000**	
Ceuthomantis-A		**AA000000**	**AA000000**	**AA000000**	
Batrachyla	AY844369	AY844546	AY844774	AY948899	AY844028
Ceratophrys	DQ679269	AY364399		AY948886	DQ347168
Lepidobatrachus	DQ679270	DQ283851	DQ282707	EF107386	
Telmatobius	AY844529	AY844757	AY844952	EF107389	DQ347182
Odontophrynus/Proceratophrys	AY844480	AY844695	AY844901	AY948897	DQ282903
Thoropa	**AA000000**	**AA000000**	**AA000000**	**AA000000**	
Rhinoderma/Cycloramphus	DQ503357	DQ283963	DQ282813	AY948895	DQ282924
Hylodes	DQ503367	DQ503253	DQ503119	**AA000000**	DQ282923
Pleurodema	AY844503	AY844721	AY844926	AY948888	
Physalaemus/Engystomops	AY844499	AY844717	DQ282875	EF107387	
Leptodactylus	AY844470	AY844681	AY844890	AY948900	DQ347193
Calyptocephalella	AY583337	DQ284036	DQ282893	EF107440	
Myobatrachidae		DQ283955	DQ282758	EF107410	DQ282965
Limnodynastidae	AY583341	DQ283954	DQ282805	AY948889	
Ranidae	AY323776	AF249119	DQ282735	EF107369	AF249182
Sequence Length (bp)	428	316	397	1111	532

Table 4-3. Times of divergence and Bayesian credibility intervals for nodes in Figure 4-7. The default analysis uses sequences of nine genes and the topology of the 17-gene ML analysis (Figure 4-4). Divergence times are also given for analyses after removal of individual calibrations, using sequence data from all 17 genes, and using the topology from the 9-gene ML analysis (Figure 4-6).

Node	Divergence Time	no 61 Ma cal.	no 35 Ma cal.	no 70 Ma cal.	no 16 Ma cal.
1	73.9 (99.7–53.0)	73.6 (100.0–52.2)	72.8 (99.4–49.7)	77.7 (115.9–53.5)	73.9 (100.0–53.2)
2	70.0 (94.5–50.2)	69.7 (94.3–49.5)	68.9 (94.2–46.9)	73.6 (110.4–50.4)	69.9 (94.5–50.5)
3	69.1 (93.2–49.6)	68.8 (93.2–48.9)	68.0 (93.0–46.2)	72.7 (108.8–49.8)	69.1 (93.2–49.9)
4	63.6 (86.6–45.3)	63.4 (86.8–44.4)	62.6 (86.3–42.0)	66.9 (101.1–45.5)	63.6 (86.8–45.6)
5	67.7 (91.4–48.6)	67.4 (91.3–47.8)	66.6 (91.2–45.3)	71.2 (106.8–48.8)	67.6 (91.4–48.8)
6	65.9 (89.1–47.1)	65.6 (89.1–46.4)	64.9 (89.1–44.0)	69.3 (104.0–47.4)	65.8 (89.0–47.4)
7	66.8 (90.2–47.9)	66.5 (90.0–47.2)	65.7 (90.1–44.6)	70.2 (105.8–48.1)	66.7 (90.2–48.2)
8	**64.9 (87.7–46.5)**	64.6 (87.7–45.7)	63.9 (87.5–43.2)	68.2 (102.7–46.6)	64.9 (87.6–46.8)
9	64.2 (86.9–45.9)	63.9 (86.6–45.2)	63.2 (86.7–42.8)	67.5 (101.7–46.2)	64.1 (86.9–46.1)
10	59.0 (80.6–41.4)	58.8 (81.1–40.7)	58.1 (80.9–38.8)	62.0 (94.3–41.8)	59.0 (80.8–41.7)
11	62.4 (84.5–44.6)	62.1 (84.4–43.9)	61.3 (84.3–41.6)	65.6 (98.8–44.8)	62.3 (84.2–44.8)
12	62.7 (84.6–44.9)	62.4 (84.5–44.4)	61.7 (84.6–41.6)	65.9 (99.2–45.2)	62.6 (84.3–45.1)
13	60.0 (81.4–42.8)	59.7 (81.5–42.1)	59.0 (81.2–40.0)	63.1 (95.2–43.0)	60.0 (81.4–42.9)
14	**60.1 (81.7–42.9)**	59.8 (81.6–42.2)	59.1 (81.4–40.0)	63.2 (95.8–43.0)	60.1 (81.7–43.1)
15	55.2 (75.4–38.8)	54.9 (75.4–38.3)	54.3 (75.6–36.5)	58.1 (88.1–39.1)	55.1 (75.7–39.1)
16	58.8 (79.9–42.0)	58.6 (79.8–41.3)	57.9 (79.7–39.1)	61.9 (93.7–42.0)	58.8 (79.8–41.9)
17	52.4 (72.2–36.7)	52.1 (72.2–36.3)	51.5 (71.7–34.3)	55.1 (84.1–37.0)	52.3 (71.7–36.8)
18	53.4 (73.4–37.5)	53.2 (73.3–36.9)	52.6 (73.4–35.2)	56.3 (85.4–37.8)	53.4 (73.3–37.7)
19	**54.2 (73.9–38.4)**	54.0 (73.7–37.9)	53.4 (73.8–36.0)	57.0 (86.2–38.7)	54.2 (73.9–38.8)
20	52.2 (71.2–36.9)	52.0 (71.1–36.4)	51.4 (71.1–34.6)	54.9 (83.0–37.2)	52.2 (71.1–37.2)
21	49.8 (68.2–35.1)	49.6 (67.9–34.6)	49.0 (68.0–32.9)	52.3 (79.4–35.4)	49.7 (68.2–35.4)
22	48.1 (65.9–33.8)	47.9 (65.9–33.5)	47.4 (65.7–31.4)	50.6 (77.1–34.3)	48.1 (66.1–34.0)
23	48.6 (66.8–34.3)	48.4 (66.4–33.7)	47.8 (66.5–32.0)	51.1 (77.8–34.6)	48.6 (66.6–34.5)
24	50.6 (67.9–36.2)	50.4 (67.9–36.0)	49.7 (68.0–33.1)	53.3 (81.5–36.4)	50.5 (67.9–36.4)
25	46.3 (65.1–31.5)	46.1 (65.3–30.9)	45.5 (65.0–29.6)	48.6 (75.4–31.6)	46.2 (65.4–31.4)
26	48.0 (65.8–33.8)	47.8 (65.4–33.2)	47.2 (65.5–31.5)	50.4 (76.7–34.1)	47.9 (65.8–34.0)
27	46.6 (64.2–32.4)	46.3 (63.9–32.0)	45.8 (64.2–30.5)	49.0 (74.7–32.7)	46.5 (64.1–32.7)
28	48.7 (67.1–34.0)	48.4 (67.3–33.4)	47.9 (66.7–31.9)	51.2 (78.2–34.3)	48.6 (66.9–34.3)
29	45.0 (62.0–31.3)	44.8 (62.1–30.8)	44.3 (61.8–29.6)	47.4 (72.4–31.9)	45.0 (62.2–31.6)
30	46.0 (63.4–32.2)	45.8 (63.3–31.7)	45.2 (63.5–29.9)	48.3 (73.8–32.3)	46.0 (63.2–32.3)
31	44.1 (60.8–30.7)	43.9 (605.–30.2)	43.3 (60.8–28.7)	46.3 (70.9–31.1)	44.0 (60.8–30.9)
32	39.9 (56.3–27.0)	39.6 (56.2–26.6)	39.2 (55.8–25.2)	41.9 (65.2–27.4)	39.8 (56.0–27.1)
33	41.5 (57.6–28.7)	41.3 (57.4–28.1)	40.8 (57.4–26.9)	43.6 (66.7–29.1)	41.4 (57.4–28.8)
34	35.5 (50.2–24.2)	35.3 (49.8–23.7)	34.9 (49.1–22.9)	37.3 (58.0–24.4)	35.4 (49.9–24.3)
35	34.5 (49.7–22.8)	34.3 (49.5–22.5)	33.9 (49.4–21.5)	36.2 (57.0–23.0)	34.4 (49.8–22.8)
36	31.8 (45.5–21.4)	31.7 (45.3–21.1)	31.3 (45.1–20.4)	33.5 (52.4–21.7)	31.8 (45.2–21.6)
37	31.3 (44.7–21.1)	31.2 (44.6–20.9)	30.8 (44.4–19.7)	32.9 (51.4–21.3)	31.3 (44.4–21.2)
38	34.1 (47.9–22.8)	33.9 (48.2–22.9)	33.6 (48.2–21.6)	35.9 (55.9–23.3)	34.1 (48.5–23.1)
39	22.2 (32.7–14.1)	22.0 (32.5–14.0)	21.7 (32.4–13.3)	23.4 (37.0–14.4)	22.1 (32.7–14.2)
40	1.2 (2.1–0.6)	1.2 (2.1–0.6)	1.2 (2.1—0.6)	1.3 (2.3–0.6)	1.2 (2.1–0.6)

Table 4-3 (continued)

Node	no 24 Ma cal.	no 15 Ma cal.	17-gene analysis	9-gene topology
1	73.9 (99.6–53.3)	73.8 (100.0–53.3)	77.4 (107.2–57.1)	73.0 (92.4–53.7)
2	69.9 (94.2–50.5)	69.9 (95.0–50.5)	71.3 (98.6–52.9)	x
3	69.1 (93.1–49.8)	69.0 (93.8–49.8)	69.1 (95.5–51.4)	x
4	63.6 (86.4–45.4)	63.6 (86.8–45.3)	67.5 (93.4–49.9)	61.7 (79.0–44.9)
5	67.6 (91.1–48.7)	67.6 (91.6–48.8)	67.2 (92.9–50.0)	x
6	65.8 (88.9–47.4)	65.8 (89.5–47.2)	66.1 (91.2–49.0)	65.6 (83.5–48.1)
7	66.7 (89.9–48.0)	66.7 (90.4–48.1)	65.6 (90.7–48.8)	x
8	64.9 (87.7–46.7)	64.8 (87.7–46.7)	64.7 (89.3–48.1)	71.9 (91.0–52.8)
9	64.1 (86.6–46.1)	64.1 (87.0–46.1)	63.8 (88.2–47.4)	68.0 (86.1–49.9)
10	59.0 (80.8–41.8)	59.0 (81.1–41.6)	62.5 (87.1–46.0)	57.0 (73.7–41.0)
11	62.3 (84.4–44.7)	62.3 (84.6–44.7)	62.3 (86.2–46.3)	66.1 (83.9–48.4)
12	62.6 (84.3–45.2)	62.6 (84.6–45.1)	61.3 (84.6–45.7)	68.0 (85.6–49.8)
13	60.0 (81.2–42.8)	59.9 (81.5–42.9)	60.9 (84.6–45.1)	63.7 (81.1–46.6)
14	60.0 (81.6–43.1)	60.0 (81.6–43.0)	58.9 (81.7–43.7)	66.7 (85.0–48.7)
15	55.1 (75.6–39.2)	55.1 (75.6–39.2)	57.0 (79.4–42.2)	60.8 (77.9–44.0)
16	58.8 (79.8–41.9)	58.7 (80.2–41.8)	56.7 (79.0–41.9)	62.5 (79.6–45.6)
17	52.3 (71.7–36.9)	52.3 (71.9–36.8)	56.1 (78.2–41.4)	55.6 (71.9–40.0)
18	53.4 (73.4–37.7)	53.4 (73.4–37.7)	53.9 (75.5–39.7)	59.0 (75.9–42.6)
19	54.2 (74.0–38.7)	54.2 (73.9–38.7)	52.5 (72.9–38.8)	60.4 (77.3–43.8)
20	52.2 (71.3–37.2)	52.2 (71.2–37.1)	51.1 (71.0–37.7)	57.9 (74.4–41.9)
21	49.7 (68.1–35.4)	49.8 (68.2–35.3)	48.5 (67.5–35.7)	54.9 (70.8–39.5)
22	48.1 (65.8–34.2)	48.1 (66.0–33.9)	47.6 (66.5–35.1)	53.0 (68.0–38.2)
23	48.6 (66.6–34.6)	48.6 (66.7–34.4)	47.4 (65.9–34.8)	53.8 (69.4–38.6)
24	50.6 (67.7–36.4)	50.5 (67.9–36.3)	47.1 (65.7–35.6)	55.9 (69.1–40.5)
25	46.2 (65.4–31.4)	46.2 (65.3–31.5)	47.0 (66.4–34.1)	48.8 (65.2–33.7)
26	47.9 (65.8–34.1)	47.9 (65.9–33.8)	46.6 (64.9–34.2)	x
27	46.6 (64.5–32.8)	46.6 (64.6–32.7)	46.1 (64.1–33.6)	45.7 (60.0–32.1)
28	48.6 (66.7–34.3)	48.6 (66.8–34.0)	45.9 (64.5–33.3)	51.7 (67.1–37.0)
29	45.0 (62.2–31.7)	45.0 (62.2–31.6)	44.7 (62.4–32.7)	x
30	46.0 (63.5–32.3)	45.9 (63.4–32.3)	44.2 (61.9–32.3)	51.3 (66.8–36.7)
31	44.0 (60.9–31.0)	44.1 (60.9–30.9)	44.0 (61.5–32.1)	49.8 (64.9–35.6)
32	39.8 (56.2–27.1)	39.8 (56.4–27.0)	41.7 (59.1–30.0)	42.2 (56.7–29.1)
33	41.4 (57.5–28.8)	41.4 (57.8–28.9)	41.3 (58.3–29.9)	45.7 (60.0–32.1)
34	35.4 (50.2–24.5)	35.4 (49.8–24.2)	36.1 (51.1–25.8)	37.5 (50.3–26.2)
35	34.4 (49.7–22.7)	34.4 (49.7–22.8)	33.5 (48.3–23.0)	33.7 (46.3–22.5)
36	31.8 (45.4–21.7)	31.8 (45.1–21.5)	33.1 (47.2–23.3)	33.8 (45.8–23.3)
37	31.3 (44.4–21.3)	31.3 (44.4–21.2)	31.2 (44.5–22.0)	35.0 (47.3–24.1)
38	34.1 (48.0–23.2)	34.1 (48.4–23.1)	31.2 (44.5–22.1)	37.9 (50.7–26.1)
39	22.2 (32.6–14.1)	22.1 (32.7–14.1)	21.1 (31.2–14.2)	23.6 (33.4–15.5)
40	1.2 (2.1–0.6)	1.2 (2.1–0.6)	1.1 (1.9–0.6)	1.4 (2.5–0.7)

CHAPTER 5

Molecular phylogeny and biogeography of West Indian frogs of the genus *Leptodactylus* (Anura: Leptodactylidae)

Note: Modified from Hedges, S. B. and Heinicke, M. P. 2007. *Molecular Phylogenetics and Evolution* **44**: 308-314. MPH carried out laboratory and computational research and co-wrote paper. SBH directed research, collected specimens in the field, and drafted paper.

5.1 Abstract

Three endemic species of the aquatic-breeding frog genus *Leptodactylus* are recognized from the West Indies: *Leptodactylus albilabris* (Puerto Rico and the Virgin Islands), *Leptodactylus dominicensis* (Hispaniola), and *Leptodactylus fallax* (Lesser Antilles). DNA sequences were obtained from several mitochondrial genes to resolve taxonomic questions involving these species and to provide insights into their origin and distribution in the islands. We found low levels of sequence divergence between *L. dominicensis* and *L. albilabris*, supporting morphological evidence that the former species is a junior synonym of the latter species. Phylogenetic analysis supported previous species-group allocations, finding that *L. albilabris* is a member of the *fuscus* group and *L. fallax* is a member of the *pentadactylus* group. Molecular time estimates for the divergence of *L. albilabris* from its closest relative in South America (24–58 million years ago, Ma) and for *L. fallax* from its closest relative in South America (23–34 Ma) indicate that they colonized the West Indies independently by over-water dispersal in the mid-Cenozoic. The absence of detectable sequence divergence between the two extant populations of *L. fallax* (Dominica and Montserrat), a species used for human food and now critically endangered, suggests that one or both arose by human introduction from an island or islands where that species originated. The relatively minor genetic differentiation of populations of *L. albilabris* can be explained by vicariance and dispersal in the Pleistocene and Holocene, although human introduction of some populations cannot be ruled out.

5.2 Introduction

Frogs of the genus *Leptodactylus* (72 species) occur in the New World tropics and have aquatic larvae (Amphibiaweb 2006; Duellman and Trueb 1986). Only three species are endemic to the West Indies (Schwartz and Henderson 1991): *Leptodactylus albilabris* (Puerto Rico and the Virgin Islands), *Leptodactylus dominicensis* (Hispaniola), and *Leptodactylus fallax* (Lesser Antilles). A fourth species, *Leptodactylus validus*, occurs in the southern Lesser Antilles (Grenada, Grenadines, and St. Vincent) and on the islands of Trinidad and Tobago, which are part of continental South America (Heyer 1994). Its origin in the West Indies has been presumed to be Pleistocene or Holocene (Hedges 1996b; Heyer 1994), including the possibility of recent human transport. In contrast, the terrestrial frog genus *Eleutherodactylus*, with direct development, has undergone a major radiation in the West Indies, with 146 endemic species known from the islands (Hedges 2006c).

West Indian *Leptodactylus* are distributed in the eastern half of the West Indies, consistent with an origin from South America and the flow of ocean currents from east to west in the Caribbean (Hedges 1996b; Hedges 2001). The Hispaniolan species, *L. dominicensis* (Cochran 1923), is known from only a small area in the extreme eastern part of the island, below the Bahia de Samaná. Morphologically, it is so similar to *L. albilabris* of Puerto Rico and the Virgin Islands that it has been considered a junior synonym of that species in some taxonomic accounts (Heyer 1978). Nonetheless, most have treated it as a distinct species since it was described (Cochran 1941; Hedges 2006c; Powell et al. 1996; Schwartz and Henderson 1988; Schwartz and Henderson 1991; Schwartz and Thomas 1975). Both species are relatively small (<50 mm snout-vent length, SVL), live and breed in shallow bodies of water such as flooded meadows and ditches, and probably use seismic signals in their intraspecific communication (Lewis et al. 2001; Schwartz and Henderson 1991). The Puerto Rican species, *L. albilabris*, is widely distributed in lowland areas of the island, and occurs throughout the Puerto Rican Bank (including the Virgin Islands) and on St. Croix (Schwartz and Henderson 1991).

Leptodactylus fallax is a large species, reaching 210 mm SVL (Daltry and Gray 1999), and occurs now on two islands: Dominica and Montserrat. It is unusual in having maternal care that includes obligatory oophagy (Gibson and Buley 2004). Historical

records have suggested that it had a wider distribution in the past, occurring also on St. Kitts, Antigua, Guadeloupe, Martinique, and St. Lucia, although museum specimens are known only from St. Kitts (Kaiser, 1994; Lescure 1979a; Lescure 1979b). The disappearance of the species from those islands has been attributed to predation by the mongoose and the introduction of the predacious toad *Bufo marinus* (Kaiser 1994). The species is also consumed by humans, and presumably this fact has had an impact on both the distribution of the species and on its current decline. In Montserrat, recent volcanic activity has affected the species range and overall health of the populations (Daltry and Gray 1999; Gibson and Buley 2004). Currently, the species is listed as "critically endangered" on the "Red List" of the International Union for the Conservation of Nature (IUCN 2006).

Each of these two lineages of *Leptodactylus* is believed to have arrived to the West Indies independently from South America. Morphologically, *L. albilabris* and *L. dominicensis* belong to the *fuscus* group (Heyer 1978) and *L. fallax* to the *pentadactylus* group (Heyer 1979). As with most frogs, the fossil record is largely silent on the origin of these species, and therefore molecular data have been collected to offer evidence on times of divergence. Estimates of amino acid substitutions in the serum albumin protein of *Leptodactylus* have been made with an immunological technique, micro-complement fixation (Maxson and Heyer 1988), but these data were only partly useful. Most species examined were too divergent to obtain comparable results, and some other results were inconsistent.

In the case of *L. albilabris*, one-way (antigen versus antibody) immunological distances (IDs) to two species in the *fuscus* group (*Leptodactylus fuscus* and *Leptodactylus labrosus*) were 76 and 66, respectively (Maxson and Heyer 1988), suggesting divergence times of approximately 40–45 Ma using an albumin calibration of 1 ID = 0.6 Ma (Maxson 1992). For *L. fallax*, the results were mixed because it exhibited very low IDs (5–11) to a species in South America (*Leptodactylus stenodema*) not considered its closest relative and similarly low IDs to *L. albilabris*, which by all other evidence is in a different species group (Maxson and Heyer 1988). Therefore, while the immunological data supported a mid-Cenozoic to late Cenozoic origin for these lineages in the West Indies, the results were inconclusive.

To clarify these taxonomic questions surrounding the endemic West Indian species of *Leptodactylus*, and to gain insights into their origin and evolution, we have conducted DNA sequence analyses. We collected samples of each of the species and sequenced portions of three mitochondrial genes. Our analyses have helped to illuminate the evolutionary history of these frogs in the West Indies.

5.3 Materials and methods

The senior author collected specimens by hand at localities in Hispaniola, Puerto Rico, the US Virgin Islands, and Montserrat. These were supplemented by other available tissue samples, and by sequence data in the public databases (Genbank). Field and laboratory research was approved by the Institutional Animal Care and Use Committee of Pennsylvania State University (#17632). Tissues were removed and frozen in liquid nitrogen, or temporarily transferred to the laboratory in 75% ethanol. The remaining specimen was preserved. Tissues were maintained in the laboratory at −80 °C. The specimens sampled, localities (Fig. 5-1), laboratory numbers, and Genbank accession numbers are listed after the acknowledgments.

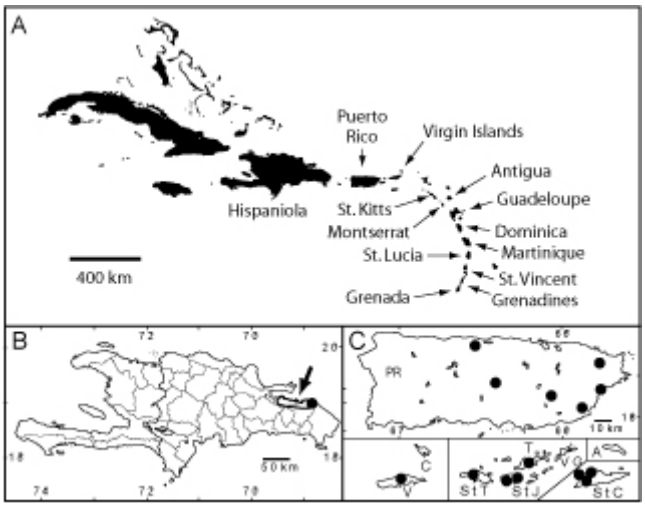

Figure 5-1. Distribution of *Leptodactylus* in the West Indies. (A) Map of West Indies showing islands mentioned in the text. (B) Distribution of *L. dominicensis* in Hispaniola (solid line indicated by arrow), showing locality sampled. (C) Puerto Rico, Culebra, Vieques, and the Virgin Islands, showing localities sampled for *L. albilabris*. The species is distributed throughout Puerto and occurs on all labeled islands except Virgin Gorda. Islands abbreviated are Anegada (A), Culebra (C), Puerto Rico (PR), St. Croix (St C), St. John (St J), St. Thomas (St T), Tortola (T), Vieques (V), and Virgin Gorda (VG).

DNA was extracted from tissue samples, amplified (PCR) with primers spanning defined regions of genes, and sequenced. Two relatively slow-evolving mitochondrial genes, 12S rRNA and 16S rRNA (~1800 bp, total) were used to determine higher-level relationships of the West Indian species to other species in the genus, and for time estimation. A portion of the faster-evolving cytochrome b mitochondrial gene (804 bp) was used for examining genetic variation among individuals and populations of the species. The primers used were (listed 5-prime to 3-prime, with gene indicated in prefix of primer name): 12L29, AAAGCRTAGCACTGAAAATGCTAAGA; 12.1L4, TACACATGCAAGTYTCCGC; 12H46, GCTGCACYTTGACCTGACGT; 12.2L4, GCTTAAAACCYAARGGAYTTGACG; 12.2H1, TCCGGTAYRCTTACCATGTTAC; 16L19, AATACCTAACGAACTTAGCGATAGCTGGTT; 16H36, AAGCTCCAWAGGGTCTTCTCGTC; 16L37, GATTAYAAGAAAAAGAAGGAACTCGGCA; 16H37, TTACTCCGGTCTGAACTCAGATC; CBL21, ACAGGHYTWTTCCTAGCDATACA; CBH22, GATGAYCCWGTTTCATGAAG; CBL20, GTYCAATGAATCTGAGGCGG; CBH15, ACTGGTTGDCCYCCRATYCAKGTKAG; CBL1, TCTGCYTGATGAAAYTTTGG; CBH1, GGAATTTTRTCTGARTTSGATT; CBL2, ATRGTMGARTGAATCTGA; CBH2, GCTACRAAGACTTATCATTT. Both strands of the PCR products were sequenced using the ABI (Applied Biosystems) BigDye sequencing kit and an ABI Prism 3100 Genetic Analyser.

Sequences were aligned using BIOEDIT (Hall 1999) and CLUSTAL (Thompson et al. 1997). Phylogenies were constructed with minimum evolution (ME) using MEGA 3.1 (Kumar et al. 2004), with maximum likelihood (heuristic search, GTR + gamma model) using PAUP* 4b10 (Swofford 2003), and with Bayesian methods of inference using MrBayes 3.1 (Ronquist and Huelsenbeck 2003). PAML 3.13d (Yang 1997) (HKY85 model) was used to find the gamma parameter in the minimum-evolution analyses (Tamura-Nei model). Optimal model parameters for likelihood analyses were estimated in PAUP using MODELTEST (Posada and Crandall 1998) and fixed before analysis. Statistical significance was evaluated with bootstrapping and Bayesian posterior probabilities.

Divergence time analyses for the rRNA dataset were conducted with the Bayesian software MULTIDIVTIME T3 (Thorne and Kishino 2002; Yang and Yoder 2003) and with the penalized-likelihood software r8S (Sanderson 2003). The MULTIDIVTIME analysis used the following priors: rttm (mean of time for ingroup root), 65 Ma; rttmsd (standard deviation of time for ingroup root), 15; rtrate (mean of rate for ingroup root), 0.003; rtratesd (standard deviation of rate for ingroup root), 0.002; brownmean (mean of variance in logarithm of the rate), 0.025; brownsd (standard deviation of variance in logarithm of the rate), 0.025; bigtime (time that is greater than that of any node in the tree), 100 Ma. The prior for rttm (65 Ma) was used based on previous time estimates from molecular analysis of serum-albumin (Maxson and Heyer 1988) showing that many interspecific divergences in *Leptodactylus* date to the early Cenozoic, although younger (45 Ma) and older (85 Ma) priors were used for comparison. Other priors chosen were based on recommendations by the authors of the software. For the r8s analysis, the gamma parameter was set using the previously determined estimate, and the smoothing factor was estimated with cross-validation. The tree used in the r8S analysis was the ML tree. Only one calibration point could be used from the serum albumin time-estimation analysis (Maxson and Heyer 1988): the divergence of *Leptodactylus labyrinthicus* and *Leptodactylus pentadactylus.* The immunological distance (35) was a reciprocal (32 and 37), yielding a divergence time of 21 million years ago (Ma), using the calibration derived from a larger vertebrate data set (Maxson 1992).

Sequences of the following species were obtained from Genbank (accession numbers in parentheses for 12S rRNA, 16S rRNA) and used in the analyses: *L. fuscus* (DQ283404), *Leptodactylus knudseni* (AY947882, AY947863), *L. labyrinthicus* (AY947875, AY947861), *L. pentadactylus* (AY326017), *Leptodactylus ocellatus* (AY843688). Corresponding sequences of the mitochondrial genome of the hylid frog *Hyla chinensis* (AY458593) were used for rooting the trees. Although DNA sequence analyses have shown that Leptodactylidae may be paraphyletic (Darst and Cannatella 2004; Ruvinsky and Maxson 1996), they also indicate that Hylidae is one of the closest lineages to the Leptodactylinae (which includes *Leptodactylus*).

5.4 Results and discussion

The phylogenetic analysis of the mitochondrial rRNA gene sequences (1661 aligned sites, excluding gaps) supports the species group allocation of endemic West Indian *Leptodactylus* based on morphology (Heyer 1978; Heyer 1979): *L. albilabris* and *L. dominicensis* cluster with a species of the *fuscus* group (*L. fuscus*), and *L. fallax* clusters with species of the *pentadactylus* group (Fig. 5-2). Both groupings are supported by high bootstrap confidence values. Although Bayesian posterior probabilities are also shown, that measure of nodal support is thought to represent an overestimate of statistical confidence (Simmons et al. 2004) and should be treated cautiously.

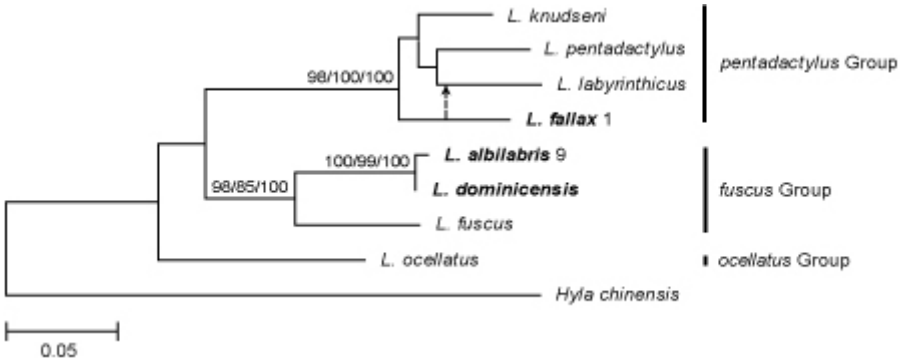

Figure 5-2. Phylogenetic relationships of endemic West Indian frogs of the genus *Leptodactylus*, including several species from South America. The tree is inferred from a maximum likelihood analysis of mitochondrial DNA sequences (12S and 16S rRNA, 1661 bp) and is identical to the Bayesian tree; dashed line and arrow indicates position of *L. fallax* in the minimum evolution tree. The tree is rooted with the hylid frog *Hyla chinensis*. Confidence values are indicated at nodes (maximum likelihood bootstrap/minimum evolution bootstrap/Bayesian posterior probabilities); no values are shown for a node if all three are <95%. Numbers next to names of species are sample reference numbers (see section 5.6). Species names in bold are those from the West Indies.

Within the *fuscus* group, the analysis also shows that *L. dominicensis* and *L. albilabris* are nearly indistinguishable at these genes. Other species in the *fuscus* group were not included, and therefore the possibility remains open that the West Indian clade may have an even closer relative on the mainland. Two South American species suggested as being closely related to *L. albilabris* based on color pattern (Heyer 1978), *Leptodactylus amazonicus* and *Leptodactylus fragilis*, would be important to examine in the future. Within the *pentadactylus* group, *L. fallax* joins an essentially unresolved polytomy with *L. knudseni*, *L. labyrinthicus*, and *L. pentadactylus.* Again, not all

members of this species group were examined, and therefore future analyses may identify a closer relative of *L. fallax*.

Sequences of the mitochondrial cytochrome b gene were collected specifically to examine genetic variation within *L. albilabris* and within *L. fallax*, because of its faster rate of evolution. The phylogenetic tree (Fig. 5-3) shows two results of taxonomic and biogeographic significance. The first involves the Greater Antillean species. The various samples of *L. albilabris* from throughout its range in Puerto Rico and the Virgin Islands show low levels of sequence divergence, and *L. dominicensis* is nested among them. This result does not support the recognition of *L. dominicensis* as a valid species, and therefore we agree with an earlier assessment based on morphological variation (Heyer 1978) that *L. dominicensis* is a junior synonym of *L. albilabris*.

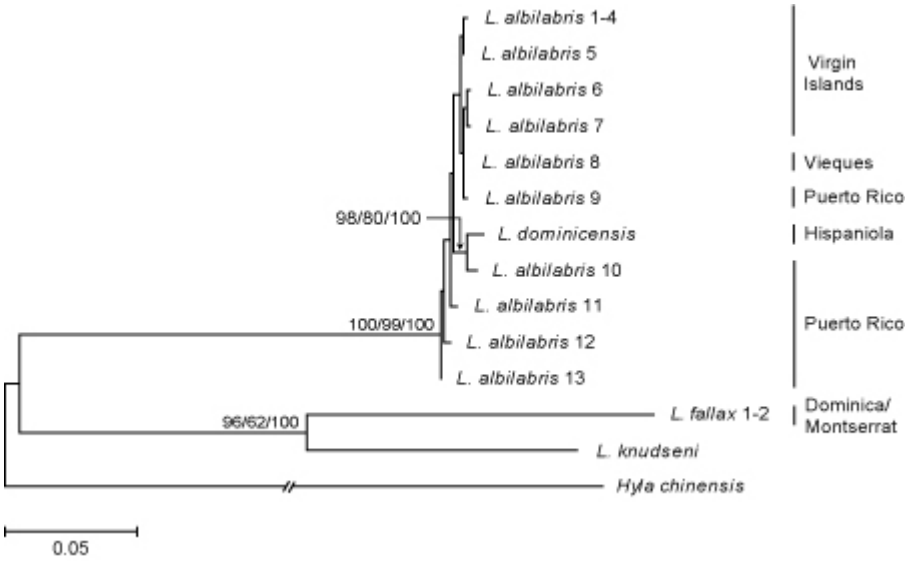

Figure 5-3. Phylogenetic relationships of endemic West Indian frogs of the genus *Leptodactylus*, including one species (*L. knudseni*) from South America. The tree is inferred from a maximum likelihood analysis of mitochondrial DNA sequences (cytochrome b, 804 bp) and is identical to the Bayesian and minimum evolution trees except for relationships among populations of *L. albilabris* which are statistically unresolved.

Among sequences of *L. albilabris*, groupings partially corresponded to geography (Fig. 5-1 and Fig. 5-3), although relationships could not be resolved statistically due to low levels of divergence and the limited number of sites. For example, the two individuals from St. John formed a cluster as did the remaining individuals from the US

and British Virgin Islands. Together, those two clades joined a more inclusive group containing the sample from Vieques and one from northeastern Puerto Rico. The remaining samples from Puerto Rico, and *L. dominicensis*, were phylogenetically outside of the group just described. Additional sequences from fast-evolving genes, or from microsatellites, will be needed to draw any additional conclusions concerning the phylogeography of *L. albilabris*.

The second noteworthy aspect of the cytochrome b tree (Fig. 5-3) involves *L. fallax*. Sequences *L. fallax* from Dominica and Montserrat are identical, a result obtained independently by R. Thorpe, University of Wales (personal communication). This result was unexpected because the two islands have never been joined and most species of amphibians and reptiles in the Lesser Antilles are endemic to single islands or island banks (Schwartz and Henderson 1991). Even some variation would be expected among individuals of a single population, and therefore the absence of detectable sequence divergence between the two extant populations of *L. fallax* suggests that one or both arose by human introduction. Unfortunately, it is not known where the species originated. It may have evolved on one or the other island, or a third island. The historical records indicating a more widespread distribution in the past (see above) make it more difficult to determine the island or island bank where this species originated. The reason for the introduction of this species to different islands is almost certainly related to human consumption, either by Amerindians or post-Columbian. At least reintroductions of populations to depleted areas could be accomplished without concern for mixing genetically distinct populations.

The estimated times of divergence from the rRNA data set (Fig. 5-4) provide evidence bearing on the historical biogeography of West Indian *Leptodactylus*. In interpreting such evidence one must realize that the inclusion of other living species from South America, or extinct species (if they were available), could substantially reduce (but not increase) the time of origin, if those missing species were found to be closest relatives of West Indian species.

The Bayesian time estimate for the divergence of *L. fallax* from its closest relative (the South American *pentadactylus* Group clade of *L. knudseni*, *L. labyrinthicus*, and *L. pentadactylus*) was 27 (23–34) Ma and the penalized likelihood estimate was 29 Ma. The

use of younger (45 Ma) and older (85 Ma) priors for rttm affected the Bayesian time estimate by only 1 million years (27–28 Ma). These dates indicate an origin by dispersal of a *pentadactylus* Group member from South America to the Lesser Antilles. As discussed above, the original island of colonization remains to be determined because of frequent introductions by humans who have used it as a food source. The data also indicate that *L. albilabris* originated by dispersal of a *fuscus* group member from South America to the Puerto Rican Bank 39 (24–58) Ma according to the Bayesian time estimation and 36 Ma according to penalized likelihood time estimation (Fig. 5-4). The use of younger (45 Ma) and older (85 Ma) priors for rttm affected the Bayesian time estimate by about 7% (36–42 Ma). There is no evidence that it inhabited any other island or island bank until relatively recently, when it appeared in St. Croix (not located on the Puerto Rican Bank) and in eastern Hispaniola.

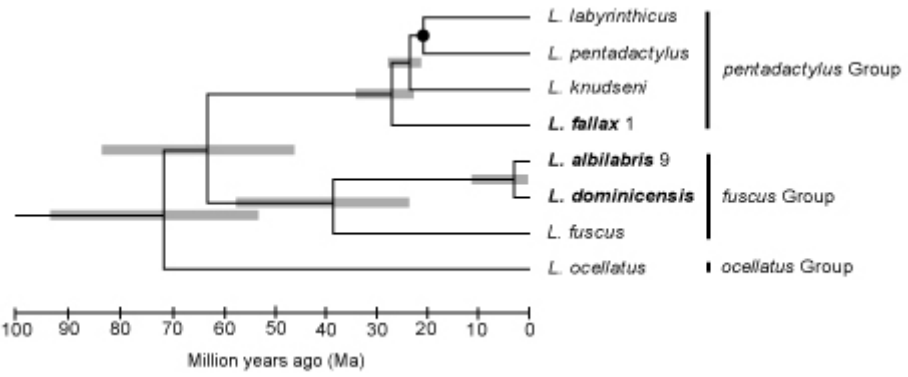

Figure 5-4. A time tree of endemic West Indian frogs of the genus *Leptodactylus* (species names in bold) using a Bayesian analysis of mitochondrial 12S rRNA and 16S rRNA gene sequences. Gray bars correspond to 95% credibility intervals for the divergence time at each node. The calibration node is denoted with a filled circle.

An origin for West Indian *Leptodactylus* by proto-Antillean vicariance is rejected because their dates of origin would need to be older than approximately 65 Ma (Hedges 2001; Hedges 2006b). Dispersal across a dry land bridge (Aves Ridge) from South America could have occurred, but geologic evidence is silent and the biological evidence argues against any dry land bridge having ever occurred (Hedges 2001; Hedges 2006b). Moreover a land bridge would not explain the presence of *Leptodactylus* in the Lesser Antilles (never connected by land) and the absence of any ancient lineage in Hispaniola (the island presumably connected by land bridge) or Cuba.

The time estimates for the divergence of *L. albilabris* (sample No. 9) from *L. dominicensis* were 2.9 (0.3–11.4) Ma using the Bayesian method and 2.2 Ma using penalized likelihood. The use of younger (45 Ma) and older (85 Ma) priors for rttm affected the Bayesian time estimate only slightly (2.8–3.1 Ma). However, considering the wide range in the Bayesian credibility interval and the recent discovery that time estimates are often inflated for shallow divergences in trees (Ho et al. 2005), little can be inferred from these estimates. Unfortunately, there were no calibrations available to estimate divergence times with the faster-evolving cytochrome b data set separately. Rates of sequence variation in the cytochrome b gene vary widely among amphibians (Babik et al. 2004; Mulcahy and Mendelson 2000; Tan and Wake 1995) and therefore use of a single rate is not justified. However, given the lowland distribution of this species and the fact that most of the Puerto Rican Bank was a continuous land area during the last glaciation suggests that the populations, including the one in Hispaniola, probably diverged in the late Pleistocene or Holocene as sea levels rose. In some cases (e.g., St. Croix), dispersal may have occurred on flotsam after storms, although human introductions cannot be ruled out.

5.5 Acknowledgments

The senior author thanks Carla Hass and Richard Thomas for assistance in the field, and the governments of the Dominican Republic, Puerto Rico, the U.S. Virgin Islands, and Montserrat for granting permission to collect and export specimens. Field work was facilitated by Sixto Inchaustegui and Yvonne Arias (Dominican Republic), and by William Coles, Douglas McNair, and Renata Platenberg (U.S. Virgin Islands). We are grateful to W. Ronald Heyer and Linda R. Maxson for providing samples and to Molly E. Means for laboratory assistance. This research was supported by grants from the National Science Foundation to S.B.H. (8906325, 9123556, 9525775, and 9615643), and to David Cannatella and David M. Hillis (0334952).

5.6 Species, localities, and sequence accession numbers

In the following list, localities are provided for each sample, followed by the laboratory tissue collection number, phylogenetic tree reference number (if applicable), and Genbank sequence accession numbers (in parentheses).

Leptodactylus albilabris.—Puerto Rico: Catalina, 101755, 9 (CytB-EF091401, 12S-EF091410, 16S-EF091413). Puerto Rico: Campamento Guavate, 101774, 10 (EF091394). Puerto Rico: Playa de Humacao, 101824, 13 (EF091396). Virgin Islands: St. Croix, 0.5 km S Canebay, 266774, 1 (EF091406). Virgin Islands: St. Croix, Hams Bay, 266796, 2 (EF091403). Virgin Islands: St. Croix, Allandale, 266803, 3 (EF091405). Virgin Islands: St. Thomas, Santa Maria, 266837, 4 (EF091404). Virgin Islands: St. John, Dever's Bay, 266869, 7 (EF091399). Virgin Islands: St. John, Carolina, 266875, 6 (EF091398). Virgin Islands: Tortola, 267840, 5 (EF091402). Puerto Rico: Isla Vieques, 267841, 8 (EF091400). Puerto Rico: Manati, 267842, 11 (EF091397). Puerto Rico: Yabucoa, 267843, 12 (EF091395).
L. dominicensis.—Dominican Republic: El Seibo, Nisibon, 192453 (CytB-EF091393, 12S-EF091411, 16S-EF091414).
L. fallax.—Montserrat: St. Peter, Spring Ghut, 192787, 1 (CytB-EF091407, 12S-EF091412, 16S-EF091415). Dominica: Coulibistri, 267838, 2 (EF091408).
L. knudseni.—Brazil: Rio Madeira, Rondonia, Calama, 267844 (EF091409).

CHAPTER 6

Lungfish evolutionary relationships and divergence times

Note: modified from Heinicke, M. P., Sander, J. M., and Hedges, S. B. 2009, pp. 348-350 in *The Timetree of Life*, S. B. Hedges and S. Kumar (Eds.). MPH performed molecular clock analyses and drafted the paper. JMS performed a molecular clock analysis. SBH directed research and co-wrote paper.

6.1 Abstract

Lungfishes (Subclass Dipnoi) number only six species in three families but are an important group of vertebrates because of their close relationship to tetrapods. Phylogenetic analyses of morphological and molecular data agree that African lungfishes (Protopteridae) and South American Lungfish (Lepidosirenidae) are closest relatives. Molecular clock analyses suggest that the divergence of these families from the Ceratodontidae (e.g., Australian Lungfish) occurred in the Permian 277 (321-234) million years ago (Ma). The divergence of South American and African lungfishes was in the Lower Cretaceous, 114 (154-94) Ma, and was probably related to the breakup of Gondwanaland.

6.2 Introduction

The six species of lungfish are the living representatives of the subclass Dipnoi. These species are divided into two suborders, Lepidosirenoidei and Ceratodontoidei, and three families: Lepidosirenidae, Protopteridae, Ceratodontidae (Nelson 2006). Several additional families are known from fossils extending back to the Devonian (416–359 Ma). Lungfishes comprise one of three extant groups of Sarcopterygii, along with tetrapods and coelacanths (Nelson 2006). Living species are characterized by stocky, eel-like bodies and fleshy fins without spines or rays. The paired pectoral and pelvic fins are paddle-like in the Australian lungfish (Ceratodontidae) and whiplike in the African and South American lungfishes (Protopteridae and Lepidosirenidae). All extant species of lungfish are obligate air-breathers, and the African and South American lungfishes have the ability to aestivate during periods of drought

(for months at a time in the case of African lungfishes) (Nelson 2006). The Australian lungfish (*Neoceratodus*) is a riverine species able to tolerate water with low oxygen content, not unlike many ray-finned fishes with the ability to breathe air, but does not aestivate. Extant lungfish are intolerant of marine conditions, and are restricted to freshwater habitats, as were most Mesozoic lungfishes (Cavin et al. 2007). Paleozoic lungfishes included numerous marine representatives, however, and the group may have originally been marine (Campbell and Barwick 1986). Here, the relationships of the three living families of lungfishes are reviewed and the first estimates of divergence times are presented based on analyses of published sequence data.

The fossil record of lungfishes is moderately complete. Tooth plates and scales are well represented, but skeletal material is relatively rare (Cavin et al. 2007; Marshall 1986). In addition to these remains, fossilized burrows are known (Berman 1976), some harboring skeletal remains. The earliest fossils of sarcopterygians on the lungfish lineage (rather than tetrapod or coelacanth lineage) are from the Devonian (Cloutier and Ahlberg 1996). True members of the Subclass Dipnoi also appear in the Devonian, and the peaks of diversity of Dipnoi were in the Devonian and Triassic (251–200 Ma) (Cloutier and Ahlberg 1996). These early lungfishes represent extinct groups, and the living families appear later in the fossil record. The three extant families are all known from the Cretaceous (146–66 Ma) (as fossils of the three modern genera) (Kemp 1997; Schultze 1991; Murray 2000). Ceratodontidae has been suggested to extend back to the Triassic (251–200 Ma), depending upon how fossil taxa are allocated (Cloutier and Ahlberg 1996; Kemp 1997). Species diversity declined in the Cenozoic, although several extinct species, including some in extinct genera, occur as late as the Pleistocene (1.81–0.01 Ma) (Kemp 1997).

The phylogenetic relationships of the living lungfish families are not controversial. It is universally accepted that Dipnoi is monophyletic, and that within Dipnoi the African and South American lungfishes (Protopteridae and Lepidosirenidae) are closest relatives. They share numerous anatomical characters, including external larval gills, fin shape, and two-lobed lungs (Nelson 2006), and are in fact often grouped together in the Family Lepidosirenidae. These relationships are strongly supported by both morphological cladistic (Schultze and Marshall 1993; Schultze 2004) and molecular

studies, including molecular studies employing nuclear or mitochondrial data (Brinkmann et al. 2004a, b; Hedges et al. 1993; Meyer and Dolven 1992; Zardoya and Meyer 1996). Molecular data also support the monophyly of Protopteridae, the only family that includes more than one living species (Tokita et al. 2005).

6.3 Methods

There have been no published molecular timing analyses among the three families of lungfish. Therefore, we conducted two molecular clock analyses using published sequence data and the Bayesian program Multidivtime (Thorne and Kishino 2002). One analysis includes all families and uses the RAG-1 and RAG-2 nucleotide sequences from Brinkmann et al. (2004a). The other analysis includes only Lepidosirenidae and Protopteridae, but uses amino acid data for six genes (RAG-1, RAG-2, TPI, GAG, ALDc, GAD65) from three studies (Brinkmann et al. 2004a; Kikugawa et al. 2004; Lariviere et al. 2002). Methodology is as described elsewhere (Heinicke et al. 2007). Several vertebrate outgroups are included in both analyses for calibration purposes (*Mus*, *Oryctolagus*, *Homo*, *Gallus*, *Xenopus*, *Danio*, and *Carcharhinus*/*Triakis* in the two-gene set; *Mus*, *Homo*, *Gallus*, *Danio* in the six-gene set), although these do not appear in the timetree.

Seven minimum and three maximum constraints were used in the two-gene dataset, based on fossil data obtained from the literature (Cloutier and Ahlberg 1996; Kemp 1997; Murray 2000; Benton et al. 2009; Blair and Hedges 2005). These include the divergence of Lepidosirenidae and Protopteridae (minimum, 92.7 Ma); the divergence of Lepidosirenoidei and Ceratodontoidei (minimum, 199 Ma); the divergence of primates and rodents (minimum, 62 Ma); the divergence of mammals and birds (minimum, 312 Ma, maximum, 370 Ma); the divergence of amniotes and amphibians (minimum, 330 Ma, maximum, 370 Ma); the divergence of tetrapods and lungfish (minimum, 404 Ma); and the divergence of ray-finned and lobe-finned fish (minimum, 416 Ma, maximum, 495 Ma). For the six-gene dataset, only the primate/rodent and mammal/bird divergences were used. The following Multidivtime parameters were employed in both analyses: rttm (450), rttmsd (100), bigtime (3000). For the two-gene analysis, rtrate was set at 0.001,

rtratesd at 0.0005, brownmean at 0.0025, brownsd at 0.0025. For the six-gene analysis, these values were 0.04, 0.04, 0.001, and 0.001, respectively.

6.4 Results and Discussion

The results of both analyses for the Protopteridae/Lepidosirenidae divergence are similar, with confidence intervals that broadly overlap (Table 6-1). The timetree (Fig. 6-1) shows that the African and South American lungfishes diverged in the early Cretaceous, 120 (165-94) Ma. This date agrees well with the fossil evidence, as it is not substantially earlier than the earliest fossils of African lungfish that appear beginning in the Cenomanian stage of the Cretaceous (100-93 Ma) (Murray 2000). The divergence between these two families and Ceratodontidae occurs much earlier, in the Permian, 277 (321-234) Ma. This date also agrees well with the fossil record, as putative ceratodontids are known from the Triassic, and several other Triassic genera of dipnoans are thought to be more closely related to lepidosirenoid lungfishes (Cavin et al. 2007; Cloutier and Ahlberg 1996; Kemp 1997).

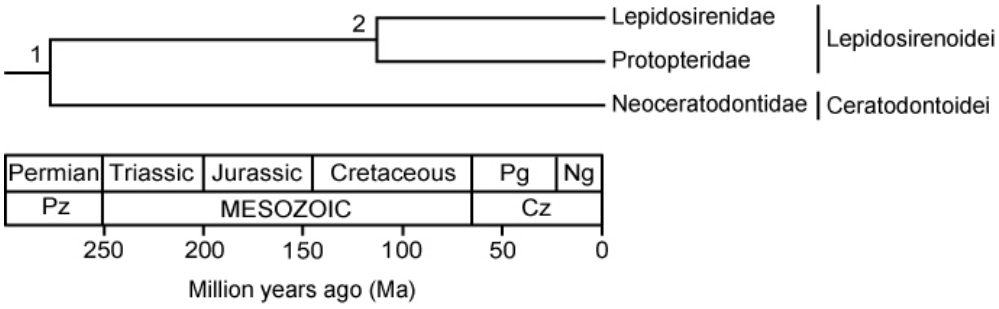

Figure 6-1. A timetree of lungfishes. Divergence times are from Table 6-1. *Abbreviations*: Ng (Neogene), P (Permian), Pg (Paleogene), and PZ (Paleozoic).

Table 6-1. Divergence times (Ma) among lungfishes and their credibility intervals (CI).

Timetree		Estimates			
Node	Time	RAG-1/RAG-2		six-gene	
(Fig. 6-1)	(Ma)	Time	CI	Time	CI
1	277.0	277	321-234		
2	120.0	114	154-94	126	165-95

The divergence of protopterid and lepidosirenid lungfishes has long been suggested to be related to Gondwanan breakup, because these families are restricted to freshwater and have fossil records extending back to the Cretaceous, but restricted to Africa and South America, respectively (Cavin et al. 2007; Lundberg 1993; Novacek and Marshall 1976). The timetree supports this hypothesis, as the South Atlantic Ocean opened largely during the Aptian and Albian stages of the Cretaceous, 125–100 Ma (Smith et al. 1994), the time period during which these families diverged according to the molecular time estimate. The ceratodontids are a much older, and formerly more widespread, group. The divergence of ceratodontid and lepidosirenoid lungfishes was too early (277 Ma) to be explained by continental vicariance, as the continents were joined into the supercontinent Pangaea at this time (Cavin et al. 2007). Further, although now restricted to Australia, fossils referable to ceratodontids have been described from Mesozoic deposits in Africa and South America, indicating a much wider distribution (Martin 1984; 1997). It is likely that additional fossils, rather than molecular data, will contribute more to elucidating the biogeographic history of the lungfishes, a relict group.

6.5 Acknowledgements

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CHAPTER 7

Cartilaginous fish evolutionary relationships and divergence times

Note: modified from Heinicke, M. P., Naylor, G. J. P., and Hedges, S. B. 2009, pp. 320-327 in *The Timetree of Life*, S. B. Hedges and S. Kumar (Eds.). MPH performed molecular clock analyses and drafted the paper. GJPN provided sequence data and comments on the manuscript. SBH directed research and co-wrote paper.

7.1 Abstract

Sharks, rays, chimaeras, and relatives (class Chondrichthyes) comprise an important component of living vertebrate diversity, with two subclasses, 18 orders, ~55 families, and ~1,200 species. Recent morphological studies have supported a position for rays deeply nested within sharks. Molecular analyses, however, support a basal divergence between rays and sharks. New molecular timing analyses presented here suggest the earliest divergences in Chondrichthyes occurred deep in the Paleozoic, 460–300 Ma, and most living families originated before the end of the Cretaceous (66 Ma). If accurate, these dates imply large ghost ranges in the fossil record for many chondrichthyan groups.

7.2 Introduction

Living members of the subclasses Holocephali (chimaeras, including ratfishes, spookfishes, and rabbitfishes, ~43 sp.) and Elasmobranchii (sharks, rays, skates, sawfishes, and guitarfishes, ~1,125 sp.) together comprise the extant representatives of the class Chondrichthyes (cartilaginous fishes) (Compagno et al. 2005). Holocephali includes only a single living order with three families (Compagno et al. 2005; Didier 1996). Elasmobranchs are more diverse, with ~17 orders and ~52 families (there is some disagreement in ordinal and familial limits, especially among rays) (Compagno et al. 2005; Shirai 1996; de Carvalho 1996; McEachran et al. 1996). Chondrichthyans can be differentiated from their closest living relatives, Osteichthyes (bony vertebrates), by possession of a skeleton of prismatic cartilage and internal fertilization via modified male pelvic fins (claspers). Other characters common to the group are possession of placoid

(toothlike) scales and, in many lineages, a heterocercal tail fin. While most sharks and chimaeras have a generally cylindrical "fishlike" body form, some sharks and all batoids (rays, skates, sawfishes, and guitarfishes) are dorsoventrally flattened and benthic in habit. Although early chondrichthyans included many freshwater forms, living species are overwhelmingly marine in distribution, excluding a few euryhaline sharks and rays and some freshwater stingrays. Here, we review the relationships of the subclasses, orders, and families of cartilaginous fishes. Additionally, molecular divergence times of these groups are estimated from publicly available sequence data and presented.

The fossil record of Chondrichthyes has been considered excellent, based largely on rich deposits of dental material (Maisey 1984). The cartilaginous skeleton of Chondrichthyes fossilizes poorly; therefore, skeletal fossil material is much rarer (Maisey et al. 2004). The earliest fossils assigned to Chondrichthyes are from the Silurian (444–416 Ma) (Coates and Sequeira 2001). Fossils become more common in the Devonian (416–359 Ma), including many representatives of extinct groups. Based on these fossils, the Subclasses Holocephali and Elasmobranchii are estimated to have diverged by 410 Ma (Coates and Sequeira 2001). Fossil evidence for modern representatives of these classes—Suborder Chimaeroidei (chimaeras) and Infraclass Neoselachii (sharks and rays)—does not occur until the Mesozoic (251–66 Ma) (Grogan and Lund 2004; Underwood 2006). Living orders and families can be identified from the Jurassic (200–146 Ma) onward, with fossil evidence of most families before the end of the Mesozoic (Maisey et al. 2004; Underwood 2006).

Division of living cartilaginous fishes into Elasmobranchii and Holocephali is strongly supported by morphological analyses, as is uniting these groups to form Chondrichthyes (Maisey 1984; Grogan and Lund 2004; Maisey 1986). Within Holocephali, it is believed that Rhinochimaeridae and Chimaeridae form a group to the exclusion of Callorhinchidae (Didier 1995). The relationships of the more species-rich elasmobranchs are more contentious. Early studies suggested a basal split between sharks and rays (Bigelow and Schroeder 1948, 1953). Shirai (1992) published an extensive and influential analysis of morphological variation among sharks and rays in which he proposed a "Hypnosqualean hypothesis" wherein the batoids fall together with the dorso-ventrally compressed sawsharks (Pristiophoriformes) and angel sharks (Squatiniformes).

These in turn group with the Orders Squaliformes, Hexanchiformes, and Echinorhiniformes in the Hypnosqualean clade. The Orders Lamniformes, Carcharhiniformes, Orectolobiformes, and Heterodontiformes are grouped as Galea (Shirai 1996; de Carvalho 1996). Minor modifications to Shirai's original 1992 hypothesis of elasmobranch inter-relationships were made by de Carvalho (1996). This hypothesis remains the consensus from morphological data.

Because the monophyly of Chondrichthyes and reciprocal monophyly of Elasmobranchii and Holocephali have not been controversial and are supported by numerous morphological characters, molecular studies have not been designed to specifically address these relationships. However, recent molecular studies that have included a broad enough sample of taxa to draw conclusions have supported the monophyly of these groups (Arnason et al. 2001; Mallatt and Winchell 2007). The interrelationships among the holocephalan families have not yet been addressed with molecular data. However, one mitochondrial gene study, using several holocephalan species as outgroups, has suggested that Rhinochimaeridae is embedded within Chimaeridae (Douady et al. 2003).

Most molecular studies have focused on elasmobranch interrelationships. Studies in the early to mid-1990s included too few taxa or sites to infer strong conclusions (Bernardi and Powers 1992; Dunn and Morrissey 1995; Kitamura et al. 1996). Since 2003, elasmobranch relationships have been inferred with more comprehensive datasets of both nuclear and mitochondrial data, including most orders and families (Maisey et al. 2004; Mallatt and Winchell 2007; Douady et al. 2003; Winchell et al. 2004; Naylor et al. 2005). These studies consistently (but weakly) reject the Hypnosqualea hypothesis, and instead suggest a basal divergence between sharks and batoids. Within the batoids, skates (Rajiformes) appear basal, followed by electric rays (Torpediniformes), then sawfishes and guitarfishes (Pristiformes, Rhinobatiformes), with stingrays (including butterfly, eagle, and manta rays; Myliobatiformes) being the most derived (Maisey et al. 2004; Mallatt and Winchell 2007; Kitamura et al. 1996; Dunn et al. 2003). In these studies, most of the batoid orders were represented by only one or a few families, but there are numerous myliobatiform families. Analyses including these families have not found significantly supported relationships, although it appears that the butterfly rays and

manta/eagle rays (Gymnuridae and Myliobatidae) form a group (Dunn et al. 2003). No studies have yet determined the relationships among the families of guitarfishes (Rhinidae, Rhyncobatidae), thornback rays (Platyrhinidae), or panrays (Zanobatidae). Based on analysis of mitochondrial 12S ribosomal RNA (rRNA) gene sequences available in GenBank, however, it appears that Rhinidae and Rhyncobatidae form a sawfish/guitarfish group with Pristidae and Rhinobatidae, while the position of thornback rays remains unresolved (results not shown).

Molecular studies of shark orders and families have led to a somewhat better understanding of relationships. The two major groups of sharks, galeomorphs and squalimorphs, are supported in most molecular studies (Maisey et al. 2004; Mallatt and Winchell 2007; Douady et al. 2003; Winchell et al. 2004; Naylor et al. 2005; Human et al. 2006). Although morphologically part of Galeomorphii, the horn sharks (Heterodontiformes) are in a basal position in molecular phylogenies, and cluster with both Squalimorphii and Galeomorphii, depending on the dataset. Within the Galeomorphii, the orders Lamniformes and Carcharhiniformes are generally recovered as closest relatives (Maisey et al. 2004; Douady et al. 2003; Naylor et al. 2005). In the Squalimorphii, Squatiniformes (angel sharks) and Echinorhiniformes (bramble sharks) are close relatives, while cow sharks (Hexanchiformes) are outside all other squalimorph orders (Maisey et al. 2004; Mallatt and Winchell 2007; Douady et al. 2003; Winchell et al. 2004; Naylor et al. 2005; Human et al. 2006). At the family level, the nominal groups Scyliorhinidae and Triakidae are estimated to be paraphyletic (Iglesias et al. 2005; Lopez et al. 2006) while the position of the hammerhead sharks is seen to fall within the Carcharhinidae. Accordingly, they are not considered a distinct family herein (Dunn et al. 2003). Carchariidae and Odontaspididae (often considered a single family) form divergent branches in Lamniformes (Maisey et al. 2004; Martin et al. 2002). The interfamilial relationships of Squaliformes remain unexplored.

Until now, no timing analyses have been performed at or above the family level using molecular sequence data. Martin et al. (1992) calculated the rate of evolution in sharks for cytochrome b sequences, but did not use this rate to infer times of divergence among different families. Batoid divergence times have been calculated, but only within families (Lovejoy et al. 1998; Valsecchi et al. 2005). Divergence times of higher

chondrichthyan taxa have been inferred using immunological distances, however (Lawson et al. 1995). These data suggest a very old divergence between sharks and batoids (392 Ma), and show divergences among sharks beginning 300 Ma.

7.3 Methods

Because there is no study reporting molecular divergence times of chondrichthyan families, we report herein the results of an analysis using published sequence data employing methodology described elsewhere (Heinicke et al. 2007). Sequence data were obtained from the most comprehensive available studies, using the nuclear protein-coding RAG-1 gene and the mitochondrial 12S and 16S rRNA genes (Maisey et al. 2004; Douady et al. 2003; Iglesias et al. 2005). Additional 12S and 16S sequences of 15 batoid families were included from GenBank, as only Rajidae and Urolophidae were included in the study of Douady *et al.* (2003). Together these data encompass a patchwork of sequences for 53 of 55 families of Chondrichthyes, excluding only Zanobatidae (panrays) and Rhincodontidae (whale shark). We note that while 53 of 55 families are represented, relatively few families are represented by all three genes, as a consequence of concatenating the data from three different studies with few overlapping taxa. In total, eight batoid families are represented only by 12S sequences, and 15 shark families by only RAG-1 sequences, while 17 families include all data and the remaining 13 families include data for two genes. Tree topology was based on the studies that reported the sequences, although branches that are not resolved or conflict among these and other published molecular phylogenies were collapsed to polytomies for the final timetree (Fig. 7-1). These polytomies mainly affect Squaliformes and the batoid orders, as molecular studies including squaliform families have very short, poorly supported internal branches, and relationships within batoid orders are similarly poorly supported (Maisey et al. 2004; Mallatt and Winchell 2004; Winchell et al. 2004; Dunn et al. 2003). An analysis of batoid 12S sequences used in the timetree did not find any significantly-supported relationships within batoid orders (results not shown).

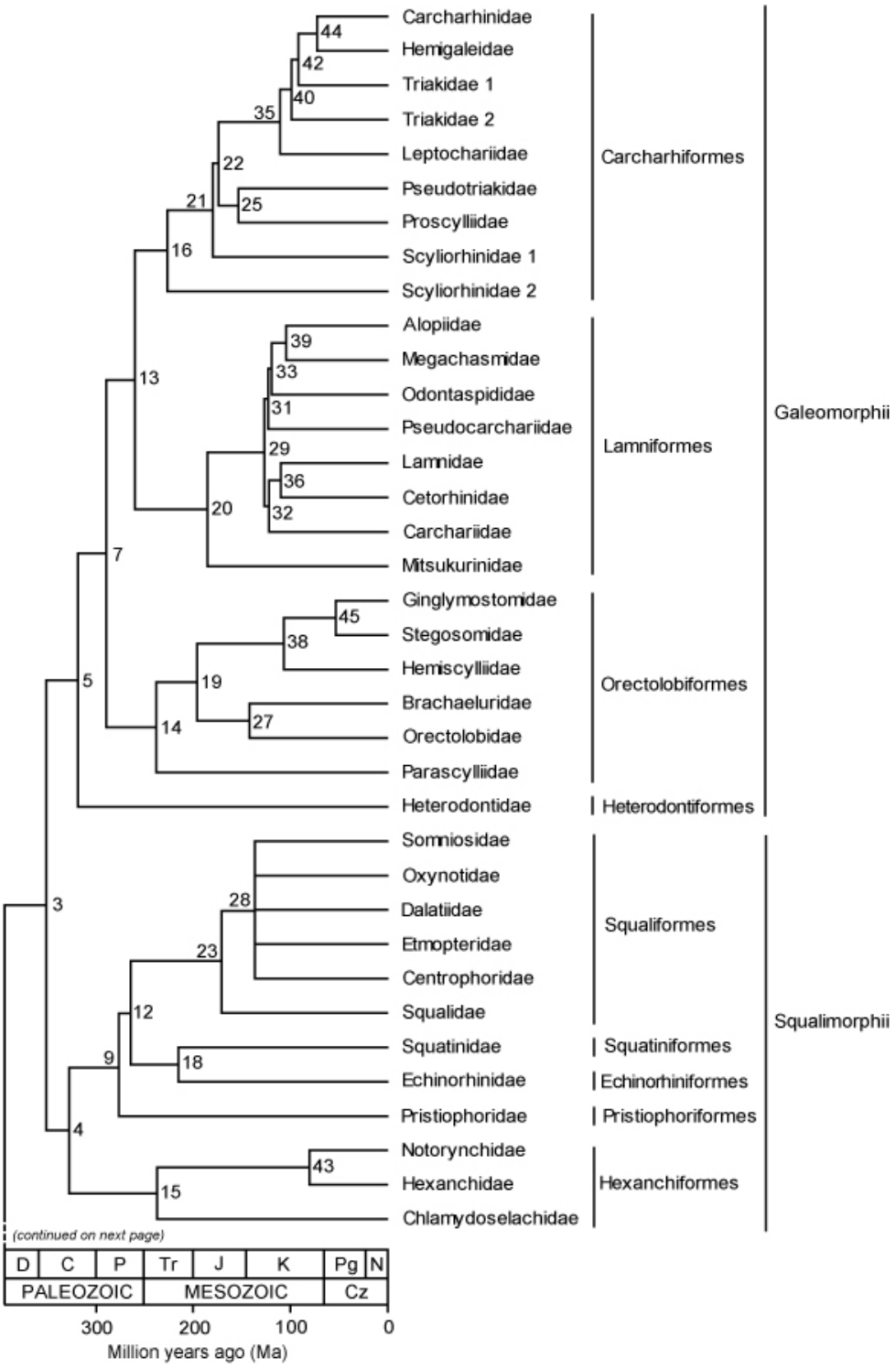
For the timetree, a combined analysis of all data was used. Analyses were also performed for the separate RAG-1 and 12S/16S data sets (Table 7-1). An amniote (*Homo*), amphibian (*Xenopus*), actinopterygian (*Danio*), cyclostome (*Petromyzon*), and

echinoderm (*Strongylocentrotus*) were used as outgroups, but these taxa are not presented in the timetree. A total of fourteen minimum (min.) and three maximum (max.) fossil constraints used to calibrate the timetree were obtained from the literature (Maisey et al. 2004; Coates and Sequeira 2001; Underwood 2006; Blair and Hedges 2005). These include the divergence of Centrophoridae from other Squaliformes (min. 89 Ma); the divergence of Squatinidae and Echinorhinidae (min. 151 Ma); the divergence of Hexanchidae and Chlamydoselachidae (min. 176 Ma); the divergence of Triakidae and Carcharhinidae (min. 89 Ma); the divergence of Scyliorhinidae and other Carcharhiniformes (min. 165 Ma); the divergence of Carchariidae and Lamnidae (min. 100 Ma); the divergence of Parascyllidae and other Orectolobiformes (min. 100 Ma); the divergence of Heterodontidae and other sharks (min. 176 Ma); the divergence of Dasyatidae and Myliobatidae (min. 100 Ma); the divergence of Rajidae and other batoids (min. 176 Ma); the divergence of sharks and batoids (min. 190 Ma); the divergence of elasmobranchs and holocephalans (min. 410 Ma, max. 495 Ma); the divergence of amniotes and amphibians (min. 340 Ma, max. 370 Ma); and the divergence of actinopterygians and sarcopterygians (min. 435 Ma, max. 495 Ma).

7.4 Results and Discussion

Times of divergence obtained from the separate RAG-1 and 12S/16S analyses differ markedly for most comparisons. Of the nodes shared between these two analyses, only the estimates for nodes within Batoidea and Lamniformes, and among derived carcharhiniform families (Carcharhinidae, Hemigaleidae, Triakidae), show noteworthy temporal concordance. In general the RAG-1 based estimates are much older than those based on 12S/16S sequences (Table 7-1). In some cases the discrepancy in age estimates is quite large. For example, RAG-1 data result in times more than 100 million years older than 12S and 16S data for divergences among the major chondrichthyan groups (chimaeras, batoids, galeomorph sharks, and squalimorph sharks). This may be caused by the large amount of branch length variation in the RAG-1 dataset (Maisey et al. 2004), while the 12S and 16S data have relatively less variation (Douady et al. 2003). Time estimates from the combined analysis, discussed below, are generally between values from the individual analyses. Conclusions based on the combined analysis must be

tempered by the knowledge that not all genes are present for all taxa (i.e., a large amount of missing data) and the large differences in times of deep branches obtained with RAG-1 as compared to 12S and 16S data.



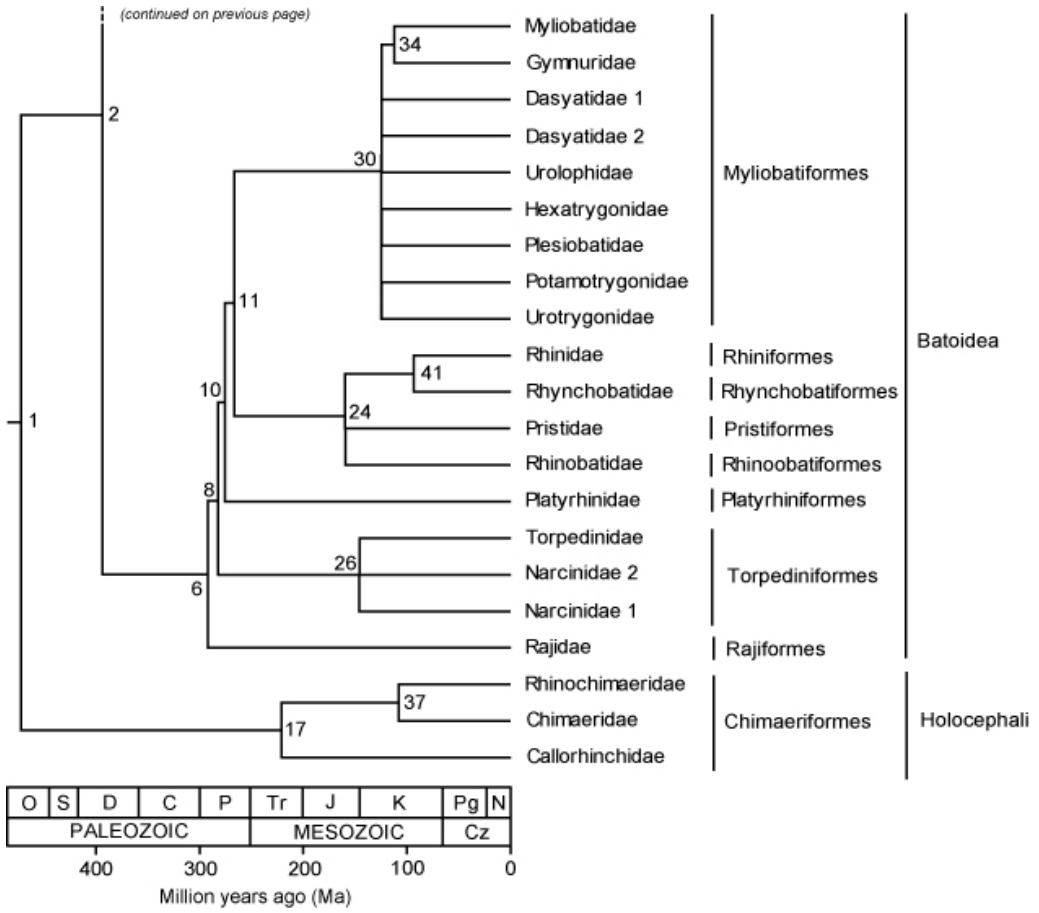


Figure 7-1. A timetree of cartilaginous fishes (Chondrichthyes). Divergence times are from Table 7-1. Galeomorphii, Squalimorphii, and Batoidea comprise the subclass Elasmobranchii. *Abbreviations*: C (Carboniferous), CZ (Cenozoic), D (Devonian), J (Jurassic), K (Cretaceous), O (Ordovician), P (Permian), Pg (Paleogene), S (Silurian), and Tr (Triassic). Codes for paraphyletic and/or polyphyletic groups are as follows: Triakidae-1 (*Mustelus*), Triakidae-2 (*Triakis*), Scyliorhinidae-1 (Pentanchinae), Scyliorhinidae-2 (Scyliorhininae), Dasyatidae-1 (*Dasyatis*), Dasyatidae-2 (*Himantura*), Narcinidae-1 (Narcininae), and Narcinidae-2 (Narkinae).

Notwithstanding the discrepancies in age estimates among genes, the timetree (Fig. 7-1) suggests that holocephalans and elasmobranchs diverged in the Ordovician, 471 (494–434) Ma. Fossil evidence indicates that these classes had diverged by at least 410 Ma (Coates and Sequeira 2001). The living families of Holocephali apparently diverged in the Mesozoic (251–66 Ma). The divergence of sharks and batoids is inferred to have occurred in the Devonian, 393 (431–354) Ma. This date is more than 100 million years older than the first appearance of neoselachian elasmobranchs in the fossil record,

and over 200 million years older than unambiguous evidence of modern orders (Underwood 2006). If these estimates are accurate, one must infer a large ghost range in the fossil record for early divergences within modern elasmobranchs. Times obtained with only 12S and 16S data are substantially younger, at 357 (402-319) Ma, but still suggests a large ghost range. Our analyses of the presented molecular data suggest that ordinal divergences were largely completed by the beginning of the Triassic, 251 Ma (whether considering the combined, RAG-1, or 12S/16S analyses) and that living families diverged throughout the Mesozoic, but especially during the Cretaceous (146–66 Ma). With the possible exceptions of Ginglymostomatidae (nurse sharks) and Stegostomatidae (zebra shark), all elasmobranch families are estimated to have appeared by the end of the Cretaceous.

Because of these apparent ancient divergences, oceanic habits of chondrichthyans, and large differences in time estimates depending upon analysis used, it is difficult to infer the biogeographic history of the living families. Most chondrichthyan families today are cosmopolitan in distribution, or found in widely divergent (i.e., separate ocean basins) areas of suitable habitat. For the many families with pelagic or deep-sea distributions, it may be impossible to infer biogeographic history due to the worldwide nature of their habitats. Extensive plate tectonic activity has contributed to substantial changes in ocean basins since the divergence of most families of Chondrichthyes. Although all ocean floor is geologically young (Mesozoic and Cenozoic), oceans differ in age when considered as bodies of water (aquatic habitat). For example, the Atlantic is relatively young (~150 Ma) compared with the Pacific (Smith et al. 1994; Gradstein et al. 2005), which may explain why no living families are restricted to the Atlantic. Other families may have their origins in basins that no longer exist. For example, many inshore, benthic families, such as batoids, diverged in the Cretaceous (based on the results of the presented analyses, as well as fossil data) when the sea level was much higher and shallow continental seaways covered large portions of North America and Asia. At the same time, the now-gone Tethys Sea existed between the northern and southern continents (Smith et al. 1994; Gradstein et al. 2005). These water bodies may have been the early sites of diversification within batoids and inshore sharks. The timetree (Fig. 7-1) is compatible with previous interpretations of shark evolution based on the fossil record, including a major radiation

of neoselachian sharks in the Jurassic and Cretaceous (200–66 Ma), possibly related to a parallel radiation of prey, actinopterygian fishes (Benton 2000). In order to better understand the factors leading to diversification in Chondrichthyes, additional fossil (especially skeletal) and paleogeographic data will be needed to complement the emerging molecular phylogenetic data. In addition, more comprehensive molecular data, including nuclear gene loci that exhibit more uniform rates of evolution among lineages,are needed to resolve poorly-known parts of the chondrichthyan tree and to estimate better-constrained times of divergence.

7.5 Acknowledgements.

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Table 7-1. Divergence times (Ma) among cartilaginous fishes (Chondrichthyes) and their credibility intervals (CI) based on analyses presented here.

Timetree	Estimates					
Node	RAG-1/12S/16S		RAG-1		12S/16S	
(Fig. 7-1)	Time	CI	Time	CI	Time	CI
1	471	494-434	486	495-463	436	482-411
2	393	431-354	440	471-403	357	402-319
3	350	392-309	419	452-380	273	319-235
4	327	372-283	392	431-345	256	304-214
5	318	359-279	374	414-330	258	301-222
6	291	333-250	308	368-248	308	357-262
7	289	329-252	344	386-300	234	276-201
8	281	324-241			294	344-249
9	276	323-232	329	385-265	219	269-178
10	274	318-235			285	334-240
11	265	307-227	283	341-228	273	321-228
12	263	311-220	312	370-247	207	256-165
13	259	297-226	304	346-262	220	260-190
14	237	287-186	280	338-215		
15	236	295-183	285	353-202		
16	226	261-195	269	310-228	189	227-166
17	220	320-125	248	351-128		
18	214	269-163	252	324-176		
19	195	249-139	231	296-160		
20	185	224-148	222	270-175		
21	179	210-153	213	251-177	155	194-124
22	173	204-149	205	242-171	141	179-111
23	170	218-128	190	268-121	142	188-106
24-Rhinobatidae/Rhinidae	175	235-121			185	248-126

24-Pristidae/Rhinidae	143	202-88			153	217-93.1
Node 24 mean	159	219-105			169	233-110
25	153	183-127	183	222-147	114	156-76.5
26-Narcinidae 1/Torpedinidae	150	210-102			159	221-105
26-Narcinidae 2/Torpedinidae	140	199-92.9			148	209-96.5
Node 26 mean	145	205-97.5			154	215-101
27	142	201-84	169	243-97		
28-Centrophoridae/Somniosidae	153	201-110	165	241-101	129	175-93.6
28-Somniosidae/Etmopteridae	136	185-93.7	153	228-92	87.1	135-51.8
28-Somniosidae/Oxynotidae	126	175-84	141	217-82		
28-Etmopteridae/Dalatiidae	126	176-83	142	215-84		
Node 28 mean	135	184-92.7	154	229-91	108	155-72.7
29	126	160-104	167	217-125	120	154-102
30-Urolophidae/Dasyatidae 2	109	143-78.8			111	150-79.8
30-Dasyatidae 1/Myliobatidae	114	142-100			117	151-100
30-Potamotrygonidae/Urotrygonidae	115	158-75.8			117	163-77.6
30-Hexatrygonidae/Plesiobatidae	121	157-90.6			123	162-90.6
30-Dasyatidae 1/Urolophidae	129	161-108			132	168-109
30-Dasyatidae 1/Hexatrygonidae	136	169-113			139	177-114
30-Dasyatidae 1/Potamotrygonidae	147	184-121			151	191-122
Node 30 mean	124	159-98.2			127	166-99
31	122	155-100	140	193-93		
32	122	154-101	122	167-100	117	150-100
33	119	151-96.6	120	172-74	117	151-98
34	111	139-97.4	115	148-100	115	149-97.7
35	110	133-95.7	112	140-96		
36	109	142-83.4	93.4	139-58.5	109	142-85.5
37	107	182-51.1			123	197-64.8
38	106	162-59.8	127	196-69.1		
39	104	139-72.8	103	157-57.6		
40	98.6	118-89.6	99	123-89.6	107	139-90.1
41	92.4	150-46.5			99.6	164-49.3
42	91.6	111-77.0	91	116-72.7	94.8	127-72.6
43	79.7	150-28.8	98.6	185-34.3		
44	72.1	93.8-53.1	70.9	96.9-50.2		
45	53.1	96.7-23.1	64.6	119-26.6		

CHAPTER 8
Concluding Remarks

The work presented above helps to shed new light on the evolution of the terraranan frogs, a group which comprises a significant component of Neotropical amphibian diversity. Through the use of molecular phylogenetic methods and divergence timing analyses, several novel discoveries were made. In review, major findings resulting from the above-presented work include the finding that most terraranans belong to one of a few clades, strongly linked with geography (*Craugastor* in Central America, *Eleutherodactylus* in the West Indies, *Pristimantis* in northern South America, *Ischnocnema* in southeast Brazil), all formerly placed in the genus *Eleutherodactylus*. Based on divergence times obtained from a Bayesian relaxed molecular clock analysis, the Central American and West Indian clades originated via separate Cenozoic oceanic dispersals out of South America. The closest relatives of the terraranan frogs are the marsupial frogs, the two groups together forming an expanded direct-developing clade. In the course of this work, a new taxonomy for terraranans was proposed to better reflect evolutionary relationships; it has been wholly adopted by the scientific community in the year since its publication (Frost 2009; AmphibiaWeb 2009; IUCN 2009). A new family of terraranan frog, Ceuthomantidae, was discovered, and its phylogenetic placement breaking the long branch leading to the other terraranan families may have been the key to uncovering the terraranan-marsupial frog relationship. Timetrees of several non-terraranan vertebrate groups were presented; these show contrasts in biogeographic history (oceanic dispersal versus vicariance) and relative rates of diversification, especially in the case of West Indian *Leptodactylus*.

The results of divergence time analyses for both *Eleutherodactylus* and *Leptodactylus* add weight to the evidence that the West Indian fauna is almost wholly derived from Cenozoic dispersals (Hedges 1996a). Both genera also illustrate additional examples of oceanic dispersal in amphibians. Coupled with other recently discovered examples (e.g. Vences et a. 2003), these results suggest that oceanic barriers are not as insurmountable as once thought, especially in groups with pre-adaptations like

dessication avoidance, or, in the case of terraranans, terrestrial egg deposition (which would allow dispersal by clutches of eggs as well as adult frogs).

The phylogenetic analysis of the position of Ceuthomantidae in regard to other terraranan families and Nobleobatrachia as a whole employed many chimeric sequences. Use of chimeric sequences appeared to have no adverse effect on the resulting phylogeny; terminal taxa that were expected to be rendered close relatives were indeed found to be close relatives. Based on these results, I would suggest a wider adoption of this strategy to take advantage of the exhaustive, but patchy, amount of data available from GenBank and to avoid problems associated with missing data in phylogeny reconstruction (Wiens 2003).

Even though the phylogenetic analyses presented above included nearly 350 species of terraranan, those are still only a fraction of the over 900 described species, and many more species are likely to exist based on current rates of discovery (Fig. 3-1). As data are collected for these species, new discoveries will occur, and new hypotheses of evolution within Terrarana will be testable, especially in regard to evolution of terraranans within South America, where the most species occur. Already, we are analyzing molecular data (not included in this thesis) for dozens of additional species, and are aware of at least one new genus of terraranan in South America. Meanwhile, data we are collecting from other species suggests local endemic clades exist in several regions of South America, including the Venezuelan Andes and Guiana Shield. Preliminary results of phylogenetic analyses we are currently conducting of *Eleutherodactylus planirostris* (the Greenhouse Frog) and relatives suggest that this Cuban species is also native to the Florida Keys, and that populations introduced to peninsular Florida are actually derived from these Key populations, and not Cuba as previously assumed. Thus, another incidence of overwater dispersal can be added to the several already demonstrated in terraranans.

The phylogenetic hypothesis of evolutionary relationships and divergence times of Terraranan frogs presented in this thesis could be used as the background for diverse comparative studies. Terraranan frogs exhibit a variety of ecologies, ranging from sea level to well above the tree line and living from the leaf litter to high forest canopy. Some groups of terraranans clearly represent adaptive radiations (Hedges 1989b). Additionally,

taking into account the wide distribution of terraranans on continents and islands of all sizes, they are obvious candidates for studies of ecomorphological diversification, and would make an excellent comparative group for the well-studied *Anolis* lizards of the West Indies, Central America, and South America (Losos 2009). The close relationship between terraranans and marsupial frogs suggests they may share developmental attributes; comparisons of developmental biology with other direct-developing amphibians may shed light on whether direct-development evolved before or after the divergence of terraranans and marsupial frogs. It is my hope that the work presented in this thesis will spur new research into these or other aspects of the biology of terraranan frogs, and amphibians in general, as well as in the fields of systematics and biogeography.

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APPENDIX

Systematic Accounts

Terrarana Hedges, Duellman, and Heinicke, 2008a

Definition.—Species in this taxon have terrestrial breeding, direct development (ovoviviparity in *Eleutherodactylus jasperi*), and embryonic egg teeth. All have arciferal (or pseudofirmisternal in a few taxa) pectoral girdles and partially fused calcanea and astragali; they lack Bidder's organs and intercalary elements in the digits. The species range in SVL from 10–11 mm in female *Brachycephalus didactylus* and *Eleutherodactylus* (*Euhyas*) *iberia* to 110 mm in female *Craugastor pelorus.*

Content.—The taxon contains five families (923 species): Brachycephalidae, Ceuthomantidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae. It corresponds to the more inclusive family Brachycephalidae of Frost et al. (2006).

Distribution.—The taxon ranges from Texas in the USA southward throughout Mexico, Central America, and the West Indies through tropical and subtropical South America to northern Argentina.

Etymology.—The name is derived from the Latin, *terra* (land) and *rana* (frog). It is used in allusion to the terrestrial breeding and direct development shared by these four families, allowing the species to successfully colonize montane forests far from standing or running water in the New World.

Remarks.— Each of the clades recognized here as families are monophyletic in the latest molecular phylogenies (see Chapters 3 and 4), has some morphological support (although no unambiguous shared derived characters are present), and occurs primarily in a different geographic region. All but Strabomantidae have receive significant support.

Dubois (2009) has objected to the spelling of the name Terrarana, proposing that it should be emended to "Terraranae" or "Terranae." His reasoning is that by doing so it would conform with his own rules of zoological nomenclature (See Dubois 2009, and references therein). In this case, it would change the name from a noun in the nominative singular to one in the nominative plural, and thus be consistent with most higher-level names. His suggestion of "Terranae" was because he thought it would be easier to pronounce. However, these proposed changes are not supported by the Code of Zoological Nomenclature and therefore we do not support them. As conceded by Dubois (2009), the name Terrarana could be considered a noun in the nominative plural if it were derived from the neuter noun *terraranum*. Moreover, other higher-level taxa of amphibians that end in the letter "A" are nouns in the nominative plural. Therefore, the name Terrarana, by itself, does not imply that it is a noun in the nominative singular or otherwise is inconsistent with the rules defined by Dubois (2009) for coining higher-level names. It was only the etymology given by Hedges et al. (2008) that created a potential conflict with Dubois' rules. To resolve that conflict, without changing the name, Heinicke

et al. (2009) proposed that that the neuter derivation of the name be assumed and that Terrarana be considered henceforth as a noun in the nominative plural.

Family Brachycephalidae Günther, 1858

Brachycephalina Günther, 1858:344. Type genus: *Brachycephalus* Fitzinger, 1826:39.
Brachycephalinae—Noble, 1931:507.
Brachycephalinae (part)—Dubois, 2005:11.
Brachycephalidae (part)—Frost et al. 2006:197.

Definition.—The following definition is derived principally from Alves et al. (2006) and Da Silva et al. (2007) for *Brachycephalus* and Lynch (1971, 1972) and Caramaschi and Canedo (2006) for *Ischnocnema*. Frogs of the family Brachycephalidae have: (1) sternum cartilaginous or absent; (2) vertebral shield present or absent; (3) transverse processes of posterior presacral vertebrae not broadly expanded; (4) cervical cotyles widely spaced; (5) usually eight presacral vertebrae, with usually the first and second not fused; (6) cranial elements co-ossified or not with overlying skin; (7) omosternum present; (8) sacral diapophyses rounded, barely or moderately dilated; (9) maxillary arch dentate or not; (10) alary processes of premaxillae broad at base, usually directed dorsally or posterodorsally; (11) palatal shelf of premaxilla broad or slender, indented or not; (12) pars facialis of maxilla deep, not exostosed; (13) palatal shelf of maxilla moderately broad, bearing pterygoid process or not; (14) maxillary arch usually complete; maxillae tapering posteriorly; quadratojugal slender or absent; (15) nasals large and in contact (or not) medially; (16) nasals in contact (or not) with maxillae; (17) nasals in contact (or not) with frontoparietals; (18) frontoparietal fontanelle usually absent; (19) frontoparietals usually not exostosed; cranial crests present or absent (20) frontoparietals fused with prootics or not; (21) temporal arcade absent; (22) epiotic eminences prominent to indistinct; (23) carotid artery passing dorsal to cranial elements; (24) zygomatic ramus of squamosal broad to slender (or short), not in contact with maxilla; (25) otic ramus of squamosal short to elongate, expanded into otic plate or not; (26) squamosal-maxilla angle 44–80˚; (27) columella present or absent; (28) vomers variable in size; dentigerous processes present or absent; (29) neopalatines broad, slender, or absent; (30) sphenethmoid usually entire; (31) anterior ramus of parasphenoid narrow to broad, not keeled; (32) parasphenoid alae at right angle to axis of skull or deflected posteriorly, usually not overlapped by pterygoids; (33) pterygoid lacking ventral flange; (34) occipital condyles small to large, stalked or not, widely separated medially; (35) mandible lacking odontoids; (36) terminal phalanges T-shaped, arrow-shaped, knobbed, or bearing hook-like lateral process; (37) one, two, or three phalanges in Finger IV; (38) Toe I fully developed, with short phalange, or with no phalange; (39) alary process of hyoid plate on slender or short stalk; (40) mandibular ramus of trigeminal nerve passing lateral or medial to the *m. adductor mandibulae* or passing between two slips of the muscle; (41) prominent external body glands usually absent; (42) males usually have a single, median, subgular vocal sac; (43) males having vocal slits and nonspinous nuptial pads or not; (44) fingers unwebbed; toes usually unwebbed, webbed basally, or rarely webbed extensively; (45) terminal digits expanded or not; circumferential grooves present or absent; digits apically pointed or not; (46) inner metatarsal tubercle present or absent; outer metatarsal tubercle present; (47) tympanic membrane and annulus well differentiated or not; (48)

amplexus usually axillary; inguinal in some species; (49) eggs deposited in terrestrial or arboreal situations and undergo direct development; (50) SVL from 10.2 mm in females of *Brachycephalus didactylus* to 54 mm in females of *Ischnocnema guentheri*.

Content.—The family contains two genera (44 species): *Brachycephalus* with 12 species and *Ischnocnema* with 32 species.

Distribution.—The family is distributed in the Atlantic Coastal Forest in eastern Brazil and in the *Aracuaria* forest in extreme southeastern Brazil and northern Argentina.

Etymology.—The familial name is derived from the Greek *brachys,* meaning short, and the Greek *kephale*, meaning head; the name refers to the small heads characteristic of the type genus.

Remarks.—The fact that both *Brachycephalus* and *Ischnocnema* occur in southeastern Brazil probably is not coincidental. However, we have yet to identify any unique morphological characters shared by these two genera. The highly derived and miniaturized bauplan of *Brachycephalus* provides a challenge to identify such characters. The species *Haddadus binotatus* (and by association, "*E.*" *plicifer*) is not closely related to either *Brachycephalus* or *Ischnocnema*. Other eastern Brazilian genera included in our analyses (*Holoaden, Barycholos,* and *Adelophryne*) likewise do not belong to this family. Our previously defined Southeast Brazil Clade is expanded here to include *Brachycephalus* and now corresponds to the family Brachycephalidae.

Genus *Brachycephalus* Fitzinger, 1826

Brachycephalus Fitzinger 1826:39. Type species: *Bufo ephippium* Spix, 1824, by monotypy.
Ephippipher Cocteau, 1835:12. Replacement name for *Brachycephalus* Fitzinger, 1826.
Psyllophryne Izecksohn, 1971:2. Type species: *Psyllophryne didactyla* Izecksohn, 1971, by original designation. Synonymy by Kaplan (2002:227).

Definition.—Members of this genus have: (1) sternum absent; (2) usually eight presacral vertebrae; Presacrals IV and V, and VI and VII fused in *B. ephippium;* (3) palatal shelf of maxilla lacking pterygoid process; (4) maxillary arch edentate, but bearing odontoids in some species; (5) neopalatines slender; absent in *B. ephippium, ferruginus, hermogenesi, pernix,* and *pombali;* (5) columella absent; fenestra ovalis directed posteriorly; (6) terminal phalanges arrow-shaped; one phalange in Finger IV, no phalange or one short phalange in Toe I; (7) terminal digits not expanded; circumferential grooves absent; digits apically pointed; (8) SVL less than 18 mm.

Content.—Twelve species are placed in this genus: *Brachycephalus alipioi, brunneus, didactylus, ephippium*, *ferruginus, hermogenesi, izecksohni, nodoterga, pernix,pitanga, pombali,* and *vertebralis*.

Distribution.—This genus is restricted to the Atlantic Coastal Forest in the states of Río de Janeiro, São Paulo, and Paraná in southeastern Brazil.

Etymology.—This is the same as that for the family; the gender is masculine.

Remarks.—Based on digital morphology, Frost et al. (2006) suggested a close relationship between *Adelophryne*, *Brachycephalus,* and *Euparkerella*. However, Da Silva et al. (2007) concluded that digital morphology does not support a relationship between *Brachycephalus* and either *Adelophryne* or *Euparkerella*. Molecular analyses have not included *Euparkerella*, but they confirm that *Brachycephalus* is not closely related to *Adelophryne* (Hedges et al. 2008a). No species groups are recognized within this genus (Alves et al. 2006).

Genus *Ischnocnema* Reinhardt and Lütken, 1862

Ischnocnema Reinhardt and Lütken, 1862:239. Type species: *Leiuperus verrucosus* Reinhardt and Lütken, 1862:171.

Basanitia Miranda-Ribeiro, 1923:851. Type species: *Basanitia lactea* Miranda-Ribeiro, 1923:851, by monotypy. Synonymy by Lynch (1968b:875).

Phrynanodus Ahl, 1933:29. Type species: *Phrynanodus nanus* Ahl, 1933:29, by monotypy. Synonymy by Lynch (1968b:876).

Definition.—This genus is characterized by: (1) sternum present; (2) eight presacral vertebrae; (3) palatal shelf of maxilla bearing pterygoid process; (4) maxillary arch dentate; (5) neopalatines broad; (5) columella present; fenestra ovalis directed laterally; (6) terminal phalanges T-shaped; full complement of phalanges in digits; (7) terminal discs expanded slightly or greatly; circumferential grooves present (8) SVL from 16 mm in females of *Ischnocnema pusilla* to 54 mm in females of *I. guentheri*.

Content.—The genus contains five species series (32 species): the *Ischnocnema guentheri, lactea, parva, ramagii,* and *verrucosa* species series.

Distribution.—The genus is widely distributed in the Atlantic Coastal Forest in eastern Brazil and in the *Aracuaria* forest in extreme southeastern Brazil and northern Argentina.

Etymology.—The generic name is derived from the Greek *ischnos,* meaning slender or weak, and the Greek *kneme,* meaning calf of the leg. The name is feminine in gender.

Remarks.—Some species previously assigned to this genus are now placed in the genus *Oreobates* (Caramaschi and Canedo 2006; Padial et al. 2008). See also the Remarks under *Oreobates*. Heinicke et al. (2007) placed all but two of the species ("*E.*" *binotatus* and "*E.*" *plicifer*) in southeastern Brazil that were previously assigned to *Eleutherodactylus* into the genus *Ischnocnema*, which they also referred to as the Southeast Brazil Clade (here expanded to include *Brachycephalus*). This was done with some trepidation because they lacked sequence information for the type species, *Ischnocnema verrucosa,* and most of the other species. Nonetheless, their decision was based on the discovery of a clade of species from southeastern Brazil (*I. guentheri*, *hoehnei*, *juipoca*, and *parva*) that by implication of prior species group affiliation contains nearly half of the species that they assigned to the genus—*I. epipeda, erythromera, gualteri, guentheri, henselii, hoehnei, izecksohni, juipoca, nasuta, oea,*

parva, pusilla, and *vinhai*. The remaining species form several clusters and previously some of those species have been placed in the same morphological species group (Lynch 1976a) along with species that Heinicke et al. (2007) assigned to *Ischnocnema*.

The important question is whether *Ischnocnema verrucosa* is part of the Southeast Brazil Clade. In his discussion of this species, Lynch (1972) noted a resemblance to several species in southeastern Brazil. Sazima and Cardoso (1978) suggested that *Ischnocnema verrucosa* resembled "*E*." *juipoca*, a species that was included in the study by Heinicke et al. (2007). The two species are similar in size, have a tuberculate dorsum (hence the Latin name *verrucosa*), short legs, small digital discs, an areolate venter, and a color pattern that includes labial and limb bars.

Heinicke et al. (2007) showed that *Holoaden* was not nested within the Southeast Brazil Clade and this conclusion is unchanged in subsequent analyses (Hedges et al. 2008; Heinicke et al. 2009). *Barycholos* contains a species, ***B. ternetzi***, that occurs in eastern Brazil, and Campos et al. (2007) suggested that it might have affinities with species here placed in the Southeast Brazil Clade, based on its karyotype. However, Heinicke et al. (2007) showed that it is not part of the Southeast Brazil Clade defined in their molecular phylogeny.

In the following definitions of species series of *Ischnocnema* we combine elements of previous species group definitions (Lynch 1968b, 1976a; Heyer 1984) but with some differences in characters and content based on the preceding discussion and a reevaluation of the importance of various characters. As we found in the Caribbean Clade (*Eleutherodactylus*), a primary character emphasized by Lynch and Duellman (1997), relative lengths of Toes III and V, does not seem to be useful in defining species series of *Ischnocnema*. Instead, body size and shape, relative lengths of legs and fingers, and size of digital discs are among the useful characters that we have identified for these species.

The number of species that have been sampled for DNA sequence data (Heinicke et al. 2007) and chromosome data (Campos et al. 2007) is too small to be of much use in defining species series. Additional molecular and chromosomal data are needed to test these series affiliations. For convenience we use the species series rank within *Ischnocnema* to allow for finer divisions (species groups and subgroups) to be defined in the future as relationships become better resolved, especially within the larger series (*I. guentheri* and *lactea* series).

Ischnocnema guentheri Species Series

Definition.—Species in this series range in SVL from 19 mm (males only, *Ischnocnema oea*) to 54 mm (females, *I. nasuta*) and have moderately slender bodies with long legs (shank length usually > 60% SVL). The snout is acuminate in dorsal view; the tympanic membrane is differentiated. The dorsum is smooth or finely granular, and the venter usually is smooth (areolate in *I. erythromera* and *vinhai*). Nuptial pads are absent in *I. hoehnei* and unknown in several species. Finger I is approximately the same length as Finger II, and the digital discs usually are small or slightly expanded (large in *I. hoehnei* and *vinhai*).

Content.—Eleven species are placed in the series: *Ischnocnema epipeda, erythromera, gualteri, guentheri, henselii, hoehnei, izecksohni, nasuta, octavioi, oea*, and *vinhai*.

Distribution.—The species series is widely distributed in the Atlantic Coastal Forest in southeastern Brazil from southern Bahia to Santa Catarina; one species, *I. henselii,* also occurs in *Aracuaria* forest in Rio Grande do Sul, Brazil, and Misiones, Argentina.

Remarks.—The definition and content of this series is based mainly on the work of Heyer (1984), who noted that a cluster of species within the more inclusive "*Eleutherodactylus*" *binotatus* Group of Lynch (1976a) shared several characters that suggested monophyly. He did not place *Ischnocnema hoehnei* and *I. vinhai* in the cluster but alluded to a relationship with the cluster. Heinicke et al. (2007) found that *I. hoehnei* was the closest relative of *I. guentheri* among the four species of the Southeast Brazil Clade they included. Therefore we have added these two species to a more inclusive *Ischnocnema guentheri* Species Series. We also include *I. octavioi* based on its smooth venter, relative finger lengths, small digital discs, and moderately long legs, and note that Bokermann (1965) associated it with an earlier version of the "*Eleutherodactylus*" *guentheri* Group. Caramaschi and Kisteumacher (1989) described *I. izecksohni* and suggested that it belonged to the "*Eleutherodactylus*" *guentheri* Group. Kwet and Solé (2005) recently resurrected *I. henselii* from the synonymy of *I. guentheri*. As more sequence data become available for this diverse species series, species groups and subgroups may be definable.

Ischnocnema lactea Species Series

Definition.—Species in this series are small to moderate in SVL and range from 18 mm (females, *I. paranaensis* and *randorum*) to 40 mm (females, *I. sambaqui*). The body is moderate or robust with short legs (shank length usually <50% SVL), and the snout is subacuminate in dorsal view. The tympanic membrane is differentiated or not; the dorsum is smooth, rugose, or tuberculate, and the venter is smooth or areolate. Nuptial pads usually are absent (minute in *I. randorum* and unknown in several species), Finger I is usually shorter than Finger II (equal in length to Finger II in several species), and at least the outer digital discs are moderate to large.

Content.—Fourteen species are placed in the species series: *Ischnocnema bilineata, bolbodactyla, conocolor, gehrti*, *holti, lactea, manezinho, melanopygia, nigriventris*, *paranaensis, randorum*, *sambaqui*, *spanios*, and *venancioi*.

Distribution.—The species series is distributed in the southern part of the Atlantic Coastal Forest from Rio de Janeiro to Santa Catarina in southeastern Brazil.

Remarks.—We concur with Castano and Haddad (2000) who suggested that *Ischnocnema manezinho* and *I. sambaqui* are members of the "*Eleutherodactylus*" *lactea* Group (roughly equivalent to our *Ischnocnema lactea* Species Series). We also added four other species (*I. bilineatus, paranaensis, randorum* and *spanios*) that conformed to our definition of the series. The last two species are most likely each others closest relatives (Heyer 1985). Three of the characters proposed by Lynch (1976a) to define the former "*E*." *lacteus* Group (tympanic membrane differentiated, smooth venter, and

rounded discs) are now known to be variable and not useful for defining this group. The tympanic membrane in six of the species (*I. holti, lactea, nigriventris*, *randorum, spanios*, and *venancioi*) is undifferentiated, and the venter of five species (*I. holti*, *nigriventris*, *randorum*, *spanios*, and *venancioi*) is areolate. Also most of the species have truncate or elliptical rather than apically rounded discs. Heinicke et al. (2007) did not include members of this group in their molecular phylogeny, but we have included one species (*I. holti*), where it clusters with other species of *Ischnocnema*. As more sequence data become available for this diverse species series, species groups and subgroups likely will be definable.

Ischnocnema parva Species Series

Definition.—Species in this series range in SVL from 16 mm (females, *I. pusilla*) to 23 mm (females, *I. parva*); the body is robust with short legs (shank length < 50% SVL) and the snout is rounded in dorsal view. The upper half of the tympanic membrane is undifferentiated. The dorsum and venter are smooth, and nuptial pads are absent; Finger I is approximately the same length as Finger II, and the digital discs are small and pointed.

Content.—Two species, *Ischnocnema parva* and *pusilla* are placed in this group.

Distribution.—The species series occurs in the Atlantic Coastal Forest in the states of Rio de Janeiro and São Paulo in southeastern Brazil.

Remarks.—Heinicke et al. (2007) included *Ischnocnema parva* in their molecular phylogeny. It clustered with two members of the *Ischnocnema guentheri* Species Series (*I. guentheri* and *hoehnei*), which because of the limited sampling only indicated that this series is closely related to the *Ischnocnema guentheri* Species Series.

Ischnocnema ramagii Species Series

Definition.—Species in this series range in SVL from 22 mm (sex unknown, *I. ramagii*) to 36 mm (females, *I. paulodutrai*). The frogs are moderate in shape (not slender or particularly robust) and have moderately long legs; the snout is subacuminate in dorsal view. The tympanic membrane is differentiated; the dorsum is finely granular, and the venter is areolate. The condition of the nuptial pads is unknown. Finger I is much longer than Finger II, and at least the outer digital discs are large.

Content.—Two species, *Ischnocnema paulodutrai* and *ramagii,* are placed in this series.

Distribution.—The species series occurs in the isolated remnants of Atlantic Coastal Forest in the states of Paraíba, Pernambuco, and Bahia in eastern Brazil.

Remarks.—As noted by Lynch (Lynch 1976a), these two species are unusual among South American eleutherodactylids in having a long first finger combined with large digital discs. With the placement of two species (*Haddadus binotatus* an *H. plicifer*) in a new genus, these are the only species of *Ischnocnema* with Finger I much longer than

Finger II. Heinicke et al. (2007) did not include a member of the *Ischnocnema ramagii* Species Series in their molecular phylogeny. Although the long first finger may indicate a close relationship with *Haddadus,* members of the *Ischnocnema ramagii* Species Series have an areolate venter, large digital discs, and a smooth dorsum, in contrast to a smooth venter, small digital discs, and longitudinal dermal ridges on the dorsum in *Haddadus*. We tentatively place these two species in *Ischnocnema*.

Ischnocnema verrucosa Species Series

Definition.—Species in this series range in SVL from 21 mm (males only, *I. juipoca*) to 26 mm (females, *I. verrucosa*). The body is moderate in shape with short legs (shank length < 55% SVL), and the snout is subacuminate in dorsal view. The tympanic membrane differentiated or not; the dorsum is tuberculate, and the venter is areolate. The condition of the nuptial pads is unknown. Finger I is approximately the same length as Finger II, and the digital discs are small.

Content.—Three species, *Ischnocnema juipoca, penaxavantinho,* and *verrucosa,* are placed in this series.

Distribution.—The distribution of the species series is disjunct in the Atlantic Coastal Forest in the states of Espírito Santo, Minas Gerais, and São Paulo in southeastern Brazil.

Remarks.—As noted above in the remarks for the genus, these two species share a suite of characters. Heinicke et al. (2007) included one species (*I. juipoca*) in their molecular phylogeny, where it appeared as a basal member of the Southeast Brazilian Clade (*Ischnocnema*).

Family Craugastoridae Hedges, Duellman, and Heinicke, 2008a

Craugastoridae Hedges, Duellman, and Heinicke, 2008a:29. Type genus *Craugastor* Cope, 1862:153.
Eleutherodactylinae (part) Lutz, 1954:157.
Eleutherodactylini—Lynch, 1971:142 [Tribe].
Brachycephalinae (part)—Dubois, 2005:4.
Brachycephalidae (part)—Frost et al., 2006.

Definition.—Frogs of the family Craugastoridae have: (1) sternum cartilaginous; (2) vertebral shield lacking; (3) transverse processes of posterior presacral vertebrae not broadly expanded; (4) cervical cotyles widely spaced; (5) eight presacral vertebrae, Presacrals I and II not fused; (6) cranial elements not co-ossified with overlying skin; (7) omosternum present; (8) sacral diapophyses rounded or barely dilated; (9) maxillary arch usually dentate; teeth blunt, pedicellate; (10) alary processes of premaxillae broad at base, usually directed dorsally or posterodorsally; (11) palatal shelf of premaxilla usually broad, indented or not; (12) pars facialis of maxilla usually deep, not exostosed; (13) palatal shelf of maxilla moderately broad, bearing pterygoid process or not; (14) maxillary arch complete; maxillae tapering posteriorly; quadratojugal slender; (15) nasals usually large with broad median contact; (16) nasals usually not in contact with maxillae or pterygoids; (17) nasals not in contact with frontoparietals; (18) frontoparietal

fontanelle usually absent; (19) frontoparietals usually not exostosed; cranial crests present or not; (20) frontoparietals fused with prootics or not; (21) temporal arcade absent; (22) epiotic eminences prominent to indistinct; (23) carotid artery passing dorsal to cranial elements; (24) zygomatic ramus of squamosal broad to slender, usually not in contact with maxilla; (25) otic ramus of squamosal short to elongate, expanded into otic plate or not; (26) squamosal-maxilla angle 44–67˚; (27) columella present; fenestra ovalis directed laterally; (28) vomers variable in size; (29) neopalatines broad; (30) sphenethmoid entire; (31) anterior ramus of parasphenoid narrow to broad, not keeled; (32) parasphenoid alae at right angle to axis of skull or deflected posteriorly, usually not overlapped by pterygoids; (33) pterygoid lacking ventral flange; anterior ramus not reaching neopalatine, except in some *Craugastor;* (34) occipital condyles small to large, stalked or not, widely separated medially; (35) mandible lacking odontoids; (36) terminal phalanges T-shaped; (37) three phalanges in Finger IV; (38) Toe I fully developed and free; (39) alary process of hyoid plate on slender stalk or not; (40) mandibular ramus of trigeminal nerve passing lateral to the *m. adductor mandibulae* ("S" condition) in *Haddadus,* passing medially ("E" condition) in *Craugastor;* (41) prominent external body glands absent; (42) males having single, median, subgular vocal sac or not (absent in some *Craugastor*); (43) males having vocal slits and nonspinous nuptial pads or not; (44) fingers unwebbed; toes usually unwebbed or webbed basally, but webbing extensive in some *Craugastor;* (45) terminal digits expanded with pads set off by distinct circumferential grooves; (46) inner and outer metatarsal tubercles present, inner tubercle not spade-like; (47) tympanic membrane and annulus usually well differentiated; (48) amplexus axillary*;* (49) eggs deposited in terrestrial or arboreal situations and undergoing direct development; (50) range in SVL from 18 mm in female *Craugastor pygmaeus* to 110 mm in female *Craugastor pelorus.*

Content.—The family contains two genera and 114 species.

Distribution.—This family is represented by one genus in southwestern USA, Mexico, Central America, and northwestern South America and another genus in southeastern Brazil.

Remarks.**—**This family joins two genera, *Craugastor* (the Middle American Clade) and a small genus of two Brazilian species (*Haddadus*), based primarily on their close relationship in our molecular phylogenies. Although their current ranges are widely separated, the divergence occurred 59–31 million years ago (Ma) (Heinicke et al. 2007), prior to the major Andean uplift and when the landscape was much different.

A long first finger (longer than Finger II) is uncommon in Terrarana, and this condition apparently is a shared derived trait of Craugastoridae. It is present in both species of *Haddadus* from Brazil and in most species of *Craugastor*. In contrast, it is present in only three species in the subgenus *Pelorius* of the large family Eleutherodactylidae. Within Brachycephalidae, a long first finger exists in only two species of *Ischnocnema* (*ramagii* series). Some earlier accounts (Lynch 1976a; Lynch and Duellman 1997) reported a long first finger for the "*Eleutherodactylus guentheri* Group" (= *Ischnocnema*), but Heyer (1984) noted that Finger I and II were subequal in many species, with "*E*". *binotatus* and "*E.*" *plicifer* being exceptions in having distinctly

long first fingers. In the large South American clade, the first finger is longer than the second in only 30–40 or so additional species in the former *conspicillatus, discoidalis, dolops, nigrovittatus,* and *sulcatus* groups of "*Eleutherodactylus*" (Lynch and Duellman 1997), which are apportioned into several genera below.

Genus *Craugastor* Cope, 1862

Craugastor Cope, 1862:153. Type species: *Hylodes fitzingeri* Schmidt, 1857:12, by subsequent designation by Dunn and Dunn, 1940:71.

Leiyla Keferstein, 1868:296. Type species *Leiyla güntherii* Keferstein, 1868:296, by monotypy. Synonymy with *Hylodes* (*sensu lato*) by Boulenger (1882:198), and with *Eleutherodactylus* by Savage (1974:290).

Microbatrachylus Taylor, 1940a:499. Type species *Eleutherodactylus hobartsmithi* Taylor, 1936, by monotypy. Synonymy by Lynch (1965:8).

Definition.—Frogs of the genus *Craugastor* are characterized by (1) head narrower than, or as wide as, body, width 31–55% of SVL; (2) tympanic membrane usually differentiated and sexually dimorphic (larger in males), although status of sexual dimorphism not determined in the subgenus *Hylactophryne* (see below); (3) cranial crests absent, except in *C. gulosus* Species Series; (4) dentigerous processes present, triangular or transverse, reduced or absent in the *C. mexicanus* Species Series; (5) "E" condition of adductor muscle; (6) digital discs narrow (with pointed discs on some toes in *C. laticeps* species series) to expanded and truncate; circumferential grooves present; terminal phalanges T-shaped; (7) Finger I longer than Finger II, except I = II or I < II in some members of the subgenus *Hylactophryne* and the *C. fitzingeri, C. mexicanus* and *C. rhodopis* Species Series; (8) Toe III longer than Toe V, except Toe V longer than Toe III in some species of the subgenus *Hylactophryne*; (9) subarticular tubercles projecting or not; (10) dorsum smooth to tuberculate; (11) venter smooth, granular, or areolate; (12) range in SVL 18 mm in female *C. pygmaeus* to 110 mm in female *C. pelorus*.

Content.—The genus contains three subgenera and 112 species.

Distribution.—The genus ranges from southern Arizona and western Texas, USA, through Mexico and Central America into northwestern Colombia.

Etymology.—The generic name presumably is derived from the Greek *kreas,* meaning fleshy, and the Greek *gaster,* meaning stomach. The name has been used as masculine.

Remarks.—This major clade of terraranan frogs has been recognized since Lynch's (1986a) discovery of the different patterns of jaw musculature in "*Eleutherodactylus.*" Independent analyses of molecular data (Crawford and Smith 2005; Frost et al. 2006; Heinicke et al. 2007) showed *Craugastor*, for the most part, to be a well-supported clade. However, the most recent analysis (Heinicke et al. 2007) discovered that some South American species assigned to this genus, those in the *C. anomalus* and *C. bufoniformis* species groups, were misclassified; thus they were removed from *Craugastor* and placed in the genus *Limnophys* (now placed in *Strabomantis*). This further strengthens the geographic distinction of the genus *Craugastor*, which is almost entirely associated with Middle America. Only four species of *Craugastor* are known to occur in South America

and in all four cases they are also distributed in Central America and their South American distribution is confined to the extreme northwest part of the continent. See Remarks under *Yunganastes* concerning the position of species allied with *Yunganastes fraudator*.

Species series and group assignments within the assemblage of species recognized herein as the genus *Craugastor* have had a complicated history, perhaps more so than in any other currently recognized genus of terraranan frogs. For example, the groups recognized by Savage (1987) differ substantially from those recognized by Lynch (2000). These and more recent arrangements (Savage 2002) are partly at odds with the molecular phylogenetic evidence (Crawford and Smith 2005; Heinicke et al. 2007), although taxon sampling was an issue in previous molecular studies. The study by Crawford and Smith (2005) and Hedges et al. (2008a) each sampled approximately one-third of the species, although the species sampled were not all the same. Different genes were used and therefore the sequences from the earlier study could not be used here. In contrast to the considerable chromosome variation in the Caribbean Clade (genus *Eleutherodactylus*) (Bogart 1981; Bogart and Hedges 1995), the chromosomes of most species of *Craugastor* that have been sampled show relatively little variation in diploid number (18–24), with most being 20 or 22 (Savage 1987). Until more species of *Craugastor* are sampled, it will be difficult to assess the diagnostic value of chromosome variation.

There is one inconsistency between two previous molecular phylogenies of *Craugastor,* in which more than 10 species were included (Crawford and Smith 2005; Heinicke et al. 2007), that requires clarification. The sequence of *C. mexicanus* used by Heinicke et al. (2007) clustered with *C. rhodopis* (*C. rhodopis* Species Series) and not with the two other species of the *C. mexicanus* Species Series (*C. montanus* and *C. pygmaeus*). That sequence came from the study of Darst and Cannatella (2004) where it was identified as "*Eleutherodactylus rhodopis.*" However, the current identification of that sequence (AY326006) in GenBank is *C. mexicanus*. Based on the tree of Heinicke et al. (2007), the original identification as a member of the *C. rhodopis* Species Series would appear to be correct, and the voucher specimen (Museum of Natural History, University of Texas, Arlington) is likewise identified as *C. rhodopis*.

In the study by Crawford and Smith (2005), an unexpected result involved the close clustering of "*E. megacephalus*" (*C. gulosus* Species Series) and "*E. ranoides*" of the former "*C. rugulosus* Group" (= herein the *C. punctariolus* Species Series). The genetic distance separating them is smaller than the distance separating some samples of the same species elsewhere in their tree and is suggestive of two closely related species of the same group rather than two species series representing a total of 38 species. Those authors did not comment on this unusual result. Unfortunately Heinicke et al. (2007) did not include a representative of the *C. gulosus* Species Series, and they used different genes. We have examined the specimen of *C. megacephalus* (FMNH 257714) used by Crawford and Smith and have confirmed its identification. Moreover, we have now sequenced a different specimen of *C. megacephalus* and six species of the *C. punctariolus* Species Series (Hedges et al. 2008a). They all cluster very tightly in the tree, agreeing with the initial result of Crawford and Smith (2005). Based on this new tree with expanded taxonomic coverage, either the *C. gulosus* and *C. punctariolus* series are distinct and extremely closely related, or there is no justification for recognizing the two clades of species. Because we included only a single species of the *C. gulosus* Species

Series, we are unable to distinguish between these two possibilities and therefore we maintain the distinction of these two species series.

We recognize subgenera, species series, and species groups within the genus *Craugastor* based largely on our new molecular phylogeny (Hedges et al. 2008a), but also considering previous morphological and molecular studies. The previous sequence analyses all agree that species allied with *Craugastor milesi* represent a basal lineage within the genus, which we designated as *Campbellius*. A second subgenus, *Hylactophryne*, is recognized for the lineage of species consisting of the former "*Eleutherodactylus alfredi*" and "*E. augusti*" species groups of Lynch (2000), which is the next most basal lineage in the genus. The remaining species are placed in the subgenus *Craugastor*.

Subgenus *Campbellius* Hedges, Duellman, and Heinicke, 2008a

Campbellius Hedges, Duellman, and Heinicke, 2008a:33. Type species: *Eleutherodactylus stadelmani* Schmidt, 1936:44, by original designation.

Definition.—Frogs of the subgenus *Campbellius* are characterized by (1) head moderate to wide, width 37–49% of SVL; (2) tympanic membrane undifferentiated in females, differentiated or not in males; (3) cranial crests absent; (4) dentigerous processes present, triangular or transverse; (5) "E" condition of adductor muscle; (6) digital discs expanded; circumferential grooves present; terminal phalanges T-shaped; (7) Finger I longer than Finger II; (8) Toe III longer than Toe V; (9) subarticular tubercles not projecting; (10) dorsum rugose and tuberculate; (11) venter smooth to slightly areolate; (12) range in SVL from 22 mm in *C. saltuarius* (males only) to 65 mm in female *C. daryi*.

In addition, species in this subgenus have a robust body, a rounded snout in dorsal view, and vocal slits (except in *C. omoaensis*). An inner tarsal fold is absent, and the toes are moderately webbed, with distinct lateral fringes or keels. Most species have pale paracloacal bars. Members of this subgenus are riparian and retreat under water when disturbed.

Content.—This subgenus includes 13 species: *Craugastor* (*Campbellius*) *adamastes, chrysozetetes, cruzi, daryi, epochthidius, fecudus, matudai, miles, myllomyllon, omoaensis, salutarius, stadelmani,* and *trachydermus*.

Distribution.—The subgenus is distributed in montane cloud forests at elevations of 150–2000 m in Guatemala and Honduras.

Etymology.—This genus group name honors Jonathan A. Campbell, University of Texas (Arlington), in recognition of his many significant contributions to the herpetology of Middle America.

Remarks.—This subgenus corresponds to the former "*Eleutherodactylus milesi*" Species Group (McCranie et al. 1989; Campbell 1994; Lynch 2000; McCranie and Wilson 2002). As with the *Craugastor punctariolus* Species Series, these frogs are largely riparian in habits. The molecular phylogenetic evidence suggests that this subgenus is the most basal lineage in the genus (Crawford and Smith 2005; Heinicke et al. 2007). We refrain from

recognizing species series or species groups because no phylogenetic analysis has been made of these species and there has been no proposal for subdivisions within the former "*Eleutherodactylus milesi*" Species Group. Crawford and Smith (2005) included two species (*C. daryi* and *C. trachydermus*) and this study and Heinicke et al. (2007) included one species (*C. daryi*) in their molecular phylogenetic analyses. Many species of these streamside frogs have disappeared from their habitats and some may be extinct (McCranie and Wilson 2002).

Subgenus *Craugastor* Cope, 1862

Craugastor Cope, 1862:153. Type species: *Hylodes fitzingeri* Schmidt, 1857:12, by subsequent designation by Dunn and Dunn, 1940:71.

Leiyla Keferstein, 1868:296. Type species *Leiyla güntherii* Keferstein, 1868:296, by monotypy. Synonymy with *Hylodes* (*sensu lato*) by Boulenger (1882:198), and with *Eleutherodactylus* by Savage (1974:290).

Microbatrachylus Taylor, 1940a:499. Type species *Eleutherodactylus hobartsmithi* Taylor, 1936, by monotypy. Synonymy by Lynch (1965:8).

Definition.—Frogs of the subgenus *Craugastor* are characterized by (1) head narrower than, or as wide as, body, width 31–55% of SVL; (2) tympanic membrane differentiated and sexually dimorphic (larger in males); (3) cranial crests absent, except in *C. gulosus* Species Series; (4) dentigerous processes present, triangular or transverse; reduced or absent in the *C. mexicanus* Species Series; (5) "E" condition of adductor muscle; (6) terminal discs narrow (with pointed discs on some toes in *C. laticeps* species series) to expanded and truncate; circumferential grooves present; terminal phalanges T-shaped; (7) Finger I longer than Finger II, except I equals II or I is shorter than II in some members of *C. fitzingeri, C. mexicanus* and *C. rhodopis* Species Series; (8) Toe III longer than Toe V; (9) subarticular tubercles not projecting, except in some members of *C. laticeps*, *C. mexicanus* and *C. rhodopis* Species Series; (10) dorsum smooth to tuberculate; (11) venter smooth, granular, or areolate; (12) range in SVL from 18 mm in female *C. pygmaeus* to 110 mm in female *C. pelorus*.

Content.—The subgenus contains 78 species placed in six species series.

Distribution.—The subgenus ranges from Mexico through Central America into northwestern Colombia.

Etymology.—As for the genus.

Remarks.—This subgenus contains all of the species of the genus *Craugastor* that are not included in the smaller subgenera *Campbellius* and *Hylactophryne*. Molecular phylogenies (Crawford and Smith 2005; Heinicke et al. 2007; Hedges et al. 2008) indicate that this subgenus is monophyletic. See Remarks for the genus *Craugastor* for discussion of the major divisions within the genus and recent taxonomic changes.

Craugastor (Craugastor) fitzingeri Species Series

Definition.—Species in this series are slender in body shape (long-limbed) and moderate to large in SVL, ranging from 31 mm (females, *C. monnichorum*) to 86 mm (females, *C. andi*). They have narrow to moderately wide heads (width 34–44% SVL) lacking cranial crests; the dorsum is smooth or slightly shagreen or tuberculate with low folds, and the venter is smooth or granular. The snout is subacuminate in dorsal view. The tympanic membrane is differentiated or not; vocal slits are present; nuptial pads are usually present. Finger I is longer or shorter than Finger II and the digits, especially on Fingers III and IV, have moderate to large digital discs; an inner tarsal fold is usually present, and plantar tubercles are absent. The toes are slightly to extensively webbed. Coloration is variable, but most have dark bars on the hind limbs. Most species are arboreal.

Content.—The species series (14 species) includes two species groups: the *Craugastor (Craugastor) fitzingeri* and *melanostictus* species groups.

Distribution.—The species series is distributed from northeastern Honduras southeastward through Nicaragua, Costa Rica, and Panama to northwestern Colombia.

Remarks.—This is one of the most studied assemblages of terraranan frogs. Previous versions, referred to as the *fitzingeri* group or series (Lynch 1976a; Lynch and Myers 1983; Lynch 1986a; Miyamoto 1986; Savage 1987; Lynch and Duellman 1997), bear almost no resemblance to the species series recognized here; some former members now reside in different genera. Savage et al. (2004) reviewed the complex taxonomic history of this group, and it is not repeated here. The content of the species series recognized here corresponds most closely to that recognized by Savage et al. (2004). Nonetheless, the relationships determined by Hedges et al. (2008a) do not agree with earlier phylogenetic analyses, including that most recent one.

Crawford and Smith (2005) analyzed five of the 13 species in their molecular phylogenetic study, although none of the seven montane species (distributions above 1000 meters) previously associated with this series (*Craugastor andi, cuaquero, emcelae, melanostictus, monnichorum, phasma, and rayo*) was included. Our molecular phylogeny (Hedges et al. 2008a) includes three of the montane species (*C. andi, emcelae*, and *melanostictus*) and three of the predominantly lowland species (*C. crassidigitus*, *fitzingeri*, and *longirostris*). Remarkably, these two groups of species form two clades in our phylogeny, even though previous analyses did not reveal such a dichotomy. An association of montane species appeared in the analysis of Savage et al. (2004), including the three in our tree, but their montane clade excluded a montane species (*C. phasma*). Their lowland species did not form a group (Savage et al. 2004).

Besides altitudinal differences, the three lowland species in our tree also have wider distributions that extend into eastern Panama and South America whereas the montane species are restricted to Costa Rica and western Panama. In morphology, the lowland species have a dorsal texture that is slightly rugose or tuberculate whereas the montane species have a mostly smooth dorsum. Also, the lowland species have moderate to extensive toe webbing (none or basal webbing in the montane species, except moderate in *C. andi*) and an unpatterned venter (marked with gray or black pigment in the montane species). These same distributional and morphological characters also hold for most of the other lowland and montane species in the *C. fitzingeri* Species Series that were not

included in our molecular phylogeny. In the West Indies, where species relationships are best known (see below), closely related species also tend to occur at similar elevations, resulting in clades of upland species and clades of lowland species. Considering all of the evidence, we recognize these two divisions within the *C. fitzingeri* Species Series as the *C. fitzingeri* Species Group and the *C. melanostictus* Species Group. Although by necessity, the names of these species groups have appeared in past literature, they should not be confused with earlier versions of these groups because their content is different.

Craugastor (Craugastor) fitzingeri Species Group

Definition.—Species in this group are slender in body shape (long-limbed) and moderate to large in SVL, ranging from 48 mm (females, *C. crassidigitus*) to 74 mm (females, *C. raniformis*). They have narrow to moderately wide heads (width 34–41% SVL) lacking cranial crests; the dorsum is finely or moderately tuberculate and rugose, and the venter is smooth (granular in *C. tabasarae*). The snout is subacuminate in dorsal view. The tympanic membrane is differentiated in both sexes; vocal slits and nuptial pads are present, except in *C. tabasarae*. Finger I is longer or shorter than Finger II, and the digits, especially on Fingers III and IV, have moderately enlarged discs. An inner tarsal fold is present or not; plantar tubercles are absent, and the toes are moderately to extensively webbed. Coloration is variable, but most are brown dorsally and have unmarked white or yellow venter. Most species are arboreal; known calls have been described as a series of chirps, mews, or clacks.

Content.—The group includes seven species: *Craugastor* (*Craugastor*) *chingopetaca, crassidigitus, fitzingeri, longirostris, raniformis, tabasarae*, and *talamancae.*

Distribution.—The species group is distributed at elevations of 0–2000 m from northeastern Honduras southeastward through Nicaragua, Costa Rica, Panama, northwestern Colombia, to southwestern Ecuador.

Remarks.—See Remarks for the *Craugastor fitzingeri* Species Series for a discussion of the taxonomic history of this assemblage and justification for erecting this species group, which should not be confused with groups of the same name used in earlier literature. All of the included species in this group occur at or near sea level, except for *C. tabasarae* (600–800 m). In contrast, all species in the *C. melanostictus* Species Group occur above 910 m elevation. Savage et al. (2004) associated *C. tabasarae* with montane species, although Crawford and Smith (2005) found it to be the closest relative of *C. longirostris*, a lowland species. Thus the group allocation of this species is uncertain.

Craugastor (Craugastor) melanostictus Species Group

Definition.—Species in this group are slender in body shape (long-limbed) and moderate to large in SVL, ranging from 31 mm (females, *C. monnichorum*) to 86 mm (females, *C. andi*). They have moderate to wide heads (width 36–44% SVL) lacking cranial crests; the dorsum is smooth or slightly shagreen or rugose, and the venter is smooth (granular in *C. melanostictus*). The snout is subacuminate in dorsal view. The tympanic membrane is

differentiated or not; vocal slits and nuptial pads are present. Finger I is longer or shorter than Finger II, and the digits, especially on Fingers III and IV, have moderate to large digital discs. An inner tarsal fold is present (absent in *C. melanostictus*); plantar tubercles are absent, and the toes are slightly webbed (moderately webbed in *C. andi* and *rayo*). Coloration is variable, but most or all have a heavily marked or mottled (with black) venter. Most species are arboreal; calls are mostly unknown, although the call of *C. andi* is described as a "deep guttural glug".

Content.—The species group includes seven species: *Craugastor* (*Craugastor*) *andi, cuaquero, emcelae, melanostictus, monnichorum, phasma, and rayo*.

Distribution.—The species group is distributed at elevations of 900–2700 m in montane Costa Rica and montane western Panama.

Remarks.—See Remarks for the *Craugastor fitzingeri* Species Series for a discussion of the taxonomic history of this assemblage and justification for erecting this species group. We include the montane species (1850 m elevation) *C. phasma* in this group, although Savage et al. (2004) did not include it in their montane clade because they considered it to be most closely related to *C. talamancae*, a lowland species. We include *C. phasma* in our *C. melanostictus* Species Group because it agrees in other ways with species in this group, as in the possession basal toe webbing (not moderate or extensive) and dark pigment on the venter.

Craugastor (Craugastor) laticeps Species Series

Definition.—Species in this series are mostly slender with long legs but *Craugastor laticeps* is robust) and range in SVL from 34 mm (females, *C. coffeus*) to 80 mm (females, *C. laticeps*). They have moderate to wide heads (width 34–49% SVL) lacking cranial crests. The dorsum is weakly granular, commonly with one or more series of distinct tubercles, including a postorbital, supratympanic, two or more paravertebrals, and two suprascapular tubercles on each side; some have a suprascapular fold across the back, and the venter is smooth. The eighth presacral and sacral vertebrae are fused. The snout is acuminate in dorsal view, but subacuminate in *C. laticeps*. The tympanic membrane is differentiated in both sexes; vocal slits and nuptial pads are absent. The digital discs are small to moderate in size; some are pointed apically. An inner tarsal fold is present; plantar tubercles are present or absent, and the toes are slightly to moderately webbed. Coloration is variable, but most have an hourglass or X-shaped middorsal blotch with smaller lateral blotches and usually a distinct dark facemask (solid or blotched) extending onto the anterior flank and bordered above by a narrow pale line. Most species are terrestrial and usually found on the forest floor, often by day. Vocalization is unknown.

Content.—The species series includes nine species: *Craugastor* (*Craugastor*) *chac, coffeus, gollmeri, greggi, laticeps, lineatus, mimus, noblei,* and *rostralis*.

Distribution.—The species series is distributed throughout Middle America, from southern Mexico to Panama.

Remarks.—This species series corresponds mostly to the "*Eleutherodactylus gollmeri*" Species Group (Savage 1987; Lynch 2000; McCranie and Wilson 2002; Savage 2002; Crawford and Smith 2005), although we use the earlier name "*laticeps*." These frogs represent a radiation of moderate to large species that inhabit the forest floor and leaf litter. The fusion of the eighth presacral and sacral vertebrae is a defining character (Lynch 2000), except that it also is present in *C. daryi*, which was shown by Crawford and Smith (2005) not to be in their "*E. gollmeri* Species Group." Although Lynch (2000) placed "*E. greggi*" in the "*E. gollmeri* Species Group," he noted that it differed in several ways from other members of the group. Crawford and Smith (2005) examined six of the nine species in this series.

Craugastor (*Craugastor*) *gulosus* Species Series

Definition.—Species in this series are robust and range from 44 mm (females, *C. aphanus*) to 103 mm (females, *C. gulosus*). They have relatively wide heads, width 39–55% SVL, with paired frontoparietal crests visible externally as cranial crests in adults of most species; the venter is smooth. In dorsal view the snout is rounded; the tympanic membrane is differentiated in both sexes, and males lack vocal slits and nuptial pads. The digital discs are small; an inner tarsal fold and plantar tubercles are absent, and the toes are not webbed. Coloration is variable, but most have dark reticulations enclosing yellow, orange, or red spots on the belly and ventral surfaces of the limbs. These species are terrestrial and inhabit leaf litter on the forest floor. Vocalizations are unknown.

Content.—The species series includes five species: *Craugastor* (*Craugastor*) *aphanus, C. gulosus, C. megacephalus, C. opimus,* and *C. rugosus*.

Distribution.—The species series is distributed from eastern Guatemala through Honduras, Nicaragua, Costa Rica, and Panama to northwestern Colombia.

Remarks.—This species series was reviewed recently, as the "*Eleutherodactylus biporcatus* Species Group," by Savage and Myers (2002), who retained the Venezuelan species "*Eleutherodactylus biporcatus*" in this largely Middle American group while recognizing that it differs substantially in structure and chromosomes from other members. We have placed "*E.*" *biporcatus* in the genus *Strabomantis* (see below) and recognize the remaining assemblage as the *Craugastor* (*Craugastor*) *gulosus* Species Series. See Remarks for the genus *Craugastor* (above) concerning the close relationship between *C. ranoides* (*C. punctariolus* Species Series) and *C. megacephalus* (*C. gulosus* Species Series) found by Crawford and Smith (2005). Crawford and Smith's (2005) analysis included one (*C. megacephalus*) of the five species in this series.

Craugastor (Craugastor) mexicanus Species Series

Definition.—Species in this series are robust with short legs, and range in SVL from 18 mm (females, *C. pygmaeus*) to 44 mm (females, *C. saltator*). They have narrow to moderately wide heads, width 38–45% SVL. The dorsum is smooth to tuberculate, and

the venter is smooth or areolate; in most species the dentigerous processes of the vomers are small, concealed, or absent, but they are well developed in *C. occidentalis*. In dorsal view the snout is rounded to subacuminate; vocal slits and nuptial pads are absent. Finger I is slightly shorter than, equal to, or longer than Finger II. The digital discs are small; an inner tarsal fold is absent. Plantar tubercles are absent or barely evident, and the toes are not webbed. Coloration is variable, but most are uniform brown or have a poorly developed pattern. Most species are active on the forest floor. The call has been described as "faint" (*C. occidentalis*).

Content.—The species series includes seven species: *Craugastor* (*Craugastor*) *hobartsmithi, mexicanus, montanus, occidentalis, omiltemanus, pygmaeus,* and *saltator*.

Distribution.—The species series is distributed primarily in western and southern Mexico, with one species extending into Guatemala.

Remarks.—This species series and the next are remnants of the former "*Eleutherodactylus rhodopis"* and "*E. omiltemanus*" species groups (Lynch 2000); he reviewed the complicated and confusing history of these groups and the relationships of species associated with "*E. bransfordii,*" "*E. mexicanus*," and "*E. rhodopis*" and resolved some nomenclatural problems. Crawford and Smith (2005) included 13 of the 18 species in the two series (as recognized here) and presented molecular phylogenetic evidence for a different arrangement of species to groups that bore little resemblance to former arrangements. They identified three clades of species: a northern clade (their "*E. rhodopis* Species Group") composed of "*E. rhodopis*" and "*E. loki*," a southern clade (their "*E. bransfordii* Species Group") composed of six described and two undescribed species, and another northern clade ("*E. mexicanus* Species Group") composed of five described and one undescribed species. The first two clades were found to be closest relatives whereas the relationship of that pair to the third clade and other species groups was unresolved.

We recognize the first and second clades as the *Craugastor rhodopis* and *C. podiciferus* species groups and place them in the *C. rhodopis* Species Series. We recognize the third clade as the *C. mexicanus* Species Series. Although Crawford and Smith (2005) did not define these three newly discovered clades morphologically, or allocate species to them that were not included in their study, we have attempted to do so here. The two series seem to differ in condition of the vomerine teeth—small, concealed, or absent (*C. mexicanus* Species Series) versus large and prominent (*C. rhodopis* Species Series). The two species groups of the *C. rhodopis* Species Series have structural and coloration differences (see below). Lynch (2000) mentioned black mesorchia (mesentery around testes) as a character of possible diagnostic value, but apparently it is not found in all of the species of the group referred herein to the *C. mexicanus* Species Series. The taxon "*Eleutherodactylus saltator*" was placed in the synonymy of "*E. mexicanus*" by Lynch (2000) but the sample of that species used by Crawford and Smith (2005) clustered with another species ("*E. pygmaeus*"), therefore supporting recognition of *C. saltator* as a valid species, assuming the identifications are correct. The *C. mexicanus* Species Series, as defined here, appears to represent the basal clade within the subgenus *Craugastor*.

Craugastor (Craugastor) rhodopis Species Series

Definition.—Species in this series are robust with short legs and range from 22 mm (females, *C. lauraster* and *C. stejnegerianus*) to 46 mm (females, *C. rhodopis*). They have narrow to moderately wide heads, width 31–44% SVL; the dorsum is rugose and tuberculate, and the venter is smooth or areolate. The dentigerous processes of the vomers are large and prominent. The snout is rounded to subacuminate in dorsal view. Vocal slits are absent (present in *C. bransfordii,* and *C. podiciferus*), and nuptial pads are absent (present in *C. bransfordii, C. underwoodi,* and in the *C. rhodopis* Species Group). The digital discs are small. An inner tarsal fold is absent or weakly developed; plantar tubercles are absent or inconspicuous, and the toes are not webbed. Coloration is variable (see below). Most species are terrestrial and usually are found on the forest floor during the day. Known vocalizations have been described as a chirp, squeak, or trill.

Content.—The species series includes two species groups (10 species): the *Craugastor* (*Craugastor*) *podiciferus* and *rhodopis* species groups.

Distribution.—The species series is distributed from eastern and southern Mexico to western Panama.

Remarks.—This species series contains remnants of the previous "*Eleutherodactylus rhodopis* Species Group" or Series (Lynch 2000; Savage 2002) as discussed in the Remarks section of the *Craugastor mexicanus* Species Series (see above).

Craugastor (Craugastor) podiciferus Species Group

Definition.—Species in this series are robust with short legs and range in body shape and SVL from 22 mm (females, *C. lauraster* and *C. stejnegerianus*) to 40 mm (females, *C. podiciferus*). They have narrow to moderately wide heads, width 31–44% SVL. The dorsum is rugose and tuberculate, and the venter is areolate. The snout is rounded to subacuminate in dorsal view; vocal slits are absent (present in *C. bransfordii,* and *C. podiciferus*), and nuptial pads are absent (present in *C. bransfordii* and *C. underwoodi*). Finger I is shorter than, or equal to, Finger II, and the digits have small discs. An inner tarsal fold is absent or inconspicuous; plantar tubercles are absent or not evident, and the toes are not webbed. Coloration is variable, but most are uniform or mottled brown with a yellow venter. Most species are active on the forest floor. Known vocalizations have been described as a chirp, squeak, or trill.

Content.—The species group includes eight species: *Craugastor* (*Craugastor*) *bransfordii, jota, lauraster, persimilis, podiciferus, polyptychus, stejnegerianus,* and *underwoodi*.

Distribution.—The species group is distributed from eastern Honduras to western Panama.

Remarks.—This species group contains remnants of the previous "*Eleutherodactylus rhodopis* Species Group" or Series (Lynch 2000; Savage 2002), and corresponds to the "*E. bransfordii* Species Group" of Crawford and Smith (2005), as discussed in the Remarks section of the *Craugastor mexicanus* Species Series (see above). Savage (2002) resurrected "*E. polyptychus*" from the synonymy of "*E. bransfordii*."

This species group is the southern counterpart of the *C. rhodopis* Species Group. Besides geography and DNA sequences, these two groups can be distinguished by ventral skin texture (weakly areolate or areolate versus smooth in the *C. rhodopis* Species Group), relative finger length (Finger I shorter than, or equal to, Finger II versus Finger I slightly longer than Finger II), and ventral ground coloration (yellow versus white). Also, with the exception of one large member of the southern group, *C. podiciferus* (40 mm SVL), the two groups are distinguished by body size (22–30 mm versus 38–46 mm in the *C. rhodopis* Species Group).

Craugastor (Craugastor) rhodopis Species Group

Definition.—Members of this group have narrow to moderately wide heads, width ±36% SVL. The dorsum is rugose and tuberculate, and the venter is smooth. The snout is subacuminate in dorsal view. Vocal slits are absent, but nuptial pads are present. Finger I is slightly longer than Finger II; the digits have small discs. An inner tarsal tubercle or vague fold is present; plantar tubercles are absent or inconspicuous, and the toes not webbed. Dorsally, the color pattern is complex and polymorphic; venter is white with dark flecks. Most species are terrestrial and usually found on the forest floor by day. Vocalization is unknown.

Content.—The species group includes two species: *Craugastor* (*Craugastor*) *loki* and *rhodopis.*

Distribution.—The species group is distributed from eastern and southern Mexico, south through Guatemala, Belize, El Salvador, and Honduras.

Remarks.—This species group contains remnants of the previous "*Eleutherodactylus rhodopis* Species Group" or Series (Lynch 2000; Savage 2002), and corresponds to the "*E. rhodopis* Species Group" of Crawford and Smith (2005), as discussed in the Remarks section of the *Craugastor mexicanus* Species Series (see above). See also the Remarks section of the *C. podiciferus* Species Group for characters distinguishing that species group from this group. Although there are only two described species in this group, molecular data support the existence of several undescribed species, including some currently recognized as *C. rhodopis*.

Craugastor (Craugastor) punctariolus Species Series

Definition.—Species in this series are robust in body shape and large in SVL, ranging from 50 mm (females, *C. emleni*) to 110 mm (females, *C. pelorus*), although one species, *E. olanchano*, is known only from small (30 mm SVL) males. They have moderate to wide heads (width 32–47% SVL) lacking cranial crests; the dorsum is smooth to rugose

to strongly tuberculate, and the venter is smooth. The snout in dorsal view is rounded, subacuminate, or subelliptical; a tympanic membrane is differentiated in both sexes. Vocal slits present or absent; nuptial pads are present or absent. The digits have small to large digital discs; an inner tarsal fold is present, but plantar tubercles are absent. The toes are slightly to nearly fully webbed. In coloration, these frogs are variable, but most have scattered areas of body with rusty or reddish color, a series of alternating pale and dark lip bars, a pale interocular spot bordered by a dark interocular bar, and sometimes a narrow cream to red middorsal line. Most species are terrestrial and are found in riparian habitats; their calls are unknown or poorly known.

Content.—The species series includes 33 species: *Craugastor* (*Craugastor*) *amniscola, anciano, angelicus, aurilegulus, azueroensis, berkenbuschii, brocchi, catalinae, charadra, emleni, escoces, fleischmanni, inachus, laevissimus, merendonensis, obesus, olanchano, palenque, pechorum, pelorus, pozo, psephosypharus, punctariolus, ranoides, rhyacobatrachus, rivulus, rugulosus, rupinius, sabrinus, sandersoni, taurus, vocalis,* and *vulcani*.

Distribution.—The species series is distributed from Mexico to western Panama.

Remarks.—This species series represents a large radiation of large, robust, riparian species formerly called the "*E. rugulosus* Group" (the series name *punctariolus* is used here because it predates *rugulosus*). Campbell and Savage (2000) divided the species into four clusters based on presence and absence of vocal slits and nuptial pads. However, they emphasized that those clusters probably do not correspond to evolutionary groups, and therefore we do not recognize any species groups within this large series. Crawford and Smith (2005) included one of the 33 species in this series in their molecular phylogeny and Heinicke et al. (2007) included two species. An additional four species have been included by Hedges et al. (2008a). As mentioned in the Remarks on the genus, this series, as currently defined, appears to be polyphyletic with respect to at least some members of the *C. gulosus* Species Series.

As of June 2007 the only known extant member of this series is a population of *Craugastor ranoides* in Costa Rica. According to J. R. Mendelson (pers. comm.), *C. punctariolus* was common in the vicinity of El Valle, Panama in 2005; this population died out from chytrid fungus infections early in 2006, but some individuals remain in captivity at the El Valle Amphibian Conservation Center, Atlanta Botanical Garden, and Zoo Atlanta. The presumed near extinction of this clade likely has been caused by the chytrid fungus.

Subgenus *Hylactophryne* Lynch, 1968

Hylactophryne Lynch, 1968a:511. Type species: *Hylodes augusti* Dugés, *in* Brocchi, 1879:21. Synonymized with "*Eleutherodactylus*" by Lynch (1968:255).

Definition.—Frogs of the subgenus *Hylactophryne* are characterized by (1) head narrower than, or as wide as, body; head width, 33–45% SVL, although broad (49%) in *C. uno*; (2) tympanic membrane differentiated; status of sexual dimorphism not established (see below); (3) cranial crests absent; (4) dentigerous processes of vomers

triangular or transverse, reduced or absent in at least two species (*C. cyanochthebius* and *C. nefrens*); (5) "E" condition of adductor muscle; (6) digital discs large (especially on outer two fingers), truncate or notched; circumferential grooves present; terminal phalanges T-shaped; (7) Finger I shorter or longer than Finger II; (8) Toe V longer than Toe III (except in *C. augusti* and *C. tarahumaraensis*); (9) subarticular tubercles not projecting; (10) dorsum usually granular, rarely smooth or tuberculate; (11) venter smooth (granular in *C. batrachylus*); (12) range in SVL 16 mm in *C. galacticorhinus* (male only) to 95 mm in female *C. augusti*.

In addition, species in this subgenus are moderate in body shape, but robust in *C. augusti* and have a subacuminate snout in dorsal view. Vocal slits and nuptial pads are present or absent. An inner tarsal fold and plantar tubercles are present or not; the toes are barely webbed if at all. Coloration is variable, but in most species the dorsum is tan or brown with an olive or green hue, and the venter is white, grayish-white, or unpigmented. These arboreal frogs are commonly encountered in trees and bushes at night, although one species (*C. augusti*) is a rock and cliff-dweller. The calls are largely unrecorded; where known they have been described as a soft "peep (*C. galacticorhinus*), barking (*C. augusti*), and a low growl, a low chuckle, single clicks, and a multi-note laugh (*C. polymniae*).

Content.—This subgenus includes 21 species in the *Craugastor* (*Hylactophryne*) *augusti* and *bocourti* species series.

Distribution.—The subgenus is distributed from southern border regions of the United States (southeast Arizona, southern New Mexico, and central and southwest Texas) through most of central and southern Mexico and central Guatemala to northwestern Honduras.

Etymology.—The subgeneric name is derived from the Greek *hylacto,* meaning barking, and the Greek *phryne,* meaning toad, in allusion to the vocalization of *C. augusti*. The name is masculine in gender.

Remarks.—This subgenus combines the previous "*Eleutherodactylus augusti*" and "*E. alfredi*" species groups (Lynch 2000). These two groups were found to be closely related in two independent molecular phylogenies (Crawford and Smith 2005; Heinicke et al. 2007). We continue to recognize these two units as species series. Because *C. bocourti* is an older name than *C. alfredi*, we refer to the larger of these two series as the *C. bocourti* Species Series. In so doing, the several recognized subgroups within the former "*E. alfredi* Species Group," namely the *alfredi*, *decoratus*, and *spatulatus* subgroups (Lynch 1966; Lynch 1967b; Smith 2005), might be considered as species groups. We refrain from doing so here because many species have been added to this assemblage since those subdivisions were described and it is unclear where some of the new additions (e.g., *C. batrachylus* and *C. uno*) would fit. Also, molecular phylogenies (Crawford and Smith 2005; Heinicke et al. 2007; Hedges et al. 2008) have included only a few of these species.

Sexual dimorphism in tympanum size, with males having a larger tympanum than females, was considered a diagnostic character in *Craugastor* that defined a monophyletic assemblage including most species except those placed here in the

subgenus *Hylactophryne* (Lynch 2000), which includes mostly the former "*E. alfredi* Species Group." Crawford and Smith (2005) also associated the lack of sexual dimorphism in tympanum size as a characteristic of the "*E. alfredi* Species Group." However, Campbell et al. (1989), in discussing the "*E. alfredi* Species Group," stated that "the relative size of the tympanum is also usually sex dependent, being larger in males of most species." It is possible that the small number of specimens of most of the species in the group (some are known only from males) has contributed to this confusion.

Craugastor uno previously has been unassigned to series or group (Lynch 2000). Our molecular phylogeny clearly shows that it is a member of the *Craugastor* (*Hylactophryne*) *bocourti* Series (Hedges et al. 2008a). Relevant characters are its large and truncate digital discs and distribution. We also include the species *Craugastor batrachylus* in this subgenus, although we have not sampled it. Previously, it was placed in the predominantly South American "*Eleutherodactylus unistrigatus* Species Group" in part because of its granular venter and long fifth toe (Lynch and Duellman 1997). However, its northern distribution (Mexico) is at odds with that assignment, and members of the *Craugastor* subgenus *Hylactophryne* typically have a long fifth toe.

Craugastor (Hylactophryne) augusti Species Series

Definition.—Species in this series are robust and range in SVL from 43 mm (females, *C. tarahumaraensis*) to 95 mm (females, *C. augusti*). They have moderately wide heads, width 41–45% SVL, lacking cranial crests. The dorsum is granular, and the venter is smooth. The snout is subacuminate in dorsal view, and the tympanic membrane is differentiated in both sexes. Vocal slits are present; nuptial pads are absent. The digital discs are small; Finger I is longer than Finger II, and Toe V is shorter than Toe III. An inner tarsal fold is absent, and plantar tubercles are present; the toes are unwebbed. Coloration is variable, but most have distinct dark spots or blotches on the body and bars on the limbs. Both species are rock and cliff-dwellers, often seen on exposed rocks. The call is a barking sound (*C. augusti*) or "quack" (*C. tarahumaraensis*).

Content.—The species series includes two species: *Craugastor* (*Hylactophryne*) *augusti* and *tarahumaraensis*.

Distribution.—The species series is distributed from the southern border regions of the United States (southeast Arizona, southern New Mexico, and central and southwest Texas) through most of central Mexico to the Isthmus of Tehuantepec.

Remarks.—This species series represents the former "*E. augusti* Species Group" (see Remarks above for subgenus *Hylactophryne*). Geographic variation in morphology, calls, and DNA sequences of the wide-ranging *C. augusti* (Zweifel 1956; Goldberg et al. 2004) suggest that it may be a complex of species.

Craugastor (Hylactophryne) bocourti Species Series

Definition.—Species in this series are moderate in body shape and range in SVL from 16 mm (males only, *C. galacticorhinus*) to 63 mm (females, *C. uno*). They have moderate to

wide heads, width 33–49% SVL, lacking cranial crests. The dorsum is smooth or granular; and the venter usually is smooth (areolate in *C. batrachylus, guerreroensis,* and *spatulatus*). The snout is subacuminate or truncate in dorsal view; the tympanic membrane is differentiated in both sexes. Vocal slits and nuptial pads are present or absent; the digital discs are large and truncate or notched. Finger I is shorter than Finger II, and Toe V is longer than Toe III. An inner tarsal fold is present, and plantar tubercles are present or absent; the toes are unwebbed or slightly webbed basally. Coloration is variable, but in most species the dorsum is tan or brown with an olive or green hue, and the venter is white, grayish-white, or unpigmented. These frogs are encountered in trees and bushes at night. The calls of most species have not been recorded; the known calls have been described as a soft "peep (*C. galacticorhinus*) or a low growl, a low chuckle, single clicks, and a multi-note laugh (*C. polymniae*).

Content.—The species series includes 19 species: *Craugastor* (*Hylactophryne*) *alfredi, batrachylus, bocourti, campbelli, cyanochthebius, decoratus, galacticorhinus, glaucus, guerreroensis, megalotympanum, nefrens, polymniae, silvicola, spatulatus, stuarti, taylori, uno, xucanebi, yucatanensis*.

Distribution.—The species series is distributed from east-central and west-central Mexico and the Yucatan Peninsula through southern Mexico and central Guatemala, to northwestern Honduras.

Remarks.—This species series represents the former "*E. alfredi* Species Group," but with the addition of several species (see Remarks above for subgenus *Hylactophryne*).

Genus *Haddadus,* Hedges, Duellman, and Heinicke, 2008a

Haddadus Hedges, Duellman, and Heinicke, 2008a:45. Type species: *Rana binotata* Spix, 1824:31, by original designation.

Definition.—This craugastorid genus is characterized by (1) head narrower than body; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous processes of vomers prominent; (5) "S" condition of adductor muscle; (6) small terminal discs on digits, bearing circumferential grooves; terminal phalanges narrow, T-shaped; (7) Finger I longer than Finger II; (8) Toe III equal in length or slightly shorter than Toe V; (9) subarticular tubercles not projecting; (10) dorsum smooth to granular with longitudinal ridges; (11) venter smooth to granular; (11) range in SVL 17 mm in only known specimen of *H. plicifer* to 64 mm in females of *H. binotatus*.

Content.—The genus contains two species, *Haddadus binotatus* and *Haddadus plicifer*.

Distribution.—The genus is distributed in Atlantic Coastal Forest in eastern and southern Brazil, from the state of Pernambuco south to the state of Rio Grande do Sul.

Etymology.—This genus is named for Célio F. B. Haddad, Universidade Estadual Paulista (UNESP), Brazil, in recognition of his contributions to the systematics of Brazilian amphibians.

Remarks.—Lynch (1968b) placed species of "*Eleutherodactylus*" from southern Brazil in four species groups (see additional discussion under *Ischnocnema*). He considered "*E.*" *binotatus* distinct enough from the other species to warrant its own species group defined primarily by a disproportionately long first finger and separate from the "*E.*" *guentheri* Species Group. Later, he placed less emphasis on the length of Finger I by combining the "*E.*" *binotatus* and "*E.*" *guentheri* species groups (Lynch 1976a). Heyer (1984) disagreed and removed "*E.*" *binotatus* from a core group of "*Eleutherodactylus*" in southeastern Brazil, which he called the "*Eleutherodactylus*" *guentheri* cluster, in effect renewing emphasis on finger length, although he did not attempt a classification of species other than those in the "*E.*" *guentheri* cluster. He pointed out that the relative lengths of Fingers I and II of species in that cluster was variable, with some having slightly longer and others slightly shorter first fingers. Frost et al. (2006) discussed the position of "*E.*" *binotatus*, but taxon sampling of terraranans was insufficient to draw any robust conclusions.

Heinicke et al. (2007) distinguished a Southeast Brazil Clade of "*Eleutherodactylus*" including "*E.*" *guentheri, hoehnei, juipoca*, and *parvus*, but not "*E.*" *binotatus*, which appeared elsewhere in the tree. They did not include "*E.*" *plicifer* or other species of the genus from southern Brazil. Since then we have added "*E.*" *holti*, which joins the Southeast Brazil Clade (Hedges et al. 2008a). In our evaluation of the Southeast Brazil Clade (*Ischnocnema*) and its content, we find that the character of relative finger length (I versus II) may be of diagnostic significance, with nearly all species in that Clade having Finger I shorter than, or equal to Finger II, except for the two species of the *Ischnocnema ramagii* Species Group, in which Finger I is longer than Finger II. Although it is possible that those two species should be transferred to *Haddadus*, they agree in other ways with *Ischnocnema* and therefore we place them in that latter genus (see above). We also note that the diploid chromosome count of *H. binotatus*, 2n=22 (Beçak and Beçak 1974), is a number commonly encountered among species of *Craugastor* (DeWeese 1976).

Family Eleutherodactylidae Lutz, 1954

Eleutherodactylinae (part) Lutz, 1954:157. Type genus *Eleutherodactylus* Duméril and Bibron, 1841:620.
Eleutherodactylini (part)—Lynch, 1971:142 [Tribe].
Brachycephalinae (part)—Dubois, 2005:4.
Brachycephalidae (part)—Frost et al., 2006.

Definition.—Frogs of the family Eleutherodactylidae have: (1) sternum cartilaginous; (2) vertebral shield lacking; (3) transverse processes of posterior presacral vertebrae not broadly expanded; (4) cervical cotyles widely spaced; (5) eight presacral vertebrae, Presacrals I and II not fused; (6) cranial elements not co-ossified with overlying skin; (7) omosternum present; (8) sacral diapophyses rounded or barely dilated; (9) maxillary arch usually dentate; teeth blunt, pedicellate; (10) alary processes of premaxillae broad at base, usually directed dorsally or posterodorsally; (11) palatal shelf of premaxilla usually broad, indented or not; (12) pars facialis of maxilla usually deep, not exostosed; (13) palatal shelf of maxilla moderately broad, bearing pterygoid process or not; (14) maxillary arch complete; maxillae tapering posteriorly; quadratojugal slender; (15) nasals

usually large with broad median contact; (16) nasals usually not in contact with maxillae or pterygoids; (17) nasals not in contact with frontoparietals; (18) frontoparietal fontanelle usually absent; (19) frontoparietals usually not exostosed; cranial crests present in some *Eleutherodactylus;* (20) frontoparietals fused with prootics or not; (21) temporal arcade absent; (22) epiotic eminences prominent to indistinct; (23) carotid artery passing dorsal to cranial elements; (24) zygomatic ramus of squamosal broad to slender, usually not in contact with maxilla; (25) otic ramus of squamosal short to elongate, expanded into otic plate or not; (26) squamosal-maxilla angle 44–67˚; (27) columella present, except in fenestra ovalis directed laterally; (28) vomers variable in size*;* dentigerous processes absent in *Eleutherodactylus* (*Syrrhophus*), and some diminutive species of *Eleutherodactylus* (*Euhyas*); (29) neopalatines usually broad; slender in *Eleutherodactylus* (*Syrrhophus*); (30) sphenethmoid usually entire, divided in some *Eleutherodactylus* (*Syrrhophus*); (31) anterior ramus of parasphenoid narrow to broad, not keeled; (32) parasphenoid alae at right angle to axis of skull or deflected posteriorly, usually not overlapped by pterygoids; (33) pterygoid lacking ventral flange; anterior ramus not reaching neopalatine; (34) occipital condyles small to large, stalked or not, widely separated medially; (35) mandible lacking odontoids; (36) terminal phalanges T-shaped; (37) usually three phalanges in Finger IV (two in some *Adelophryne*); (38) Toe I fully developed and free; (39) alary process of hyoid plate on slender stalk or not; (40) mandibular ramus of trigeminal nerve passing lateral to the *m. adductor mandibulae* (condition unknown in *Adelophryne*); (41) prominent external body glands usually absent; lumbar glands in some *Eleutherodactylus*; (42) males having single or paired subgular vocal sac, single pectoral vocal sac, or no vocal sac; (43) males having vocal slits (or not) and no nuptial pads; (44) fingers unwebbed; toes usually unwebbed or webbed basally, but webbing extensive in some *Eleutherodactylus;* (45) terminal digits usually expanded with pads set off by distinct circumferential grooves; digits apically pointed in *Adelophryne* and some *Diasporus;* grooves present only laterally in *Phyzelaphryne;* (46) inner and outer metatarsal tubercles present, inner tubercle not spade-like; (47) tympanic membrane and annulus well differentiated or not; (48) amplexus axillary*;* (49) eggs deposited in terrestrial or arboreal situations and undergoing direct development; ovoviviparity exists in at least *Eleutherodactylus* (*Eleutherodactylus*) *jasperi;* (50) range in SVL from 10.5 mm in female *Eleutherodactylus* (*Euhyas*) *iberia* to 88 mm in female *Eleutherodactylus* (*Pelorius*) *inoptatus.*

Content.—There are 201 species placed in four genera, and five subgenera.

Distribution.—The family is distributed throughout the West Indies, peninsular Florida (either native or introduced) and from southern Texas (USA) south to northwestern Ecuador; other genera are discontinuously distributed in northeastern South America and in the Amazon Basin.

Remarks.—Support for the family in molecular phylogenies (Hedges et al. 2008; Heinicke et al. 2009) is significant. Use and authorship of the family-group name needs to be clarified. The first name created for Eleutherodactylidae was Cornuferinae by Noble (1931). Based on the proposal by Zweifel (1966) the International Commission of Zoological Nomenclature changed the type species of the genus *Cornufer*, which

antedates *Eleutherodactylus*, and thus transferred Cornuferinae to Ranoidea. Taylor (1940b) used the name eleutherodactylid. However, Taylor did not explicitly use it as a family-group name; the name could have been validated under Article 11.7.2 of the Code of Zoological Nomenclature only if it had been published before 1900, Latinized later (e.g., by Lutz), and credited to Taylor by subsequent authors. Because the name was published in 1940 and was never credited to Taylor, it cannot be credited to him now.

Our recent molecular phylogeny (Hedges et al. 2008a) contain three taxa that were not present in any earlier study including our own (Heinicke et al. 2007), and they proved to be critical for defining this family. "*Eleutherodactylus diastema*" turned out to be a close relative of the Caribbean Clade (*Eleutherodactylus*), and therefore we placed most species of the former "*Eleutherodactylus diastema* Group" in a new genus, *Diasporus*, with the exception of two species identified as belonging to the genus *Pristimantis* (see below). Together, the *Diasporus* and *Eleutherodactylus* are placed in the subfamily Eleutherodactylinae. We also included representatives of *Adelophryne* and *Phyzelaphryne*. They form a closely related pair, which in turn is the closest relative of the subfamily Eleutherodactylinae.

Subfamily Eleutherodactylinae Lutz 1954

Eleutherodactylinae (part) Lutz, 1954:157. Type genus *Eleutherodactylus* Duméril and Bibron, 1841:620.

Definition.—These are eleutherodactylid frogs that have expanded terminal digits on the fingers and toes; the discs are rounded or truncate (apically pointed in some members of *Diasporus*), and the circumferential grooves are complete; Finger IV always has three phalanges. The species are terrestrial or arboreal (some *Pelorius* are fossorial) and range in size from 10.5 mm in female *Eleutherodactylus* (*Euhyas*) *iberia* to 88 mm in female *Eleutherodactylus* (*Pelorius*) *inoptatus.*

Content.—The 194 currently recognized species are placed in two genera—*Diasporus* and *Eleutherodactylus,* the latter with five subgenera.

Distribution.—The subfamily is distributed throughout the West Indies, peninsular Florida (either native or introduced) and from southern Texas (USA) south to northwestern Ecuador.

Remarks.—With the exception of the inclusion of *Diasporus*, this subfamily is equivalent to the genus *Eleutherodactylus* in the sense of Heinicke et al. (2007).

Genus *Diasporus* Hedges, Duellman, and Heinicke, 2008a

Diasporus Hedges, Duellman, and Heinicke, 2008a:49. Type species: *Lithodytes diastema* Cope, 1876:155, by original designation.

Definition.—Frogs of the genus *Diasporus* are characterized by: (1) head distinct from body; head width 32–41% SVL; (2) tympanic membrane usually differentiated; membrane not differentiated but annulus visible beneath skin in *Diasporus gularis;* (3) cranial crests absent; (4) dentigerous processes of vomer usually prominent (absent in *E.*

hylaeformis); (5) "S" condition of adductor musculature (contra Starrett 1968); (6) digital discs expanded with or without lanceolate or papillate tips; circumferential grooves present; terminal phalanges T-shaped; (7) Finger I shorter than Finger II; (8) Toe V much longer than Toe III; (9) subarticular tubercles not projecting; (10) dorsum smooth to rugose; (11) venter coarsely areolate; (12) range in SVL from 10.9 mm in male *E. quidditus* to 26 mm in female *E. hylaeformis.* Additionally, the left lobe of the liver is long and pointed whereas the right lobe is smaller and rounded (liver shape examined in *D. diastema*, *hylaeformis*, and *vocator.*

Content.—Nine species—*Diasporus anthrax, diastema, gularis, hylaeformis, quidditus, tigrillo, tinker, ventrimaculatus,* and *vocator*—are assigned to this genus.

Distribution.—These frogs inhabit humid lowland and montane forests from eastern Honduras through Panama to the Pacific versant of Colombia and northwestern Ecuador.

Etymology.—The generic name is from the Greek *diaspora* (a dispersion from). The gender is masculine. It is used here in allusion to the close relationship of this mainland group to the Caribbean Clade, inferring an ancient dispersal event.

Remarks.—Hedges et al. (2008a) showed that *Diasporus diastema* is the closest relative of the Caribbean clade (= *Eleutherodactylus*), whereas *Pristimantis chalceus*, previously associated with *D. diastema* in the "*Eleutherodactylus diastema* Group" (Lynch 2001), is deeply imbedded in the South American clade containing *Pristimantis*, and most closely related to species in the subgenus *Pristimantis*. This led us to erect a genus for *diastema* while retaining *chalceus* in *Pristimantis*. However, we were faced with allocating the remaining species of the former "*E. diastema* Group" to these two genera. Fortunately, some morphological characters provided the needed guidance. Members of the genus *Diasporus* have oval palmar tubercles and prominent vomerine teeth; also they are like some West Indian *Eleutherodactylus* by having)(-shaped gular folds. Lynch (2001) noted that: "Such folds also are found in several small species from Cuba and Hispaniola, which caused Dunn (1926) to posit a relationship between the Middle American and some Caribbean taxa." However, Lynch (2001) noted that "*E. chalceus*" and "*E. scolodiscus*" differed from the others by having bifid palmar tubercles, weakly developed vomerine teeth, and by not having)(-shaped gular folds. We recognize these two species as members of the *Pristimantis* (*Pristimantis*) *chalceus* Species Group.

Genus *Eleutherodactylus* Duméril and Bibron, 1838

Cornufer Tschudi, 1838:28. Type species: *Cornufer unicolor* Tschudi, 1838:28, by monotypy. Synonymy by Zweifel, 1966:23.

Eleutherodactylus Duméril and Bibron, 1841:620. Type species: *Hylodes martinicensis* Tschudi, 1838:37, by monotypy. Official list of generic names, 1978.

Definition.—Members of the genus *Eleutherodactylus* can be defined as eleutherodactylid frogs having: (1) head narrow or moderate; (2) tympanic membrane differentiated; (3) cranial crests absent (present in *E. inoptatus* Group of subgenus *Pelorius*); (4) dentigerous process of vomers present (absent in subgenus *Syrrhopus* and

several diminutive Cuban species of the subgenus *Euhyas*); (5) "S" condition of the adductor muscles; (6) terminal discs on digits present, bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I usually shorter than Finger II (about equal to Finger II in the subgenus *Syrrhophus* and longer than Finger II in some species of the subgenus *Pelorius*); (8) Relative length of Toe III and Toe V variable, but Toe V longer than Toe III in most species of subgenera *Eleutherodactylus* and *Pelorius* and Toe V shorter than Toe III in most species of the subgenera *Euhyas* and *Syrrhophus*; (9) subarticular tubercles prominent; (10) texture of skin on dorsum variable; (11) texture of skin on venter variable; (12) range in SVL from 11 mm in female *E. iberia* to 88 mm in female *E. inoptatus.*

Content.—One hundred and eighty-five species are placed in five subgenera.

Distribution.—The genus is distributed throughout the West Indies, peninsular Florida (either native or introduced) and southern Texas, USA, Mexico, Belize, and Guatemala.

Etymology.—The generic name is derived from the Greek *eleutheros,* meaning free, and the Greek *dactylos,* meaning finger or toes, in reference to the absence of webbing between the digits. The generic name is masculine in gender.

Remarks.— This was once the largest genus of vertebrates, but its content was restricted to a smaller Caribbean-centered clade of species in a recent molecular phylogenetic analysis (Heinicke et al. 2007). The four subgenera proposed in an earlier allozyme analysis (Hedges 1989a) were largely corroborated in that recent analysis, and therefore they are maintained here (the group recognized as the subgenus *Eleutherodactylus* here was considered the "*auriculatus* section" of the subgenus *Eleutherodactylus* in that earlier study). Frost et al. (2006) considered those subgenera to be distinct genera, but that was under a prior assumption of phylogenetic relationships unsupported by the analyses of Heinicke et al. (2007) and Hedges et al. (2008a). Our DNA sequence analyses (Heinicke et al. 2007) also revealed that one species from Hispaniola, *E. counouspeus*, is not a member of those subgenera. Morphologically, it also does not neatly fit into any of the named subgenera. For these reasons, we erected a fifth subgenus for this species (see below).

Definitions and content for the pre-existing subgenera are modified from that proposed by Lynch and Duellman (1997). The differences mainly involve species in which conflicts existed among external morphological characters, internal morphological characters, and molecular data sets and these are discussed in the remarks section of each subgenus. The major difference between the previous classifications (Savage 1987; Joglar 1989; Lynch and Duellman 1997; Frost et al. 2006) and this classification is the recognition here of the Caribbean Clade (genus *Eleutherodactylus*) that excludes a close relationship between the eastern Caribbean clade (formerly *E. auriculatus* Group or *E. auriculatus* Section) and a large component of South American species (formerly the "*E.*" *unistrigatus* Group). DNA sequence analyses (Heinicke et al. 2007) revealed that the external morphological characters that had previously suggested a link between these groups, such as enlarged digital tips, Toe V longer than Toe III, and an areolate venter are convergent.

The molecular phylogeny (Hedges et al. 2008a) defines an Eastern Caribbean Clade (99% support) consisting of the subgenera *Eleutherodactylus, Pelorius,* and *Schwartzius,* and a Western Caribbean Clade (100% support) consisting of the subgenera *Euhyas* and *Syrrhophus*. At least one morphological character, liver shape, is consistent with this higher-level arrangement of subgenera. The liver shape character showed an association with the allozyme data (Hedges 1989a). Species of the Western Caribbean Clade have a long and pointed left lobe of the liver whereas species in the Eastern Caribbean Clade have shorter and rounded left lobes, similar to their right lobes. The phylogenetic position of *Diasporus*, as the closest relative of the Caribbean Clade, provides the opportunity to examine polarity of this liver shape character. *Diasporus* has a long and pointed left lobe suggesting that the alternative condition, rounded lobes of equal size (Eastern Caribbean Clade), is derived in *Eleutherodactylus*. Liver shape has yet to be surveyed extensively outside of Eleutherodactylidae. Immunological data supported the Western Caribbean Clade of *Syrrhophus* and *Euhyas* (Hass and Hedges 1991); support also appeared in earlier sequence analyses of small numbers of species (Crawford and Smith 2005; Frost et al. 2006). Even prior to the molecular studies, the association of *Syrrhophus* with a Cuban *Eleutherodactylus* was found in a phylogenetic analysis of morphological data (Heyer 1975).

Several other characters may not be diagnostic but nonetheless help to define the Eastern and Western Clades. Species of the Western Caribbean Clade are generally ground-dwelling (terrestrial) or saxicolous, lack an external vocal sac, and often have inguinal glands and Toe V shorter than Toe III. In contrast, species of the Eastern Caribbean Clade are mostly arboreal, have external vocal sacs (except the subgenus *Pelorius*), lack inguinal glands, and usually have Toe V longer than Toe III. In vocalization, most species in the Western Caribbean Clade have soft chirping-type calls whereas most in the Eastern Caribbean Clade have loud whistle-type calls. Not all species conform to this generalization, but the difference can be striking if, for example, one compares the species in Jamaica (17 native species, all of the Western Caribbean Clade) with those in Puerto Rico (16 native species, all of the Eastern Caribbean Clade). This difference even appears to extend to the sex of the parent that guards the egg clutch—male in Puerto Rico and female in Jamaica (Townsend 1996).

The definition of species series and species groups of West Indian *Eleutherodactylus* was last attempted nearly two decades ago (Hedges 1989a). Unfortunately, allozyme data were available for only about one-half of the species and therefore many species were left unassigned to species group or series. This problem was rectified in the recent DNA study (Heinicke et al. 2007), where nearly all species were sampled. These new data, together with morphological data, have allowed us to completely reorganize and redefine the classification of the West Indian species in this genus; this reorganization is presented below in the accounts of the subgenera. In doing so, we have emphasized clades that are strongly supported in the molecular phylogenies (Hedges et al. 2008a), and which, additionally, have morphological and geographic support.

Subgenus *Eleutherodactylus* Duméril and Bibron, 1838

Cornufer Tschudi, 1838:28. Type species: *Cornufer unicolor* Tschudi, 1838:28, by monotypy. Synonymy by Zweifel (1966:23).

Eleutherodactylus Duméril and Bibron, 1841:620. Type species: *Hylodes martinicensis* Tschudi, 1838:37, by monotypy. Official list of generic names, 1978.

Ladailadne Dubois, 1987:23. Type species: *Eleutherodactylus jasperi* Drewry and Jones, 1976:161. Synonymy by Hedges (1989a:327).

Definition.—Members of the subgenus *Eleutherodactylus* can be defined as eleutherodactylid frogs having: (1) head narrow; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous process of vomers present; (5) "S" condition of the adductor muscles; (6) terminal discs on digits expanded, bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I shorter than Finger II; (8) Toe V longer than Toe III; (9) subarticular tubercles prominent; (10) texture of skin on dorsum usually smooth; (11) texture of skin on venter usually areolate; (12) range in SVL 17 mm in female *E. brittoni* to 80 mm in female *E. karlschmidti.* Additionally, the two lobes of the liver are approximately the same length and shape.

Content.—Fifty-four species are placed in the subgenus.

Distribution.—The subgenus is distributed naturally throughout the West Indies, excluding Jamaica, although its natural occurrence on some islands in the Lesser Antilles is not yet established (distinguished from human introductions). In terms of species density, it is the only (or predominate) group on the eastern islands (Lesser Antilles, Virgin Islands, Puerto Rico, and the North Paleoisland (region north of the Cul de Sac-Valle de Neiba) of Hispaniola.

Etymology.—As for genus.

Remarks.—See the Remarks under the genus *Eleutherodactylus* for a discussion of the taxonomic history of the subgenera. The ground-dwelling Puerto Rican species *E. richmondi* was originally placed in the *E. ricordii* Group (now subsumed into the subgenus *Euhyas*) based on morphological traits (Schwartz 1976; Joglar 1989), but was placed in the *E. auriculatus* section of the subgenus *Eleutherodactylus* by Hedges (1989a). Lynch and Duellman (1997) claimed that this was done "inexplicably" and assigned it once again to the subgenus *Euhyas*. However, the reason that Hedges (1989a) placed it in the *E. auriculatus* section was because *E. richmondi* was found to cluster in his molecular phylogenetic tree with other members of the *E. auriculatus* section, and was found to have the same liver shape (short and rounded left lobe) and vocal sac condition (external) as species in the *E. auriculatus* section (subgenus *Eleutherodactylus*). The recent DNA sequence analysis also places this species in the subgenus *Eleutherodactylus*. The unusual ground-dwelling habits of *E. richmondi* (nearly all other Puerto Rican species are arboreal) probably influenced a suite of morphological traits that led to this confusion, causing it to converge with the predominantly terrestrial species of the Western Caribbean Clade.

In their assignment of species to subgenus, Lynch and Duellman (Lynch and Duellman 1997) listed assignments for nine other West Indian species (besides *E. richmondi*) that differed from the assignments made by Hedges (Hedges 1989a; Hedges and Thomas 1992) based on allozyme data and liver shape. They moved the following species of the subgenus *Euhyas* to the subgenus *Eleutherodactylus*: *E. amadeus*, *bakeri*,

corona, *eunaster*, *glanduliferoides*, and *thorectes*. They also moved the following species of the subgenus *Eleutherodactylus* to the subgenus *Euhyas*: *E. minutus*, *parabates*, and *unicolor*. In all of these cases, the species possess lengths of Toe V (relative to Toe III) that disagree with their taxonomic placement by allozyme data and liver shape. However, the recent DNA sequence results (Hedges et al. 2008a) support the original assignments and show substantial homoplasy in the character of the relative lengths of Toes III and V. Most of the nine species have ecological habits that are unusual for their subgenus—arboreal for the normally terrestrial subgenus *Euhyas* and terrestrial (*E. unicolor*) for the normally arboreal subgenus *Eleutherodactylus.* Therefore the relative lengths of the toes seems to be an adaptive feature related to climbing, which is perhaps not surprising. Therefore, based on DNA sequences, relative toe lengths do not seem to be as closely correlated with phylogeny in *Eleutherodactylus* as they do in *Pristimantis.*

Eleutherodactylus (Eleutherodactylus) auriculatus Species Series

Definition.—Species in this series are mostly small, ranging in SVL from 19 mm (female *E. minutus*) to 36 mm (female *E. mariposa*). They lack a distinct narrowing of the body in the neck region (see *Eleutherodactylus varians* Species Series). Most are tan or brown and lack bright colors or bold markings. They are arboreal but usually are found on small bushes and herbaceous plants and rarely high in trees. Most have a repetitious mating call made up of hollow- or metallic-sounding clicks and snaps, constant in frequency and rarely whistle-like.

Content.—Three species groups (16 species) are placed in this series: the *Eleutherodactylus* "*Eleutherodactylus*" *abbotti*, *auriculatus,* and *minutus* species groups.

Distribution.—This species series is distributed on the islands of Cuba and Hispaniola (including Haiti and the Dominican Republic).

Remarks.— A comparison between this series and the *E. varians* Species Series (see below) is pertinent because both are broadly sympatric on Cuba and Hispaniola. The average maximum size of the species in the *E. auriculatus* Species Series is 26.5 mm, compared with 36.5 mm for the *E. varians* Species Series. Also, species in the latter series have a distinct neck (narrowing of the body posterior to the head). In addition to these differences in size and body shape, these two large radiations have segregated ecologically; the former occurs on small plants and the latter primarily higher in trees and bromeliads. The vocalizations of the two species series also differ; species in the *E. auriculatus* Species Series usually produce a continuous series of hollow or metallic clicks that are constant in frequency, whereas species in the other series usually have a loud whistle-like call composed of notes that change frequency (Hedges et al. 1992).

Eleutherodactylus (Eleutherodactylus) abbotti Species Group

Definition.—Species in this group are uniformly small in size, ranging in SVL from 21 mm (female *E. haitianus*) to 29 mm (female *E. pituinus*), have a finely and irregularly

granular dorsum that often includes a pair of slightly concave dorsolateral folds, and relatively narrow heads (34.1–37.4% SVL).

Content.—Seven species are placed in the group. Five of those were previously recognized as species: *Eleutherodactylus abbotti, audanti, haitianus, parabates,* and *pituinus*. Two were described as subspecies of *E. audanti* (Schwartz 1966) and have been elevated to species status: *Eleutherodactylus* (*Eleutherodactylus*) *melatrigonum* and *Eleutherodactylus* (*Eleutherodactylus*) *notidodes*. An additional species is being described from the Sierra de Neiba (SBH, in preparation).

Distribution.—The species group is distributed in Haiti and the Dominican Republic on the island of Hispaniola.

Remarks.—This group is the major radiation of species of the *Eleutherodactylus auriculatus* Species Series on Hispaniola. Six of the species, including the most divergent, occur only in upland areas of the North Paleoisland, suggesting that this paleoisland was important in the evolution of the group.

Schwartz (1966) described two subspecies of *E. audanti* on Hispaniola, and one of us (SBH) has field experience with both taxa and the nominate subspecies. All three subspecies have disjunct distributions, separated by intervening areas with no known populations and no evidence of intergradation among the subspecies. Besides consistent pattern and structural differences (Schwartz 1966), they also have different mating calls. By criteria currently used to distinguish different species of the genus *Eleutherodactylus*, these three subspecies should be recognized as distinct species; therefore we have elevated them to species level.

This leaves the species *E. audanti* as occurring only in three disjunct regions on the Hispaniolan South Paleoisland—the Sierra de Baoruco, the Massif de La Selle, and The Massif de al Hotte. These require further study to determine if differentiation has occurred among these isolates. One of us (SBH) has noticed that most individuals collected from the Massif de la Selle are erythristic. Also, individuals of *E. audanti*, normally an abundant species in its habitat, were rarely encountered on several expeditions to both the north and south slopes of the Massif de la Hotte. Instead, arboreal species of the normally terrestrial subgenus *Euhyas,* of the same size as *E. audanti*, were abundant; this suggests possible competition.

Schwartz (1964) considered *E. parabates* to be most closely related to *E. jugans* (here placed in the subgenus *Euhyas*) because both are small, dark, and have robust body shapes. However, liver shape and phylogenetic analyses of proteins (Hedges 1989a) indicated that it was convergent with *E. jugans* and a member of the subgenus *Eleutherodactylus*. Lynch and Duellman (1997) placed *E. parabates* in the subgenus *Euhyas*. However, phylogenetic analyses of DNA sequences (Heinicke et al. 2007) confirm that it belongs in the subgenus *Eleutherodactylus* and is convergent with *E. jugans*. Also, despite its robust body shape inferring terrestrial habits (typical of the subgenus *Euhyas*), it has short dentigerous processes of the vomers, an external vocal sac, and arboreal habits, all typical of the subgenus *Eleutherodactylus* and not the subgenus *Euhyas*.

Eleutherodactylus (Eleutherodactylus) auriculatus Species Group

Definition.—Species in this group are small to moderate in size, ranging in SVL from 23 mm (female *E. principalis*) to 36 mm (female *E. mariposa*), have a finely and evenly granular dorsum, and have relatively wide heads (39.5–43.8% SVL).

Content.—Seven species are placed in the group: *Eleutherodactylus* (*Eleutherodactylus*) *auriculatus, bartonsmithi, eileenae, glamyrus, mariposa, principalis,* and *ronaldi*.

Distribution.—The species group is endemic to Cuba.

Remarks.—This species group represents one of the two major radiations of species of the subgenus *Eleutherodactylus* on Cuba, the other being the *E. varians* Species Group of the *E. varians* Species Series. See Remarks under *E. auriculatus* Species Series (above) for a discussion of their morphological and ecological differences.

The distinction here between the Cuban radiation (*E. auriculatus* Species Group) and major Hispaniolan radiation (*E. abbotti* Species Group) of the *E. auriculatus* Species Series, as reflected in the molecular phylogeny is born out in the non-overlapping difference in head shape and to a lesser degree in dorsal skin textures.

Eleutherodactylus (Eleutherodactylus) minutus Species Group

Definition.—Species in this group are variable in SVL, with one being small (female *E. minutus*, 19 mm) and the other being moderate in size (female *E. poolei*, 34 mm). They have relatively narrow heads (34.1–37.5% SVL).

Content.—Two species are placed in the group: *Eleutherodactylus* (*Eleutherodactylus*) *minutus* and *poolei*.

Distribution.—The species group is distributed in Haiti and the Dominican Republic on the island of Hispaniola.

Remarks.— A comparison between this species group and the *E. abbotti* Species Group is pertinent because they are sympatric on Hispaniola and their distributions are primarily centered on the North Paleoisland (north of the Cul de Sac, Valle de Neiba). In body size, the two species of this group are both smaller (*E. minutus*) and larger (*E. poolei*) than those of the *E. abbotti* Species Group. Also, their calls are different from each other and from those of the *E. abbotti* Species Group. The latter have calls typical of the *E. auriculatus* Species Series in being constant in frequency and repetitious. The call of *E. minutus* is a high-pitched rising whistle and that of *E. poolei* is a two-note electronic-sounding “eenk-eenk;” both calls are unusual for species in the *E. auriculatus* Species Series.

Eleutherodactylus (Eleutherodactylus) martinicensis Species Series

Definition.—Species in this series are moderate in body shape and variable in SVL, ranging from 17 mm (female *E. juanriveroi*) to 58 mm (female *E. coqui*). Except for one species (*E. hedricki*), they lack a distinct narrowing of the body in the neck region (present in the *Eleutherodactylus varians* Species Series). They are variable in coloration; some are brightly colored (red, yellow, or green) although most are tan or brown with variable patterns. Most species are arboreal, but *E. barlagnei* is riparian and *E. cooki* is cavernicolous. Most species have a relatively loud mating call that is whistle-like or has a whistle-like component.

Content.—Three species groups (20 species) are placed in this series: the *Eleutherodactylus* (*Eleutherodactylus*) *antillensis*, *flavescens*, and *martinicensis* species groups.

Distribution.—This species series is distributed on islands in the eastern Caribbean, including eastern Hispaniola, the Puerto Rican Bank, St. Croix, and the Lesser Antilles. Two species (*E. coqui* and *E. johnstonei*) have been widely introduced outside this range.

Remarks.— The phylogenetic position of the Lesser Antillean radiation, the *E. martinicensis* Species Group, is somewhat surprising from a biogeographic standpoint because of its nested position within the Eastern Caribbean Clade. This suggests dispersal from west to east, estimated to have occurred 15–20 Ma (Heinicke et al. 2007). However, there is no geologic evidence that the Lesser Antilles, which are volcanic, were ever interconnected by land and therefore dispersal was most likely by flotsam. Moreover, ocean currents now and in the past have flowed predominantly from east to west (Hedges 2006). This suggests that currents flowed differently in the past, perhaps associated with a clockwise Caribbean gyre, or that larger areas of land were exposed (facilitating dispersal) during sea level low stands, or both.

Eleutherodactylus (Eleutherodactylus) antillensis Species Group

Definition.—Species in this group have 26 chromosomes (Bogart 1981). They range in SVL from 17 mm (female *E. juanriveroi*) to 58 mm (female *E. coqui*) and are arboreal in habits (*E. cooki* is cave-dwelling).

Content.—Four species subgroups (14 species) are placed in the group: the *Eleutherodactylus* (*Eleutherodactylus*) *antillensis, gryllus, locustus,* and *wightmanae* subgroups.

Distribution.—The species group is distributed on the Puerto Rican Bank and St. Croix. One species (*E. coqui*) has been introduced into Florida, Hawaii, Guam, and other locations, and *E. antillensis* has been introduced into Panama.

Remarks.—This group is the major radiation of species of the subgenus *Eleutherodactylus* on Puerto Rico. All species have large digital discs and climb on vegetation or, in the case of *E. cooki*, on rock faces in boulder caves. It shares the island with the three species of the *Eleutherodactylus* (*Eleutherodactylus*) *richmondi* Species

Group, which have 30 chromosomes and are more terrestrial in habits. We recognize four species subgroups of this species group, primarily based on the phylogenetic analysis (Heinicke et al. 2007; Hedges et al. 2008). The first of those is not well defined based on other information, but the remaining three subgroups have some support from body shape, coloration, and vocalization.

Eleutherodactylus (Eleutherodactylus) antillensis Species Subgroup

Definition.—Species in this subgroup are small to moderate, ranging in SVL from 19 mm (female *E. brittoni*) to 35 mm (female *E. antillensis* and *E. hedricki*). They are moderate in body shape and have small to large digital discs, rounded to ovate in shape. They are variable in coloration and vocalization.

Content.—Four species are placed in the subgroup: *Eleutherodactylus* (*Eleutherodactylus*) *antillensis, brittoni, cochranae,* and *hedricki*.

Distribution.—The species subgroup is distributed on the Puerto Rican Bank and St. Croix; *E. antillensis* has been introcuced into Panama.

Remarks.—This is the most weakly-defined subgroup from the standpoint of non-molecular information. The call of *Eleutherodactylus brittoni* consists of sharply rising whistles (Drewry and Rand 1983), and the second note of the *E. antillensis* call is also a sharply rising whistle (more so than the corresponding notes of *E. coqui*, *portoricensis*, and *schwartzi*).

Eleutherodactylus (Eleutherodactylus) gryllus Species Subgroup

Definition.—Species in this subgroup are small, ranging in SVL from 17 mm (female *E. juanriveroi*) to 23 mm (female *E. jasperi*). They are dorsoventrally flattened in body shape and have relatively short legs and rounded digital discs. They usually have green or yellow on the body. Their calls are variable.

Content.—Three species are placed in the subgroup: *Eleutherodactylus* (*Eleutherodactylus*) *gryllus, jasperi*, and *juanriveroi*.

Distribution.—The species subgroup is distributed on Puerto Rico.

Remarks.—This subgroup is a small radiation of small, green or yellow species that have depressed bodies and occupy arboreal niches, including bromeliads. The group contains an ovoviviparous species (*Eleutherodactylus jasperi*) which is considered critically endangered and possibly extinct (IUCN 2006).

Eleutherodactylus (Eleutherodactylus) locustus Species Subgroup

Definition.—Species in this subgroup are small to large, ranging in SVL from 24 mm (female *E. locustus*) to 54 mm (female *E. cooki*). They are moderate in body shape with

relatively large eyes, long legs, and large, ovate digital discs. In coloration, they are usually dark (brown or dark brown). In vocalization, they emit a call with multiple (usually >5), evenly spaced notes of the same frequency.

Content.—Three species are placed in the subgroup: *Eleutherodactylus* (*Eleutherodactylus*) *cooki, eneidae, and locustus*.

Distribution.—The species subgroup is restricted to Puerto Rico.

Remarks.—This subgroup is a small radiation of dark, gracile species with large eyes and ovate digital discs.

Eleutherodactylus (Eleutherodactylus) wightmanae Species Subgroup

Definition.—Species in this subgroup are small to large, ranging in SVL from 23 mm (female *E. wightmanae*) to 58 mm (female *E. coqui*). They are moderate in body shape (almost robust in *E. wightmanae*), and have rounded to slightly ovate digital discs. In coloration, they are variable, but often have red, reddish, or salmon color on the body. The call consists of two types of notes, including one or more initial low frequency monotonic notes followed by one or more higher frequency notes, each rising moderately in frequency.

Content.—Four species are placed in the subgroup: *Eleutherodactylus* (*Eleutherodactylus*) *coqui, portoricensis, schwartzi,* and *wightmanae*.

Distribution.—The species subgroup is distributed on the Puerto Rican Bank and St. Croix. One species (*E. coqui*) has been introduced into Florida, Hawaii, Guam, and other locations.

Remarks.—This subgroup is a small radiation of mostly large species with loud, two-note calls ("co-qui"). *Eleutherodactylus wightmanae* does not fit neatly into that description. However, the call structure of that species, which is a variation on the basic two-note call (Drewry and Rand 1983), and reddish coloration (in some specimens) could be viewed as characters tying it to this group, in addition to the evidence from molecular phylogeny.

Eleutherodactylus (Eleutherodactylus) flavescens Species Group

Definition.—The single species is moderate in SVL (females, 41 mm), has a yellow vocal sac, large and indented digital discs, and is the only representative of the *Eleutherodactylus martinicensis* Species Series in Hispaniola.

Content.—A single species is placed in this group: *Eleutherodactylus* (*Eleutherodactylus*) *flavescens*.

Distribution.—The species group occurs in the Dominican Republic on the eastern one-third of Hispaniola.

Remarks.—This species is the closest relative of the *E. antillensis* Species Group in phylogenetic analyses of DNA sequences (Heinicke et al. 2007).

Eleutherodactylus (Eleutherodactylus) martinicensis Species Group

Definition.—Species in this group have 28 chromosomes (Kaiser et al. 1994). They range in SVL from 20 mm (female *E. pinchoni*) to 50 mm (female *E. amplinympha*) and are arboreal, except that *E. barlagnei* is stream-dwelling.

Content.—Five species are placed in the group: *Eleutherodactylus* (*Eleutherodactylus*) *amplinympha, barlagnei, johnstonei, martinicensis,* and *pinchoni*.

Distribution.—The species group is distributed in the Lesser Antilles. One species (*E. johnstonei*) has been introduced into Jamaica and Venezuela.

Remarks.—This group is a small radiation of species of the genus *Eleutherodactylus* in the Lesser Antilles. Two species, *E. johnstonei* and *E. martinicensis*, occur on multiple islands and their wider distributions are believed to be the result of introductions (Kaiser 1992), although their natural distributions have yet to be determined. Two species in the genus *Pristimantis* (see below) occur on islands in the southernmost Lesser Antilles (St. Vincent and Grenada).

Eleutherodactylus (Eleutherodactylus) richmondi Species Series

Definition.—Species in this series have 30 chromosomes (Bogart 1981). They are robust in body shape and variable in SVL, ranging from 17 mm (female *E. unicolor*) to 80 mm (female *E. karlschmidti*). They lack a distinct narrowing of the body in the neck region (see *Eleutherodactylus varians* Species Series). They range from dark brown (*E. unicolor*) to reddish brown (*E. richmondi*) to dark brown with yellow mottling (*E. karlschmidti*). These frogs are terrestrial; *E. karlschmidti* occupies rocky stream-side habitats. The mating calls are variable, although none is whistle-like.

Content.—Three species are placed in this series: *Eleutherodactylus* (*Eleutherodactylus*) *karlschmidti, richmondi,* and *unicolor*.

Distribution.—This species series is endemic to Puerto Rico.

Remarks.—This species series is the smaller of two radiations of species of the subgenus *Eleutherodactylus* on Puerto Rico. The major radiation is the *Eleutherodactylus antillensis* Species Group. Besides sharing the same chromosome number, similarities in their karyotype suggest a close relationship. All other Puerto Rican species have 26 chromosomes (Bogart 1981).

See Remarks for the subgenus *Eleutherodactylus* (above) for discussion of confusion surrounding the classification of *E. richmondi*. Morphologically, that species and *E. unicolor* are similar with stocky bodies, narrow snouts, and small digital discs. The third species, *E. karlschmidti*, is much larger and has large digital discs, not outwardly similar to the other two species. However, the chromosomes of *E. karlschmidti* and *richmondi* suggest a close relationship (Bogart 1981). One of the three species in this series, *E. karlschmidti*, is a large riparian species that is considered critically endangered and possibly extinct (IUCN 2006).

Eleutherodactylus (Eleutherodactylus) varians Species Series

Definition.—Species in this series are moderate in SVL, ranging from 28 mm (*E. olibrus* and *E. staurometopon*, males only) to 44 mm (females of *E. montanus*). They have a narrowing of the body in the neck region distinguishing their relatively wide head from the rest of the body. Most are tan, brown, or greenish-brown, uniform or mottled, and usually lack bright colors or bold markings (except *E. lamprotes* which has orange flash markings). These frogs are arboreal and usually are found high in trees, frequently in bromeliads. Most have a whistle-like call, commonly composed of a note that rises in frequency.

Content.—Four species groups (15 species) are placed in this series: the *Eleutherodactylus* (*Eleutherodactylus*) *lamprotes, montanus, varians,* and *wetmorei* species groups.

Distribution.—This species series is distributed on the islands of Cuba and Hispaniola (including Haiti and the Dominican Republic).

Remarks.—This species series is one of two major assemblages of species of the subgenus *Eleutherodactylus* on Cuba and Hispaniola, with the other being the *E. auriculatus* Species Series. See Remarks for the *E. auriculatus* Species Series (above) about how these two species series differ in morphology and ecology.

Eleutherodactylus (Eleutherodactylus) lamprotes Species Group

Definition.—Species in this group are moderate in SVL, ranging from 29 mm (females, *E. lamprotes*) to 33 mm (females, *E. fowleri*), have large eyes, and large, rounded digital discs. The dorsum is mostly tan or brown, occasionally with mottling (*E. lamprotes*). Both species dwell in bromeliads.

Content.—Two species are placed in the group: *Eleutherodactylus* (*Eleutherodactylus*) *fowleri* and *lamprotes*.

Distribution.—The species group occurs in southern Haiti and southern Dominican Republic on the island of Hispaniola.

Remarks.— The two included species are allopatric; *E. lamprotes* occupies the Massif de la Hotte and *E. fowleri* occurs on the Massif de la Selle and western portion of the Sierra de Baoruco. These three massifs make up the Hispaniolan South Paleoisland.

Eleutherodactylus (Eleutherodactylus) montanus Species Group

Definition.—Species in this montanus are moderate in SVL, ranging from 33 mm (females, *E. auriculatoides*) to 44 mm (females, *E. montanus*), and have moderately expanded and rounded digital discs. In dorsal coloration, they vary from being uniformly tan or brown to having yellowish-green vermiculations. One species (*E. auriculatoides*) inhabits bromeliads in trees, whereas the two high elevation species are often found under objects on the ground, and call from the ground, rocks, or low on vegetation.

Content.—Three species are placed in the group: *Eleutherodactylus* (*Eleutherodactylus*) *auriculatoides, montanus*, and *patricae*.

Distribution.—The species group is endemic to the Cordillera Central of the Dominican Republic on the island of Hispaniola.

Remarks.— The three included species are regionally sympatric, although one species (*E. auriculatoides*) occurs at lower elevations than the other two species. The Cordillera Central is part of the Hispaniolan North Paleoisland.

Eleutherodactylus (Eleutherodactylus) varians Species Group

Definition.—Species in this group are moderate in SVL, ranging from 28 mm (males only, *E. olibrus* and *staurometopon*) to 40 mm (females, *E. ionthus*), moderate in shape, and have large and rounded digital discs. In dorsal coloration, most are tan, brown, or greenish-brown, and some have mottling or a bold pattern. All but one species (*E. leberi*) have been found in bromeliads during the day, males have been observed calling from bromeliads and leaves of trees (or rarely rocks) at night, often high above the ground.

Content.—Two species subgroups (seven species) are placed in the group: the *Eleutherodactylus* (*Eleutherodactylus*) *leberi* and *varians* subgroups.

Distribution.—The species group is endemic to Cuba.

Remarks.—This species group is one of the two major radiations of species of the subgenus *Eleutherodactylus* on Cuba, the other being the *E. auriculatus* Species Group of the *E. auriculatus* Species Series. See Remarks under *E. auriculatus* Species Series (above) for a discussion of the morphological and ecological differences of this species group and the *E. auriculatus* Species Group.

Species in the two subgroups of the *Eleutherodactylus (Eleutherodactylus) varians* Species Group are remarkably similar in appearance and habits. They are highly arboreal and frequent bromeliads. The molecular phylogeny defines these two subgroups, and the *E. leberi* subgroup is further supported by a shared chromosome number (2N =

24), unique among Cuban species (Bogart 1981; Hedges et al. 1992). The species in the *E. varians* subgroup are united by their calls, which are higher in frequency and similar in quality. The calls of *E. leberi* and *E. melacara* differ in number of notes, yet they possess lower frequency calls than other species in the group (Hedges et al. 1992). Species within each of the two subgroups are allopatric, yet the two species subgroups are sympatric.

Eleutherodactylus (Eleutherodactylus) leberi Species Subgroup

Definition.—Species in this subgroup are moderate in SVL, ranging from 33 mm (females, *E. leberi*) to 36 mm (females, *E. melacara*). They have 24 chromosomes and a relatively low frequency call (2.0–2.3 kilohertz). If the call of *E. melacara* is shown to have two components, as is suggested by an audiospectrogram (Hedges et al. 1992), this would be another character shared with *E. leberi*.

Content.—Two species are placed in this subgroup: *Eleutherodactylus* (*Eleutherodactylus*) *leberi* and *melacara*.

Distribution.—The species group is restricted to the Sierra Maestra in eastern Cuba.

Remarks.— See remarks below under *Eleutherodactylus (Eleutherodactylus) varians* Species Subgroup for a comparison of the two subgroups of the *E. varians* Species Group.

Eleutherodactylus (Eleutherodactylus) varians Species Subgroup

Definition.—Species in this subgroup are moderate in SVL, ranging from 28 mm (males only, *E. olibrus* and *staurometopon*) to 40 mm (females, *E. ionthus*). They have 18, 26, or 28 chromosomes and a relatively high frequency call (2.4–2.8 kilohertz) composed of a series of multiple notes, each with one frequency component, compared with other Cuban species in the subgenus where individual notes have multiple components (Hedges et al. 1992).

Content.—Five species are placed in the subgroup. Three of those were previously recognized as species: *Eleutherodactylus guantanamera, ionthus,* and *varians*. Two were described as subspecies of *E. varians* (Schwartz 1958c, 1960) and have been elevated to species status: *Eleutherodactylus* (*Eleutherodactylus*) *olibrus* and *Eleutherodactylus* (*Eleutherodactylus*) *staurometopon.*

Distribution.—The species subgroup is endemic to Cuba.

Remarks.— Schwartz (1958c, 1960) described three subspecies of *E. varians* on Cuba. Hedges et al. (1992) discussed their differences and elevated one of those taxa (*E. ionthus*) to full species status. The decision to leave the other taxa unchanged was made only because the focus of that study was on species from eastern Cuba. The other two taxa, elevated here, occur in western Cuba (*E. olibrus*) and on Isla de Juventud (*E.*

staurometopon). Their specific status is supported by morphological, pigmentation, and call differences, and their ranges are disjunct with no evidence of intergradation.

Eleutherodactylus (Eleutherodactylus) wetmorei Species Group

Definition.—Species in this group are moderate in SVL, ranging from 33 mm (females, *E. wetmorei*) to 36 mm (females, *E. sommeri*), and have large and rounded digital discs. They are uniformly tan or grayish-tan dorsally, and the concealed areas of the groin and hindlimbs are orange or red. All species call from bromeliads or leaves of trees, often high above the ground, and have a loud, two-note call.

Content.—Three species are placed in the group. One of these, *Eleutherodactylus wetmorei,* was previously recognized as a species. The other two were described as subspecies of *E. wetmorei* (Schwartz 1968, 1973, 1977) but have been elevated to species status: *Eleutherodactylus* (*Eleutherodactylus*) *diplasius* and *Eleutherodactylus* (*Eleutherodactylus*) *sommeri*.

Distribution.—The species group is distributed in Haiti and the Dominican Republic on the island of Hispaniola.

Remarks.—This species group is a small radiation of closely related species in Hispaniola. A fourth taxon, *Eleutherodactylus wetmorei ceraemerus*, was described by Schwartz (1968). One of us (SBH) has had field experience with all four taxa. They have minor but significant structural and call differences and differ especially in the pattern of their flash markings on the concealed areas of the groin and hindlimbs. The ranges of two of the species, *E. diplasius* and *E. wetmorei*, contact in the Massif de la Hotte of Haiti, and specimens from the area of contact show no signs of intergradation. Another species, *E. sommeri*, occurs far to the north in Haiti and the Dominican Republic, on the North Paleoisland of Hispaniola. There is no evidence of intergradation with the species to the south (Schwartz 1977). For these reasons, we recognize these three taxa as full species. However, we leave the status of *E. w. ceraemerus* unchanged because of the identification of two populations showing intergradation with *E. w. wetmorei* (Schwartz 1977). Nonetheless, the overall differences between those two subspecies exist, and further study may justify recognition of *E. w. ceraemerus* as a full species.

Subgenus *Euhyas* Fitzinger, 1843

Euhyas Fitzinger, 1843:31. Type species: *Hylodes ricordii* Duméril and Bibron, 1841:623, by original designation.

Sminthillus Barbour and Noble, 1920:402. Type species: *Phyllobates limbatus* Cope, 1862:154, by original designation. Synonymy by Hedges (1989a:318).

Definition.—Members of the subgenus *Euhyas* can be defined as eleutherodactylid frogs having: (1) head narrow; (2) tympanic membrane differentiated, prominent, and large in most species; (3) cranial crests absent; (4) dentigerous processes of vomers present (absent in several diminutive Cuban species); (5) "S" condition of the adductor muscles; (6) terminal discs on digits expanded, bearing well-defined circumferential grooves,

supported by T-shaped terminal phalanges; (7) Finger I usually shorter than Finger II; (8) Toe V usually shorter than Toe III, although longer than Toe III in several arboreal species (e.g., *E. amadeus*, *E. bakeri*, *E. corona*, *E. eunaster*, *E. glanduliferoides*, and *E. thorectes*); (9) subarticular tubercles prominent; (10) texture of skin on dorsum variable; (11) texture of skin on venter usually smooth; (12) range in SVL from 11 mm in female *E. iberia* to 64 mm in female *E. greyi.* Additionally, the left lobe of the liver is long and pointed whereas the right lobe is smaller and rounded.

Content.—The subgenus contains eight species series, 20 species groups, five species subgroups, and 95 species.

Distribution.—*Euhyas* is widely distributed throughout the Greater Antilles (except mainland Puerto Rico), Bahamas Islands, Virgin Islands, and Cayman Islands. It has been introduced into Florida, Louisiana, and Hawaii in the USA.

Etymology.—The generic name is derived from the Greek *eu,* meaning true, and the Greek mythological character *hyas,* used in reference to a treefrog. The name is feminine in gender.

Remarks.—See the Remarks under the genus *Eleutherodactylus* for a discussion of the taxonomic history of the subgenera. See above (under subgenus *Eleutherodactylus*) for discussion of the ten species whose placement in this subgenus has been controversial. Sexual size dimorphism is more pronounced in this subgenus than in the other West Indian subgenera; males of most species are considerably smaller than females. Moreover, species in the subgenus *Euhyas* often lack an external vocal sac and even vocal slits, and their mating calls tend to be less noisy and include irregular chirps and clicks rather than whistles common to the subgenus *Eleutherodactylus*. Calling sites also differ; most species are terrestrial (e.g., ground, rocks, and streams) rather than arboreal as in most members of the other West Indian subgenera (more than half of the species of *Peorius* call from the ground or below ground). The major difference in liver shape noted previously (Hedges 1989a) agrees virtually completely with recent DNA sequence evidence (Heinicke et al. 2007). Members of this subgenus and those species in the genus *Diasporus* and the subgenus *Syrrhophus* that have been examined have livers with long, pointed left lobes whereas species in the Eastern Caribbean Clade (subgenera *Eleutherodactylus*, *Pelorius*, and *Schwartzius*) have livers in which both lobes are short and rounded (apparently the derived state).

Eleutherodactylus (Euhyas) armstrongi Species Series

Definition.—Species in this series are moderate in body shape and moderate in SVL, ranging from 37 mm (females, *E. alcoae*) to 45 mm (females, *E. leoncei*). All have large, ovate digital discs, and most have long legs (*E. alcoae* has short legs). In coloration, they are variable (yellows, reds, greens, and browns), although two species (*E. darlingtoni* and *leoncei*) have a pair of pale scapular bars resembling quotation marks. In habits, they vary from ground dwelling (*E. darlingtoni* and *leoncei*) to rock dwelling (*E. alcoae*) and tree and bromeliad dwelling (*E. armstrongi*). Vocalization (unknown in *E. darlingtoni*)

ranges from soft, irregular chirps (*E. alcoae* and *leoncei*) to a loud, metallic "peng" (*E. armstrongi*).

Content.—Four species are placed in the species series: *Eleutherodactylus* (*Euhyas*) *alcoae, armstrongi, darlingtoni,* and *leoncei*.

Distribution.—The species series is distributed in southern Hispaniola, including Haiti and the Dominican Republic.

Remarks.— The evolutionary history of this series seems to be confined to the region of the Massif de la Selle and Sierra de Baoruco, including the Barahona Peninsula. This has resulted in a pair of allopatric species (*E. darlingtoni* and *leoncei*) at high elevations and a pair of mostly allopatric (partly sympatric) species (*E. alcoae* and *armstrongi*) at low to moderate elevations. Previously, *E. darlingtoni* and *leoncei* were considered to be sympatric (Schwartz and Henderson 1991) but the status of the two species was reassessed and the distributions were revised (Hedges 1992). The distribution of one species (*E. armstrongi*) consists of two isolated regions separated by tens of kilometers. The habits of the two ground-dwelling species (*E. darlingtoni* and *E. leoncei*) are not well known, and their large digital discs suggest that they climb and probably do so mostly on rocks.

Eleutherodactylus (Euhyas) dimidiatus Species Series

Definition.—Species in this series are robust in body shape and moderate to large in SVL, ranging from 27 mm (females, *E. emiliae*) to 58 mm (females, *E. dimidiatus*). Most are tan, brown, or greenish-brown, and one (*E. albipes*) has limbs with red or orange. The calls of these terrestrial species are variable, but most emit a faint, short sound.

Content.—Two species groups (seven species) are placed in this series: the *Eleutherodactylus* (*Euhyas*) *dimidiatus* and *schmidti* species groups.

Distribution.—This species series occurs on Cuba and Hispaniola (including Haiti and the Dominican Republic).

Remarks.— The series includes two island radiations (species groups) of related, robust, ground-dwelling species. The Cuban radiation (*E. dimidiatus* Species Group) contains forest floor species whereas the Hispaniolan radiation (*E. schmidti* Species Group) contains streamside species.

Eleutherodactylus (Euhyas) dimidiatus Species Group

Definition.—Species in this group are robust in body shape and small to moderate in SVL, ranging from 27 mm (females, *E. emiliae*) to 45 mm (females, *E. dimidiatus*). All have short fingers with small digital discs. Two species (*E. albipes* and *E. emiliae*) have short hind limbs, whereas the other two (*E. dimidiatus* and *E. maestrensis*) have long hind limbs. Typically these frogs are tan or brown, and have a weakly or well-developed

dark face mask that may extend posterior to the forelimb. Most of these terrestrial species emit a faint chirping sound (Díaz et al. 2005).

Content.—Four species are placed in the group: *Eleutherodactylus* (*Euhyas*) *albipes*, *dimidiatus*, *emiliae*, and *maestrensis*.

Distribution.—The species group is endemic to Cuba.

Remarks.— Schwartz (1958b) described the subspecies *Eleutherodactylus dimidiatus amelasma* from Western Cuba, but Díaz et al. (2005) did not consider the taxon to be valid based on data from morphology and vocalization. Nonetheless, there seems to be a geographic gap between the eastern and western populations, and molecular analyses are needed to determine if there has been genetic differentiation.

Eleutherodactylus (Euhyas) schmidti Species Group

Definition.—Species in this group are robust in body shape and moderate to large in SVL, ranging from 43 mm (females, *E. limbensis*) to 58 mm (females, *E. rucillensis*). They have slight webbing present between their toes and are variable in coloration. They are most commonly encountered on rocks and the ground adjacent to mountain streams; some individuals have been found in the water. The call of most species is a short, faint, "mew" noise.

Content.—Three species are placed in the group. One of those, *Eleutherodactylus* (*Euhyas*) *schmidti*, was previously recognized as a species, whereas two have been recognized as subspecies of *E. schmidti* (Schwartz 1970) and have been elevated to species status—*Eleutherodactylus* (*Euhyas*) *limbensis* and *Eleutherodactylus* (*Euhyas*) *rucillensis*.

Distribution.—The species group is distributed in central and northern Hispaniola (including Haiti and the Dominican Republic).

Remarks.—This species group represents a small island radiation of streamside frogs in Hispaniola. Schwartz (1970) reviewed the available material and redefined the previously described subspecies. The Haitian taxon (*E. limbensis*), which is isolated from the other two, is slightly smaller, has a different color pattern, and males have shorter legs than males of the other two species. The two species inhabiting the Cordillera Central of the Dominican Republic, *E. rucillensis* and *E. schmidti*, differ greatly in body size (58 mm versus 46 mm, respectively), have different leg proportions, and different coloration (Schwartz 1970). Their ranges are in broad contact; yet there is no substantial evidence of intergradation. Accordingly, we recognize *E. limbensis* and *rucillensis* as full species. One of us (SBH) is aware of an undescribed species belonging to this group from the Sierra de Neiba in the Dominican Republic.

Eleutherodactylus (Euhyas) greyi Species Series

Definition.—The single species in this series is moderate in body shape and large in SVL (females, 64 mm). It has a tuberculate dorsum, long legs, and large, ovate digital discs. The dorsal coloration is variable but usually tan, yellowish-grey, or greenish gray with small dark spots. It is primarily rock dwelling, but also has been encountered on the forest floor and on river talus. It has a two-note call, with the second note higher in frequency than the first.

Content.—This species series contains a single species, *Eleutherodactylus* (*Euhyas*) *greyi*.

Distribution.—This species occurs in central Cuba.

Remarks.—The vocalization and habits of this species were recently described by Díaz et al. (2007).

Eleutherodactylus (Euhyas) luteolus Species Series

Definition.—Species in this series are robust to moderate in body shape and small to large in SVL, ranging from 18 mm (females, *E. griphus* and *sisyphodemus*) to 59 mm (females, *E. cuneatus*). Leg length and digital disc size varies among species. Coloration is variable, and many taxa exhibit pattern polymorphism. The Cuban species are associated with streams, whereas the Jamaican species occupy diverse habitats. The calls include the typical chirps and mews characteristic of the subgenus *Euhyas* as well as some with hollow rapping or knocking noises.

Content.—Four species groups (22 species) are placed in this species series: the *Eleutherodactylus* (*Euhyas*) *cuneatus*, *luteolus*, *riparius*, and *toa* species groups.

Distribution.—The species series occurs on Cuba and Jamaica.

Remarks.—This species series includes the Jamaican radiation of *Eleutherodactylus* (*E. luteolus* Species Group) and the five Cuban species that are their closest relatives. Because Jamaica and Cuba were not connected geologically during the Cenozoic, the only way that the Jamaican radiation could have originated was from dispersal over water of a Cuban species. A riparian species would be more likely than others to be washed out of a river with flotsam, and therefore it is relevant that the five Cuban species in this series are the only riparian species in Cuba.

Eleutherodactylus (Euhyas) cuneatus Species Group

Definition.—Species in this group are robust in body shape and large in SVL, ranging from 53 mm (females, *E. turquinensis*) to 59 mm (females, *E. cuneatus*). They have moderate to long legs, moderate to large digital discs, and toe webbing (*E. turquinensis*). They are primarily reddish-brown, grayish brown, and greenish brown with pattern polymorphism. They occupy mountain streams and adjacent habitats; *E. cuneatus* is less aquatic than *E. turquinensis* and also occurs in forests away from streams. The call is a

"chirp" that either descends (*E. cuneatus*) or rises slightly (*E. turquinensis*) in frequency (Hedges et al. 1995).

Content.—Two species are placed in the group: *Eleutherodactylus* (*Euhyas*) *cuneatus* and *E. turquinensis*.

Distribution.—The species group is restricted in eastern Cuba.

Remarks.—This species group includes a pair of closely related, sympatric riparian species. The confused taxonomic history of *Eleutherodactylus cuneatus* was reviewed elsewhere (Estrada and Hedges 1998). Other stream-associated species in Cuba are *E. riparius* and *E. rivularus*, (*E. riparius* Species Group) and *E. toa* (*E. toa* Species Group). Although it might seem appropriate to place all five riparian species in the same group, they differ considerably in morphology. The species in this group are larger than those in the *E. riparius* Species Group, have a tubercular dorsum (not rugose), and have large digital discs (not small). The single species in the *E. toa* Species Group is small and has a tuberculate dorsum and areolate venter. Neither these two groups nor the *E. toa* group are closest relatives in the molecular phylogeny, but more sequence data will be needed to resolve the details of those relationships.

Eleutherodactylus (Euhyas) luteolus Species Group

Definition.—Species in this group are robust to moderate in body shape and small to moderate in SVL, ranging from 18 mm (females, *E. griphus* and *E. sisyphodemus*) to 49 mm (females, *E. nubicola*). Leg length and digital disc size varies among species. They are variable in coloration; distinctive pattern polymorphisms are shared among the species (Schwartz and Fowler 1973; Crombie 1977, 1986). These species occupy a diversity of habitats, including leaf litter, caves, bromeliads, and streams. The calls are variable and include the typical chirps and mews characteristic of the subgenus *Euhyas* as well as some with hollow rapping or knocking noises.

Content.—Five species subgroups (17 species) are placed in this species group: *Eleutherodactylus* (*Euhyas*) *cundalli, gossei, jamaicensis, luteolus,* and *nubicola* species subgroups.

Distribution.—The species group is endemic to Jamaica.

Remarks.— This species group includes all native species of the genus *Eleutherodactylus* on Jamaica and represents an adaptive radiation in the true sense, inasmuch as the included species occupy a great diversity of habitats and show obvious adaptations to those habitats (Hedges 1989b). Although originally identified by protein variation (Hedges 1989a, 1989b), this species group has support from albumin immunology (Hass and Hedges 1991), chromosome variation (Bogart and Hedges 1995), and DNA sequence analyses (Hedges et al. 2008a). The DNA sequence data were insufficient to resolve relationships of species within the species group; longer sequences

will be needed. The subgroups recognized here are based on relationships defined in the earlier analyses of protein variation, albumin immunology, and chromosomes.

Eleutherodactylus (Euhyas) cundalli Species Subgroup

Definition.—Species in this group are moderate in body shape and moderate in SVL, ranging from 38 mm (females, *E. cavernicola* and *E. glaucoreius*) to 45 mm (females, *E. cundalli*). They have relatively large eyes, long legs, long digits, and moderate to large digital discs. They are variable in coloration. All are commonly found on rocks, and at least two (*E. cavernicola* and *E. cundalli*) are encountered in caves. Calls consist of irregular series of chirps and ticks.

Content.—Three species are placed in the species subgroup: *Eleutherodactylus* (*Euhyas*) *cavernicola, cundalli,* and *glaucoreius*.

Distribution.—The species subgroup is restricted to Jamaica.

Remarks.—This species subgroup represents a trio of long-legged, large-disced, allopatric species that occupy rocky and cave habitats on Jamaica. At least one species (*E. cundalli*) has a unique reproductive behavior (froglet transport) among terraranan frogs (Diesel et al. 1995).

Eleutherodactylus (Euhyas) gossei Species Subgroup

Definition.—Species in this subgroup are robust in body shape and small to moderate in SVL, ranging from 18 mm (females, *E. griphus*) to 44 mm (females, *E. pantoni*). They have relatively short legs, short digits, and small digital discs. The dorsal ground color usually is tan, brown, grayish-brown, or reddish-brown; they have red or orange in the concealed areas of the groin and hindlimbs. These frogs are usually encountered on the ground, or more rarely, on low vegetation and rocks. The calls include a muffled whistle or series of hollow sounding notes.

Content.—Five species are placed in the subgroup: *Eleutherodactylus* (*Euhyas*) *fuscus, gossei, junori, pantoni,* and *pentasyringos*.

Distribution.—The species subgroup is endemic to Jamaica.

Remarks.—This species subgroup includes a pair of mostly allopatric (partially sympatric in one small region) species, *E. pantoni* and *E. pentasyringos,* that have yellow or orange bellies. It also includes a trio of sympatric species, *E. fuscus*, *E. gossei*, and *E. junori* that differ in body size and lack yellow or orange bellies.

Eleutherodactylus (Euhyas) jamaicensis Species Subgroup

Definition.—The single species in this group has a depressed body and moderate SVL (females, 30 mm). It has large, rounded digital discs. The dorsal ground color is dark

brown, tan, or gray, commonly with a pair of pale dorsolateral marks, and capable of changing colors (dark to pale). This frog lives exclusively in bromeliads. The call is a series of short chirps.

Content.—One species is placed in the subgroup: *Eleutherodactylus* (*Euhyas*) *jamaicensis*.

Distribution.— The species subgroup occurs on Jamaica.

Remarks.—This species was found to be most closely related to the *Eleutherodactylus cundalli* and *E. nubicola* subgroups (Hedges 1989b; Bogart and Hedges 1995).

Eleutherodactylus (Euhyas) luteolus Species Subgroup

Definition.—Species in this subgroup are robust in body shape and small to moderate in SVL, ranging from 18 mm (females, *E. sisyphodemus*) to 29 mm (females, *E. grabhami*). They have short limbs and small to large digital discs. They are variable in coloration. One species (*E. sisyphodemus*) lives in leaf litter; the other two climb on vegetation and rocks. The calls are a faint, insect like buzz (*E. sisyphodemus*) or whistle-like peep.

Content.—Three species are placed in the subgroup: *Eleutherodactylus* (*Euhyas*) *grabhami, luteolus,* and *sisyphodemus.*

Distribution.— The species subgroup is restricted to Jamaica.

Remarks.—This species subgroup is the most divergent of the subgroups and contains species that are the least similar to one another, yet they are united by several types of genetic data (Hedges 1989b; Bogart and Hedges 1995), including partial support from DNA sequence data (Heinicke et al. 2007). Their greater morphological divergence may reflect their longer period of diversification on Jamaica (i.e., earlier divergences among species compared with the other species subgroups in Jamaica).

Eleutherodactylus (Euhyas) nubicola Species Subgroup

Definition.—Species in this subgroup are robust in body shape and small to moderate in SVL, ranging from 18 mm (females, *E. griphus*) to 49 mm (females, *E. nubicola*). Most have relatively short legs and small digital discs, although one species (*E. orcutti*) has large digital tips and webbing between the toes. They are variable in coloration. All are terrestrial, except for *E. orcutti,* which inhabits streams. The calls vary from chirps to raspy notes and faint whistles.

Content.—Five species are placed in the subgroup: *Eleutherodactylus* (*Euhyas*) *alticola, andrewsi, griphus, nubicola,* and *orcutti*.

Distribution.—The species subgroup is endemic to Jamaica.

Remarks.—Based on distribution, the diversification of this species group occurred in eastern Jamaica (the Blue Mountains), with a single species (*E. griphus*) occurring in the Cockpit Country of western Jamaica.

Eleutherodactylus (Euhyas) riparius Species Group

Definition.—Species in this group are robust in body shape and moderate in SVL, ranging from 31 mm (females, *E. rivularis*) to 42 mm (females, *E. riparius*). They have a rugose dorsum and W-shaped suprascapular fold, long legs, and small digital discs. They are primarily gray, grayish brown, or olive brown and exhibit pattern polymorphism (uniform, mottled, and striped) in one species. These frogs usually occur along the edges of streams. They emit one or a few chirps (Estrada and Hedges 1998; Díaz et al. 2001).

Content.—Two species are placed in the group: *Eleutherodactylus riparius* and *rivularis*.

Distribution.—The species group is distributed in Cuba.

Remarks.—This species group includes a pair of closely related, sympatric riparian species. *Eleutherodactylus riparius* is a common, wide-ranging species found throughout most of Cuba and formerly called *E. cuneatus*. The confused taxonomic history of *E. cuneatus* was reviewed elsewhere (Estrada and Hedges 1998). Other Cuban stream-associated species are *E. turquinensis (E. cuneatus* Species Group*)* and *E. toa* (*E. toa* Species Group). See Remarks (above) under *Eleutherodactylus (Euhyas) cuneatus* Species Group concerning relationships and differences among the five riparian species of Cuba.

Eleutherodactylus (Euhyas) toa Species Group

Definition.—The species in this group is robust in body shape and moderate in SVL (females, 33 mm). It has a tuberculate dorsum, an areolate venter, long legs, and digital discs of moderate size. The dorsum is either pale whitish tan, yellowish green, or greenish gray, with several pattern polymorphisms. Most individuals of this terrestrial species have been collected in pine forest, although some have been found along streams; this leads to the impression, along with its streamlined and robust habitus, that it may be adapted to streamside situations. Vocalization is unknown.

Content.—A single species is placed in the group: *Eleutherodactylus* (*Euhyas*) *toa.*

Distribution.—The species is distributed in eastern Cuba.

Remarks.—Other Cuban stream-associated species are *E. cuneatus* and *E. turquinensis* (*E. cuneatus* Species Group) and *E. riparius* and *E. rivularis* (*E. riparius* Species Group). See Remarks (above) under *Eleutherodactylus (Euhyas) cuneatus* Species Group concerning relationships and differences among the five riparian species of Cuba.

Eleutherodactylus (Euhyas) oxyrhyncus Species Series

Definition.—Species in this series are variable in body shape and in SVL, ranging from 15 mm (females, *E. thorectes*) to 43 mm (females, *E. apostates*). They are variable in coloration. These frogs are terrestrial or arboreal; they inhabit streams, marshes, bromeliads, rocks, and caves. Their calls span the spectrum of variation from faint chirps to loud whistles.

Content.—Six species groups (21 species) are placed in this series: the *Eleutherodactylus* (*Euhyas*) *bakeri, glandulifer*, *jugans, oxyrhyncus*, *paulsoni,* and *rufifemoralis* species groups.

Distribution.—This species series is distributed in southern Hispaniola, including Haiti and the Dominican Republic.

Remarks.—This species series is the major adaptive radiation of species of the subgenus *Euhyas* on Hispaniola. Compared with the similar-sized major radiation on Cuba, the *Eleutherodactylus* (*Euhyas*) *planirostris* Species Series (23 species), species in this Hispaniolan radiation seem to be adapted to more habitats. In particular, this radiation of frogs has exploited arboreal habitats, an aspect of the environment not utilized by the *E. planirostris* Species Series (see below). A possible explanation for this difference is that the arboreal subgenus *Eleutherodactylus* colonized Cuba and filled arboreal niches, thereby excluding the *E. planirostris* series from those niches prior to the colonization of the South Paleoisland of Hispaniola by the *E. oxyrhynchus* Species Series. Thus, the *E. oxyrhyncus* Species Series was free to expand into those vacant arboreal niches on the South Paleoisland. The comparatively modest presence of the arboreal subgenus (*Eleutherodactylus*) on the South Paleoisland probably can be attributed to its later arrival, after *Euhyas* had filled most of the arboreal niches.

Eleutherodactylus (Euhyas) bakeri Species Group

Definition.—Species in this group are moderate in body shape and small to moderate in SVL, ranging from 15 mm (females, *E. thorectes*) to 35 mm (females, *E. bakeri*). Most have moderate to large digital discs (small in *E. glanduliferoides* and *E. thorectes*). Coloration is variable; most are shades of tan, brown, and reddish brown and are polymorphic in pattern (Hedges et al. 1987). All of the species are arboreal, and several frequent bromeliads. Surprisingly, the two species with small digital tips also climb, but only in low vegetation. One species, *E. glaphycompus*, also calls from limestone rocks. Their calls vary, but most species emit a loud whistle-like noise.

Content.—Eleven species are placed in the group: *Eleutherodactylus* (*Euhyas*) *amadeus, bakeri, caribe, corona, dolomedes, eunaster, glanduliferoides, glaphycompus, heminota, semipalmatus*, and *thorectes*.

Distribution.—This species group is distributed in southern Hispaniola, including Haiti and the Dominican Republic.

Remarks.—This species group is an unusual radiation of arboreal species within the otherwise predominantly ground- and rock-dwelling subgenus *Euhyas*. The enlarged, rounded digital discs seem to be associated with climbing abilities. Their resemblance to species in the mostly arboreal subgenus *Eleutherodactylus* also includes the short dentigerous processes of the vomers and loud, whistle-like calls of some of the species. It has been suggested that the size of the vomerine processes is correlated with feeding habits: short for soft-bodied prey such as dipterans and lepidopterans that might be encountered in arboreal situations and longer (with more teeth) for hard-bodied prey such as coleopterans and orthopterans that might be encountered more frequently in terrestrial situations (Hedges 1989a).

Protein analyses and liver shape (Hedges 1989a) first revealed this radiation and its remarkable convergence, although Lynch and Duellman (Lynch and Duellman 1997) were not convinced and relegated more than half of the species in this group (*Eleutherodactylus amadeus, bakeri, corona, eunaster, glanduliferoides, glaphycompus, and thorectes*) to the subgenus *Eleutherodactylus*. They did this primarily based on variation in relative length of the fifth toe compared to the third toe, a character that they singled out as being of high systematic value. However, the recent DNA sequence analyses (Heinicke et al. 2007) now confirm the original allocations using proteins and liver shape, indicating that the toe character is part of the convergence and thus not a useful character in this group.

The two small species with small digital tips, *E. glanduliferoides* and *E. thorectes*, are least like other members of this group, and not surprisingly they are the most divergent members in the molecular phylogeny (Hedges et al. 2008a). However, more molecular data are needed to resolve the relationships of these species.

Eleutherodactylus (Euhyas) glandulifer Species Group

Definition.—Species in this group are robust in body shape and small to moderate in SVL, ranging from 21 mm (females, *E. sciagraphus*) to 36 mm (females, *E. glandulifer*). Three have short snouts, short legs, and small digital discs in contrast to *E. glandulifer.* Coloration is mostly dark shades of brown and green. These frogs are terrestrial, although the large digital discs of *E. glandulifer* suggest that it also climbs. Vocalization is variable.

Content.—Four species are placed in the group: *Eleutherodactylus* (*Euhyas*) *brevirostris, glandulifer, sciagraphus, and ventrilineatus*.

Distribution.—The species group is restricted to the Massif de la Hotte, on the eastern end of the Tiburon Peninsula of Haiti, on the island of Hispaniola.

Remarks.—This is a small radiation of robust, dark, terrestrial species in the Massif de la Hotte.

Eleutherodactylus (Euhyas) jugans Species Group

Definition.—The single species in this group is robust in body shape and moderate in SVL (females, 33 mm). It has short legs and small digital discs. It is usually brown, reddish brown, or orange-brown. The call of this terrestrial species is a series of soft, raspy notes.

Content.—A single species is placed in the group: *Eleutherodactylus* (*Euhyas*) *jugans*.

Distribution.—The species is distributed in the Massif de la Selle of Haiti and the western end of the adjacent Sierra de Baoruco in the Dominican Republic, in southern Hispaniola.

Remarks.—For discussion of the confusion in relationships between this species and *E. parabates*, see above in Remarks of *Eleutherodactylus (Eleutherodactylus) abbotti* Species Group. In addition, this species was considered to be closely related to *E. ventrilineatus*, a similarly dark, robust species in the Massif de al Hotte (Schwartz 1964). However, the DNA sequence evidence (Hedges et al. 2008a) shows that the two species are not close relatives and are convergent in appearance.

Eleutherodactylus (Euhyas) oxyrhyncus Species Group

Definition.—Species in this group are robust in body shape and moderate to large in SVL, ranging from 43 mm (females, *E. apostates*) to 55 mm (females, *E. oxyrhyncus*). They have relatively long snouts, long legs, and small digital discs. Sexual size dimorphism is pronounced; males are much smaller than females (e.g., approximately one-half the length and a small fraction of the mass) and may represent the most extreme sexual size dimorphism within the family. They are variable in color and pattern, although the ground color is usually tan, brown, or gray-brown. The calls of these terrestrial frogs are either soft, raspy notes (*E. apostates*) or chirps (*E. oxyrhyncus*).

Content.—Two species are placed in the group: *Eleutherodactylus* (*Euhyas*) *apostates* and *E. oxyrhyncus*.

Distribution.—The species group is distributed on the Tiburon Peninsula in southern Hispaniola, including the Massif de la Hotte and the Massif de la Selle of Haiti.

Remarks.—This is a pair of long-snouted, long-legged, terrestrial species that in many ways resemble, convergently, species in the *Eleutherodactylus* (*Euhyas*) *dimidiatus* Species Group of Cuba. In the Massif de la Hotte, *E. oxyrhyncus* is the more abundant of the two species on the North Slope whereas *E. apostates* is the most abundant, if not the only species of the pair, on the South Slope. The two species are sympatric at least at Castillon in the northern portion of mountain range. One of us (SBH) has noted differences in the geographic isolates of *E. oxyrhyncus* in the Massif de la Hotte compared with those in the Massif de al Selle that may warrant recognition of the latter isolate as a separate species.

Eleutherodactylus (Euhyas) paulsoni Species Group

Definition.—The single species in this group is moderate in body shape and small in SVL (females, 26 mm). It has small (slightly expanded) digital discs and a noticeably and evenly tuberculate dorsum. The dorsum is mottled brown, usually with a pinkish wash in the posterior half of the body. The call of this terrestrial frog is unknown.

Content.—A single species is placed in the group: *Eleutherodactylus* (*Euhyas*) *paulsoni*.

Distribution.—This species is restricted to Haitian Tiburon Peninsula in southern Hispaniola.

Remarks.—This is one of two predominantly lowland species in the entire *Eleutherodactylus oxyrhyncus* Species Series; the other is *E. caribe*, known only from a single brackish marsh in Haiti.

Eleutherodactylus (Euhyas) rufifemoralis Species Group

Definition.—Species in this group are moderate in body shape and small to moderate in SVL, ranging from 18 mm (females, *E. rufifemoralis*) to 37 mm (females, *E. furcyensis*). They have small digital discs. The ground color is gray or brown; the snout is bluish gray, and the concealed areas of the limbs and groin are red or orange. These frogs are terrestrial. The call of *E. furcyensis* is an irregular series of soft ticks and peeps.

Content.—Two species are placed in the group: *Eleutherodactylus* (*Euhyas*) *furcyensis* and *rufifemoralis*.

Distribution.—This species group is distributed in southern Hispaniola, including Haiti and the Dominican Republic.

Remarks.—This is an allopatric pair of closely related species occupying a connected pair of mountain ranges, the Massif de la Selle (Haiti) and Sierra de Baoruco (Dominican Republic) in Hispaniola.

Eleutherodactylus (Euhyas) planirostris Species Series

Definition.—Species in this series are variable in body shape and small to large in SVL, ranging from 11 mm (females, *E. iberia*) to 62 mm (females, *E. pinarensis*). The included species have small to large digital discs. They are variable in coloration. Most are terrestrial or saxicolous and have calls consisting of a series of chirps.

Content.—Six species groups (23 species) are placed in this series: the *Eleutherodactylus* (*Euhyas*) *atkinsi, gundlachi, limbatus, pezopetrus, pinarensis,* and *planirostris* species groups.

Distribution.—The species series is distributed on Cuba, the Cayman Islands, and the Bahamas. Whether the presence of *E. planirostris* in southern Florida is natural or

through human introduction is debated. However, the presence of *E. planirostris* in other areas (e.g., northern Florida, Georgia, Louisiana, Mississippi, Hawaii, and the Lesser Antilles) is from human introduction.

Remarks.—This is a large and diverse radiation of terrestrial and saxicolous species that evolved almost entirely on Cuba. As such, it is the largest single adaptive radiation of frogs on Cuba. All other adaptive radiations of frogs of the subgenus *Euhyas* in Cuba include only 2–4 species. See Remarks above under *Eleutherodactylus (Euhyas) oxyrhyncus* Species Series for ecological and evolutionary comparison of these two major species series on neighboring islands.

Eleutherodactylus (Euhyas) atkinsi Species Group

Definition.—The single species in this group is robust in body shape and moderate in SVL (females, 43 mm). It has small digital discs. It is reddish-brown, tan, or gray with dark spots in the groin and on the thighs. This terrestrial species emits a series of chirps.

Content.—A single species is placed in the group: *Eleutherodactylus* (*Euhyas*) *atkinsi*.

Distribution.—The species is restricted to Cuba.

Remarks.—This species has an unusually broad distribution throughout Cuba and has adapted well to human modifications of natural habitats. A subspecies, *E. a. estradai*, occurs in extreme eastern Cuba, adjacent to the range of *E. a. atkinsi*. The status of this taxon is in need of assessment.

Eleutherodactylus (Euhyas) gundlachi Species Group

Definition.—Species in this group are robust in body shape and small in SVL, ranging from 14 mm (females, *E. tetajulia*) to 23 mm (females, *E. gundlachi*). They have short snouts, short legs, short digits, and small digital discs, although *E. gundlachi* is moderate in all of those characters. Several species (*E. adelus*, *gundlachi*, and *varleyi*) have a distinctly tuberculate dorsum. The dorsum is mostly gray, brown, or grayish brown with or without pale dorsolateral stripes. Most species are found on the ground, but *E. adelus* and *E. varleyi* inhabit grasses, and *E. gundlachi* occurs on the ground or among rocks. The calls of most species consist of a faint chirping noise with species-specific differences (Estrada and Hedges 1996b; Díaz et al. 2003).

Content.—Five species are placed in the group: *Eleutherodactylus* (*Euhyas*) *adelus, gundlachi, intermedius, tetajulia,* and *varleyi*.

Distribution.—The species group is distributed on Cuba.

Remarks.— The soft calls and relatively cryptic behavior of these species, which may call from cavities (e.g., *E. intermedius* and *E. tetajulia*) or beneath grass and leaf litter (*E.*

adelus and *E. varleyi*) suggest that there are more, as yet unnamed species in this group awaiting discovery.

Eleutherodactylus (Euhyas) limbatus Species Group

Definition.—Species in this group are mostly robust in body shape and small in SVL, ranging from 11 mm (females, *E. iberia*) to 19 mm (females, *E. etheridgei*). Body shapes vary greatly, from robust (most species) to indented at midbody (*E. orientalis*) to slender with a long snout (*E. jaumei*); all have short legs. In all species, digital discs are small, and in all except *E. etheridgei* digits are distinctly short. All species except *E. etheridgei* have pale dorsolateral stripes. Most of these terrestrial species emit a faint chirping noise that is species-specific.

Content.—Six species are placed in the group: *Eleutherodactylus* (*Euhyas*) *cubanus, etheridgei, iberia, jaumei, limbatus*, and *orientalis*.

Distribution.—The species group is distributed on Cuba; all species except *E. limbatus*, which is islandwide, are endemic to eastern Cuba.

Remarks.—This species group is a radiation of diminutive, short-legged, terrestrial species that evolved in eastern Cuba and have one wide-ranging member (*E. limbatus*). The inclusion here of *E. etheridgei* differs from previous characterizations of the group (Estrada and Hedges 1996a; Estrada and Alonso 1997) and is based primarily on the molecular phylogeny (Heinicke et al. 2007). However, that species also is small, short-legged, and has a slight midbody constriction that is more or less evident in other members of the group. One species in the group, *E. iberia*, is the smallest tetrapod in the northern hemisphere and is similar in size to the smallest tetrapod in the southern hemisphere, *Brachycephalus didactylus* (Estrada and Hedges 1996a).

Eleutherodactylus (Euhyas) pezopetrus Species Group

Definition.—The single species in this group is moderate in body shape and SVL (females, 49 mm). It has long legs, long digits, and large, ovate digital discs. The dorsal ground color is tan or greenish tan with brown mottling, pale dorsolateral stripes, or pale sacral blotches, and patches of orange tubercles on the dorsum. It is encountered on and around rocks and cliff faces. The call (a chirping sound) is a one- to three-notes (Díaz et al. 2007).

Content.—A single species is placed in the group: *Eleutherodactylus* (*Euhyas*) *pezopetrus*.

Distribution.—The species is endemic to eastern Cuba.

Remarks.—In size and body shape, this species resembles members of the *Eleutherodactylus cuneatus* Species Group of the *E. luteolus* Species Series, and has been compared with *E. cuneatus* (Díaz et al. 2007). However, the molecular phylogeny

(Heinicke et al. 2007) indicates that the resemblance is the result of convergence, and that this species is an unusually large member of the *E. planirostris* Species Series. The pattern polymorphisms (mottling and dorsolateral stripes) bear a greater resemblance to those in the *E. planirostris* Species Series and thereby support the molecular phylogeny.

Eleutherodactylus (Euhyas) pinarensis Species Group

Definition.—Species in this group are moderate in body shape and small to large in SVL, ranging from 30 mm (females, *E. blairhedgesi*) to 62 mm (females, *E. pinarensis*). They have a tuberculate dorsum, long digits, and large, ovate digital discs. The dorsum usually is tan, yellowish tan, or greenish brown with mottling or other markings. These primarily rock-dwelling frogs emit a series of one- to three-note trills (Díaz et al. 2007).

Content.—Three species are placed in the group: *Eleutherodactylus* (*Euhyas*) *blairhedgesi, pinarensis, and thomasi*.

Distribution.—The species group is distributed on Cuba.

Remarks.—This is a small radiation of tuberculate, saxicolous species that emit trills. One species (*E. thomasi*) is composed of three subspecies with disjunct distributions; these may be distinct species.

Eleutherodactylus (Euhyas) planirostris Species Group

Definition.—Species in this group are somewhat flattened in body shape and small to moderate in SVL, ranging from 23 mm (females, *E. guanahacabibes*) to 37 mm (females, *E. goini*). They have a tuberculate dorsum with or without a slight middorsal ridge, moderate to long legs, and digital discs of moderate size. The dorsum usually is tan, yellowish tan, greenish tan, or brown with two major pattern polymorphisms—mottled or pale dorsolateral stripes. They are primarily ground dwelling and rock dwelling, and most species emit an irregular series of faint chirps.

Content.—Seven species are placed in the group: *Eleutherodactylus* (*Euhyas*) *casparii, goini, guanahacabibes, planirostris, rogersi, simulans,* and *tonyi*.

Distribution.—The species group is distributed on Cuba, the Cayman Islands, and the Bahamas. Whether the presence of *E. planirostris* in southern Florida is natural or through human introduction is debated. However, the presence of *E. planirostris* in other areas (e.g., northern Florida, Georgia, Louisiana, Mississippi, Hawaii, and the Lesser Antilles) is from human introduction.

Remarks.—This species group is a moderate-sized radiation of mostly small, tuberculate, and ground-dwelling species with pattern polymorphisms and which are almost entirely allopatric in distribution. Their wide distribution and ability to successfully invade new areas (at least in one species, *E. planirostris*) probably derives from desiccation resistance (Stewart and Martin 1980). Several of the included species

were previously considered subspecies of *E. planirostris* (Schwartz 1974) but have since been elevated to species status (Estrada and Hedges 1997; Heinicke et al. 2007).

Eleutherodactylus (Euhyas) ricordii Species Series

Definition.—Species in this series are robust or moderate in body shape and small to moderate in SVL, ranging from 24 mm (females, *E. lucioi*) to 48 mm (females, *E. michaelschmidi*). They have small to slightly expanded digital discs. The dorsum usually is tan, yellowish tan, greenish tan, or reddish tan with either dark mottling or pale dorsolateral stripes. These frogs are primarily ground dwelling and rock dwelling. Most species emit a series of faint chirps that differ among species.

Content.—Two species groups (14 species) are placed in this series: the *Eleutherodactylus* (*Euhyas*) *lentus* and *ricordii* species groups.

Distribution.—This species series is distributed on Cuba, Hispaniola, Mona Island, and the Virgin Islands.

Remarks.— The two included species groups of this series each have more restricted geographic distributions, with the *Eleutherodactylus lentus* Species Group occupying the eastern islands in the Antilles and the *E. ricordii* Species Group distributed in the west (Cuba). Given this allopatric distribution and their close relationships (Heinicke et al. 2007), it can be inferred that they arose by either dispersal or vicariance between Cuba and Hispaniola.

Eleutherodactylus (Euhyas) lentus Species Group

Definition.—Species in this group are moderate in body shape and small to moderate in SVL, ranging from 24 mm (male only, *E. lucioi*) to 43 mm (females, *E. pictissimus*). Most have depressed bodies and small to slightly expanded digital discs. The dorsal ground color commonly is yellowish tan, with a reddish wash in some species, and a polymorphic pattern (typically dark mottling or pale dorsolateral lines). Most of these terrestrial species emit a faint chirping noise that is species specific.

Content.—Ten species are placed in the group. Nine of those previously were recognized as species—*Eleutherodactylus* (*Euhyas*) *grahami, lentus, lucioi, monensis, pictissimus, probolaeus, rhodesi, warreni*, and *weinlandi.* One was described as a subspecies of *E. weinlandi* and was elevated to species status—*Eleutherodactylus* (*Euhyas*) *paralius*.

Distribution.—The species group is distributed on Hispaniola, Mona Island, and the Virgin Islands.

Remarks.—This is a moderate (probably large when fully understood) radiation of terrestrial species; their distributions center on the Hispaniolan North Paleoisland, Mona Island, and the Virgin Islands. The absence of a species in this group from Puerto Rico is odd, because it leaves a major distributional hiatus between Mona Island (*E. monensis*)

and the Virgin Islands (*E. lentus*). Previously, *E. richmondi* was considered to be that Puerto Rican link, but that species is unquestionably a member of the subgenus *Eleutherodactylus* (see discussion above under Remarks for that subgenus). It is possible that a species of this group once occurred on Puerto Rico (not unlikely considering land connections during the Pleistocene) and was outcompeted by *E. richmondi*, which is somewhat similar ecologically, or other members of the subgenus *Eleutherodactylus*.

Schwartz (1965a; Schwartz 1976) described two subspecies of *E. weinlandi* on Hispaniola, both of which "are exceptionally distinct in numerous details of size, pattern, and coloration" (Schwartz 1976). In the case of *E. w. chersonesodes*, he found evidence of intergradation with *E. w. weinlandi* in a "compact" region where their distributions joined. However, the taxon recognized here as a full species, *E. paralius*, has not been found to intergrade with either of the other taxa despite close proximity of their ranges. Two subspecies of *E. pictissimus* also were described (Schwartz 1965a); additional study is needed to determine their taxonomic status. One of us (SBH) has collected both *E. pictissimus* and *weinlandi* throughout Hispaniola, and has encountered many specimens that do not conform to either species, suggesting that species group is larger than currently recognized.

Eleutherodactylus (Euhyas) ricordii Species Group

Definition.—Species in this group are robust in body shape and small to moderate in SVL, ranging from 25 mm (females, *E. acmonis*) to 48 mm (females, *E. michaelschmidi*). They have an evenly, mildly tuberculate (granular) dorsum with a slightly raised middorsal ridge, small to slightly expanded digital discs, and relatively large and dark eyes. The dorsum usually is tan, yellowish tan, greenish tan, or reddish tan with distinctive dark brown or black spots, blotches, or mottling, with or without pale dorsolateral stripes. Most of these primarily ground-dwelling and rock-dwelling species emit a series of faint chirps, with species-specific differences (Díaz et al. 2007).

Content.—Four species are placed in the group: *Eleutherodactylus* (*Euhyas*) *acmonis, bresslerae, michaelschmidi, and ricordii*.

Distribution.—The species group is restricted to eastern Cuba.

Remarks.— In many ways these species resemble those in the *Eleutherodactylus planirostris* Species Group of the *E. planirostris* Species Series. For many years *E. planirostris* was considered a subspecies of *E. ricordii*. Both groups consist of small- to moderate-sized, ground- and rock-dwelling species with patterns of mottling and dorsolateral stripes. The members of the *E. planirostris* Species Group appear more flattened in body shape, although that trait is difficult to measure. Also, members of the *E. ricordii* Species Group appear to have darker eyes that are relatively larger than those of species in the other group.

Eleutherodactylus (Euhyas) zugi Species Series

Definition.—Species in this series are robust in body shape and small in SVL, ranging from 19 mm (females, *E. zugi*) to 27 mm (females, *E. klinikowskii*). The dorsum has irregular, low tubercles and commonly a slight middorsal ridge. The legs are relatively short, and digital discs are small to moderate in size. The dorsum is pinkish tan, tan, or brown, and with pattern polymorphism (dorsolateral stripes or dark mottling). These frogs have been encountered on the ground and climbing on rocks and vegetation around rocky outcrops. Their calls consist of a series of faint insect-like clicks or chirps.

Content.—Three species are placed in the group. Two of these were previously recognized as species: *Eleutherodactylus* (*Euhyas*) *klinikowskii* and *zugi.* The third was described as a subspecies of *E. zugi* (Schwartz 1960) and was elevated to species status—*Eleutherodactylus* (*Euhyas*) *erythroproctus*.

Distribution.—This species series is restricted to western Cuba.

Remarks.—This is a radiation of small ground and rock-dwelling species in western Cuba. We recognize *Eleutherodactylus erythroproctus* as a full species because of its structural differences from *E. zug*i; it has short, rather than long, vomerine tooth rows (Schwartz 1960). Also it is geographically isolated from *E. zugi* with no evidence of intergradation. We place *E. klinikowskii* in this species series based on a close relationship with *E. zugi* in the DNA sequence analyses (Heinicke et al. 2007), even though the two species have not been considered close relatives in the past (additional material of both species needs to be compared, because the genetic difference is unusually low for valid species). However, certain structural and pattern elements are shared among the three species, but not necessarily in all specimens. These include a narrow middorsal ridge, a wide shank bar, dorsolateral stripes, and narrow dorsal cross-bars that are slightly chevron shaped.

Subgenus *Pelorius* Hedges, 1989

Pelorius Hedges, 1989:329. Type species: *Leptodactylus inoptatus* Barbour, 1914:252, by original designation.

Definition.—Members of the subgenus *Pelorius* can be defined as eleutherodactylid frogs having: (1) head narrow; (2) tympanic membrane differentiated; (3) cranial crests present or absent; (4) dentigerous process of vomers present; (5) "S" condition of the adductor muscles; (6) terminal discs on digits expanded, bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I longer than Finger II (shorter than Finger II in *E. chlorophenax*, *E. hypostenor*, and *E. nortoni*); (8) Toe V longer than Toe III; (9) subarticular tubercles prominent; (10) texture of skin on dorsum usually smooth (tuberculate in *E. nortoni*); (11) texture of skin on venter smooth; (12) range in SVL from 48 mm in female *E. aporostegus* to 88 mm in female *E. inoptatus.* All have internal, subgular vocal sacs (Hedges and Thomas 1987) and the two lobes of the liver are approximately the same length and shape.

Content.—The subgenus contains two species series (nine species): the *Eleutherodactylus* (*Pelorius*) *inoptatus* and *ruthae* Species Series.

Distribution.—The genus is endemic to the island of Hispaniola in the West Indies.

Etymology.—The subgeneric name is derived from the Greek adjective *pelorios,* meaning huge or prodigious; the name, which is masculine, was proposed in reference to the comparative large sizes of the included species.

Remarks.—See the Remarks under the genus *Eleutherodactylus* for a discussion of the taxonomic history of the subgenera. Lynch (1996b) and Lynch and Duellman (1997) questioned the monophyly of *Pelorius*, but that subgenus is supported by allozyme data (Hedges 1989a) and by DNA sequence data (Heinicke et al. 2007). Even before those studies, it was recognized as a monophyletic species group of *Eleutherodactylus* based on external morphological traits (Schwartz 1965b; Hedges and Thomas 1987).

Eleutherodactylus (Pelorius) inoptatus Species Series

Definition.—Species in this series are large, ranging in SVL from 66 mm (females, *E. nortoni*) to 88 mm (females, *E. inoptatus*), sexually dimorphic in size and robust in shape. They have moderately to greatly enlarged digital discs, cranial crests, an otic shelf on the cranium, and bars on the shanks that are not chevron-shaped; they lack dermal cornification on the tip of the snout. Normally they do not burrow in the ground or call from underground cavities.

Content.—Three species are placed in the series: *Eleutherodactylus* (*Pelorius*) *chlorophenax, inoptatus, and nortoni*.

Distribution.—The species series is distributed throughout the countries of the Dominican Republic and Haiti on the island of Hispaniola.

Remarks.— This series was initially defined by Hedges (1989a), based on protein variation and body size. Lynch (1996b) identified additional osteological characters, and Heinicke et al. (2007) added DNA sequence evidence. One species, *Eleutherodactylus chlorophenax*, is poorly known. It was described from a single specimen from the north slope of the Massif de La Hotte, and no additional specimens have been collected in that region. Hedges and Thomas (1987) collected a specimen on the south slope of the La Hotte but were unable to record its call.

Eleutherodactylus (Pelorius) ruthae Species Series

Definition.—Species in this series are moderate to large in SVL, ranging from 48 mm (females, *E. aporostegus*) to 58 mm (females, *E. ruthae*), and apparently are not sexually dimorphic in size, although females of only one species are known. They are robust in shape with a shovel-shaped snout with an unpigmented dermal cornification on the tip, moderately enlarged digital discs, and bars on the shanks that are chevron-shaped. They lack cranial crests and an otic shelf on the cranium. Normally they burrow in the ground or call from underground cavities

Content.—Six species are placed in the series. Three of those were previously recognized as a species: *Eleutherodactylus* (*Pelorius*) *hypostenor, parapelates,* and *ruthae.* Three additional taxa have been recognized as subspecies of *E. ruthae* (Schwartz 1965b) and were elevated to species status—*Eleutherodactylus* (*Pelorius*) *aporostegus, Eleutherodactylus* (*Pelorius*) *bothroboans,* and *Eleutherodactylus* (*Pelorius*) *tychathrous*.

Distribution.—The species series is distributed in disjunct populations within the countries of the Dominican Republic and Haiti on the island of Hispaniola.

Remarks.— Schwartz (1965b) described three subspecies of *E. ruthae* from Hispaniola. These have disjunct distributions separated by intervening areas with no known populations and no evidence of intergradation among the subspecies. They have pattern differences, non-overlapping structural differences (e.g., leg proportions), and different mating calls (Schwartz 1965b). By criteria currently used to distinguish different species of the genus *Eleutherodactylus*, these three subspecies are recognized as distinct species here. Based on call differences in other isolated populations of this complex, it is likely that additional species remain to be discovered and described (Hedges and Thomas 1987).

This series was initially defined by Hedges (1989a) as a species group based on protein variation, body size, and leg pattern. Lynch (1996b) identified additional osteological characters, and sequence evidence was added by Heinicke et al. (2007). The largest species, *E. tychathrous*, is known only from the holotype collected 45 years ago, and the distributions of all of the species are spotty. Males construct and call from enclosed underground chambers that have no surface evidence or exit hole. One of us (SBH) observed this behavior in a captive specimen, in which it used its snout and all four limbs to construct the chamber. Hatchlings of *E. aporostegus* were encountered inside a chamber that was opened (Schwartz 1965b); presumably they lay eggs in the chambers. Only a few females, all of *E. ruthae,* are known; these were encountered above ground. All known specimens of the remaining five species are males that were secured while they were vocalizing from their underground chambers.

Subgenus *Schwartzius* Hedges, Duellman, and Heinicke, 2008a

Schwartzius Hedges, Duellman, and Heinicke, 2008a:87. Type species: *Eleutherodactylus counouspeus* Schwartz, 1964:2, by original designation.

Definition.—The sole member of the subgenus *Schwartzius* can be defined as an eleutherodactylid frog having: (1) head narrow; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous process of vomers present; (5) "S" condition of the adductor muscles; (6) terminal discs on digits expanded, bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I shorter than Finger II; (8) Toe V shorter than Toe III; (9) subarticular tubercles prominent; (10) texture of skin on dorsum smooth; (11) texture of skin on venter smooth; (12) maximum SVL 57 mm in female *Eleutherodactylus (Schwartzius) counouspeus.*

Content.—This subgenus includes only one Haitian species, *Eleutherodactylus (Schwartzius) counouspeus*.

Distribution.—The subgenus is found only at the western end of the Haitian Tiburon peninsula of southwestern Hispaniola.

Etymology.—This subgenus is named in memory of Albert Schwartz (1923–1992) for his contributions to the herpetology of the West Indies.

Remarks.—This subgenus is required to accommodate *Eleutherodactylus (Schwartzius) counouspeus*, which branches basally within the Eastern Caribbean Clade of *Eleutherodactylus* according to DNA sequence analysis (Heinicke et al. 2007). Morphologically, it has a suite of characters that support this basal position and exclude it from either subgenus in the clade (*Eleutherodactylus* or *Pelorius*). In the original description, (Schwartz 1964) placed it in the "*Eleutherodactylus*" *ricordii* Group (now part of the subgenus *Euhyas*), probably because of its smooth venter and rock-dwelling habits, although this was not explicitly stated. In the allozyme analysis by Hedges (1989a), it was too divergent from all other species to associate it with any subgenus, and therefore it was left "unassigned to series" within the *auriculatus* section (= subgenus *Eleutherodactylus*). This was done because the vomerine tooth rows are short (usually long in the subgenus *Euhyas*), the vocal sac is external (although not prominent; vocal sac is internal or absent in most species of *Euhyas*), and the liver has a short, rounded left lobe (long and pointed in *Euhyas*). Joglar (1989) also associated *E. counouspeus* with the subgenus *Eleutherodactylus* (the West Indian portion of his "*Eleutherodactylus*" *unistrigatus* Group). However, Lynch and Duellman (Lynch and Duellman 1997) returned the species to the subgenus *Euhyas*, apparently based on the presence of a short Toe V relative to Toe II. From this discussion, it can be seen that this species does not fit readily in any named subgenus, and therefore its phylogenetic position as a basal branch in the Eastern Caribbean Clade was not unexpected. The characters allying it with *Euhyas*, such as a smooth venter, inguinal glands (contra Schwartz 1964), and short Toe V, can be interpreted as primitive characters shared with the Western Caribbean Clade.

Subgenus *Syrrhophus* Cope, 1878

Epirhexis Cope, 1866b:96. Type species: *Batrachyla longipes* Baird, 1859:35, by original designation. Suppression of generic name requested by Lynch (1967a:313–315); officially suppressed 1974.

Syrrhophus Cope. 1878:253. Type species: *Syrrhophus marnockii* Cope, 1878:253, by monotypy. Official list of generic names 1974.

Malachylodes Cope, 1879:264. Type species: *Malachylodes guttilatus* Cope, 1879:264, by monotypy. Synonymy by Boulenger (1888:206).

Tomodactylus Günther, 1900:219. Type species: *Tomodactylus amulae* Günther, 1900:219. Synonymy by Hedges (1989a:318).

Definition.—Members of the subgenus *Syrrhophus* can be defined as eleutherodactylid frogs having: (1) head narrow or wide; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous process of vomers small or absent; (5) "S" condition of the adductor muscles; (6) terminal discs on digits expanded, bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I about

equal in length to Finger II (slightly shorter or slightly longer than Finger II in various species); (8) Toe V shorter than Toe III; (9) subarticular tubercles prominent; (10) texture of skin on dorsum variable; (11) texture of skin on venter variable; (12) range in SVL from 19 mm in males of *E. pallidus* to 83 mm in females of *E. zeus.* Additionally, the left lobe of the liver is long and pointed whereas the right lobe is smaller and rounded.

Content.—Two species series (six species groups and 26 species) are recognized: the *Eleutherodactylus* (*Syrrhophus*) *longipes* and *symingtoni* species series.

Distribution.—The subgenus occurs from southern Texas, USA through Mexico to Belize and Guatemala. Two species, *Eleutherodactylus* (*Syrrhophus*) *symingtoni* and *zeus,* occur in western Cuba.

Etymology.—The subgeneric name is derived from the Greek *syrrhaptos*, meaning sewn together, in reference to the united outer metatarsals, or in reference to the condition of "the nasal bones [in forming] a close continuous roof" (Cope 1878).

Remarks.—See the Remarks under the genus *Eleutherodactylus* for a discussion of the taxonomic history of the subgenera. In the previous DNA sequence study, it was discovered that two species from western Cuba formerly placed in the subgenus *Euhyas*, *E. symingtoni* and *E. zeus*, clustered with *E. marnockii* in the molecular phylogeny (Heinicke et al. 2007). For this reason, they were transferred to the subgenus *Syrrhophus*. Their presence in Cuba supports the inference that mainland members of the subgenus *Syrrhophus* arose through dispersal from Cuba. Because in both Cuban species (each others' closest relatives) the dentigerous processes of the vomers are present (absent in mainland species of the subgenus), it can be inferred that they represent a basal branch of the *Syrrhophus* radiation. Our newer phylogeny (Hedges et al. 2008a) now includes representatives of three mainland species groups of the subgenus and they form a monophyletic group (100% support), further supporting the definition of a mainland clade (*E. longipes* Species Series). Although it removes the absence of dentigerous processes as defining character of the subgenus, it is relevant that those two Cuban species have short processes, whereas most species of *Euhyas* have long processes. They are so short that the original describer (Schwartz 1958a) debated as to whether they should be placed in the "*Eleutherodactylus auriculatus*" group (= current subgenus *Eleutherodactylus*). A second character allying them with the mainland species of the subgenus *Syrrhophus* is the relative length of the first and second fingers. In the subgenus *Euhyas*, the first finger is normally shorter than the second, whereas in mainland *Syrrhophus* it is variable but the two fingers are approximately equal in length (Lynch and Duellman 1997). In the two Cuban species of the subgenus, they are approximately equal in length as well. Lynch and Duellman (1997) defined species in the subgenus *Syrrhophus* as lacking sexual size dimorphism, yet females are larger than males in all nine species that had measurements of four or more specimens of each sex (Lynch 1970).

The species groups currently recognized for this subgenus were defined by Lynch (1970), with the addition of the species formerly placed in the genus *Tomodactylus*. Hedges (1989a) recognized two higher level groupings as species series: the *longipes* species series (species formerly placed in the genus *Syrrhophus*) and the *nitidus* species

series (species formerly placed in the genus *Tomodactylus*). Our new molecular phylogeny (Hedges et al. 2008a) shows that the distinction of the former genera *Syrrhophus* and *Tomodactylus* was artificial, because *E. nitidus* ("*Tomodactylus*") appears in a nested position among species of the former genus "*Syrrhophus*". Therefore, we recognize only a mainland clade (*E. longipes* Species Series, with six species groups) and a Cuban clade (*E. symingtoni* Species Series).

Eleutherodactylus (Syrrhophus) longipes Species Series

Definition.—Species in this series are robust to moderate in body shape and small to moderate in SVL, ranging from 19 mm (males only, *E. pallidus*) to 40 mm (females, *E. longipes*). Dentigerous process of the vomers and compact lumbar glands are absent.

Content.—Six species groups (24 species) are placed in this species series: the *Eleutherodactylus* (Syrrhophus) *leprus*, *longipes, marnockii, modestus, nitidus,* and *pipilans* species groups.

Distribution.—The species series is distributed from southern Texas, USA through Mexico to Belize and Guatemala. Most species occur at low to moderate elevations.

Remarks.—This species series corresponds to the content of the combined genera *Syrrhophus* and *Tomodactylus,* as was previously recognized (Lynch 1970). In the accounts of the species groups, we use the same character definitions as were used by Lynch (1970).

Eleutherodactylus (Syrrhophus) leprus Species Group

Definition.—Species in this group are robust to moderate in body shape and small in SVL, ranging from 24 mm (males only, *E. rubrimaculatus*) to 29 mm (females, *E. leprus*). The snout is acuminate or subacuminate, the first finger is slightly shorter or slightly longer than the second, the digits lack distinct lateral fringes, the digital discs are small, and the outer metatarsal tubercle is conical.

Content.—Three species are placed in the group: *Eleutherodactylus* (*Syrrhophus*) *cystignathoides, leprus,* and *rubrimaculatus*.

Distribution.—The species group is distributed from southern Texas (USA) and eastern Mexico to the Isthmus of Tehuantepec and northern Guatemala and Belize.

Remarks.—The definition of this species series is adapted from Lynch (1970).

Eleutherodactylus (Syrrhophus) longipes Species Group

Definition.—Species in this group are robust to moderate in body shape and moderate in SVL, ranging from 32 mm (females, *E. dennisi*) to 40 mm (females, *E. longipes*). The snout is acuminate, the first finger is slightly shorter than the second, the digits bear

lateral fringes, the digital discs are large and ovate, and the outer metatarsal tubercle is not conical.

Content.—Two species are placed in the group: *Eleutherodactylus* (*Syrrhophus*) *dennisi* and *longipes*.

Distribution.—The species group occurs in the Sierra Madre Oriental from central Nuevo Léon to northern Hidalgo in eastern Mexico.

Remarks.—The definition of this species group is adapted from Lynch (1970).

Eleutherodactylus (Syrrhophus) marnockii Species Group

Definition.—Species in this group are robust in body shape and small to moderate in SVL, ranging from 20 mm (*E. verruculatus*) to 35 mm (females, *E. marnockii*). The snout is rounded, the first finger is slightly shorter or equal in length to the second, the digits lack lateral fringes, the digital discs are moderate to large in size and rounded or truncate in outline, and the outer metatarsal tubercle is not conical.

Content.—Four species are placed in the group: *Eleutherodactylus* (*Syrrhophus*) *guttilatus, marnockii, verrucipes*, and *verruculatus*.

Distribution.—The species group is distributed primarily on the Mexican Plateau and in the Sierra Madre Oriental of central and eastern Mexico, from southern Texas (USA) west to central Durango and south to Hidalgo and west-central Veracruz, Mexico.

Remarks.—The definition of this species group is adapted from Lynch (1970). Firschein (1954) considered "*Syrrhophus*" *verruculatus* to be a *nomen dubium* which should be omitted from lists of valid species. Lynch and Duellman (1997) followed this recommendation, although Frost et al. (2006) recognized it as valid. Firschein (1954) considered it a *nomen dubium* because he found errors in the type locality and because all specimens allocated to this species, other than the type, were done so in error. Firschein (1954) said that he was "inclined to believe that the type belonged to some other genus of the family Leptodactylidae." However, he did not examine the type (nor have we done so) and noted that E. R. Dunn's examination of the type revealed that it lacks vomerine teeth and lumbar glands. However, considering the type locality, these characters would place it within *Syrrhophus*. For these reasons, we continue to recognize this species as valid. It may well be a valid species represented by a single specimen. Also, the error in the type locality (Huatusco, Veracruz, Mexico) was relatively minor (a misspelling of one letter), and there was good evidence, as Firschein conceded, that it was collected in that region of Mexico. We tentatively assign *E. verruculatus* to the *E. marnockii* Species Group based on its large digital discs and skin texture (tuberculate dorsum and areolate belly), as noted in the original description. The only other species of the *E. longipes* Species Series with a tuberculate dorsum and areolate venter is *E. verrucipes*, also in this species group.

Eleutherodactylus (Syrrhophus) modestus Species Group

Definition.—Species in this group are robust to moderate in body shape and small in SVL, ranging from 19 mm (males only, *E. pallidus*) to 27 mm (females, *E. interorbitalis*). The snout is subacuminate, the first finger is slightly shorter than the second, the digits bear poorly defined lateral fringes, the digital discs are moderate to large and truncate in outline, and the inner metatarsal tubercle is twice as large (or larger) as the outer metatarsal tubercle.

Content.—Five species are placed in the group: *Eleutherodactylus* (*Syrrhophus*) *interorbitalis, modestus, nivicolimae, pallidus,* and *teretistes*.

Distribution.—The species group is distributed in the Pacific lowlands and the Sierra Madre Occidental of western Mexico, from Sinaloa and Durango south to Colima, and includes the Tres Marias Islands.

Remarks.—The definition of this species group is adapted from Lynch (1970).

Eleutherodactylus (Syrrhophus) nitidus Species Group

Definition.—Species in this group are robust to moderate in body shape and small to moderate in SVL, ranging from 23 mm (males, *E. rufescens*) to 32 mm (males, *E. saxatilis*). They have relatively short legs and a granular (areolate) venter. Dentigerous processes of the vomers are absent. Compact lumbar glands are present.

Content.—Nine species are placed in this species group: *Eleutherodactylus* (*Syrrhophus*) *albolabris*, *angustidigitorum, dilatus, grandis, maurus, nitidus, rufescens, saxatilis,* and *syristes*.

Distribution.—Central, west-central, and southern Mexico from the Sierra Madre Occidental in southwest Durango south Oaxaca.

Remarks.—This species group corresponds to the content of the genus *Tomodactylus* as was previously recognized (Lynch 1970). It was distinguished from "*Syrrhophus*" (= the *Eleutherodactylus (Syrrhophus) longipes* Species Series) primarily by the presence of compact lumber glands (Lynch 1968a, 1971). It is recognized as a species group here rather than a species series because of the phylogenetic results showing that it is more closely related to one species group (*E. pipilans*) of the former genus *Syrrhophus* than to another species group (*E. marnockii*) of that former genus.

Hedges (1989a) provided the replacement name *Eleutherodactylus maurus* (for *E. fuscus* Davis and Dixon 1955, preoccupied by *E. fuscus* Lynn and Dent 1943). Dixon (1957) suggested four groupings within the assemblage that is referred to here as the *Eleutherodactylus* (*Syrrhophus*) *nitidus* Species Group. One of those groups included the species *E. angustidigitorum*, *E. grandis*, and *E. maurus*. However, the remaining three groups included single species, and the subsequently described species, *E. rufescens* and *E. saxatilis*, also do not fit readily into those four groups. Rather than recognize five or

six species subgroups for these nine species, we have chosen not to recognize any divisions within the species group until a review or phylogenetic analysis is undertaken.

Eleutherodactylus (Syrrhophus) pipilans Species Group

Definition.—The single species in this group is robust in body shape and small in SVL (females, 29 mm). The snout is subacuminate, the first finger is equal in length to the second, the digits lack lateral fringes, the digital discs are small, and the metatarsal tubercles are subequal in size.

Content.—A single species is placed in the group: *Eleutherodactylus* (*Syrrhophus*) *pipilans*.

Distribution.—The species is distributed from south-central Mexico (states of Mexico, Guerrero, Oaxaca, and Chiapas) to southwestern Guatemala.

Remarks.—The definition of this species group is adapted from Lynch (1970).

Eleutherodactylus (Syrrhophus) symingtoni Species Series

Definition.—Species in this series are moderate in body shape and large in SVL, ranging from 69 mm (females, *E. symingtoni*) to 83 mm (females, *E. zeus*). The dentigerous processes of the vomers are short. Lumbar glands are absent. The dorsum is tuberculate (heavily so in *E. symingtoni*), with one or more canthal spines. They have long digits, and digital disc size varies from small (*E. symingtoni*) to large (*E. zeus*). The dorsum is either dark brown to brown (*E. symingtoni*) or olive-brown to bluish-brown (*E. zeus*). These terrestrial frogs mostly inhabit rocks. The calls consist of a low-frequency whistle-like noise (Díaz et al. 2007).

Content.—Two species are placed in this species series: *Eleutherodactylus* (*Syrrhophus*) *symingtoni* and *zeus*.

Distribution.—The species series is restricted to low to moderate elevations in western Cuba.

Remarks.— These two sympatric species are large, have short vomerine dentigerous processes, and share distinctive canthal tubercles, traits that indicate that they are close relatives (Schwartz 1958a).

Subfamily Phyzelaphryninae, Hedges, Duellman, and Heinicke, 2008a

Phyzelaphryninae Hedges, Duellman, and Heinicke, 2008a:93. Type genus: *Phyzelaphryne* Heyer, 1977:152.

Type genus.—*Phyzelaphryne* Heyer, 1977:152.

Definition.—In these small eleutherodactylid frogs the terminal digits are not or barely expanded; the digits are pointed apically; the circumferential grooves are weak or in *Phyzelaphryne* evident only laterally; Finger IV has three phalanges (only two in some *Adelophyne*). The species are inhabitants of terrestrial leaf litter and none exceeds 20 mm in SVL.

Content.—The two genera contain seven species.

Distribution.—The species have discontinuous distributions in northeastern Brazil, the Guianan Region, and the Amazon Basin in South America.

Remarks.—The molecular support (Hedges et al. 2008a) for this subfamily is significant (99%). It supports the suggestion of a close relationship of the two genera by Hoogmoed and Lescure (1984), based on sharing of slightly expanded terminal discs that have incomplete circumferential grooves and pointed tips.

Genus *Adelophryne* Hoogmoed and Lescure, 1984

Adelophryne Hoogmoed and Lescure, 1984:92. Type species: *Adelophryne adiastola* Hoogmoed and Lescure, 1984:95, by original designation.

Definition.—These minute eleutherodactylid frogs are characterized by: (1) head no wider than body; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous processes of vomers small, transverse; (5) condition of adductor muscle unknown; (6) terminal discs on digits barely expanded, apically pointed, with circumferential grooves and discs; terminal phalanges knobbed or barely T-shaped; (7) Finger I shorter than Finger II; Finger IV with two (*A. adiastola* and *pachydactyla*) or three (*A. baturitensis, gutturosa,* and *maranguapensis*) phalanges; (8) Toes III longer than Toe V; (9) subarticular tubercles not projecting; (10) dorsum smooth; (11) venter smooth; (12) maximum SVL in females 17 mm.

Content.—Six species are presently recognized: *Adelophryne adiastola, baturitensis, gutturosa, maranguapensis, pachydactyla,* and *patamona*.

Distribution.—The genus has a discontinuous distribution in eastern and northeastern Brazil and in the Guiana Shield Region in northeastern South America, and in the upper Amazon Basin.

Etymology.—The generic name is derived from the Greek *adelos,* meaning unseen, unknown, or obscure, and the Greek *phryne,* meaning toad. The genus is feminine in gender.

Remarks.—These minute frogs inhabit leaf litter. Hoogmoed et al. (1994) provided a review of the genus and a key to the species.

Genus *Phyzelaphryne* Heyer, 1977

Phzelaphryne Heyer, 1977:152. Type species: *Phyzelaphryne miriamae* Heyer,1977:153, by original designation.

Definition.—This genus of eleutherodactylid is characterized by: (1) head not as wide as body; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous processes of vomers distinct, transverse; (5) "S + E" condition of adductor muscle; (6) terminal discs on digits not expanded, acuminate on Fingers III and IV and on toes; circumferential grooves present laterally; terminal phalanges T-shaped; (7) Finger I slightly shorter than Finger II about equal in length; (8) Toe III longer than Toe V; (9) subarticular tubercles protruding moderately; (10) dorsum shagreen; (11) venter smooth; (12) SVL to 20 mm in females.

Content.—One species is recognized: *Phyzelaphryne miriamae*.

Distribution.—The single species occurs in the drainages of the Rio Madeira and Rio Tapajos in Amazonian Brazil.

Etymology.—The generic name is derived from the Greek *phyzelos,* meaning shy, and the Greek *phryne,* meaning toad. The name is feminine in gender.

Remarks.—See comments in the subfamily account concerning the close relationship of this genus to *Adelophryne*.

Family Strabomantidae, Hedges, Duellman, and Heinicke, 2008a

Strabomantidae Hedges, Duellman, and Heinicke, 2008a:95. Type genus *Strabomantis* Peters, 1863:405.
Eleutherodactylinae (part) Lutz, 1954:157. Type genus *Eleutherodactylus* Duméril and Bibron, 1841:620.
Eleutherodactylini (part)—Lynch, 1971:142 [Tribe].
Brachycephalinae (part)—Dubois, 2005:4.
Brachycephalidae (part)—Frost et al. 2006.

Definition.—Frogs of the family Strabomantidae have: (1) sternum cartilaginous; (2) vertebral shield lacking; (3) transverse processes of posterior presacral vertebrae not broadly expanded; (4) cervical cotyles widely spaced; (5) eight presacral vertebrae, Presacrals I and II not fused; (6) cranial elements not co-ossified with overlying skin; (7) omosternum present; (8) sacral diapophyses rounded or barely dilated; (9) maxillary arch usually dentate; teeth blunt, pedicellate; (10) alary processes of premaxillae broad at base, usually directed dorsally or posterodorsally; (11) palatal shelf of premaxilla usually broad, indented or not; (12) pars facialis of maxilla usually deep, not exostosed; (13) palatal shelf of maxilla moderately broad, bearing pterygoid process or not; (14) maxillary arch complete; maxillae tapering posteriorly; quadratojugal slender; (15) nasals usually large with broad median contact; (16) nasals usually not in contact with maxillae or pterygoids; (17) nasals not in contact with frontoparietals; (18) frontoparietal fontanelle usually absent; (19) frontoparietals usually not exostosed; cranial crests present in *Strabomantis,* and some *Pristimantis;* (20) frontoparietals fused with prootics or not; (21) temporal arcade absent; (22) epiotic eminences prominent to indistinct; (23) carotid artery passing dorsal to cranial elements; (24) zygomatic ramus of squamosal broad to slender, usually not in contact with maxilla; (25) otic ramus of squamosal short to

elongate, expanded into otic plate or not; (26) squamosal-maxilla angle 44–67˚; (27) columella present, except in *Euparkerella* and *Holoaden*; fenestra ovalis directed laterally; (28) vomers variable in size, greatly reduced in *Euparkerella;* dentigerous processes absent in *Euparkerella, Noblella,* and most *Phrynopus* and *Psychrophrynella*; (29) neopalatines usually broad; slender in *Euparkerella, Holoaden, Phrynopus,* and *Psychrophrynella;* bearing odontoid ridge in *Oreobates;* (30) sphenethmoid usually entire, divided in *Euparkerella*; (31) anterior ramus of parasphenoid narrow to broad, not keeled; (32) parasphenoid alae at right angle to axis of skull or deflected posteriorly, usually not overlapped by pterygoids; (33) pterygoid lacking ventral flange; anterior ramus not reaching neopalatine, except in *Oreobates;* (34) occipital condyles small to large, stalked or not, widely separated medially; (35) mandible lacking odontoids; (36) terminal phalanges T-shaped, knobbed, or bearing hook-like lateral process (*Euparkerella*); (37) usually three phalanges in Finger IV (two in *Noblella myrmecoides*); Finger IV reduced or absent in *Euparkerella*; (38) Toe I fully developed and free; (39) alary process of hyoid plate on slender stalk or not; process absent in *Euparkerella* and *Holoaden;* (40) mandibular ramus of trigeminal nerve passing lateral to the *m. adductor mandibulae,* passing medially in some *Strabomantis,* anterior to the *m. adductor mandibulae* in *Pristimantis* (*Yunganastes*), passing between two slips of the muscle in *Noblella myrmecoides;* (41) prominent external body glands usually absent, entire dorsum with glands in *Holoaden,* and inguinal glands in *Euparkerella*, and inguinal and axillary glands in *Oreobates*; (42) males usually having single, median, subgular vocal sac (absent in *Holoaden,* unknown in *Atopophrynus*); (43) males having vocal slits and nonspinous nuptial pads or not; (44) fingers unwebbed; toes usually unwebbed or webbed basally, but webbing extensive in some *Strabomantis;* (45) terminal digits usually expanded with pads set off by distinct circumferential grooves; digits apically pointed in *Euparkerella, Geobatrachus* and *Noblella*; grooves absent in *Barycholos, Bryophryne, Euparkerella, Geobatrachus, Holoaden, Lynchius, Noblella, Oreobates, Phrynopus,* and *Psychrophrynella;* (46) inner and outer metatarsal tubercles present, inner tubercle not spade-like; (47) tympanic membrane and annulus well differentiated or not; (48) amplexus axillary, inguinal in at least some *Phrynopus;* (49) eggs deposited in terrestrial or arboreal situations and undergoing direct development; (50) range in SVL from 13 mm and 14 mm in male *Pristimantis imitatrix* and *Pyschrophrynella boettgeri,* respectively, to 106 mm in female *Strabomantis cheiroplethus.*

Content.—There are 527 species placed in two subfamilies and 16 genera, one of which contains three subgenera.

Distribution.—Fourteen genera are restricted to tropical and subtropical South America as far south as northwestern Argentina; the family is most diverse in western South America and is meagerly represented in eastern Brazil. *Pristimantis* and *Strabomantis* extend into Central America (to Honduras and Costa Rica, respectively) and the former extends into the Lesser Antilles.

Remarks.—This family is a more inclusive South American Clade than was defined in our earlier work (Heinicke et al. 2007) and includes all South American terraranans except a few species in the Craugastoridae and Eleutherodactylidae and the Southeast

Brazil Clade (Brachycephalidae) of 40 species. The vast majority of strabomantids are associated with the Andean uplift in the western and northwestern part of the continent. Five of the recognized genera—*Atopophrynus* (1 species), *Dischidodactylus* (2 species), *Euparkerella* (4 species), *Geobatrachus* (1 species), and *Niceforonia* (3 species)—are not included in the molecular analyses because tissues were not available for our use. *Niceforonia* and the monotypic *Atopophrynus* and *Geobatrachus* are endemic to Andean Colombia, and the two species of *Dischidodactylus* are known from two tepuis in southern Venezuela. Until now, *Niceforonia* has been a synonym of the strabomantid genus *Phrynopus*. The remaining four genera are placed here in Strabomantidae principally on the basis of geography.

Each of the four species of *Euparkerella* has a restricted distribution in the Atlantic Coastal Forest in southeastern Brazil. Members of this genus differ from other strabomantids in several morphological characters (absence of a columella, greatly reduced vomers, divided sphenethmoid, structure of terminal phalanges, and reduction or loss of Finger IV), whereas they share some features, especially phalangeal reduction, with *Brachycephalus* (Izecksohn 1988; Giaretta and Sawaya 1998). In contrast, Heyer (1975) found that *Euparkerella* and *Holoaden* (a strabomantid) were closest relatives in a phylogenetic analysis of morphological data. Molecular sequence data will be needed to confirm the position of this enigmatic genus.

Subfamily Holoadeninae, Hedges, Duellman, and Heinicke, 2008a

Holoadeninae Hedges, Duellman, and Heinicke, 2008a:97. Type genus *Holoaden* Miranda-Ribeiro, 1920:319.

Definition.—These are strabomantid frogs that have narrow terminal digits on the fingers and toes and lack circumferential grooves (present distally in *Noblella*); the toes are apically pointed in *Euparkerella* and some *Noblella,* and the terminal phalanges are knob-shaped (*Bryophryne, Holoaden,* and *Psychophrynella*), hook-shaped (*Euparkerella*), or weakly T-shaped (*Barycholos* and *Noblella*). The tympanic membrane is differentiated only in *Barycholos, Noblella,* and *Psychophrynella boettgeri.* These terrestrial frogs range in SVL from 14 mm in male *Psychrophrynella boettgeri* to 48 mm in female *Holoaden.*

Content.—The 45 currently recognized species are placed in six genera.

Distribution.—The subfamily is confined to South America; it occurs on the Pacific lowlands of Ecuador and southern Colombia, in the Andes of southern Ecuador, Peru, and Bolivia, and in the Amazon Basin; two genera (*Euparkerella* and *Holoaden* are endemic to the Atlantic Coastal Forest in southeastern Brazil.

Remarks.—This subfamily received moderately strong support (94%) in a ML molecular phylogeny and the Bayesian posterior probability was 100% (see Chapter 4).

Genus *Barycholos* Heyer, 1969

Barycholos Heyer, 1969:6. Type species: *Leptodactylus pulcher* Boulenger, 1898:122, by original designation.

Definition.—This genus is characterized by: (1) head as broad as body; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous processes of vomers small, transverse; (5) "S" condition of adductor muscle; (6) terminal discs on fingers not expanded, those of toes slightly expanded, round; circumferential grooves absent; terminal phalanges weakly T-shaped; (7) Finger I longer than Finger II; (8) Toe III longer than Toes V; (9) subarticular tubercles projecting; (10) dorsum smooth with short, longitudinal ridges; (11) venter smooth; (12) SVL to 31 mm in females.

Content.—Two species are placed in this genus: *Barycholos pulcher* and *ternetzi*.

Distribution.—One species occurs on the Pacific lowlands of Ecuador and the other inhabits highlands and lowlands in eastern Brazil from Maranháo to Goiás and Mato Grosso.

Etymology.—The generic name is derived from the Greek *baruxolos,* meaning savage, in reference to Jay M. Savage. The genus is masculine in gender.

Remarks.—This genus was originally placed in Leptodactylinae by Heyer (1969) and Lynch (1971), because of the condition of the body style of the pectoral girdle. Heyer (1975) found that *Barycholos* was allied with "*Eleutherodactylus*" and, later, Lynch (1980) determined that *Barycholos* was most closely related to a then member of the "*Eleutherodactylus*" *discoidalis* Group, "*E*." *nigrovittatus* (here placed in the strabomantine genus *Hypodactylus*). *Barycholos ternetzi* was included in the phylogenetic analyses of molecular data by Heinicke et al. (2007). Hedges et al. (2008a) included both species and they were found to be each others closest relatives (100% support), supporting the continued recognition of this genus. Based on the molecular phylogeny, *Barycholos* is closely related to *Noblella*. Unlike other holoadenines, these genera display weakly T-shaped terminal phalanges.

Genus *Bryophryne,* Hedges, Duellman, and Heinicke, 2008a

Bryophryne Hedges, Duellman, and Heinicke, 2008a:99. Type species *Phrynopus cophites* Lynch, 1975a:16 by original designation.
Phrynopus (in part)—Lynch, 1975a:8.

Definition.—This genus is characterized by (1) head narrow, not as wide as body; (2) tympanic membrane, tympanic annulus, columella, and cavum tympanicum absent (3) cranial crests absent; (4) dentigerous processes of vomers absent; (5) "S" condition of adductor muscle; (6) tips of digits narrow, rounded; circumferential groves absent; terminal phalanges knob-shaped; (7) Finger I shorter than Finger II; (8) Toes III and V about equal in length; (9) subarticular tubercles not projecting; (10) dorsum finely areolate; (11) venter coarsely areolate; (12) SVL to 29.3 mm.

Content.—This genus contains six species: *Bryophryne bustamantei*, *cophites, gymnotis, hanssaueri, nubilosus,* and *zonalis.*

Distribution.—The genus occurs at elevations of 2900–4120 m in the Cordillera Oriental in the Departamento de Cusco in southern Peru.

Etymology.—The generic name is derived from the Greek *bryon* meaning moss and the Greek *phrynos,* meaning toad. The name is feminine in gender and refers to a common habitat of these species.

Remarks.—At a time when some frogs now placed in *Bryophryne, Lynchius, Niceforonia, Phrynopus,* and *Psychrophrynella* were considered to be congeneric, *Bryophryne* (then *Phrynopus*) *cophites* was considered to be closely related to species of "*Phrynopus*" now placed in *Psychrophrynella* (Lynch 1975a; Cannatella 1984). Our analyses of sequence data (Hedges et al. 2008a) reveal that *Bryophryne* is the closest relative of a clade containing *Barycholos* and *Noblella lochites* within the Holoadeninae, with moderately strong support (81%) in the ML phylogeny and significant (97%) Bayesian support. The Holoadeninae also contains *Psychrophrynella* but not *Lynchius, Niceforonia,* and *Phrynopus*; the last three genera are in Strabomantinae.

Genus *Euparkerella* Griffiths, 1959

Euparkerella Griffiths, 1959:477. Type species: *Sminthillus brasiliensis* Parker, 1926:201, by original designation.

Definition.—This genus of relatively small species is characterized by: (1) head narrower than body; (2) tympanic membrane and annulus absent; (3) cranial crests absent; (4) dentigerous processes of vomers absent; (5) "S" condition of adductor muscle; (6) discs on digits small, pointed; circumferential grooves absent; terminal phalanges with small, hook-like lateral processes; Finger IV reduced or absent; (7) Fingers I and II about equal in length; (8) Toe III slightly longer than Toe V; (9) subarticular tubercles not projecting; (10) dorsum finely granular; (11) venter areolate; (12) SVL to 20 mm in females.

Content.—The genus contains four species: *Euparkerella brasiliensis, cochranae, robusta,* and *tridactyla*.

Distribution.—The distribution is restricted to the Atlantic Coastal Forest in southeastern Brazil.

Etymology.—The generic name, a patronym for H. W. Parker, who named the type species, has the Greek prefix *eu-,* meaning true, and the Greek suffix *–ella,* a diminutive form. The gender is feminine.

Remarks.—The phylogenetic relationships of these small frogs are unknown, and this genus has not been included in any molecular phylogeny. The similarity in reduction of Finger IV is like that in some species of *Noblella.* The presence of inguinal glands is shared with some species of *Syrrhophus.* The reduction in the number of phalanges and

of entire digits, as seen in *Euparkerella,* was compared with the even greater reduction in *Brachycephalus* (including *Psyllophryne*) by Izechsohn (1988) and Giaretta and Sawaya (1998). These possible relationships await much needed analyses of molecular data. Until then, we place this genus tentatively in the subfamily Holoadeninae, in part based on its association with *Holoaden* in some early phylogenetic analyses of morphological data (Heyer 1975).

Genus *Holoaden* Miranda-Ribeiro, 1920

Holoaden Miranda-Ribeiro, 1920:319. Type species: *Holoaden lüderwaldti* Miranda-Ribeiro, 1920:319, by monotypy.

Definition.—Frogs of the genus *Holoaden* are characterized by: (1) Head not as wide as body; (2) tympanic membrane and annulus absent; (3) cranial crests absent; (4) dentigerous processes of vomers prominent, transverse; (5) "S" condition of adductor muscle; (6) discs on digits small, rounded; circumferential grooves absent; terminal phalanges knob-shaped; (7) Finger I longer than Finger II; (8) Toe III longer than Toe V; (9) subarticular tubercles not protuberant; (10) dorsum highly glandular; (11) venter areolate; (12) SVL to 48 mm in females.

Content.—Three species are recognized in the genus: *Holoaden bradei, luederwaldti,* and *phloeter*.

Distribution.—*Holoaden* is endemic to the Atlantic Coastal Forest in southeastern Brazil.

Etymology.—The generic name is derived from the Greek *holos,* meaning whole or entire, and the Greek *aden,* meaning gland. The name refers to the dorsum being covered with pustular glands. The gender is neuter.

Remarks.—In the phylogenetic analyses of molecular data presented by Heinicke et al. (2007), *Holoaden bradei* was associated with various species of *Phrynopus* or *Phrynopus* and *Barycholos ternetzi.* Hedges et al. (2008a) included the other species, *H. luederwaldti*, and it clusters with *H. bradei* as expected. In their tree of mitochondrial and nuclear gene sequences the genus *Holoaden* clusters, with significant ML (96%) and Bayesian (100%) support, with the clade containing *Barycholos*, *Noblella*, and *Bryophryne*. The genus is unique among strabomantid frogs in having many rounded glands on the dorsum.

Genus *Noblella* Barbour, 1930

Noblella Barbour, 1930:81. Type species *Sminthillus peruvianus* Noble, 1921:1, by original designation.
Phyllonastes Heyer, 1977:151. Type species: *Euparkerella myrmecoides* Lynch, 1976b:50, by original designation. Synonymy by De la Riva et al., 2008:0.

Definition.—Species of *Noblella* are strabomantid frogs having: (1) head no wider than body; (2) tympanic membrane differentiated (except in *N. duellmani*); (3) cranial crests absent; (4) dentigerous processes of vomers absent; (5) "S" condition of adductor muscle;

(6) terminal discs on digits not or barely expanded; discs and circumferential grooves present distally (except in *N. duellmani*); terminal phalanges narrowly T-shaped; (7) Finger I shorter than, or equal in length to, Finger II; Finger IV containing only two phalanges in *N. carrascoicola, lochites, myrmecoides,* and *ritarasquinae;* (8) Toe III shorter than Toe V; tips of at least Toes III–IV acuminate; (9) subarticular tubercles not protruding; (10) dorsum pustulate or shagreen; (11) venter smooth; (12) SVL less than 22 mm.

Content.—Ten species are recognized: *Noblella carrascoicola, coloma, duellmani, heyeri, lochites, lynchi, myrmecoides, peruviana, pygmaea* and *ritarasquinae*.

Distribution.—Seven species occur in the Andes from extreme southern Ecuador to central Bolivia, and one species occurs in the Amazonian lowlands of Ecuador, Peru, and extreme western Brazil.

Etymology.—The generic name is a patronym for Gladwyn K. Noble, who described the first species (*N. peruviana*). The name is feminine in gender.

Remarks.—For the past three decades these small frogs have been recognized as *Phyllonastes,* a generic name proposed by Heyer (1977) for the small Amazonian species formerly known as *Euparkerella myrmecoides* Lynch (1976). Subsequently additional species were discovered and named. De la Riva et al. (2008) discovered that *Sminthillus peruvianus* Noble, 1921, was not a *Phrynopus,* a genus in which it has been placed for many years (Lynch 1975a), but instead possessed the features characteristic of frogs recognized as *Phyllonastes*. Consequently, they considered *Smithillus peruvianus* Noble, the type species of *Noblella* Barbour, 1930, to be congeneric with *Phyllonastes,* for which *Noblella* is an earlier name.

As noted by Lehr et al. (2004), *Noblella duellmani* lacks some features characteristic of other members of the genus—viz. discs and circumferential grooves on digits, tympanum, and suprainguinal spots. In these regards this species is like members of the genus *Phrynopus;* however, it has pointed tips of digits and an inner tarsal tubercle, features unique to *Noblella.*

The generic status and phylogenetic relationships of *Noblella* are unresolved. Using molecular data (12s and 16s mitochondrial genes), Lehr et al. (2005) created a maximum likelihood tree of 13 species of "*Phrynopus,*" in which an undetermined species of "*Phyllonastes*" from Departamento de San Martín, Peru, was the closest relative of *Phrynopus* (= *Hypodactylus*) *brunneus* from Ecuador. This species is closest to *Noblella lochites* in our molecular phylogeny (Hedges et al. 2008a). We have included only *N. lochites* in our more comprehensive analyses and it appears as the closest relative of *Barycholos* in Holoadeninae, with significant ML (100%) and Bayesian (100%) support.

Genus *Psychrophrynella,* Hedges, Duellman, and Heinicke, 2008a

Psychrophrynella Hedges, Duellman, and Heinicke, 2008a:102. Type species *Phrynopus bagrecito* Lynch, 1986b:428, by original designation.
Phrynopus (part)—Lynch, 1975a:8.

Type species.—*Phrynopus bagrecito* Lynch, 1986b:428.

Definition.—The small frogs of the genus *Psychrophrynella* are characterized by (1) head narrow, not as wide as body; (2) differentiated tympanic membrane and tympanic annulus usually absent (annulus visible beneath skin in some species; differentiated tympanic membrane in *P. boettgeri*); (3) cranial crests absent; (4) dentigerous processes of vomers usually absent; (5) "S" condition of adductor muscle; (6) tips of digits narrow, rounded, or bulbous, not expanded; circumferential groves absent; terminal phalanges knob-shaped; nuptial pads are absent; (7) Finger I shorter, equal to, or greater than Finger II; (8) Toe V usually slightly longer than Toe III; (9) subarticular tubercles not projecting; (10) dorsum smooth, granular, or shagreen; (11) venter finely granular, granular, or coarsely granular, although smooth in *P. pinguis*; (12) SVL in ranging from 14.0 mm in *P. boettgeri* to 33.4 mm in *P. wettsteini.*

Content.—Twenty species are recognized at this time: *Psychrophrynella adenopleura, ankohuma, bagrecito, boettgeri, chacaltaya, condoriri, guillei, harveyi, iani, iatamasi, illampu, illimani, kallawaya, katantika, kempffi, pinguis, quimsacruzis, saltator, usurpator,* and *wettsteini.*

Distribution.—The genus, as now recognized, occurs at elevations of 1830–4190 m in the Cordillera Oriental of the Andes in southern Peru and Bolivia.

Etymology.—The generic name is derived from the Greek *psychros* meaning cold and the Greek *phrynos* meaning toad with the Greek diminutive suffix *ella.* The name is feminine in gender and is used in allusion to the cold environments inhabited by these small frogs.

Remarks.—Of the species herein placed in *Psychrophrynella, P. iatamasi* and *wettsteini,* plus three unnamed species are contained in a southern Peru-Bolivian clade that is distinct from *Phrynopus* in central Peru in our molecular phylogeny (Hedges et al. 2008a). In an analysis of 12S and 16S mitochondrial genes of more species of *Phrynopus* (*sensu lato*) these same species plus *Psychrophrynella boettgeri* form a well-supported clade (E. Lehr, pers. comm.). *Psychrophrynella* is associated with *Barycholos, Bryophryne,* and *Holoaden* in Holoadeninae, in contrast to the association of *Phrynopus* with *Oreobates* in Strabomantinae. *Psychrophrynella* appears to be the basal genus within the Holoadeninae.

For the past three decades our definition of "*Phrynopus peruvianus*" has been based on the description by Lynch (1975a) of specimens from Abra Acanacu, Departamento de Cusco, Peru. According to De la Riva et al. (pers. comm.), who compared the type series of *Sminthillus peruvianus* with specimens from Abra Acanacu, the latter are not conspecific with *Sminthillus peruvianus,* which they consider to be congeneric with *Phyllonastes.* Thus the specific name *peruvianus* is not applicable to the frogs herein referred to that species, nor is the generic name *Noblella* available for these high Andean frogs, because *Noblella* is a senior synonym of *Phyllonastes* (De la Riva et al. 2008).

Subfamily Strabomantinae, Hedges, Duellman, and Heinicke, 2008a

Strabomantinae Hedges, Duellman, and Heinicke, 2008a:103. Type genus *Strabomantis* Peters, 1863:405.

Type genus.—*Strabomantis* Peters, 1863:405.

Definition.—These are strabomantid frogs that have expanded terminal digits on the fingers and toes (except *Hypodactylus, Lynchius, Niceforonia,* and *Phrynopus*) and have circumferential grooves (absent in *Lynchius, Niceforonia,* and *Phrynopus*). The terminal phalanges are T-shaped (knob-shaped in *Lynchius, Niceforonia, Oreobates,* and *Phrynopus*). The tympanic membrane usually is differentiated. Most species are arboreal, but others (e.g., *Geobatrachus, Niceforonia, Lynchius,* and *Phrynopus* are secretive and terrestrial, whereas some of the large species of *Strabomantis* are riparian; SVL varies from 13 mm in male *Pristimantis imitatrix* to 106 mm in *Strabomantis cheiroplethus.*

Content.—The 516 currently recognized species are placed in 10 genera, one of which has two subgenera.

Distribution.—This subfamily is widespread in tropical South America, where it is most diverse in the Andean regions of Colombia, Ecuador, and Peru. It extends as far south as northwestern Argentina; although it is reasonably diverse in northeastern South America, it does not occur in the Atlantic Coastal Forest of Brazil. *Pristimantis* and *Strabomantis* extend into Central America (to Honduras and Costa Rica, respectively) and the former extends into the Lesser Antilles.

Remarks.—This is largest clade of Terrarana, but because of the absence of tissues several small, but distinctive, genera (e.g., *Atopophrynus, Dischidodactylus, Geobatrachus, Niceforonia*) are not included in the molecular analyses, so their relationships are unknown. Likewise, because of the absence of tissues, *Pristimantis* and *Phrynopus* of diverse phenetic species groups are not represented. Moreover, we are aware of many undescribed species, especially of *Pristimantis.* Therefore, the present analyses and resulting classification must be regarded as an initial effort waiting to be expanded and refined.

Genus *Atopophrynus* Lynch and Ruiz-Carranza, 1982

Atopophrynus Lynch and Ruiz-Carranza, 1982:557. Type species: *Atopophrynus syntomopus* Lynch and Ruiz-Carranza, 1982:557, by original designation.

Definition.—This genus is characterized by (1) head narrow; (2) tympanic membrane and annulus absent; (3) cranial crests absent; (4) dentigerous processes of vomers absent; (5) "S = E" condition of adductor muscle; (6) terminal discs expanded (absent on Finger I); circumferential groove present; terminal phalanges T-shaped; (7) Finger 1 shorter than Finger II; (8) Toes III and V about equal in length; Toe I weak, concealed externally and adherent to Toe II; toes three-fourths webbed; (9) subarticular tubercles not projecting; (10) dorsum smooth; (11) venter smooth; (12) SVL less than 20 mm in females.

Content.—The genus contains a single species: *Atopophrynus syntomopus*.

Distribution.—*Atopophrynus* is known only from the crest of the Cordillera Central in Departamento de Antioquia, Colombia.

Etymology.—The generic name is derived from the Greek *atopos,* meaning strange or out of place, and the Greek *phryne,* meaning toad. The genus is masculine in gender.

Remarks.—This monotypic genus originally was placed in Dendrobatidae by Lynch and Ruiz-Carranza (1982). Myers and Ford (1986) unequivocally removed it from that family; their detailed observations on myology and osteology led them to consider the genus to be a sister taxon to *Geobatrachus.* Both monotypic genera share certain unique features among strabomantine frogs—concealed Toe I and a pair of slender anterior processes on each hyale of the hyoid. Neither genus has been included in any molecular phylogenetic analysis, so their placement in Strabomantinae is tentative. However, we place this pair of genera in this subfamily because one or both has T-shaped terminal phalanges, expanded terminal digits, and digital disks with circumferential grooves.

Genus *Dischidodactylus* Lynch, 1979

Dischidodactylus Lynch, 1979:5. Type species: *Elosia duidensis* Rivero, 1968:1, by original designation.

Definition.—This genus is characterized by: (1) head not as wide as body; (2) tympanic membrane not differentiated; tympanic annulus visible below skin; (3) cranial crests absent; (4) dentigerous processes of vomers small, oblique; (5) "S" condition of adductor muscle; (6) terminal discs expanded, rounded, bifurcate; circumferential groove present; terminal phalanges T-shaped; (7) Finger I shorter than Finger II; (8) Toe III longer than Toe V; (9) subarticular tubercles not protruding; (10) dorsum granular; (11) venter areolate; (12) SVL to 43 mm in females.

Content.—Two species are known: *Dischidodactylus colonnelloi* and *duidensis*.

Distribution.—One species is confined to Cerro Duida and the other to Cerro Marahuaca in the Guiana Highlands of southeastern Venezuela.

Etymology.—The generic name is derived from the Greek *dischidos,* meaning divided, and the Greek *dactylos,* meaning finger or toe, in reference to the divided ungual flap. The generic name is masculine.

Remarks.—Neither species of *Dischidodactylus* has been included in phylogenetic analyses; consequently, the relationships of the genus are unknown. However, we tentatively place it in the Strabomantinae because of its possession of expanded terminal disks with circumferential grooves. *Dischidodactylus* differs from other strabomantines mainly by having bifurcate discs on the digits. However, at least two species of terraranan from Tamacuari Tepui in southern Venezuela (*Ceuthomantis cavernibardus* and *Pristimantis memorans*) have notably notched anterior margins of the digital discs (Myers and Donnelly 1997). Possibly there is a radiation of cleft-digited strabomantines

on the tepuis in the Guiana Highlands like that in some other groups of anurans (e.g., *Stefania*: MacCulloch and Lathrop 2002; MacCulloch et al. 2006).

Genus *Geobatrachus* Ruthven, 1915

Geobatrachus Ruthven, 1915:1. Type species: *Geobatrachus walkeri* Ruthven, 1915:2, by original designation.

Definition.—This genus is characterized by: (1) head narrower than body; (2) tympanic membrane not differentiated; tympanic annulus visible beneath skin; (3) cranial crests absent; (4) dentigerous processes of vomers absent; (5) "S" condition of adductor muscle; (6) discs not expanded; tips of digits pointed; circumferential grooves absent; terminal phalanges narrowly T-shaped; (7) Finger I barely longer than Finger II; (8) Toe III slightly longer than Toes V; Toe I concealed externally and adherent to Toe II; (9) subarticular tubercles not projecting; (10) dorsum smooth with low longitudinal ridges; (11) venter smooth; (12) SVL to 24 mm in females.

Content.—The monotypic genus contains *Geobatrachus walkeri*.

Distribution.—*Geobatrachus* is endemic to the Sierra Nevada de Santa Marta in northern Colombia.

Etymology.—The generic name is derived from the Greek nouns *ge* and *batrachos,* meaning earth and frog, respectively. The generic name is masculine in gender.

Remarks.—The morphology, ecology, and life history of this small frog that originally was assigned to Dendrobatidae, were thoroughly examined by Ardila-Robayo (1979), who placed it in Leptodactylidae. The relationships of *Geobatrachus* are unknown, but it is worth noting that *Geobatrachus* and *Atopophrynus* from the Cordillera Central in northern Colombia are unique among strabomantids by having Toe I externally fused with Toe II. See comments under *Atopophrynus* concerning placement in Strabomantinae.

Genus *Hypodactylus,* Hedges, Duellman, and Heinicke, 2008b

Hypodactylus Hedges, Duellman, and Heinicke 2008b:67. Type species: *Eleutherodactylus elassodiscus* Lynch, 1973:222, by original designation. Replacement name for *Isodactylus*.
Isodactylus Hedges, Duellman, and Heinicke 2008a:108. Type species: *Eleutherodactylus elassodiscus* Lynch, 1973:222, by original designation. Junior homonym of *Isodactylus* Gray, 1845.

Definition.—This genus is characterized by (1) head narrower than body; (2) tympanic membrane differentiated; only tympanic annulus visible under skin in *H. latens, manipus, nebulanastes,* and *peraccai.* (3) cranial crests absent; (4) dentigerous processes of vomers prominent; (5) "S" condition of adductor muscle; (6) terminal discs on digits not expanded, usually bearing weak circumferential grooves; terminal phalanges narrow, T-shaped; (7) Finger I equal to, or longer than Finger II; (8) Toes III and V about equal in length; (9) subarticular tubercles not projecting; (10) dorsum smooth to weakly

tuberculate; (11) venter smooth; (12) range in snout–vent length 18.8 mm in males of *I. adercus* to 48.8 mm in females of *lundbergi*.

Content.—The genus contains 13 species, nine of which formerly were placed in *Eleutherodactylus,* whereas four species formerly were placed in *Phrynopus*: *Hypodactylus adercus, araiodactylus, babax, brunneus, dolops, elassodiscus, fallaciosus, latens, lucida, lundbergi, mantipus, nigrovittatus,* and *peraccai*.

Distribution.—The genus ranges from the northern parts of the Cordillera Occidental and Cordillera Oriental in Colombia southward through Ecuador to the Cordillera Oriental in central Peru; most species occur at elevations of 1500–3710 m, but *H. nigrovittatus* inhabits the Amazon Basin in Ecuador and northern Peru.

Etymology.—The masculine generic name is derived from the Greek *hypo* meaning less than and the Greek *daktylos* meaning toe; the name applies to the narrow digital discs characteristic of this genus.

Remarks.—Support for the genus in the molecular phylogeny (Hedges et al. 2008a) was significant in the ML (98%) and Bayesian (100%) analyses. The relationship of this genus to other genera in the Subfamily Strabomantinae is poorly resolved and will require additional gene sequences. As noted by Lynch (1994) and Lehr (2005), circumferential grooves in narrow-toed eleutherodactylids are difficult to distinguish and are not necessarily present on all digits. However, the presence of T-shaped terminal phalanges distinguishes species of *Hypodactylus* from those of *Phrynopus, Noblella,* and most *Oreobates.*

Genus *Lynchius,* Hedges, Duellman, and Heinicke, 2008

Lynchius Hedges, Duellman, and Heinicke 2008a:109. Type species: *Phrynopus parkeri* Lynch, 1975a:21, by original designation.

Definition.—The small frogs of the genus *Lynchius* are characterized by (1) head narrow, not as wide as body; snout inclined anteroventrally in profile; (2) differentiated tympanic membrane and tympanic annulus present in *L. flavomaculatus,* membrane absent in other species; (3) cranial crests absent, except in *L. flavomaculatus*; (4) dentigerous processes of vomers prominent, oblique; (5) "S" condition of adductor muscle; (6) tips of digits narrow, rounded, or bulbous; circumferential groves absent or weakly developed; terminal phalanges knob-shaped or weakly T-shaped; (7) Finger I longer than Finger II; (8) Toe V usually slightly longer than Toe III (toes equal in length in *L. parkeri*); (9) subarticular tubercles not projecting; (10) dorsum smooth; (11) venter smooth; (12) SVL to 43 mm in *L. flavomaculatus.*

Content.—Three species are placed in this genus: *Lynchius flavomaculatus, nebulanastes,* and *parkeri.*

Distribution.—*Lynchius* is known from elevations of 2215–3100 m in the Cordillera Oriental in southern Ecuador and the Cordillera de Huancabamba in northern Peru.

Etymology.—The masculine generic name is a patronym for John D. Lynch, who has devoted his professional life to the study of "eleutherodactylid" frogs and described the type species of this genus.

Remarks.— The structure of the terminal phalanges is somewhat intermediate between the knob-shaped phalanges of *Phrynopus* and the T-shaped phalanges of *Pristimantis.* The similarities of the digital structure led Lehr (2005, 2006) to place *Lynchius flavomaculatus* and *L. nebulanastes* in "*Eleutherodactylus.*" Independent analyses of gene sequences by us (Hedges et al. 2008a) and by E. Lehr (pers. comm.) revealed that the three species here assigned to *Lynchius* are in a clade well separated from true *Phrynopus.* A fourth (undescribed) species is represented in the tree by GenBank sequence AM039707 (Lehr et al. 2005).

Genus *Niceforonia* Goin and Cochran, 1963

Niceforonia Goin and Cochran, 1963:499. Type species: *Niceforonia nana* Goin and Cochran, 1963:499. *Phrynopus* (in part)—Lynch, 1975a:8.

Definition.—The small frogs of the genus *Niceforonia* are characterized by (1) head narrow, not as wide as body; (2) differentiated tympanic membrane and tympanic annulus usually absent (present in *N. columbiana*); (3) cranial crests absent; (4) dentigerous processes of vomers usually present and dentate; (5) "S" condition of adductor muscle; (6) tips of digits narrow, rounded; circumferential groves absent; terminal phalanges knob-shaped; (7) Finger I usually shorter than Finger II (equal in length in *N. columbiana*); (8) Toe V slightly longer than Toe III; (9) subarticular tubercles not projecting; (10) dorsum smooth; (11) venter smooth or areolate; (12) SVL to 20.9 mm in *N. nana.*

Content.—In addition to several undescribed species in Colombia (J. D. Lynch, pers. comm.) three species are recognized at this time: *Niceforonia adenobrachia, columbiana,* and *nana.*

Distribution.—With the exception of the questionable locality of *Niceforonia columbiana* at an elevation of 1000–1300 m on the eastern slopes of the Cordillera Oriental, this genus is known only from paramos at elevations of 3000–3600 m in the Cordillera Central and Cordillera Oriental in Colombia.

Etymology.—The feminine generic name is for the late Colombian herpetologist, Hermano Nicéforo María.

Remarks.—No species of Colombian *Niceforonia* has been included in molecular analyses. Species of *Niceforonia* differ from *Lynchius* and most *Phrynopus* by lacking vomerine teeth. *Niceforonia* shares the distinction of having knobbed, rather than T-shaped, terminal phalanges with a clade that includes *Phrynopus*, *Oreobates*, and *Lynchius*. Because the other strabomantine genera have T-shaped phalanges, this may be considered a shared derived character uniting *Niceforonia* with these three genera.

Genus *Oreobates* Jiménez de la Espada, 1872

Oreobates Jiménez de la Espada, 1872:87. Type species: *Oreobates quixensis* Jiménez de la Espada, 1872:87, by monotypy.

Teletrema Miranda-Ribeiro, 1937:67. Type species *Teletrema heterodactylum* Miranda-Ribeiro, 1937:67, by monotypy. Synonymy with *Eleutherodactylus* by Myers (1962:198). **New synonymy.**

Definition.—Frogs of the genus *Oreobates* can be defined as strabomantid frogs having (1) head about same width as body; (2) tympanic membrane differentiated; (3) cranial crests absent; (4) dentigerous processes of vomers prominent; (5) "S" condition of adductor muscle; (6) terminal segments of digits usually rounded with reduced, or absent, disc structure, when present only on Finger III and IV, and always with incomplete circumferential grooves and poorly defined ungual flap; terminal phalanges knob-shaped; (7) Finger I longer than, or equal to, Finger II; (8) Toe V equal in length to, or shorter than Toe III; (9) subarticular and supernumerary tubercles large, conical or subconical, projecting; (10) dorsum smooth to tuberculate; (11) venter smooth; (12) range in SVL 20 mm in males of *O. cruralis* to 63 mm in females of *O. quixensis.*

Content.—Sixteen species are recognized: *Oreobates barituensis, choristolemma, cruralis, discoidalis, granulosus, heterodactylus, ibischi, lehri, madidi, pereger, quixensis, sanctaecrucis, sanderi, saxatilis, simmonsi,* and *zongoensis.* Most of these were recognized by Padial et al. (2008); we have added "*Phrynopus*" *pereger*, which shares morphological characters with species of *Oreobates* (E. Lehr, pers. comm.).

Distribution.—The genus occurs in western South America from southern Colombia to southwestern Brazil and northwestern Argentina. Most species occur in the Andes to elevations of 2830 m but two inhabit the upper Amazon Basin; one species inhabits the Brazilian Shield between Brazil and Bolivia.

Etymology.—The generic name is derived from the Greek *oreos,* meaning mountain, and the Greek *bates,* meaning one that treads or haunts; the name refers to the mountainous region on the lower slopes of the Andes where the type species was found. The gender is masculine.

Remarks.— Formerly, some of these species were assigned to the genus *Ischnocnema,* the type species of which, *Leiuperus verrucosus* Reinhardt and Lütken (1862), was shown to possess T-shaped terminal phalanges and was placed in the synonymy of "*Eleutherodactylus*" by Caramaschi and Canedo (2006); Heinicke et al. (2007) recognized *Ischnocnema* as a distinct genus, which herein is placed in the Brachycephalidae. Analyses of molecular and morphological data by Padial et al. (2008) provide a well-supported phylogenetic tree of *Oreobates,* a genus that also includes all species formerly placed in the "*Eleutherodactylus" discoidalis* Group sensu Lynch (Lynch 1989). The advertisement call of members of *Oreobates* is composed of a single pulsed note or a rapid series of pulse-like consecutive notes modulated in amplitude (Padial et al. 2007b). Call structure has been proposed as a putative shared derived character of the genus (Padial et al.2008).

Genus *Phrynopus* Peters, 1873

Phrynopus Peters, 1873:416. Type species: *Phrynopus peruanus* Peters,1873:416, by monotypy.

Definition.—Frogs of the genus *Phrynopus* are characterized by (1) head narrow, not as wide as body; (2) differentiated tympanic membrane and tympanic annulus usually absent (present in *P. auriculatus* and *P. peruanus*); (3) cranial crests absent; (4) dentigerous processes of vomers usually absent (present and dentate in *P. auriculatus, bracki, dagmarae, kauneorum,* and *peruanus*); (5) "S" condition of adductor muscle; (6) tips of digits narrow, rounded, or bulbous; circumferential groves absent; terminal phalanges knob-shaped; (7) Finger I usually shorter than Finger II (equal in length in *P. juninensis,* and *P. thompsoni*); (8) Toe V usually slightly longer than Toe III (toes equal in length in *P. thompsoni;* Toe V shorter than Toe III in *P. juninensis* and *P. peruanus*); (9) subarticular tubercles not projecting; (10) dorsum smooth to pustulate; (11) venter smooth or areolate; (12) SVL ranging from 14.5 in *P. auriculatus* to 54 mm in *P. kauneorum.*

Content.—Twenty-two species are recognized: *Phrynopus auriculatus, ayacucho, barthlenae, bracki, bufoides, dagmarae, heimorum, horstpauli, juninensis, kauneorum, kotosh, lechriorhynchus, miroslawae, montium, nicoleae, oblivius, paucari, peruanus, pesantesi, tautzorum, tribulosus,* and *thompsoni.*

Distribution.—The genus occurs mainly at elevations of 2200–4400 m in upper humid montane forests and supra-treeline grasslands in the Cordillera Oriental in central Peru and at one locality at an elevation of 3290 m in the Cordillera Occidental in Peru.

Etymology.—The generic name is derived from the Greek *phrynos,* meaning toad, and the Latin *pusillus,* meaning small. The name is masculine in gender.

Remarks.— The placement of several former species of *Phrynopus* in other genera (*Bryophryne, Lynchius, Hypodactylus, Noblella, Niceforonia,* and *Psychrophrynella*) restricts *Phrynopus* to a clade of 21 described species in the high Andes of central Peru, the type species of which, *Phrynopus peruanus*, was recently rediscovered and described by Lehr (2007). In this region the genus does not overlap the distributions of other genera that formerly were placed in *Phrynopus.* Loss of a tympanic membrane and diminution and eventual loss of the tympanic annulus are derived character states in many groups of anurans. A molecular phylogeny including many species, including *P. peruanus,* which has a fully developed ear, has *P. peruanus* as the basal species (E. Lehr, pers. comm.). Thus, phylogenetic analyses of molecular data corroborate the existence of an ear as primitive.

Genus *Pristimantis* Jiménez de la Espada, 1871

Pristimantis Jiménez de la Espada, 1871:61. Type species: *Pristimantis galdi* Jiménez de la Espada, 1871:61, by monotypy.

Cyclocephalus Jiménez de la Espada, 1875:pl. 3. Type species: *Cyclocephalus lacrimosus* Jiménez de la Espada, 1875:pl. 3, by monotypy. Synonymy with *Eleutherodactylus* by Lynch and Schwartz (1971:109). **New synonymy.**

Pseudohyla Andersson, 1945:86. Type species *Pseudohyla nigrogrisea* Andersson, 1945:86, by monotypy. Synonymy with *Eleutherodactylus* by Lynch (1969:219). **New synonymy.**

Trachyphrynus Goin and Cochran, 1963:502. Type species: *Trachyphrynus myersi* Goin and Cochran, 1963:502, by original designation. Synonymy with *Eleutherodactylus* by Lynch (1968c:295). **New synonymy.**

Definition.—Members of the genus *Pristimantis* can be defined as strabomantid frogs having: (1) head about as wide as body; (2) tympanic membrane differentiated or not; (3) cranial crests usually absent; (4) dentigerous process of vomers usually present; (5) "S" condition of the adductor muscles, except in the *subgenus* Yunganastes; (6) terminal discs on digits expanded (with apical papillae in members of the *P. chalceus* Group), bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) comparative lengths of Fingers I and II variable; (8) Toe V as long as, or longer than, Toe III; (9) subarticular tubercles not protruding; (10) texture of skin on dorsum variable; (11) venter smooth or areolate; (12) range in SVL 13 mm in male *P. imitatrix* to 73 mm in female *P. lymani.*

Content.—As now recognized, the genus includes two subgenera and 433 species.

Distribution.—*Pristimantis* is most diverse in northwestern South America, where the distribution of the genus includes the lowlands to elevations of about 4000 m in the Andes in Colombia, Ecuador, and Peru (except for the arid coastal regions and semi-arid Pacific slopes of the Andes). The genus also occurs throughout much of Venezuela, except the arid coastal region and the llanos, as well as in Bolivia and south-central Brazil. *Pristimantis* occurs throughout the Amazon Basin, where it is most diverse in Ecuador; it also occurs in the Guianas, Trinidad, and Tobago. Ten species occur in lower Central America (*P. cerasinus* as far north as eastern Honduras); three of these (*P. altae, cerasinus,* and *pirrensis*) are endemic to Central America, and the others range principally into Chocoan South America. Two species exist on islands in the Lesser Antilles closest to the mainland of South America (*P. euphronides* on Grenada and *P. shrevei* on St. Vincent).

Etymology.— As stated by Jiménez de la Espada (1871 "1870"), the name *Pristimantis* is derived from two Greek words meaning sierra and treefrog. The earliest generic name ending with *mantis* is *Platymantis* coined by Günther (1859 "1858"-a), wherein he specifically stated that the name was derived from the Greek *platys* meaning flat and *mantis* meaning treefrog. The Greek word *mantis* normally is translated as meaning prophet and is masculine. But as pointed out by Kraus and Allison (2007), according to Liddell and Scott (1996), the masculine term was applied by ancient Greeks to treefrogs in reference to their calls prophesizing the advent of rains. Günther (1859 "1858"-a) did not state the gender of *Platymantis* and included two species, one with a masculine ending and the other with a feminine ending.

Inasmuch as Jiménez de la Espada (1871 "1870") described only one species as a genitive name, his determination of gender cannot be ascertained. However, Peters (1863) in his description of *Strabomantis* also referred to *mantis* as a frog and used the

generic name as masculine, as did Laurent and Combaz (1950) for *Phlyctimantis.* Throughout most of the 20th Century, authors treated *Platymantis* as masculine, but Günther (1999) erroneously used *Platymantis* as a feminine name; this usage was followed by Frost (2007) and Global Amphibian Assessment (IUCN 2006) and has been forced upon some recent authors by editors of some journals. The gender of generic names ending in *mantis* definitely is masculine; as emphasized by Kraus and Allison (2007); recent usage of specific names of *Platymantis* as feminine are unjustified and should be rendered masculine.

Remarks.—Support for the monophyly of this genus in the taxon-dense molecular phylogeny of Hedges et al. (2008a). It appears as a close relative of the clade containing *Lynchius*, *Oreobates*, and *Phrynopus*, with moderate support (67%) in the ML analysis and significant support (100%) in the Bayesian analysis.

Heinicke et al. (2007) resurrected the generic name *Pristimantis* for this large "South American Clade" that they discovered, centered primarily in the Andes. Several well-supported groups within the genus are evident, but there is not complete agreement with the previously defined morphological species groups (Lynch and Duellman 1997). These discrepancies and the limited taxon-sampling (104 species) make it difficult to define subdivisions within *Pristimantis*. Nonetheless, the molecular phylogeny of Hedges et al. (2008a) defines several strongly supported and large groups of species that show some, but not complete, agreement with previous groups defined by morphology (e.g., the former "*Eleutherodactylus*" *conspicillatus* and "*E.*" *unistrigatus* species groups, among others). Using this new perspective, we recognize three subgenera within *Pristimantis*. One includes species previously placed mostly in the "*Eleutherodactylus cruentus*" and "*E. cerasinus*" species groups, and one mostly includes species assigned to the "*E. fraudator*" Species Group. The other assemblage, the subgenus *Pristimantis*, contains the species placed previously in the enormous "*E.*" *unistrigatus* Species Group, as well as species placed in fifteen other species groups. Based on our molecular phylogenies (Hedges et al. 2008a) many of the species groups retained here are demonstrably not monophyletic. Additional subgenera may be definable within the genus, especially those assigned herein to the subgenus *Pristimantis*, but we refrain from defining additional subgenera until DNA sequence data become available for a larger proportion of the subgenus. One species, *P. dendrobatoides*, is unassigned to subgenus.

The recognition of *Pristimantis* necessitates the removal of four nominal genera from the synonymy of *Eleutherodactylus* and their placement in the synonymy of *Pristimantis*, or as subgenera of *Pristimantis.* Furthermore, DNA sequence data show that some species were incorrectly placed in *Pristimantis* by Heinicke et al. (2007); several of the species placed in the "*Eleutherodactylus diastema*" Group by Lynch and Duellman (1997) and Lynch (2001) actually belong in the family Eleutherodactylidae as discussed above.

Subgenus *Hypodictyon* Cope (1885)

Hypodictyon Cope, 1885:383. Type species: *Phyllobates ridens* Cope, 1866a:131. Synonymy with *Eleutherodactylus* by Taylor (1952:690). **New synonymy.**

Definition.—Members of the subgenus *Hypodictyon* can be defined as strabomantid frogs having: (1) head moderately narrow; (2) tympanic membrane differentiated; (3) cranial crests usually absent; (4) dentigerous process of vomers well developed; (5) "S" condition of the adductor muscles; (6) terminal discs on digits expanded, bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I slightly shorter or longer than Finger II; (8) Toe V longer than Toe III; (9) subarticular tubercles prominent; (10) texture of skin on dorsum variable; (11) texture of skin on venter variable; (12) range in SVL from 16 mm in males of *Pristimantis ridens* to 72 mm in females of *P. w-nigrum.*

Content.—The subgenus includes two species series (28 species): the *Pristimantis* (*Hypodictyon*) *ridens* and *P.* (*H.*) *rubicundus* species series.

Distribution.—Members of this subgenus range from Honduras through Central America onto the Pacific versant of Colombia and Ecuador, as well as on the eastern Andean slopes and in the upper Amazon Basin of Colombia, Ecuador, northern Peru, and extreme western Brazil.

Etymology.—The subgeneric name is derived from the Greek *hypo* meaning under or beneath and the Greek *diktyon* meaning net. Cope used the name in reference to the granulate skin on the belly.

Remarks.—The included species of this subgenus are variable with respect to most morphological features. There is no consistency in the presence or absence of tarsal folds or lateral fringes on the digits; furthermore, the venter may be smooth or areolate. However, within *Hypodictyon* there are two well-supported clades, recognized here as species series, that differ consistently in relative lengths of Toes III and V.

Pristimantis (*Hypodictyon*) *ridens* Species Series

Definition.—Frogs in this series are small to moderate in size with proportionately short limbs; the range in SVL is from 16 mm in male *Pristimantis ridens* to 45.2 mm in *P. jorgevelosai.* Head width is 35–43% SVL. Cranial crests are absent except in female *P. jorgevelosai*. The tympanic membrane and annulus are distinct, except in *P. pirrensis* and *cruentus.* The dorsum is smooth, shagreen, or tuberculate; the venter is coarsely areolate. The toes lack webbing, and Toe V is much longer than Toe III; an inner tarsal fold or elongate tubercle is usually present. Lateral fringes are usually present on the fingers and toes. Vocal slits and nuptial pads are present or absent. Species in this series usually are found on low vegetation at night.

Content.—The species series includes 16 species: *Pristimantis* (*Hypodictyon*) *altae, bicolor, caryophyllaceus, colomai, cremnobates, cruentus, jorgevelosai, laticlavius, latidiscus, moro, museosus, pardalis, pirrensis, ridens, rosadoi,* and *sanguineus*.

Distribution.—Species in this series mostly occur at elevations less than 2000 m in lower Central America and on the Pacific versant of Colombia and Ecuador. *Pristimantis*

ridens extends northward into Honduras. Two species (*P. cremnobates,* and *jorgevelasoi*) occur at elevations of 100–2050 m on the eastern slopes of the Andes in Colombia and Ecuador.

Remarks.—Several species in this series (e.g., *Pristimantis cruentus* and *ridens*) were among the most common eleutherodactylids in lower Central America prior to the amphibian declines of recent decades.

Pristimantis (*Hypodictyon*) *rubicundus* Species Series

Definition.—Frogs in this series have moderately robust bodies and proportionately long limbs; the range in SVL is from 17 mm in male *Pristimantis cerasinus* to 72 mm in *P. w-nigrum.* Head width is 37–42% SVL. Cranial crests are absent except in female *P. orpacobates*. The tympanic membrane and annulus are distinct. The dorsum is shagreen or tuberculate; the venter usually is smooth, but it is weakly areolate in *P. cerasinus, labiosus, orpacobates, rubicundus,* and *tenebrionis.* The toes lack webbing, and Toe V is only slightly longer than Toe III; an inner tarsal fold is absent, except in *P. actities* and *cerasinus*. Lateral fringes are absent on the fingers and toes, except in *P. achatinus, actites, ocellatus, w-nigrum,* and toes of *P. rubicundus*. Vocal slits are present, except in *P. orpacobates* and *rubicundus;* nuptial pads are present except in *P. crenunguis, labiosus, and tenebrionis*. Most species in this series are found on low vegetation at night; *P. actites* and *w-nigrum* are most common near streams.

Content.—The species series includes 12 species: *Pristimantis* (*Hypodictyon*) *actites, cerasinus, crenunguis, epacrus, ixalus, labiosus, lanthanites, ocellatus, orpacobates, rubicundus, tenebrionis,* and *w-nigrum*.

Distribution.—Most species occur at elevations of 200–2700 m on the Pacific versant of the Andes in Colombia and Ecuador; three species occur at elevations of 1000–1700 m on the Amazonian slopes of the Andes in Colombia and Ecuador, and *Pristimantis w-nigrum* exists at elevations up to 3300 in the Andes of southern Colombia, Ecuador, and extreme northern Peru. One species, *P. cerasinus*, ranges from eastern Honduras to western Panama, and *P. achatinus*, although primarily distributed in Chocoan Colombia and Ecuador, also occurs in eastern Panama and in the Cauca and Magdalena valleys in Colombia.

Remarks.—Conceivably, other species (e.g., *Pristimantis fallax*) will be assigned to this series once molecular data become available.

Subgenus *Pristimantis* Jiménez de la Espada, 1871

Pristimantis Jiménez de la Espada, 1871:61. Type species: *Pristimantis galdi* Jiménez de la Espada (1871:61), by monotypy.

Mucubatrachus La Marca, 2007:68. Type species: *Hylodes briceni* Boulenger (1903:481), by original designation.

Paramophrynella La Marca, 2007:84. Type species: *Eusophus ginesi* Rivero (1964:299), by original designation.

Definition.—Members of the subgenus *Pristimantis* can be defined as strabomantid frogs having: (1) head about as wide as body; (2) tympanic membrane differentiated or not; (3) cranial crests present or absent; (4) dentigerous process of vomers usually present; (5) "S" condition of the adductor muscles; (6) terminal discs on digits expanded (with apical papillae in *P. chaceus*), bearing well-defined circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I usually shorter than Finger II variable; (8) Toe V usually much longer than Toe III; (9) subarticular tubercles not protruding; (10) texture of skin on dorsum variable; (11) venter usually areolate; (12) range in SVL 13 mm in male *P. imitatrix* to 73 mm in female *P. lymani*.

Content.—The subgenus includes 16 species groups and 34 species unassigned to group (total, 404 species).

Distribution.—The distribution of the subgenus *Pristimantis* is essentially the same as that of the genus except that it barely enters Central America.

Etymology.—As for the genus.

Remarks.—There is no clear resolution of this subgenus in the taxon-dense molecular phylogeny of Hedges et al. (2008a). Although it received moderately strong support (80%) in the ML analysis and significant support (100%) in the Bayesian analysis of their character-dense data set, many species appearing basally within *Pristimantis* in the other phylogenies were not included in this analysis. Within what we now recognize as the subgenus *Pristimantis* several species groups were identified by Lynch and Duellman (1997) and others have been proposed subsequently. None of these phenetic groups has been clearly distinguished in the various phylogenetic analyses (Hedges et al. 2008a). In part, this presumed lack of distinction is because of insufficient taxon sampling. Many of these groups are moderately well defined by morphological characters, and some have restricted geographic ranges, principally in the Andes. However, in other cases, the phylogenetic trees clearly show instances of paraphyly and polyphyly. Because of this discordance between molecular and morphological definitions of groups, it would not be possible to allocate species lacking sequence data to groups defined only in the molecular phylogeny. For this reason we postpone the reclassification of the subgenus *Pristimantis* until a sufficient number of species is sampled with DNA sequences. Thus, except for two of the groups having strong support from the molecular phylogeny (*P. conspicillatus* and *P. peruvianus* species groups), the species groups listed below should not be assumed to be monophyletic.

Recently, La Marca (2007) described two new genera of terraranans from Venezuela, *Mucubatrachus* and *Paramophrynella*. He assigned seven species (*briceni, culatensis, flabellidiscus, lancinii, paramerus, rhigophilus, and thyellus*) to the former and three species (*boconoensis, ginesi, and jabonensis*) to the latter. Five of the species were newly described, three of the others (*briceni, paramerus,* and *boconoensis*) were previously placed in groups (*conspicillatus* and *unistrigatus*) assigned here to *Pristimantis* (*Pristimantis*), and the remaining two species (*lancinii* and *ginesi*) have characteristics that would also lead us to place them in the subgenus *Pristimantis*. We

have unpublished sequence data suggesting at least some of these species belong in the subgenus *Pristimantis*. Also, it is not clear from the description (La Marca 2007) that terraranans were surveyed broadly for the diagnostic morphological characteristics of those new genera. For these reasons we place *Mucubatrachus* and *Paramophrynella* in the synonymy of the subgenus *Pristimantis* and leave the ten species unassigned to species group. New morphological and/or molecular analyses will be needed to clarify the status of these taxa.

Because of incomplete descriptions or peculiar combinations of characters, we are unable to assign an additional 32 species to a species group within the subgenus *Pristimantis.* Thus, to summarize, the 42 species that have not been assigned to groups are *Pristimantis* (*Pristimantis*) *achuar, acutirostris, aemulatus, altamnis, bicumulus, boconoensis, briceni, caliginosus, culatensis, factiosus, fallax, fetosus, flabellidiscus, ganonotus, ginesi, incertus, jabonensis, kichwarum, kirklandi, lancinii, lentiginosus, megalops, melanoproctus, orcus, paramerus, piceus, pleurostriatus, polychrus, pruniatus, pulvinatus, restrepoi, reticulatus, rhigophilus, ruedai, ruthveni, sanctaemartae, stenodiscus, thyellus, veletis, viridis, yukpa,* and *yuruaniensis*.

Pristimantis (*Pristimantis*) *bellona* Species Group

Definition.—This is a small group of medium-sized frogs in which females attain a maximum SVL of 46 mm. The body is robust with a relatively broad head, short snout, and long limbs. Finger I is slightly shorter than Finger II; Toe V is much longer than Toe III and extends to the distal edge of the distal subarticular tubercle on Toe IV. The digital discs are expanded. A tympanic annulus and membrane are present. Cranial crests and cranial co-ossification are present in large females. Vocal slits and vomerine teeth are present.

Content.—Three species are included in this group—*Pristimantis* (*Pristimantis*) *bellona, mars,* and *polemistes.*

Distribution.—These frogs inhabit humid montane forest on the Pacific versant of the Cordillera Occidental in Colombia.

Remarks.—This group is recognized by adult females having cranial crests and co-ossification of the dermis with underlying bones of the skull. This combination of characters is unknown elsewhere in the genus. No species were included in our molecular phylogenetic analyses.

Pristimantis (*Pristimantis*) *chalceus* Species Group

Definition.—In these small frogs with SVLs to 31.2 mm in females, the bodies are moderately robust with short snouts, narrow heads, and short limbs. Finger I is shorter than Finger II; Toe V is much longer than Toe III and extends to the distal margin of the distal subarticular tubercle on Toe IV. The discs on the digits are expanded with terminal papillae (at least on Finger III). A tympanic membrane is not differentiated, but the

tympanic annulus is visible beneath the skin. Cranial crests are absent. Vocal slits are present, and vomerine teeth are weak or absent.

Content.—Two species, *Pristimantis* (*Pristimantis*) *chalceus* and *P.* (*P.*) *scolodiscus,* are placed in this group.

Distribution.—Members of this group are arboreal in humid tropical forests on the Pacific lowlands and adjacent Andean slopes to 2000 m in Colombia and Ecuador.

Remarks.—These species formerly were placed in the "*Eleutherodactylus diastema*" Group by Lynch (2001) and Lynch and Duellman (1997). Our molecular data revealed *P. chalceus* to be imbedded in *Pristimantis,* whereas *Eleutherodactylus diastema* is a basal branch in the West Indian Clade. Morphological features, such as the absence of)(-shaped gular folds and bifid palmar tubercle also distinguish members of the *Pristimantis* (*Pristimantis*) *chalceus* Species Group from the *E. diastema* and its relatives, now recognized as the genus *Diasporus.*

Pristimantis (*Pristimantis*) *conspicillatus* Species Group

Definition.—Frogs in this group are moderate to large in size with proportionately long hind limbs; the range in SVL is from 19 mm in male *Pristimantis skydmainos* to 72.9 mm in *P. lymani.* Head width is 30–43% SVL, and shank length is 45–64% SVL. Cranial crests are absent. The tympanic membrane and annulus are distinct, except in *P. carmelitae* and *P. johannesdei.* The dorsum is smooth or shagreen; a dorsolateral fold is present or absent. The venter usually is smooth, but it is weakly granular (areolate) in some species. The toes commonly have basal webbing, and Toe V is only slightly longer than Toe III; an inner tarsal fold is present or absent. Lateral fringes are present or absent on the fingers and toes. Vocal slits usually are present. A dark face mask is apparent in many species; the most common dorsal color pattern consists of two or three chevron-shaped marks on the back. Species in this group are primarily terrestrial, but they ascend low vegetation at night.

Content.—The species group includes 38 species: *Pristimantis* (*Pristimantis*) *achatinus, asiastolus, avicuporum, bipunctatus, buccinator, caprifer, carlossanchezi, carmelitae, carranguerorum, charlottevillensis, chiastonotus, citriogaster, condor, conspicillatus, fenestratus, gaigeae, gutturalis, illotus, insignitus, johannesdei, koehleri, lymani, malkini, medemi, meridionalis, metabates, padrecarlosi, pedimontanus, phalaroinguinis, samaipatae, savagei, skydmainos, stegolepis, terraebolivaris, thectopternus, vilarsi, viridicans,* and *zeuctotylus*.

Distribution.—Members of this species group are principally distributed in northern South America from Colombia eastward to the Guianas and Isla Taboga. One species ranges northward into Costa Rica, and four species occur as far south as Bolivia.

Remarks.—Support for a monophyletic core of this species group in the taxon-dense molecular phylogeny (was moderately strong (87%) and it received significant support

(100%) in the ML and Bayesian analyses of the character-dense data set (Hedges et al. 2008a). This group contains most of the species recognized in the "*Eleutherodactylus*" *conspicillatus* Group by Lynch and Duellman (1997). In the phylogenetic analyses of mitochondrial and nuclear gene sequences by Heinicke et al. (2007) as augmented by Hedges et al. (2008a) by further analyses including more taxa, frogs formerly associated with the "*Eleutherodactylus*" *conspicillatus* Group were contained in two well-supported clades: the *Pristimantis* (*Pristimantis*) *conspicillatus* Species Group and the *P.* (*P.*) *peruvianus* Species Group. Individually they represent perhaps the two best-supported species groups within the subgenus *Pristimantis*. Two species assigned to the *conspicillatus* Group included in the molecular phylogeny (*P. zeuctotylus* and *P. caprifer*) are not part of this clade.

Pristimantis (*Pristimantis*) *curtipes* Species Group

Definition.—These are small to medium-sized frogs with a maximum SVL of 50 mm in females. These frogs have robust bodies, short snouts, relatively narrow heads, and proportionately short limbs. Finger I is shorter than Finger II; Toe V is only slightly longer than Toe III and does not extend to the proximal edge of the distal subarticular tubercle on Toe IV. The digital discs are narrow and rounded. A tympanic membrane and annulus are absent (present in *P. buckleyi*). Cranial crests are present. Vocal slits are absent, and vomerine teeth are present.

Content.—There are six species in this group—*Pristimantis* (*Pristimantis*) *buckleyi, cryophilus, curtipes, gentryi, satagius,* and *xestus*.

Distribution.—Members of this group occur in the Cordillera Occidental of the Andes from southern Colombia to central Ecuador, where they are terrestrial in paramos and humid upper montane forest.

Remarks.—The taxonomy of this group was summarized by Lynch (1995). This species group is not monophyletic (Hedges et al. 2008a). However, there is a well-supported (95% bootstrap) clade that unites most members of the *curtipes*, *devillei*, and *surdus* species groups, as well as a member of the *unistrigatus* group (*P. thymalopsoides*). The *curtipes*, *devillei*, and *surdus* groups (and *P. thymalopsoides*) share the presence of cranial crests, which is an otherwise rare trait within *Pristimantis*. These three groups are also distributed sympatrically and probably should be treated as a single species group (a more inclusive *devillei* Species Group).

Pristimantis (*Pristimantis*) *devillei* Species Group

Definition.—In these medium-sized frogs with SVLs to 52 mm in females; the bodies are slender to moderately robust with short snouts, narrow heads, and moderately short to relatively long limbs. Finger I is shorter than Finger II; Toe V is only slightly longer than Toe III and does not extend to the proximal edge of the distal subarticular tubercle on Toe IV. The discs on the digits are expanded. A tympanic membrane and annulus are present (absent in *P. siopelus*). Cranial crests are present (absent in *P. acatatelus* and

appendiculatus). Vocal slits are absent (present in *P. acatatelus* and *appendiculatus*); vomerine teeth are present.

Content.—Thirteen species—*Pristimantis* (*Pristimantis*) *acatallelus, appendiculatus, cacao, chrysops, devillei, quinquagesimus, silverstonei, siopelus, sulculus, susaguae, truebae, vertebralis,* and *xylochobates*—are recognized in this group.

Distribution.—Collectively, these species inhabit humid montane forests in the Andes in Colombia and Ecuador.

Remarks.—The relative lengths of the toes are like those of species in the *Pristimantis* (*Pristmantis*) *conspicillatus* and *P.* (*P.*) *peruvianus* species groups. One of the two species lacking cranial crests and having vocal slits (*P. appendiculatus*) was included in the molecular phylogeny by Hedges et al. (2008a); it appears to be unrelated to the other species. The other species appear to be related to members of the *curtipes* and *surdus* Groups (see Remarks under *Pristimantis (Pristimatnis) curtipes* Species Group).

Pristimantis (*Pristimantis*) *frater* Species Group

Definition.—These are small frogs with females attaining a SVL of 32.5 mm; they have moderately robust bodies with relatively narrow heads, short round to subacuminate snouts, and moderately long limbs. Finger I is shorter than Finger II; Toe V is much longer than Toe III and extends to the distal margin of the distal subarticular tubercle on Toe IV. The digital discs are expanded. The tympanic annulus and membrane are distinct. Cranial crests are absent. Vocal slits and vomerine teeth are present. Snout length is sexually dimorphic, being longer in males than in females.

Content.—Fourteen species—*Pristimantis* (*Pristimantis*) *frater, incomptus, librarius, martiae, miyatai, ockendeni, paisa, pecki, ptochus, quaquaversus, suetus, taeniatus, viejas, zophus*—are placed in this group.

Distribution.—Members of this group inhabit humid lowland and montane forests throughout the Pacific lowlands, Cauca and Magdalena valleys, and the Andes of Colombia, including the Sierra de Macarena but not the Sierra Nevada de Santa Marta, and the eastern slopes of the Andes and Amazon Basin in Colombia, Ecuador, Peru, and Bolivia. One species, *Pristimantis taeniatus,* ranges from the Pacific lowlands of Colombia into central Panama.

Remarks.—This rather poorly defined group includes the "*Eleutherodactylus taeniatus* Complex" of Lynch and Ardila-Robayo (1999). The single species included in the molecular phylogeny by Hedges et al. (2008a), *P. ockendeni*, is embedded within a section of the *unistrigatus* Group.

Pristimantis (*Pristimantis*) *galdi* Species Group

Definition.—These are small to medium-sized frogs with SVLs to 34 mm in females; the bodies are rather robust with broad heads, long limbs, and long, acuminate snouts. Finger I is shorter than Finger II; Toe V is much longer than Toe III and extends to the distal edge of the distal subarticular tubercle on Toe IV. The digital discs are expanded. A tympanic annulus and differentiated tympanic membrane are present. Cranial crests are present, and the edges of the frontoparietals and squamosals are serrate. Vocal slits and vomerine teeth are present.

Content.—Four species—*Pristimantis* (*Pristimantis*) *delicatus, douglasi, galdi,* and *tribulosus*—are placed in this group.

Distribution.—The distribution is disjunct—Sierra Nevada de Santa Marta, extreme northern part of the Cordillera Oriental in Colombia, Pacific slopes of the Cordillera Occidental in southwestern Colombia, and Amazonian slopes of the Andes in Ecuador and northern Peru.

Remarks.—The unusual condition of bony tubercles (serrations) along the lateral edges of the frontoparietals and dorsal edge of the squamosal are unique to this group (Lynch 1996a).

Pristimantis (*Pristimantis*) *lacrimosus* Species Group

Definition.—In these small to medium-sized frogs, females of the largest species attain a SVL of 34 mm. The body is moderately robust with a broad, flat head and acuminate, round, or truncate snout; the limbs are moderately long. Dorsal skin shagreen or smooth; belly areolate. Finger I is shorter than Finger II; Toe V is much longer than Toe III and extends to the distal edge of the distal subarticular tubercle on Toe IV. The digital discs are expanded. A tympanic annulus is present, and the tympanic membrane usually is differentiated. Cranial crests are absent; Vocal slits and vomerine teeth are present.

Content.—The group contains 18 species—*Pristimantis* (*Pristimantis*) *apiculatus, aureolineatus, boulengeri, brevifrons, bromeliaceus, dorsopictus, eremitus, lacrimosus, mendax, olivaceus, pardalinus, petersorum, prolixodiscus, royi, schultei, tayrona, waoranii,* and *zimmermanae*.

Distribution.—Members of this group are arboreal and commonly inhabit bromeliads; the group is widespread in the upper Amazon Basin and adjacent slopes of the Andes from Colombia to Bolivia; other species inhabit humid forests on the Pacific versant of Ecuador and Colombia and the Sierra Nevada de Santa Marta in northern Colombia. At least two species, *Pristimantis aureolineata* and *P. waoranii,* are inhabitants of the canopy in lowland rainforest (Guayasamin et al. 2006; McCracken 2007).

Remarks.—The generic name *Cyclocephalus* (type species *C. lacrimosus*) Jiménez de la Espada (1875) is available for this group. In the molecular phylogeny of Hedges et al. (2008a), the included species form a monophyletic group.

Pristimantis (*Pristimantis*) *leptolophus* Species Group

Definition,—The frogs in this group are small with females attaining a maximum SVL of less than 30 mm; they have robust bodies, narrow heads, short snouts, and moderately long legs. Finger I is shorter than Finger II, and Toe V is much longer than Toe III and extends to the distal edge of the distal subarticular tubercle on Toe IV. The discs on the digits are expanded. A tympanic membrane and annulus usually are present but weakly defined (absent in *P. peraticus*). Cranial crests are absent; vocal slits and vomerine teeth are present.

Content.—The group contains seven species—*Pristimantis* (*Pristimantis*) *lasallorum, leptolophus, maculosus, parectatus, peraticus, scoloblepharus,* and *uranobates*.

Distribution.—These small terrestrial frogs inhabit paramo and subparamo throughout the length of the Cordillera Central in Colombia, but one species, *Pristimantis lasallorum,* is known only from a paramo in the northern part of the Cordillera Occidental in Colombia.

Remarks.—The resemblance of *Pristimantis lasallorum* to other members of the group is superficial; that species may not be related to the others. Species of this group have not been included in molecular phylogenies.

Pristimantis (*Pristimantis*) *loustes* Species Group

Definition.—These are medium-sized frogs with SVLs to 56 mm in females; the bodies are slender with narrow heads and long snouts and limbs. Finger I is slightly shorter than Finger II, but slightly longer in *P. loustes;* Toe V is much longer than Toe III and extends to the distal edge of the distal subarticular tubercle on Toe IV. Cranial crests are present in females of *P. hybotragus* and *jaimei;* vocal slits and vomerine teeth are present.

Content.—Only three species—*Pristimantis* (*Pristimantis*) *hybotragus, jaimei,* and *loustes*—are recognized in this group.

Distribution.—These frogs inhabit lowland and lower montane humid forest in southwestern Colombia and northwestern Ecuador, where they have been found on rocks and low vegetation in and near streams.

Remarks.— An apparently unique condition exists in members of this group; the ventral edge of the zygomatic ramus of the squamosal is expanded and evident externally as a knob immediately anterior to the tympanic annulus (Lynch and Duellman 1997).

Pristimantis (*Pristimantis*) *myersi* Species Group

Definition.— Frogs in this group are small (females less than 28 mm) with short snouts, robust bodies, with short snouts and relative narrow heads; the limbs are short to moderately long. Finger I is shorter than Finger II, and Toe V is only slightly longer than

Toe III and does not extend to the proximal edge of the distal subarticular tubercle of Toe IV the digital discs are narrow and rounded. The tympanic membrane is differentiated (except in *P. leoni* and *ocreatus*). Cranial crests are absent. Vocal slits are present (except in *P. floridus*); vomerine teeth are present.

Content.—Twelve species—*Pristimantis* (*Pristimantis*) *bicantus, festae, floridus, gladiator, hectus, leoni, myersi, ocreatus, pyrrhomerus, repens, scopaeus,* and *xeniolum*—are recognized in this group.

Distribution.—These terrestrial frogs inhabit paramos and upper humid montane forests in Ecuador and southern Colombia.

Remarks.—The generic name *Trachyphrynus* Goin and Cochran (1963) (type species *T. myersi*) is available for species in this group. The four species included in the molecular phylogeny of Hedges et al. (2008a) form a monophyletic group which is part of a larger, strongly-supported assemblage (99% bootstrap) including the *curtipes*, *devillei*, and *surdus* groups, as well as some species in the *unistrigatus* Group.

Pristimantis (*Pristimantis*) *orcesi* Species Group

Definition.—These small to medium-sized frogs (SVL in females to 36 mm) have robust bodies, narrow heads, short snouts, and moderately short limbs. Finger I is shorter than Finger II; Toe V is much longer than Toe III and extends to the distal edge of the distal subarticular tubercle on Toe IV. The digital discs are expanded. The tympanic annulus and membrane are differentiated (absent in *P. thymelensis*). Cranial crests are absent, except weakly developed in *P. thymelensis.* Vocal slits are present; vomerine teeth are present (absent in *P. orcesi*).

Content.—There are eight species—*Pristimantis* (*Pristimantis*) *huicundo, obmutescens, orcesi, ortizi, racemus, simoteriscus, simoterus,* and *thymelensis*—in this group.

Distribution.—Members of this group are terrestrial in paramos and subparamo in the Cordillera Occidental in Colombia and northern Ecuador.

Remarks.—This group was defined by Lynch (1981a) and revised by Guayasamin (2004). Two species are represented in the molecular phylogeny (*P. orcesi* and *P. thymelensis*) of Hedges et al. (2008). They appear to be unrelated.

Pristimantis (*Pristimantis*) *orestes* Species Group

Definition.—Frogs in this group are small (females less than 34 mm) with short snouts, robust bodies, relative narrow heads, and proportionately short limbs. Finger I is shorter than Finger II, and Toe V is only slightly longer than Toe III inasmuch as it barely extends to the proximal edge of the distal subarticular tubercle of Toe IV; the digital discs are narrow and rounded. The tympanum is small with a differentiated tympanic membrane except in *P. orestes, pataikos, simonbolivari,* and *vidua;* both tympanic

membrane and annulus absent in *P. simonsii.* Cranial crests are absent. Vocal slits usually are present (absent in *P. melanogaster* and *simonsii*); vomerine teeth are present or absent.

Content.—Fourteen species—*Pristimantis* (*Pristimantis*) *atrabracus, chimu, cordovae, corrugatus, melanogaster, orestes, pataikos, pinguis, seorsus, simonbolivari, simonsii, stictoboubonus, ventriguttatus,* and *vidua*—currently are placed in this group.

Distribution.—These frogs are terrestrial in paramo and humid upper montane forest in the Andes of southern Ecuador and northern Peru.

Remarks.—Lynch and Duellman (1997) defined this group for three species in southern Ecuador, but within the last decade many new species in this group have been discovered in northern Peru (Duellman and Pramuk 1999; Duellman et al. 2006). Frogs in the *Pristimantis orestes* Group resemble those in the *Pristimantis myersi* Group in size, robustness, general proportions, relative lengths of Fingers I and II, and size of digital discs. Furthermore, the two groups are parapatric in the Andes of western Ecuador. Toe V is slightly longer in species in the *Pristimantis orestes* Group than it is in members of the *Pristimantis myersi* Group. Four species were included in the molecular phylogeny of Hedges et al. (2008a). Whereas *P. orestes* and *P. simonbolivari* cluster together, the other species sampled (*P. simonsii* and *P. melanogaster*) do not. Nor do any of these species cluster with members of the *myersi* Group. Thus, the shared morphologies of these species may represent convergent evolution to cope with similar habitats.

Pristimantis (*Pristimantis*) *peruvianus* Species Group

Definition.—Frogs in this series are small to moderate in size with proportionately long hind limbs; the range in SVL is from 15.7 mm in male *Pristimantis peruvianus* to 45.8 mm in *P. danae.* Head width is 38–44% SVL, and shank length is 49–70% SVL. Cranial crests are absent. The tympanic membrane and annulus are distinct. The dorsum is smooth or shagreen; a dorsolateral fold is present or absent. The venter usually is smooth, but it is areolate in *P. danae, pharangobates, rhabdolaemus, sagittulus, stictogaster,* and *toftae.* The toes usually lack even basal webbing, and Toe V is only slightly longer than Toe III; an inner tarsal fold is present or absent. Lateral fringes are present or absent on the fingers and toes. Vocal slits are present. A dark face mask is present in some species, and the dorsal color pattern is highly variable, but two or three dark brown chevrons are present on the back of most species. Some of the frogs in this series are active on the ground by day, but all are found on low vegetation at night.

Content.—The species series includes 14 species: *Pristimantis* (*Pristimantis*) *albertus, aniptopalmatus, crepitans, cuneirostris, danae, dundeei, ornatus, pharangobates, peruvianus, rhabdolaemus, sagittulus, stictogaster, tanyrhynchus,* and *toftae*.

Distribution.—Members of this species series occur in humid forests on the Amazonian slopes of the Andes and in the Amazon Basin in Ecuador, Peru, and Bolivia; two species

(*P. crepitans* and *P. dundeei*) inhabit shrub and dry forest in Mato Grosso in southwestern Brazil.

Remarks.— See comments above in the account of the *Pristimantis* (*Pristimantis*) *conspicillatus* Species Group. Adults in the *P.* (*P.*) *peruvianus* Species Group generally are smaller than those in the *P.* (*P.*) *conspicillatus* Species Group. The former also has a more southern distribution than the latter, but the two groups broadly overlap in Peru and Bolivia. Recently, cryptic species have been discovered from among specimens identified as *P. peruvianus*, including those in lowland Amazonian Peru (Padial and De la Riva 2009). Sequence data are needed from topotypic *P. peruvianus* (our sample is not from the type locality) to resolve whether this group retains its current name or takes on another name. Additionally, two species placed here in the *P. peruvianus* group, *P. crepitans* and *P. dundeei*, may belong in the *P. conspicillatus* Group (J. M. Padial, pers. comm.).

Pristimantis (*Pristimantis*) *surdus* Species Group

Definition.—In these medium-sized frogs with SVLs in females to 55 mm, the head is narrow, snout short, and limbs relatively long. Finger I is shorter than Finger II; Toe V is only slightly longer than Toe III and extends to the proximal edge of the distal subarticular tubercle of Toe IV. The digital discs are expanded. The tympanic annulus and membrane are absent. Cranial crests are present. Vomerine teeth are present, and vocal slits are absent.

Content.—There are four species—*Pristimantis* (*Pristimantis*) *duellmani, hamiotae, sobetes,* and *surdus*—in this putative group.

Distribution.—These frogs inhabit humid montane forest in the Cordillera Occidental in Ecuador, where individuals are primarily terrestrial and associated with streams.

Remarks.—For restriction of the content of this group, see Lynch and Duellman (1997). This group is not monophyletic, but part of a larger clade of crested species (Hedges et al. 2008a). See Remarks under *Pristimantis (Pristimantis) curtipes* Species Group.

Pristimantis (*Pristimantis*) *unistrigatus* Species Group

Definition.—In these small to medium sized frogs (SVL in females to 45 mm), the bodies are slender to robust with narrow heads, short snouts, and usually moderately long limbs (shorter in some high montane terrestrial species). Finger I is shorter than Finger II; toe V is much longer than Toe III and extends to the distal edge of the distal subarticular tubercle on Toe IV. The digital discs are expanded. The tympanic annulus and tympanic membrane usually are present, but they are absent in a few species (e.g., *P. acuminatus, altamazonicus,* and *ventrimarmoratus*). Cranial crests usually are absent (present in a few species, such as *P. ruidus* and *thymalopsoides*). Vomerine teeth and vocal sacs usually are present.

Content.—There are 202 species assigned to this group—*Pristimantis* (*Pristimantis*) *aaptus, acerus, actinolaimus, acuminatus, affinis, alalocophus, alberico, altamazonicus, amydrotus, andinognomus, anemerus, angustilineatus, angustilineata, anolirex, anotis, aquilonaris, ardalonychus, atratus, aurantiguttatus, auricarens, avius, bacchus, baiotis, balionotus, baryecuus, batrachites, bearsei, bellator, bernali, bogotensis, cabrerai, caeruleonotus, cajamarcensis, calcaratus, calcarulatus, cantitans, capitonis, carvalhoi, celator, ceuthospilus, chloronotus, colodactylus, colonensis, colostichos, corniger, coronatus, cosnipatae, cristinae, croceoinguinus, crucifer, cruciocularis, cryptomelas, cuentasi, degener, deinops, delius, diadematus, diaphonus, diogenes, dissimulatus, duende, elegans, eriphus, ernesti, erythropleura, esmeraldas, eugeniae, euphronides, eurydactylus, exoristus, fasciatus, flavobracatus, gagliardoi, glandulosus, gracilis, grandiceps, guaiquinimensis, helvolus, hernandezi, ignicolor, imitatrix, incanus, infraguttatus, inguinalis, inusitatus, jester, juanchoi, jubatus, karelinae, kaptoptroides, kelephas, lemur, leucopus, leucorrhinus, lichenoides, lindae, lirellus, lividus, llojsintuta, lucasi, luscombei, luteolateralis, lutitus, lynchi, lythrodes, marahuaka, marmoratus, memorans, merostictus, minutulus, mnionaetes, modipeplus, molybrignus, mondolfi, muricatus, muscosus, myops, nephophilus, nervicus, nicefori, nigrogriseus, nyctophylax, ornatissimus, orphnolaimus, palmeri, parvillus, pastazensis, paululus, penelopus, percnopterus, percultus, permixtus, petrobardus, phalarus, philipi, phoxocephalus, phragmipleuron, platychilus, platydactylus, prolatus, proserpens, pseudoacuminatus, pteridophilus, pugnax, pycnodermis, quaiquinimensis, quantus, reclusas, reichlei, renjiforum, repens, rhabdocnemus, rhodoplichus, rhodostichus, riveroi, riveti, roseus, rozei, rufioculus, ruidus, salaputium, saltissimus, sarisarinama, scitulus, serendipitus, shrevei, signifer, spectabilis, spilogaster, spinosus, sternothylax, subsigillatus, supernatis, taciturnus, tamsitti, tantanti, telefericus, tepuiensis, thymalopsoides, torrenticola, trachyblepharis, tubernasus, turik, turpinorum, turumiquirensis, uisae, unistrigatus, urichi, vanadise, variabilis, ventrimarmoratus, verecundus, vermiculatus, versicolor, vicarius, vilcabambae, wagteri, walkeri, wiensi, yaviensis, yustizi, zoilae*.

Distribution.—This group is distributed throughout most of northwestern South America, where it occurs from lowland tropical rainforests to supra-treeline habitats in the Andes; it occurs southward to Bolivia and eastward into the Guianas, Trinidad, and Tobago; two species occur in the Lesser Antilles—*P. euphronides* on Grenada and *P. shrevei* on St. Vincent.

Remarks.—This is demonstrably not a natural group (Hedges et al. 2008a), but rather an assemblage of species of *Pristimantis* that do not fit clearly in other groups. The phylogenetic trees show a well-supported structure among species in this group. For example, it is clear that the two Lesser Antillean members, *P. euphronides* and *P. shrevei*, form a clade distinct from other clades in the subgenus, and show affinity with the *conspicillatus* Group. But without having a sufficient sampling of nearby Venezuelan taxa, it would be premature to erect a species group for that clade. The same logic applies for the many other well-supported clades. Some of these species (e.g., *P. thymalopsoides*) have both morphological and molecular support for placement near or in other species groups. We have sequence data for less than a quarter of these species. As more data

become available, it will be possible to divide this group into more manageable, named monophyletic units.

Genus *Yunganastes* Padial, Castroviejo-Fisher, Köhler, Domic and De la Riva 2007

Yunganates Padial, Castroviejo-Fisher, Köhler, Domic and De la Riva, 2007: 219. Type species: *Eleutherodactylus pluvicanorus* De la Riva and Lynch (1997).

Definition.—Members of the subgenus *Yunganastes* can be defined as medium to large strabomantid frogs with females attaining a SVL of 63 mm, having: (1) head wider or equal than long; (2) tympanic membrane and annulus differentiated; (3) cranial crests absent; (4) dentigerous processes of vomers present; (5) "E'" condition of the adductor muscles (different from the standard "E" condition); (6) terminal discs on Finger III and IV and on toes broad, bearing poorly-defined and incomplete circumferential grooves, supported by T-shaped terminal phalanges; (7) Finger I slightly longer than, or equal to, Finger II; (8) Toe V equal or slightly shorter than Toe III, not reaching distal subarticular tubercle of Toe IV; (9) subarticular tubercles round, protruding; tarsal fold present in one species; (10) texture of skin on dorsum finely shagreen to smooth, with dorsolateral folds present or absent; (11) venter smooth to granular; (12) range in SVL 26 mm in male *P. bisignatus* to 63 mm in female *P. mercedesae*.

Content.—Five species—*Yunganastes ashkapara, bisignatus, fraudator, mercedesae,* and *pluvicanorus*—are placed in this genus.

Distribution.—Members of this subgenus inhabit humid montane forests on the Andean slopes from central Bolivia to southern Peru.

Etymology.— According to Padial et al. (2007), the name *Yunganastes* is derived from the Quechua word *yunga* applied to the humid forests in the Andean valleys and the Greek *nastes*, meaning dweller and refers to the habitat of these frogs.

Remarks.—Three members of this genus (*Y. ashkapara*, *fraudator*, and *pluvicanorus*) were formerly assigned to the *Eleutherodactylus fraudator* group by Köhler (2000). Based on molecular and morphological chracters, Padial et al. (2007a) proposed and described *Yunganastes* to include these three species and two others (*P. bisignatus* and *mercedesae*); they described a new arrangement of the mandibular ramus of the adductor and the trigeminal nerve for *Yunganastes* and proposed it as a shared derived character for this taxon; they also rejected the hypothesis of relationship of *Craugastor* with members of the former *E. fraudator* Species Group. Only a short (~500 bp) sequence of a representative species of this subgenus (*Pristimantis pluvicanorus*) was available to Hedges et al. (2008) (I. de la Riva, pers. comm.). It appeared as the most divergent (basal) subgenus within *Pristimantis*, although support levels were not significant. Padial et al. (2009) used additional sequence data to demonstrate that *Yunganastes* represents a distinct genus.

Genus *Strabomantis* Peters, 1863

Strabomantis Peters, 1863:405. Type species: *Strabomantis biporcatus* Peters, 1863:405, by monotypy.
Limnophys Jiménez de la Espada, 1871:59. Type species: *Limnophys cornutus* Jiménez de la Espada, 1871:59, by subsequent designation (Myers, 1962:197). **New synonymy.**
Ctenocranius Melin, 1941:49. Type species: *Limnophys cornutus* Jiménez de la Espada, 1871:59, by original designation. Synonymy by Myers, 1962:198. **New synonymy.**
Amblyphrynus Cochran and Goin, 1961:543. Type species: *Amblyphrynus ingeri* Cochran and Goin, 1961:543, by original designation. Synonymy by Lynch (1981b:318). **New synonymy.**

Definition.—This genus of strabomantid frogs is characterized by (1) head much wider than body, up to 54% of SVL; (2) tympanic membrane and annulus distinct; (3) cranial crests usually present, except in *S. anatipes, anomalus, cheiroplethus,* and *zygodactylus*; (4) dentigerous processes of vomers prominent, triangular, or arched; (5) "E" or "S" condition of adductor muscle; (6) terminal discs on digits expanded, except in *S. biporcatus,* bearing circumferential grooves; terminal phalanges T-shaped; discs absent on fingers of *S. heleonotus, ingeri, ruizi,* and *sulcatus*, and absent on fingers and toes of *S. heleonotus* and *S. ingeri;* (7) Finger I longer than Finger II; (8) Toe III longer than Toe V; (9) subarticular tubercles projecting in *S, biporcatus,* not projecting in other species; (10) dorsum tuberculate with or without prominent longitudinal ridges (11) venter smooth in most species, areolate in *S. biporcatus, helonotus, ingeri, ruizi,* and *sulcatus;* (12) SVL in adult females from 30 mm in *S. sulcatus to* 106 mm *in S. cheiroplethus*.

Content.—Two species series (17 species) are placed in the genus: the *Strabomantis biporcatus* and *bufoniformis* species series. One species (*S. aramunha*) is not assigned to a series or group.

Distribution.—The genus occurs predominately on the Pacific lowlands and slopes of the Cordillera Occidental in Ecuador and Colombia, but also occurs in the Cordillera Central of Colombia and Cordillera Oriental of Colombia and Ecuador. One species (*S. biporcatus*) has a restricted range in the Cordillera de la Costa, Serranía del Interior, and Peninsula de Paria in northern Venezuela. Two species (*S. bufoniformis* and *S. laticorpus*) extend into Costa Rica and Panama, respectively; one species (*S. sulcatus*) occurs in the upper Amazon Basin of Ecuador, Peru, and western Brazil. The recently described *S. aramunha* is restricted to eastern Brazil.

Etymology.—The generic name is derived from the Greek *strabos,* meaning oblique, and the Greek *mantis,* meaning frog; the gender is masculine (see Etymology of *Pristimantis*).

Remarks.—Support for the monophyly of this genus is significant (100%) in all analyses of Hedges et al. (2008a). Most of the species in this genus formerly were recognized as the "broad-headed eleutherodactyline frogs" (Lynch 1975b) or the "*Eleutherodactylus sulcatus* Group" (Lynch and Duellman 1997). In the various analyses of molecular data by Heinicke et al. (2007), four species ("*E.*" *anomalus, bufoniformis, necerus,* and *sulcatus*) formed a well-supported clade with support values of 99% in each analysis; Heinicke et al. (2007) resurrected the generic name *Limnophys* for this clade. In the more inclusive analyses reported herein, *Strabomantis biporcatus* is shown to be in the same clade. *Strabomantis* Peters, 1863, has priority over *Limnophys* Jiménez de la Espada, 1871, and therefore is used as the generic epithet for this clade.

Strabomantis biporcatus Peters (1863) is the correct name for the species known for more than half a century as *Eleutherodactylus maussi* Boettger (1893) (Savage and Myers 2002). This species was included in the *Eleutherodactylus* (*Craugastor*) *biporcatus* Group by Lynch and Duellman (1997) and Savage and Myers (2002), but the latter authors questioned the putative relationship of the species to the "*Eleutherodactylus biporcatus* Group." By inference the species was included in the genus *Craugastor* by Crawford and Smith (2005). *Strabomantis biporcatus* differs from other members of the "*Eleutherodactylus biporcatus* Group" as defined by Savage and Myers (2002) by having coarsely areolate (instead of smooth) skin on the venter, distinct inner tarsal fold, accessory palmar and plantar tubercles, and vocal slits in adult males; furthermore, the karyotype of 2N = 36 differs from the diploid number of 2N = 20 known for other members of the group (DeWeese 1976; Schmid et al. 1992); in fact, the karyotype of 2N =36 is more like that of species of *Pristimantis* than species of *Craugastor.*

The phylogenetic analyses of molecular data reveal that those species of *Strabomantis* having the "E" condition of the adductor muscle are not closely related to *Craugastor,* all of which have the "E" condition. The placement of species having the "E" condition of the adductor muscle and formerly assigned to *Craugastor* (*Strabomantis anatipes, anomalus, biporcatus, bufoniformis, cheiroplethus, necerus,* and *zygodactylus*) together with other species having the "S" condition" of the adductor muscle (*S. cadenai, cerastes, cornutus, heleonotus, ingeri, laticorpus, necopinus, ruizi,* and *sulcatus*) is contradictory to the morphological assessment of Lynch (1986a). Savage and Myers (2002) postulated that the two conditions of the adductor musculature were derived independently from the plesiomorphic state in which both the *m. adductor subexternus posterior* and *m adductor externus superficialis* are present, as in caecilians and most salamanders. Inasmuch as the "E" condition has evolved independently in disparate families of anurans (e.g., Rhinophrynidae, Bufonidae, Microhylidae) (Starrett 1968), the independent evolution of that state in different clades of eleutherodactylids is not unreasonable.

Strabomantis biporcatus Species Series

Definition.—Frogs in this series are moderately large with robust bodies and proportionately short limbs; the range in SVL is from 30 mm in female *Strabomantis sulcatus* to 74 mm in *S. biporcatus.* Head width is 45–62% SVL. Cranial crests are present and are prominent in most species. The dorsum is tuberculate with longitudinal ridges in some species (*S. biporcatus, cerastes. ingeri, laticorpus, ruizi,* and *sulcatus*); the venter usually is smooth, but it is areolate in *S. biporcatus, heleonotus, ingeri, ruizi,* and *sulcatus.* The toes lack webbing. Lateral fringes are present on the fingers and toes, except in *S. cerastes* and *S. laticorpus;* discs are absent on the fingers of *S. heleonotus, ingeri, ruizi,* and *sulcatus.* Vocal slits and nuptial pads usually are absent. All species, except *S. biporcatus,* have the "S" condition of the adductor musculature. Frogs in this group are terrestrial and are found on the ground and amidst leaf litter on the forest floor.

Content.—Two species groups (10 species)—the *Strabomantis biporcatus* Group and the *Strabomantis cornutus* Group—are placed in this series.

Distribution.—With the exception of *Strabomantis biporcatus,* which is restricted to northern Venezuela, *S. sulcatus,* which occurs in the upper Amazon Basin, and *S. laticorpus* known only from the Cerro Tacarcuna area on the Colombian-Panamanian border, all species are confined to the Andes in Colombia and Ecuador.

Remarks.— This species series mostly represents the former "*Eleutherodactylus*" *sulcatus* Species Group as discussed above in the Remarks for this genus.

Strabomantis biporcatus Species Group

Definition.—This monotypic species group is characterized by a robust body attaining a maximum SVL of 74 mm in females and having a tuberculate dorsum and coarsely areolate venter. The terminal discs on the digits are barely expanded, and the lateral fringes on the toes are weak. Vocal slits are present. The species has the "E" condition of the adductor musculature.

Content.—The group consists of a single species, *Strabomantis biporcatus*.

Distribution.—The species is restricted to the Cordillera de la Costa, Serranía del Interior, and the Peninsula de Paria in northern Venezuela.

Remarks.—In the analyses of Hedges et al. (2008a), *Strabomantis biporcatus* is in a clade with *S. sulcatus,* which differs by having the "S" condition of the adductor musculature; therefore we place *S. biporcatus* in a separate species group.

Strabomantis cornutus Species Group

Definition.—Frogs in this series are moderately large with robust bodies and proportionately short limbs; the range in SVL is from 30 mm in female *Strabomantis sulcatus* to 70 mm in *S. heleonotus.* The terminal digits are slightly to moderately expanded, and lateral fringes are present on the fingers and toes, except in *S. cerastes* and *S. laticorpus.* Vocal slits and nuptial pads are absent. These species have the "S" condition of the adductor musculature.

Content.—Nine species are placed in the species series: *Strabomantis cadenai, cerastes, cornutus, helonotus, ingeri, laticorpus, necopinus, ruizi,* and *sulcatus*.

Distribution.—With the exception of *Strabomantis sulcatus,* which occurs in the upper Amazon Basin, and *S. laticorpus* known only from the Cerro Tacarcuna area on the Colombian-Panamanian border, all species are confined to the Andes in Colombia and Ecuador.

Remarks.—This species group represents the former "*Eleutherodactylus*" *sulcatus* Species Group as discussed above in the Remarks for this genus.

Strabomantis bufoniformis Species Series

Definition.—Frogs in this series are large with robust bodies and relatively short limbs; the range in SVL is from, 83 mm in females of *Strabomantis zygodactylus* to 106 mm in females of *S. cheiroplethus.* Head width is 37–58% of SVL. Cranial crests are low in *S. bufoniformis* and *S. necerus* and absent in the other species. The dorsum usually has distinct longitudinal dermal ridges (only low warts in *S. anatipes* and *S. zygodactylus*); the venter is smooth. With the exception of *S. necerus,* the toes have various degrees of webbing—basal in *S. bufoniformis* to nearly entirely webbed in *S. anatipes* and *S. zygodactylus.* Lateral fringes are absent on the fingers, except in *S. zygodactylus;* discs are present on all digits, and inner tarsal folds are absent. Vocal slits are present, except in *S. anomalus,* and nuptial pads are present in breeding males. All species have the "E" condition of the adductor musculature. These terrestrial frogs are usually in riparian situations; they are found at night on stones in streams and in the spray zones of waterfalls.

Content.—Six species are placed in the species series: *Strabomantis anatipes, anomalus, bufoniformis, cheiroplethus, necerus,* and *zygodactylus*.

Distribution.—With the exception of *Strabomantis bufoniformis,* which ranges northward into Panama and Costa Rica, all members of this series are restricted to the Chocoan lowlands and adjacent slopes of the Cordillera Occidental on the Andes in Colombia and northwestern Ecuador.

Remarks.— This species series combines the former "*E*." *bufoniformis* and "*E.*" *anomalus* species groups as discussed above in the Remarks for this genus. The adductor musculature condition can be considered a derived character in this group.

Family Ceuthomantidae Heinicke, Duellman, Trueb, Means, MacCulloch, and Hedges, 2009.

Type genus.—*Ceuthomantis* Heinicke, Duellman, Trueb, Means, MacCulloch, and Hedges, 2009.

Diagnosis.—A member of Terrarana (Hedges et al. 2008) based on direct development of terrestrial eggs (inferred), T-shaped terminal phalanges, "S" condition of adductor musculature as defined by Lynch (1986), and its lacking intercalary elements. It differs from other families in that group in having paired dorsal gland-like protrusions of unknown function in the post-temporal, and sacral regions. Although these protrusions appear to have contained lipids, they are not true glands. Body glands, similar in external appearance to these structures, are present in some species of *Eleutherodactylus* (Eleutherodactylidae) but they are located in the inguinal and flank regions. Also, computed tomography scans of the holotype show that the neurocranium is extraordinarily poorly ossified, and the neopalatine is unusually massive.

Content.—One genus, *Ceuthomantis.*

Distribution.—Known only from the Guiana Highlands, northeastern South America.

Genus *Ceuthomantis* Heinicke, Duellman, Trueb, Means, MacCulloch, and Hedges, 2009.

Type species.—*Ceuthomantis smaragdinus* Heinicke, Duellman, Trueb, Means, MacCulloch, and Hedges, 2009.

Diagnosis.—Same as for family. Members of the genus *Ceuthomantis* are unique compared to the strabomantid genera *Dischidodactylus* and *Pristimantis* in the Guiana Highlands by having notched digital discs on the fingers and toes and by lacking dentigerous processes of vomers.

Content.—Tentatively three species, *C. aracamuni* (Barrio Amorós and Molina) and *C. caveribardus* (Myers and Donnelly), new combinations, plus *C. smaragdinus* n. sp. described below, are assigned to the genus.

Distribution.—The genus is known only from elevations of 493–1540 m in the southern and eastern parts of the Guiana Highlands. These include Mt. Ayanganna and the Wokomung Massif in Guyana, Cerro Aracamuni and Sierra Tapirapecó in the Cerro Neblina Massif on the Venezuela-Brazil border, and possibly Sarisariñama Tepui in southern Venezuela (see Remarks). The species are known from the slopes of the mountains and the tops of tepuis.

Etymology.—The generic name is masculine and derived from the Greek noun *mantis,* meaning treefrog and the Greek adjective *keuthos,* meaning hidden and alludes to its hidden existence in the tepuis of the Guiana Shield, which became known as the *Lost World* through the writings of Arthur Conan Doyle (1912).

Ceuthomantis smaragdinus Heinicke, Duellman, Trueb, Means, MacCulloch, and Hedges, 2009.

Holotype.—KU 300000, an adult male, from top of Kamana Falls on Mt. Kopinang, part of the Wokomung Massif, Potaro-Siparuni District, Guyana (05°00'08" N, W 59°52'47" W, ~1540 m elevation), obtained on 18 July 2007 by D. Bruce Means. Field number CPI 10559.

Paratype.—KU 315000, a subadult female collected with the holotype.

Referred specimen.—ROM 40161, a juvenile, from Mt. Ayanganna, Potaro-Siparuni District, Guyana, 1490 m (05°24' N 59°57' W, 1490 m elevation), obtained on 20 October 2000 by Amy Lathrop and Carter Cox.

Diagnosis.—This small frog has: (1) skin on dorsum smooth, that on belly areolate; dorsolateral folds absent; pair of dorsal protrusions in sacral region and small pair in scapular region; discoidal fold not evident; (2) tympanic membrane differentiated;

tympanic annulus low, smooth, round, its diameter about 40% length of eye; (3) snout rounded in dorsal view, bluntly rounded in profile; (4) upper eyelid bearing prominent subconical tubercle; width of eyelid slightly less than interorbital distance; cranial crests absent; (5) dentigerous processes of vomers absent; (6) vocal slits present; nuptial excrescences absent; (7) Finger I shorter than Finger II; discs on outer fingers broadly expanded with terminal notch; (8) fingers lacking lateral fringes; (9) ulnar tubercles absent; (10) heel bearing prominent subconical tubercle; row of conical tubercles on outer edge of tarsus; (11) inner metatarsal tubercle elliptical 3x subconical outer metatarsal tubercle; plantar supernumerary tubercles absent; (12) toes lacking lateral fringes; webbing absent; Toe V slightly longer than Toe III; discs about same size as those on fingers; (13) dorsum olive brown with diffuse black markings and prominent bright green (in life) interorbital bar, subcanthal stripe, and diagonal bars in scapular region; venter pale gray with black mottling; (14) SVL in one male 19.8 mm, in one subadult female 19.5 mm.

Ceuthomantis smaragdinus shares a unique combination of five characters with two other species from elevated areas of the Guiana Shield that we tentatively place in *Ceuthomantis*: *C. aracamuni* and *C. cavernibardus*. These characters are notched digital discs, narrow heads, green coloration, and the absence of vomerine teeth and nuptial pads (Barrio-Amorós and Molina 2006; Myers and Donnelly 1997). Separately, each of these characters is found in other species of terraranans (Hedges et al. 2008; Lynch 1979; Lynch and Duellman 1997; Duellman and Pramuk 1999), but their combination in species from the same region suggests a close relationship. Nonetheless, *C. smaragdinus* differs from both in having paired dorsal gland-like protrusions, prominent subconical tubercle on the upper eyelid and the heel, and a row of conical tubercles on the outer edge of the tarsus.

Other terraranans known from the highlands in the southwestern part of the Guiana Highlands are *Pristimantis avius* (Myers and Donnelly 1997) and *P. memorans* (Myers and Donnelly 1997). These, like all other *Pristimantis* known from the highlands, have vomerine teeth and both lack tubercles of the heels. Furthermore, *P. avius* differs from *C. smaragdinus* by having weak dorsolateral folds, marginate discs on the digits, no eyelid tubercle, a brown dorsum, and a pale orange or yellow venter. *Pristimantis memorans* differs from *C. smaragdinus* by having small tubercles on the eyelid, shallowly indented digital discs, a brown dorsum with dark brown markings, and a gray venter.

Description of the holotype.—Small frog with head much longer than wide, head length 40.9% SVL, head width 33.3% SVL; head narrower than body; snout moderately long, rounded in dorsal view (Fig. A-1), bluntly rounded in profile; eye-nostril distance 80.0% length of eye; loreal region concave; nostrils barely protruding, directed laterally at level well behind anterior margin of lower lip; canthus rostralis slightly curved, rounded in section; lips rounded; width of upper eyelid 85.7% interorbital distance; side of head vertical. One rounded postrictal tubercle posteroventral to tympanum; supratympanic fold weak, barely obscuring posterodorsal edge of tympanum; tympanic membrane differentiated; tympanic annulus low, smooth, round, its diameter 40.0% length of eye; tympanum separated from eye by distance about twice diameter of tympanum.

Skin smooth on dorsum, weakly granular on throat, areolate on belly; discoidal fold not evident; cloacal sheath short, not bordered laterally by fold or tubercles. Prominent subconical tubercle on upper eyelid and heel; row of conical tubercles on outer edge of tarsus; inner tarsal fold absent; inner metatarsal tubercle ovoid, elliptical, three times size of subconical outer metatarsal tubercle; ulnar tubercles absent; thenar tubercle elliptical, slightly elevated, much larger that low, bifid palmar tubercle; plantar supernumerary tubercles absent; subarticular tubercles low, rounded; nuptial excrescences absent; pairs of what appear to be small glandular structures in the post-temporal and sacral regions (Fig. A-1).

Finger I shorter than Finger II; Finger III very long; relative lengths of fingers: I < II < IV < III; discs on outer fingers broadly expanded, rounded with terminal notch (Fig. A-1), lacking lateral fringes; circumferential grooves present; Toe V slightly longer than Toe III; Toe IV very long; discs on toes expanded, rounded with terminal notch, about equal in size to those on fingers; toes not webbed, lacking lateral fringes; relative lengths of toes: I < II < III < V < IV; tip of Toe V extending to base of penultimate subarticular tubercle of Toe IV; tip of Toe III extending to point midway between antepenultimate and penultimate subarticular tubercles on Toe IV. When hind limbs flexed perpendicular to axis of body, heels broadly overlap; shank 59.6% SVL; foot length 40.1% SVL.

Vocal slits and single, median, subgular vocal sac present; vocal slits extending from midlateral base of tongue to point about two-thirds distance to angle of jaw; tongue ovoid, broadest posteriorly, not notched behind, free posteriorly for nearly half of its length; choanae ovoid, not obscured by palatal shelf of maxillary; cranial crests and dentigerous processes of vomers absent.

In life, dorsum dull olive-brown with diffuse black markings on body; black transverse bars on limbs; black longitudinal stripe on inner surface of forearm; black labial bars; broad black canthal stripe; bright, almost phosphorescent green interorbital bar; pair of diagonal marks in scapular region; spot on anterior surfaces of upper arm; distinct green bar below black canthal stripe (Fig. 4-1); dorsal surfaces of discs on fingers white; dorsal surfaces of toe pads creamy white with black suffusion in terminal notch; venter creamy gray, heavily mottled in black; throat nearly entirely black (Fig. 4-1); belly mottled black and gray; iris greenish bronze heavily flecked with black.

In preservative, dorsum tan with irregular paravertebral marks extending from occiput to sacrum; bright green marks in life now pale gray; limbs tan with brown transverse bars; posterior surfaces of thighs brown; belly cream with irregular brown spots; throat black; ventral surfaces of hind limbs brown with cream spots; palmar and plantar surfaces black.

Figure A-1. Dorsal view of female paratype of *Ceuthomantis smaragdinus,* KU 315000 SVL 19.5 mm. Arrows point to the dorsal glandlike structures. The third finger of the right hand is enlarged to show the notched anterior margin of the disc. Photographs by A. Campbell.

Measurements of holotype.—Measurements and proportions of the three known specimens are given in Table A1, below.

TABLE A1. Measurements and proportions of *Ceuthomantis smaragdinus.*

Character	KU 300000 (Male)	KU 315000 (Female)	ROM 40161 (Juvenile)
Snout-vent length	19.8	19.5	14.8
Shank length	11.8	11.7	8.2
Foot length	8.9	9.1	7.1
Head length	8.1	7.8	6.1
Head width	6.6	6.3	4.5
Interorbital distance	2.1	2.0	1.3
Eyelid width	1.8	1.8	1.1
Internarial distance	1.6	1.6	1.2
Eye length	2.5	2.4	2.0
Eye-nostril distance	2.0	1.9	1.5
Tympanum diameter	1.0	1.0	0.8
Head length/SVL	40.9%	40.0%	41.2%
Head width/SVL	33.3%	32.3%	30.4%
Eyelid/IOD	85.7%	90.0%	84.6%
Tympanum/Eye	40.0%	41.7%	40.0%
Shank/SVL	59.6%	60.0%	55.4%
Foot/SVL	40.1%	40.0%	47.9%

Variation.—Both adults (KU 300000 and 315000) and the one juvenile (ROM 40161) are alike structurally, except that glandlike protrusions are less pronounced in the juvenile. The dorsal color pattern is the same in all specimens; the bright green markings are distinct not only in adults but also in the juvenile. The throat in the female and in the juvenile are mottled like the belly, not black as in the male.

The holotype (KU 300000) and female paratype (KU 315000) both bear what appear to be small glandular structures in the post-temporal and sacral regions (Fig. A-1). Close examination reveals the skin to be slightly elevated and to lack melanophores. A section through the structure in KU 315000 shows a disassociation between the connective tissue and the overlying unpigmented skin, whereas the surrounding skin is loosely connected to the underlying muscles by the connective tissue. It is possible that the "bubble" of unpigmented skin might have been filled with adipose cells, which have dissolved in preservative.

Osteology.—The head is widest anterior to angle of jaw at the level of the articulation of the quadratojugal and maxilla, at which level, the medial head length is 98% the head width. The overall width of the head diminishes gradually in the orbital region, being 86% of the greatest width (HWG of Trueb 1977) at the mid- orbit level and 76% of this measure at the anterior margin of the orbit. The rostrum seems especially massive, with its medial length composing 25% of the length of the skull (HLM of Trueb 1977), and its posterior and anterior widths, composing 68% and 27%, respectively, of the greatest width of the skull (Fig. 4-1).

The braincase is poorly ossified. Sphenethmoid ossification is limited to a narrow girdle of bone in the anterolateral walls of the braincase; the anterior limit of the bone is the orbitonasal canal, which has a complete margin in bone. There is an asymmetrical structure apparent dorsomedially that probably represents mineralization of ethmoidal cartilage. The prootic forms the bony anterior, anterodorsal, and anteroventral walls of the otic capsule; the bony posterior walls are formed by the exoccipital. These bones are so poorly ossified that epiotic eminences, as well as most of the lateral parts of the otic capsule, remain cartilaginous. The stapes are exceedingly delicate and small, but there is a large, bony operculum. The bony parts of the exoccipitals and prootics are widely separated from one another and their counter members.

The massive frontoparietals completely roof the central braincase from the anterior level of the orbit to the tectum synoticum posteriorly. The lamina perpendicularis is particularly well developed along the entire orbital margin of the frontoparietal. In the posterior part of the orbit, there is small, knoblike orbital process on the frontoparietal. In lateral profile, a ventral process extends into the orbital fenestra from the lamina perpendicularis at the same level. Posterolaterally, the frontoparietal expands to form a flangelike process that extends dorsally along the anteromedial margin of the anterior epiotic eminence.

The parasphenoid floors the braincase (Fig. 4-1). The long, narrow cultriform process extends from the anterior margin of the sphenethmoid to the otic capsules posteriorly. The alae completely floor the otic capsules and are approximately perpendicular to the cultriform process. The posteromedial process of the parasphenoid is broadly acuminate and does not reach the margin of the foramen magnum.

The nasal region is remarkable for its lack of bony armament. The small, slender nasals are broadly separated—apparently poised along the anterolateral margins of the olfactory capsules leaving the central portions of the capsules exposed in cartilage. Ventrally, the vomers are revealed as a pair of L-shaped bones that seem to lack a dorsal flange. The vomers seem to consist only of pre- and postchoanal bony process to support

the internal choana. The paired septomaxillae are minute and lie dorsal to the partes palatinae and the articulation between the maxilla and premaxilla.

In contrast to the seemingly weak construction of the endocranium, the suspensory apparatus, maxillary arcade, and its support is robust. The otic and ventral rami of the squamosal are especially well developed, with the otic ramus seeming to extend along the entire lateral margin of the cartilaginous crista parotica. The zyogmatic ramus is short and acuiminate in lateral profile. The quadratojugal is particularly robust and bears a broadly overlapping articulation with the maxilla. The maxillae and premaxillae bear teeth, and both have moderately well developed partes palatinae; that of the premaxilla is medially notched to produce prominent medial and lateral flanges. The pars facialis of the maxilla is well developed and bears a large, acuminate preorbital process that extends nearly to the ventral margin of the nasal lateral to the planum antorbitale at the anterior margin of the eye. Anteriorly, the pars facialis overlaps the lateral margin of the pars dentalis of the premaxilla slightly. The pterygoid is stout, triradiate element. The anterior ramus extends toward the braincase from the maxilla at the mid-orbit level and braces against the anteroventral margin of the otic capsule via the short medial ramus. The posterolateral ramus lies in the same plane as the anterior ramus and is about half its length; it provides support for the palatoquadrate cartilage and the jaw articulation. One of the most extraordinary features of the skull is the massive neopalatine, which seems to have encased completely the planum antorbitale and extends from the sphenethmoid laterally to the lingual margin of the maxilla.

The main component of the mandible is the stout angulosplenial, which is weakly sigmoid, bears scarcely no coronoid flange, and extends nearly to the mentomecklian bone anteriorly. The dentary is fused to the mentomecklian anteriorly and extends along the lateral surface of the mandible to terminate in the posterior part of the orbit. The only part of the hyoid revealed are the posteromedial processes, which are long, slender elements that are slightly expanded proximally and distally; the proximal expansion is slightly greater than the distal expansion. There is no mineralization in the hyoid corpus.

The vertebral column is composed of eight nonimbricate, procoelous vertebrae. The atlantal cotylar arrangement is stalked and Type I of Lynch (1973). The tranverse processes are short and not expanded. There is little variation in the overall width of vertebrae with the vertebral profile being as follows: III > Sacrum > II > IV > VII > V ≅ VI > VIII > I. The neural arches are well developed and bear neural spines that are most prominent on Presacrals I–IV; however, the neural arches are exceeding narrow, with the result that much of the spinal column is exposed dorsally. The short, round sacral diapophyses are nearly uniform in width and directly slightly posterolaterally. The sacrum has a bicondylar articulation with the urostyle. The urostyle is short, being only 84% of the length of the presacral vertebral column. It bears a well-developed dorsal crest and one pair of nerve foramina; there is no other evidence of postsacral vertebrae.

The pectoral girdle likely is arciferal. The clavicles are robust, curved, and moderately broadly separated from one another medially; the bones are separated from the adjacent scapulae and coracoids by cartilage. The posterior margin of the stout coracoid is straight, whereas the anterior margin is convex; the long axis of the coracoid is nearly perpendicular to the longitudinal axis of the body. The glenoid and sternal ends of the coracoid are about equally expanded and slightly more than twice as wide as the midshaft width of the bone. A distinct notch separates the pars acromialis from the pars

glenoidalis of the scapula, which is long and slender, with shallowly concave anterior and posterior margins. The suprascapular margin is about twice the width of the narrowest part of the bone, and the length is slightly more than three times the width of the suprascapular margin. The cleithrum is a dagger-shaped element; there is no indication of mineralization of the suprascapular cartilage. Ossified or mineralized pre- and postzonal elements are absent.

The head of the humerus is cartilaginous. There is a moderate crista ventralis or deltoid crest extending along the proximal third of the bone. The cristae medialis and lateralis are not evident, but the eminentia capitata and ulnar and radial condyles are relatively well developed. The radio-ulna has a low olecranon and shallow sulcus intermedius; the epiphyses of the ulna and radius are cartilaginous. All carpal elements and the prepollex, if it is present, are cartilaginous.

The phalangeal formula is 2-2-3-3, and the relative lengths of the digits in increasing order is: II > III > V > IV. Concerning the phalangeal formula: fingers are numbered preaxially to postaxially from II–V, in consistency with the hypothesis that Digit I was lost in anurans (Alberch and Gale 1985; Fabrezi and Alberch 1996; Shubin and Alberch 1986); the reader is cautioned that in older accounts, fingers are numbered from I to IV. The relative lengths of the metacarpals in increasing order is: II > V > III > IV. The phalangeal elements are well ossified with cartilaginous epiphyses. The terminal phalanges are stout, thick elements that are almost hourglass-shaped, with T-shaped distal ends (Fig. A-1).

The postsacral trunk region is short and narrow. The dorsal width of the pelvis at the sacrum is 57% of its overall length, and the angle of expansion is about 33°. The internal margin of the pelvis in dorsal view describes a narrow U-shape. The ilial shaft is smooth and bears a scant indication of low, rounded dorsal ridge that terminates posteriorly in a low knob of a posterior prominence. The preacetabular angle is about 90°. The pubes are lightly mineralized. The ischium is well ossified. The acetabulum is round; about two thirds of it is formed in bone by equal contributions of the ilium and ischium.

There is nothing particularly remarkable in the hind limb except for the lack of ossification (but presence of scattered mineralization) of the epiphyses of the femur, tibiofibula, and tibiale and fibulare. The tibiale and fibulare seem especially long, being about 58% of the length of the tibiofibula. Tarsal elements and a prehallux, if present, are cartilaginous. The phalangeal formula is 2-2-3-4-3, and the relative lengths of the digits in increasing order is: I > II > III = V > IV. The relative lengths of the metacarpals in increasing order is: I > II > III = V > IV. The phalangeal elements are well ossified with cartilaginous epiphyses. The terminal phalanges are stout, thick elements that are almost hourglass-shaped, with T-shaped distal ends (Fig. A-1).

Distribution and ecology.—*Ceuthomantis smaragdinus* is known from two of the easternmost mountains in the Guiana Shield, Mt. Ayanganna and Mt. Kopinang in the Wokomung Massif (Fig. A-2). These mountains are separated by 37 km of uplands that support lower montane forest (Huber *et al* 1995). At the type locality, the forest consists of shrubs, including Melastomataceae (Myrtales), broad-leafed trees about 12 m high, and a few small tree ferns (Cyatheales); the trunks, boles, and limbs of all are festooned with epiphytes, especially dense olive-green moss and many bromeliads. The ground is deep organic peat covered with the same moss and bromeliads as on the trees. The

holotype and paratype were collected after dark in cloud forest at an elevation of about 1540 m. The holotype was sitting on a leaf 1.5 m above the ground about 5 m from a cascading stream; another leaf sheltered it from a heavy rain. The paratype was found 30 min later during a light rain. The juvenile from Mt. Ayanganna was collected at night amidst leaf litter on the ground in dense low-canopy forest at an elevation of 1490 m.

At the type locality 18 other species of anurans were found—*Oreophrynella* cf. *macconnelli* Boulenger, *Anomaloglossus beebei* (Noble), *A. kaiei* (Kok, Sambhu, Roopsind, Lenglet and Bourne), *Pristimantis saltissimus* Means and Savage, *P. dendrobatoides* Means and Savage, *Pristimantis* sp., *Leptodactylus lutzi* Heyer, *Stefania ayangannae* MacCulloch and Lathrop, *S. coxi* MacCulloch and Lathrop, *S. roraimae* Duellman and Hoogmoed, *Vitreorana gorzulae* (Ayarzagüena), *Hypsiboas sibleszi* (Rivero), *Myersiohyla kanaima* (Goin and Woodley), *Osteocephalus* cf. *cabrerai* (Cochran and Goin), *O.* cf. *exophthalmus* (Smith and Noonan), *Otophryne steyermarki* Rivero, and two species of "*Bufo.*" Only six of these are represented in the other 16 species that were found at 1490 m on Mt. Ayanganna—*Anomaloglossus beebei* (Noble), *A. tepuyensis* (La Marca), *Oreophrynella dendronastes* Lathrop and MacCulloch, *Stefania ackawaio* MacCulloch and Lathrop, *S. ayangannae* MacCulloch and Lathrop, *S. coxi* MacCulloch and Lathrop, *S. roraimae* Duellman and Hoogmoed, "*Hyla*" *warreni* Duellman and Hoogmoed, *Hypsiboas roraima* (Duellman and Hoogmoed), *Myersiohyla kanaima* (Goin and Woodley), *Osteocephalus phasmatus* MacCulloch and Lathrop, *Leptodactylus lutzi* Heyer, *Pristimantis inguinalis* (Parker), *P. jester* Means and Savage, *P. marmoratus* (Boulenger) and *P. pulvinatus* (Rivero).

Etymology.—The specific name (*smaragdinus*) is a Latin adjective meaning emerald green and refers to the distinctive marks on the head and body.

Figure A-2. Distribution of the family Ceuthomantidae. Lowlands are indicated by green and uplands by brown. Known localities of the new family are indicated in the northeastern and southwestern portions of elevated areas on the Guiana Shield, in Venezuela, Brazil, and Guyana. (1) Mt. Kopinang, Guyana (*C. smaragdinus*, type locality), (2) Mt. Ayanganna, Guyana *C. smaragdinus*, referred specimen), (3) Pico Tamacuari, Venezuela and Brazil (*C. cavernibardus*), (4) Cerro Aracamuni, Venezuela (*C. aracamuni*), and (5) Sarisariñama Tepui, Venezuela (*C.* cf. *cavernibardus*).

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