FACULDADE DE BIOCIÊNCIAS

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MOLECULAR PHYLOGENETICS OF *Crossodactylus* DUMÉRIL & BIBRON, 1841 (ANURA: HYLODIDAE)

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DISSERTAÇÃO DE MESTRADO

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Danielle Angelini Fabri
Orientador: Dr. Taran Grant

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SUMMARY

Àqueles que atravessam mares e montanhas, florestas e
desertos, sob chuva e contra o vento...
Àqueles que negam o grandioso e abraçam o microscópico...
Àqueles que trilham caminhos diferentes, mas seguem
sempre em frente, motivados por uma mesma paixão:
Ciência.

Can you hear the calling of the raving wind and water?
We just keep dreaming of the land 'cross the river
We are always on the way to find the place we belong
Wandering to nowhere, we're paddling
Down the raging sea
Kajiura Yuki – To Nowhere

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RESUMO

Hylodidae é uma família de anuros composta dos gêneros *Crossodactylus*, *Hylodes* e *Megaelosia*, conhecidos popularmente como “rãzinhas-do-riacho”, e cuja área de distribuição conhecida vai do nordeste do Brasil até o norte da Argentina, através do sul do Paraguai. *Crossodactylus*, conhecido como o gênero de taxonomia mais problemática dos três, é composto de 11 espécies de pequeno tamanho (exceto por *C. grandis*), atualmente divididas entre três grupos de espécies: os grupos *C. gaudichaudii*, *C. trachystomus* e *C. schmidti*, o primeiro dos quais contém a maioria das espécies reconhecidas.

O relacionamento entre Hylodidae e outras famílias de anuros tem sido extensamente discutido, com hipóteses variadas. Ainda assim, a monofilia do grupo parece bem corroborada e tem sido recuperada em diversos estudos filogenéticos independentes. Contudo, apesar das recorrentes menções à sistemática problemática de *Crossodactylus*, suas relações filogenéticas permanecem não testadas. Além disso, a única sinapomorfia proposta para o grupo é a ausência do osso quadradojugal, hipótese já refutada na literatura.

Tendo em vista os problemas ainda presentes em torno de *Crossodactylus*, o presente estudo objetivou testar a monofilia do gênero e seus grupos de espécies, ao mesmo tempo buscando esclarecer os relacionamentos entre espécies do gênero e entre esse e os demais gêneros de Hylodidae. Para tanto, uma análise filogenética de três genes mitocondriais e cinco genes nucleares de diferentes graus de variabilidade foi realizada através do software POY 4.1.2.1, sob a implementação de homologia dinâmica, empregando o critério de otimalidade de máxima parcimônia. 72 táxons do grupo externo e 88 terminais do grupo interno foram incluídos. Do grupo externo, 21 táxons — compostos de 61 terminais — foram sequenciados nesse estudo. Todas as sequências do grupo interno foram geradas nesse estudo, exceto por aquelas de *C. schmidti*, para o qual sequências já estavam disponíveis no GenBank.

Um total de 14 árvores igualmente maximamente parcimoniosas de 25.508 passos foi encontrado, os conflitos das quais se restringiam a relações entre terminais do grupo interno. A monofilia de Hylodidae mais uma vez foi corroborada. O gênero *Megaelosia* foi encontrado como parafilético em relação a *Hylodes*, o qual é monofilético. *Crossodactylus* foi recuperado como um grupo monofilético, irmão do clado composto pelos dois outros gêneros. Descobriu-se que os grupos de espécies como definidos atualmente não refletem os relacionamentos entre espécies, com o grupo *C. gaudichaudii* sendo parafilético com respeito ao grupo *C. schmidti* — e, provavelmente, ao grupo *C. trachystomus*. Além disso, diversos complexos de espécies foram encontrados em *Crossodactylus* e descobriu-se que espécies cuja distribuição acreditava-se ser extensa são na verdade compostas de várias espécies de distribuição restrita. 14 espécies putativas foram descobertas em adição às seis espécies reconhecidas amostradas. O posicionamento das cinco espécies reconhecidas não amostradas nesse estudo permanece desconhecido e, como a maioria destas não é coletada desde os anos 1970–1980, estudos futuros necessitarão de evidência morfológica de modo a endereçar essa questão.

ABSTRACT

Hylodidae is an anuran family composed of genera *Crossodactylus*, *Hylodes*, and *Megaelosia*, commonly known as “torrent frogs”, and known to range from northeastern Brazil through southern Paraguay and northern Argentina. *Crossodactylus*, previously referred to as the most taxonomic problematic of the three, is comprised of 11 small-sized (but for *C. grandis*) species, currently divided among three species groups: the *C. gaudichaudii*, *C. trachystomus*, and *C. schmidti* groups, the first of which contains the majority of recognized species.

The relationship between Hylodidae and other anuran families has been extensively discussed, and hypotheses have been varied. Nonetheless, the monophyly of the group seems well corroborated, and has been recovered in several independent phylogenetic studies. However, despite recurrent mentions to the problematic systematics of *Crossodactylus*, its phylogenetic relationships remain untested. Furthermore, the only proposed synapomorphy for the group is the absence of the quadratojugal bone, a hypothesis which has already been refuted in literature.

In view of the problems still revolving around *Crossodactylus*, this study aimed to test the monophyly of the genus and its species groups, while clarifying relationships among its species, and among itself and the remainder of hylodid genera. For that, a phylogenetic analysis of 3 mitochondrial and 5 nuclear genes of different degrees of variability was performed on software POY 4.1.2.1 under dynamic homology, employing the maximum parsimony optimality criterion. 72 outgroup taxa, and of 88 ingroup terminals were included. Of the outgroup, 21 taxa—comprised of 61 terminals—were sequenced by this study. All ingroup sequences were generated in this study, except for those of *C. schmidti*, for which sequences were already available on GenBank.

A total of 14 equally most parsimonious trees of 25,508 steps were found, the conflicts of which were restricted to relationships between terminals of the ingroup. The monophyly of Hylodidae was corroborated once more. *Megaelosia* was found to be paraphyletic with respect to *Hylodes*, which is monophyletic. *Crossodactylus* was recovered as a monophyletic group, sister to the clade comprising the other two hylodid genera. The species groups as currently defined were found not to reflect the actual relationships among species, with the *C. gaudichaudii* group being paraphyletic with respect to *C. schmidti*, and likely to *C. trachystomus*. Also, several species complexes were found within *Crossodactylus*, and species believed to be widespread were found to be actually several narrowly distributed species. 14 putative species were discovered in addition to the six recognized species sampled. The placement of the five recognized species not sampled by this study remains unknown and, as most of these were last collected in the 1970–1980s, future studies will require morphological evidence in order to address this question.

INTRODUCTION

Hylodidae Günther, 1859 is an anuran family composed of genera *Crossodactylus*, *Hylodes*, and *Megaelosia*, commonly referred to by english names “spinythumb frogs”, torrent frogs”, and “big-tooth frogs”, respectively, or, more generally, as “torrent frogs” in Brazil. The family’s currently known distribution ranges from northeastern Brazil through southern Paraguay to northern Argentina (Frost, 2011; Figure 1).

Though morphologically very similar at first glance, these genera differ greatly in size and composition. *Hylodes*, the most speciose of the three, comprises 24 medium- to small-sized species divided in four species-group; *Megaelosia*, the least speciose, comprises 7 species of relatively large size; and *Crossodactylus*, the most taxonomically problematic hylodid genera (as first noted by Heyer *et al.,* 1990), comprises 11 species, all of them small-sized with the exception of *C. grandis*, which has snouth-vent length comparable to most *Hylodes* and external morphology quite similar to *Megaelosia* (view Figures 2–4).

Crossodactylus was described by Duméril & Bibron (1841) with *C. gaudichaudii* as type species by monotypy. Currently, another ten species of *Crossodactylus* are recognized: *C. trachystomus* (Reinhardt & Lütken, 1862 “1861”); *C. aeneus* Müller, 1924; *C. dispar* A. Lutz, 1925; *C. grandis* B. Lutz, 1951; *C. schmidti* Gallardo, 1961; *C. bokermanni* Caramaschi & Sazima, 1985; *C. dantei* Carcerelli & Caramaschii, 1993 “1992”; *C. lutzorum* Carcerelli & Caramaschii, 1993 “1992”; *C. caramaschii* Bastos & Pombal, 1995; and *C. cyclospinus* Nascimento, Cruz & Feio, 2005.

When describing *C. bokermanni*, Caramaschi & Sazima (1985) divided the species known at that time among three species groups on the basis of two morphological characters of unknown polarity: snout length and shape of *canthus rostralis*. As such, species were divided in groups: (1) the *C. gaudichaudii* species group, comprised of *C. aeneus*, *C. bokermanni*, and *C. gaudichaudii*, and characterized by an acuminate snout and well-defined *canthus rostralis*; (2) the *C. trachystomus* species group, comprised of *C. dispar*, *C. grandis,* and *C. trachystomus,* and characterized by a short, rounded snout and poorly defined *canthus rostralis*; and the monotypic group of (3) *C. schmidti*, separated from other species by its “very short snout, rounded *canthus rostralis* and great interorbital space” (Caramaschi & Sazima, 1985: 48). All species described subsequently—*C. dantei*, *C. lutzorum*, *C. caramaschii* and *C. cyclospinus*—were allocated to the *C. gaudichaudii* species group and, except for Pimenta *et al.*’s (2008) questioning of the inclusion of *C. bokermanni* in that group, at no time were the applicability or the very definition of the

groups criticized.

Despite several authors' warnings about the problematic systematics of *Crossodactylus* (Heyer *et al.*, 1990; Haddad *et al.*, 2003; Ribeiro *et al.*, 2005; Izecksohn & Carvalho-e-Silva, 2001; Pimenta *et al.*, 2008), the phylogenetic relationships have not yet been submitted to a rigorous testing. Pimenta *et al.* (2008), in their publication on morphological and acoustical variation in *C. bokermanni*, employed external morphological characters to compare 55 unidentified specimens of *Crossodactylus* from several localities and museum collections with *C. trachystomus and* species of the *C. gaudichaudii* species-group (*i.e., C. aeneus, C. bokermanii, C. caramaschii, C. cyclospinus*, *C. dantei*, *C. lutzorum*, and *C. gaudichaudii*). These characters, however, were not described or listed in the publication and, up to this moment, the only known synapomorphy for *Crossodactylus* is the absence of the quadratojugal bone (Nuin & do Val, 2005), a character coded by Ponssa (2008) as present in *C. gaudichaudii*.

Lynch (1971: 165), in his study of Leptodactylidae, considered *Crossodactylus* as primitive relative to the other species of Elosiinae (= Hylodidae *sensu* Grant *et al.*, 2006; *i.e.*, *Crossodactylus* + [*Hylodes* + *Megaelosia*]), due to secondary sexual and larval phenotypic characteristics: (1) median, subgular vocal sac, (2) nuptial asperities, and (3) median vent tube; but considered the genus as specialized for the loss of the quadratojugal (although it is visible in his Figure 108, of the skull of *C. gaudichaudii*; also see above). Lynch's (1971) observation of the vent tube of *Crossodactylus*, however, was disputed by Weber & Caramaschi (2006), who reported to have found a dextral vent tube in all specimens examined in their study. Lynch (1971) also cited the thigh musculature of *Crossodactylus* as having a ranoid pattern of attachment of the distal tendons, distinct from the pattern observed in *Hylodes* and *Megaelosia*. Nonetheless, the ranoid and bufonoid patterns as defined by Noble (1922) have been extensively discussed in studies of dendrobatids (*e.g*. Ford, 1993; Grant *et al.*, 1997; Grant *et al.*, 2006) and Grant *et al.* (1997: 31) reported *Crossodactylus* as having a bufonoid pattern, as observed in several specimens examined—citing two specimens of *C. dispar* (AMNH 103756 and 103760) and another unidentified specimen (AMNH 103789). Thus, available evidence does not clearly indicate a distinction between *Crossodactylus* and *Hylodes* and *Megaelosia* with respect to the insertion of the distal tendon of the *m. semitendinosus* (Grant *et al.*, 1997, footnote 20).

The relationship between Hylodidae and other anuran families, in turn, has been extensively discussed and phylogenetic hypotheses have varied extensively. Such discrepancy could hardly be considered surprising, as taxonomy of *Hylodes* alone was sufficient for great controversy (see below), and specially as the first phylogenetic studies

of anurans considered only a few, determined morphological aspects for analyses, being therefore subject to erroneous interpretation (*e.g.* taking homoplasies for synapomorphies [see de Pinna, 1996]) or incomplete and/or inadequate character coding. For instance, Noble (1922) already admitted the little reliability of osteological characters in his introductory chapter on de inadequacy of certain characters for phylogenetic studies. Bogart (1970) specifically criticized the usage of determined characters for delimiting genera of Leptodactylidae, while Ford (1993) and Grant *et al.* (2006) provided multiple examples where poor character-coding affected the phylogenetic placement of Dendrobatoidea (*sensu* Grant *et al.*, 2006) relative to other anuran families. As such, Hylodidae, after originally recognized as a family by Günther (1858; see below), has been known as subfamily Elosiinae in Bufonidae (Noble, 1931; see below), as family Elosiidae (Miranda-Ribeiro, 1926), again as subfamily Elosiinae, in Leptodactylidae (Lynch, 1971), as subfamily Hylodinae in Cycloramphidae (Frost *et al.*, 2006), and was finally brought to family status again by Grant *et al.* (2006). The monophyly of the group was been tested and corroborated several times as part of ample studies (*e.g.*, Lynch, 1971; Heyer, 1975; Haas, 2003; Frost *et al.*, 2006; Grant *et al.*, 2006; Pyron & Wiens, 2011), and specifically by Nuin & do Val (2005). As detailing of hypotheses of phylogenetic relationships involving this group would be far too long and unnecessarily confusing, the summary provided in this study is restricted so as to reflect only those hypotheses which are most relevant or were most influential in other studies of Hylodidae, while considering the objectives of this study; references cited herein can be consulted for a more thorough retrospect.

SYSTEMATIC HISTORY

Günther (1858) originally proposed Hylodidae to accommodate genera *Crossodactylus*, *Hylodes*, *Phyllobates,* and *Platymantis*, with *Hylodes* Fitzinger, 1826 as type-genus (Lynch, 1971; Savage, 1986). Miranda-Ribeiro (1926), proposed Elosiidae for genera *Crossodactylus*, *Elosia* (= *Hylodes*) and *Megaelosia*, with *Elosia* Tschudi, 1838 as type-genus. Despite great taxonomic confusion generated by Fitzinger and his 1826 and 1843 publications (see Lynch, 1971; Savage, 1986), the name Hylodidae had precedence over Elosiidae, being synonymized by Savage (1973; *apud* Frost, 2011; see Savage, 1986).

Noble (1926), when commenting on the structure of the pectoral girdle of his Brachycephalidae, asserted that the family included at least three distinct groups,

independently originated from bufonid ancestrals (see Grant *et al.*, 1997: 31, footnote 18, on Noble's vision of natural non-monophyletic groups), pointing out the second group, made of *Hyloxalus*, *Phyllobates*, and *Dendrobates*—which he later recognized as Dendrobatinae (Noble, 1931: 507)—as directly descendant from *Crossodactylus*, based on the presence of dermal scutes on the dorsal surface of digits. Furthermore, he (Noble, 1926: 9) affirmed that the pectoral girdle of *Crossodactylus* showed "an approach to the firmisternal condition", present in the second group, "in the great reduction and slight overlap of the coracoid cartilages", and that the genus "gave rise to *Hyloxalus* by merely a fusion of the coracoid cartilages". Later, Noble (1931: 504) defined Elosiinae as "Bufonidae with a pair of scute-like structures on the upper surface of each digit tip" and referred to *Crossodactylus* as "merely an *Elosia* without vomerine teeth" (see Figure 8). It is interesting to highlight that Bufonidae *sensu* Noble (1931) included numerous groups that would later be recognized as phylogenetically distant families (see Frost, 2011).

Ardila-Robayo (1979), in her revision of the systematic status of *Geobatrachus* (Strabomantidae), codified 67 morphological characters and number of chromosomes for her analyses, incorporating data published by Lynch (1971) and Heyer (1975) for species of Leptodactylidae (*sensu* Lynch, 1971). Two equally most parsimonious topologies (Figure 11) were found: (1) *Megaelosia* + (*Crossodactylus* + *Hylodes*) as sister-group to Phyllobatinae (= Dendrobatoidea *sensu* Grant *et al.*, 2006), and *Thoropa* as sister-group to that clade; (2) *Thoropa* + (*Crossodactylus* + *Hylodes*) as sister-group to *Megaelosia* + Phyllobatinae (Ardila-Robayo, 1979).

Haas (2003) coded 152 characters from 81 species of Anura and four species of Caudata: 136 larval, and 14 adult morphology characters and six reproductive biology characters. Only two hylodines were included, namely *Crossodactylus schmidti* and *Hylodes meridionalis*, but the monophyly of Hylodinae was supported (Figure 12) based on two synapomorphies: (1) T-shaped terminal phalanges, and (2) complex reproductive behavior, in which a territorial male guides the female to a suitable oviposition site (Zimmermann & Zimmermann, 1988; Weygoldt & Carvalho-e-Silva, 1992; *apud* Haas, 2003). Haas (2003) found Hylodinae to be the sister-group of Dendrobatidae, asserting that the diurnal habits cited by Weygoldt & Carvalho-e-Silva (1992), as well as hand musculature aspects pointed out by Burton (1998), could serve as additional synapomorphies for such phylogenetic relationship.

Nuin & do Val (2005) used 49 morphological characters in their analysis of Hylodinae. Of these, 44 were defined by Heyer (1973, 1975) in his studies of *Leptodactylus* and Leptodactylidae, respectively; the remaining five characters were defined by Lobo (1994)

in an osteological study of *Physalaemus* (Leiuperidae; also in Leptodactylidae at the time of the study). Nuin & do Val (2005) only found two synapomorphies for Hylodinae: (1) dorsal scutes on adhesive discs, and (2) extensive tarsal fold. The sister-group of Hylodinae could not be determined due to problems on the resolution of the outgroup (Nuin & do Val, 2005: 3–4). *Megaelosia* was recovered as sister-group to *Crossodactylus* + *Hylodes*, with all genera, as well as species-groups of the latter two, considered monophyletic (Figure 13). However, only nine species of two species-groups of *Hylodes* (*H. lateristrigatus*, *H. phyllodes*, *H. ornatus*, and *H. sazimai* of the *H. lateristrigatus* species-group; *H. asper*, *H. dactylocinus*, *H. nasus*, *H. meridionalis*, and *H. perplicatus* of the *H. nasus* species-group), three species of two groups of *Crossodactylus* (*C. caramaschii* and *C. dantei*, of the *C. gaudichaudii* group, and *C. schmidti*, of the monotypic *C. schmidti* group) and one single species of *Megaelosia* (*M. goeldii*) were included in the analysis. Nuin & do Val (2005: 143) recognized that increased taxon sampling could overturn their findings and suggested that molecular evidence might be a better approach to further clarify intergeneric relationships in Hylodinae.

Frost *et al*. (2006) included *C. schmidti*, *H. phyllodes* and M. *goeldii* in their expansive analysis of Amphibia. Molecular characters codified from the mitochondrial H-strand transcription unit 1 (H1; including the 12S ribosomal, $tRNA^{Valine}$ ($tRNA^{val}$), and 16S ribosomal sequences), the nuclear protein coding genes histone H3 (H3), tyrosinase (tyr), rhodopsin (rhod), and *seventh in absentia* (SIA), and the nuclear 28S ribosomal gene were added to Haas's (2003) matrix of morphological characters and analyzed to produce four equally most parsimonious trees for the 532 terminal taxa included. The strict consensus tree (Figure 14; see their Figure 50) showed Hylodinae nested in Cycloramphidae (excluding *Thoropa*, which was recovered as sister-group to Dendrobatidae), and *Crossodactylus* as sister-group to *Megaelosia* + *Hylodes.* Hylodinae was supported by 70 molecular transformations, and diagnosed by three morphological synapomorphies: the presence of (1) a lateral vector to the alary processes, (2) T-shaped terminal phalanges, and (3) dermal scutes on the top of digital discs (Lynch, 1971, 1973; *apud* Frost *et al.*, 2006). Still the authors considered (Frost *et al.*, 2006: 128) the monophyly of the group to be poorly supported by molecular evidence, but noted the morphological evidence suggested by Lynch as additionally corroborating the close relationship of those genera (1971, 1973).

Also in 2006, Grant *et al.* published their findings on the phylogenetic relationships of Dendrobatoidea, expanding on the character-sampling of Frost *et al*. (2006) by adding fragments for the mitochondrial genes cytochrome *b* (cytb), and cytochrome oxidase *c*

subunit I (COI), the nuclear recombination activating gene 1 (RAG1), and morphological and behavioral characters—though naturally restricting their taxon-sampling to fewer (414) taxa, more relevant to the problem of dendrobatoids; their sampling of Hylodidae was the same as Frost *et al.*'s (2006). In addition to 103 molecular transformations, nine morphological synapomorphies were listed for Hylodidae: origin of (1) preaxial fringe on finger II, (2) preaxial fringe on finger III, (3) tarsal fringe, (4) preaxial fringe on toe I, and (5) postaxial fringe on toe V, loss of (6) oocyte pigmentation, (7) fibers of *m. depressor mandibulae* originating from the *annulus tympanicus*, (8) origin of paired lateral vocal sacs, and (9) gain of lateral line stitches (Grant *et al.*, 2006). Once again, the monophyly of the group was corroborated (Figure 15), and *Crossodactylus* was recovered as sister-group to *Megaelosia* + *Hylodes*, only they diverged from those of Frost *et al.* (2006) on the relationship between Hylodinae and Cycloramphidae (*sensu* Frost *et al.*, 2006). Hylodinae was recovered outside of Cycloramphidae, as sister-group to superfamily Dendrobatoidea —being once again elevated to family status—making the unranked clade Nobleobatia. The sister-group of Nobleobatia, in turn, was Bufonidae (Grant *et al.*, 2006)

Finally, Pyron & Wiens (2011) published the most extensive phylogeny of Amphibia to date. Although they did not provide any new data, they expanded on Frost *et al*.'s (2006) taxon sampling, with 2,871 species (versus the 522 species sampled for that study), and targeted 12 genes for inclusion in their exclusively molecular analysis: nuclear genes (1) C-X-C chemokine receptor type 4 (CXCR4), (2) histone 3a, (3) sodium–calcium exchanger (NCX1), (4) pro-opiomelanocortin (POMC), (5) recombination activating gene 1, (6) rhodopsin, (7) *seventh in absentia*, (8) solute-carrier family 8 (SLC8A3), (9) and tyrosinase, as well as mitochondrial genes (10) cytochrome *b*, and the (11) large and (12) small subunits of the mitochondrial ribosome genes (12S/16S). Their sampling of Hylodidae was also increased in relation to Frost *et al.* (2006) and Grant *et al.* (2006), with the inclusion of *C. caramaschii*, *H. dactylocinus*, *H. meridionalis*, *H. ornatus*, *H. perplicatus*, and *H. sazimai*[1] in addition to the three hylodids sampled in those studies. Some worrisome comments were made in their Material and Methods (Pyron & Wiens, 2011: 545), however, such as:

> We removed a few (<10) taxa with identical sequence data for all genes (arbitrarily retaining the first in alphabetical order), to avoid potentially misidentified or otherwise confounded specimens or sequences.

[1] Sequences by P.A.S. Nuin (unpublished results) available on GenBank.

The selection of retained terminals solely based on the Alphabet not only left plenty of room for those terminals to be the ones of mistaken identity, but put the rigor of their methods in question. Pyron & Wiens (2011) did not seem to consider this potentially problematic, though, as they never clarified which terminals were removed or retained, moving on to say (on page 545):

> We selected *Homo* as an outgroup because data were available for *Homo* from all 12 genes, and the sister group to Amphibia is Amniota (*e.g.*, Alfaro et al., 2009; Hugall et al., 2007; Pyron, 2010).

A procedure which rendered the outgroup of very little evidential value—as *Homo* was the sole outgroup taxa—not to mention forced the monophyly of Amphibians. Their maximum-likelihood tree recovered a monophyletic Hylodidae, with *Crossodactylus* as sister to *Hylodes*, in which *M. goeldii* was embedded. Hylodidae, in turn, was embedded in a mixture of Ceratophryidae and Cycloramphidae, the paraphyly of those two families (and the support of the clades recovered within them) being used to support splitting them into seven families (Pyron & Wiens, 2011).

Considering all lines of evidence analyzed so far, as well as philosophical and methodological aspects of each study, that of Grant *et al.* (2006) is the most rigorous context in which *Crossodactylus* has been analyzed. Yet, as this study focused on the phylogenetic relationships of species of Dendrobatoidea, only three species of hylodids (once again, *Crossodactylus schmidti, Hylodes phyllodes*, and *Megaelosia goeldii*) were analyzed, and as admitted by authors themselves (Grant *et al*., 2006: 50), character-sampling was “strongly biased to reflect variation among dendrobatid terminals”.

BIOLOGICAL BACKGROUND

In general terms, species of Hylodidae are known to live along streams where males call—with the exception of those *Megaelosia*, for which call’s remain unknown (see Giaretta *et al.*, 1993). Most publications in the area concern *Hylodes* (*e.g.* Haddad & Pombal, 1995; Haddad & Giaretta, 1999; Nascimento *et al.*, 2001; Haddad *et al*., 2003; Pombal *et al.*, 2002; Wogel *et al.*, 2004; Narvaes & Rodrigues, 2005; Lingnau & Bastos, 2007; Lingnau *et al*., 2008; Hatano *et al.*, 2009) and, although there are interesting reports of visual signaling in this genus (*e.g.* Haddad & Giaretta, 1999; Wogel *et al.*, 2004; Narvaes & Rodrigues, 2005), there is very little information available on reproductive

habits, behavior, or other biological aspects for the great majority of hylodid species. Still, a few reproductive aspects of *Crossodactylus* are known and noteworthy.

Caramaschi & Sazima (1985: 48) claimed that, with the exception of thicker forearms in male specimens, secondary sexual characteristics were inconspicuous in *Crossodactylus*. Such assertion may not necessarily be due to lack of sexual dimorphism, but possibly due to the fact that sexual characteristics usually considered exclusively of males, namely the presence of cornified spines at the base of finger I (Miranda-Ribeiro, 1926; Lynch, 1971; Caramaschi & Sazima, 1985) can be found in both male and female of most species of this genus (Caramaschi & Sazima, 1985; Carcerelli & Caramaschi, 1993; Cochran, 1955; Nascimento *et al*., 2005; personal obs.). Notwithstanding, Pimenta *et al.* (2008) disputed such claim, arguing that more prominent tarsal and toe fringes on males was a common dimorphic characteristic in *Crossodactylus*, as they observed in all examined specimens of the *gaudichaudii* species-group and in *C. trachystomus*. The same had been already noticed by Duméril & Bibron (1841) when describing *C. gaudichaudii*, and by Nascimento *et al.* (2005), when describing *C. cyclospinus*. Jordão-Nogueira *et al*. (2006: 38) report that "[m]ature males were readily distinguished from females by their more developed tarsal folds and toe fringes" in *C. aeneus*, while Cochran (1955: 247), when commenting on *C. dispar* noticed that "[t]he most apparent structural differences between male and female [were] the blunt snout and swollen head of the former, together with its greatly thickened forearm".

Weygoldt & Carvalho-e-Silva (1992) reported the reproductive behavior of *C. gaudichaudii* specimens collected in the city of Rio de Janeiro and kept in captivity in terraria with small, artificial streams. The authors detailed (Weygoldt & Carvalho-e-Silva, 1992: 37) male calling behavior, which included the use of different calls, visual signaling and aggressive encounters, with specimens showing great territoriality. Oviposition took place in narrow openings excavated in the rocky bottom of the streams by calling males (and sometimes by receptive females) and clutches were later hidden by male specimens, some of which would then return to the rock above where the clutches laid and guard the place from invaders for some days; poorly concealed clutches were soon found and consumed by conspecific tadpoles (Weygoldt & Carvalho-e-Silva,1992). Weygoldt & Carvalho-e-Silva (1992) also emphasized the similarities in the reproductive behavior of *Crossodactylus* and Dendrobatidae (*sensu lato*), such as: (1) possible absence, or very short time of amplexus, as mating pairs were never observed in amplexus when choosing mating sites (the dimensions of which could serve as an impediment); (2) the fact that the male leaves oviposition site before the female does, and (3) returns after the female has

left.

Aguiar-Jr. *et al.* (2006) analyzed the ultrastuctural characteristics of sperm of *H.phyllodes*, *Crossodactylus* sp., and *M. massarti*, finding it to be very similar in all three species, specially with regards to the acrosomal complex and midpiece. The structure of the acrosomal complex was also very similar to that of Leptodactylidae (of which Hylodidae was considered to be a subfamily at the time), Bufonidae and Dendrobatidae (*sensu lato*), however, as it was considered to be a plesiomorphic trait, it did not add much to the understanding of relationships of hylodids and the other groups. Furthermore, *H. phyllodes* and *M. massarti* showed a distinctive condition in their axial and juxtaxonemal fibers, while *Crossodactylus* showed conditions also believed to be plesiomorphic of leptodactylids.

The larvae of five species of *Crossodactylus* are known and have been described: *C. bokermanni* (Caramaschi & Sazima, 1985), *C. dispar* (Bokermann, 1963 [see Faivovich, 1998]), *C. gaudichaudii* (Francioni & Carcarelli, 1993), *C. schmidti* (Faivovich, 1998), and *C. trachystomus* (Caramaschi & Kisteumacher, 1989). Weber & Caramaschi (2006) described the internal oral morphology of *C. dispar*, *C. gaudichaudii*, and *C. trachystomus*, and concluded that system was not sufficient to corroborate the species-groups proposed by Caramaschi & Sazima (1985). Additionaly, they (Weber & Caramaschi, 2006) and Faivovich (1998) demonstrated that as pigmentation of ventral fin, shape of spiracle, presence or absence of inframarginal papillae, presence or absence of a constriction behind the eyes, and shape of ventral *velum* are useful in diagnosing the different species of the genus.

Another interesting morphological particularity of *Crossodactylus* is the presence of small, keratinous spines along the upper lip (Cochran, 1955; Gallardo, 1961; Caramaschi & Sazima, 1985; Carcerelli & Caramaschi, 1992; Bastos & Pombal, 1995; Nascimento *et al.*, 2005). Gallardo (1961: 37; freely translated from the Spanish) went so far as to speculate such spines could be “interpreted as the persistence of the uppermost larval tooth row (though with a function different from that of the larval teeth)”. Those structures are reportedly present in *C. cyclospinus*, *C. grandis*, *C. schmidti*, *C. trachystomus* (Cochran, 1955; Gallardo, 1961; Lutz, 1952; Nascimento *et al.*, 2005; Reinhardt & Lütken, 1862), and controversially in *C. gaudichaudii*, for which it has been reported as present by Steindacher (1907; *apud* Gallardo, 1961) and as absent by Nascimento *et al*. (2005). Indeed, Caramaschi & Sazima (1985) alleged that the presence or absence of such spines was quite variable, both inter- and intraspecifically, ranging from a few, very small white spines to a complete row of sparse, large, and dark spines. Interestingly, several species

of *Hylodes* are known to have a row of small (sometimes minuscule), unkeratinized tubercles along the upper lip (*e.g. H. fredi*, *H. meridionalis*, *H. otavioi*, *H. pipilans*, *H. phyllodes*, *H. uai*; personal obs.), but a hypothesis of homology of such tubercles and the supralabial spines of *Crossodactylus* has never been formally proposed, let alone tested.

Considering available evidence (*e.g.* Haas, 2003; Nuin & do Val, 2005; Frost *et al.*, 2006; Grant *et al.*, 2006), the monophyly of Hylodidae seems strongly corroborated. However, the same cannot be said about the phylogenetic relationships of its genera: hypotheses diverge when it comes to the relationship among genera and their monophyly remains poorly tested. The species-groups proposed by Caramaschi & Sazima (1985) for *Crossodactylus* species also remain very poorly tested. Once very few, and basically the same, hylodid species have been included in phylogenetic analyses up to this moment, further taxon- and character-sampling could not only clarify those relationships, but also overturn previous hypotheses. As such, this study aimed to test the monophyly of *Crossodactylus* and its species groups, while also clarifying the relationships among species of *Crossodactylus,* and among this and other hylodid genera—*i.e.*, *Hylodes* and *Megaelosia*.

MATERIALS AND METHODS

TAXON SAMPLING

Ingroup Selection

Inclusion of ingroup *taxa* (*i.e.*, *Crossodactylus* specimens) in the analyses was guided by availability of (1) tissues for DNA extraction, and (2) sequences deposited on GenBank. With previous mentions on the problematic taxonomy of *Crossodactylus* (*e.g.* Heyer *et al.*, 1990; Haddad *et al.*, 2003; Ribeiro *et al.*, 2005; Izecksohn & Carvalho-e-Silva, 2001) in mind, whenever possible, I sequenced multiple specimens from several localities. Figure 5 shows localities where samples were collected.

For this study, I was able to obtain tissue samples for six of the 11 currently recognized species of *Crossodactylus*, namely *C. aeneus*, *C. bokermanni*, *C. caramaschii*, *C. gaudichaudii*, *C. schmidti* (sequences already available on GenBank), and *C. trachystomus*. Additionally, there was a great number of tissue samples from unidentified specimens from various localities, ranging from the state of Santa Catarina, in southern Brazil, to the state of Bahia, in the northeast. The identified and unidentified samples comprised a total of 88 ingroup specimens included in the analyses. Among species not sampled, *C. dispar* and *C. grandis* have not been collected for the past 30–40 years despite fieldwork in localities where specimens had been found previously (B. Pimenta, personal commun.; personal obs.).

Outgroup Selection

In very simple terms, the inclusion of an outgroup in an analysis serves to the root the tree—and, consequently, the polarization of character-states—and to test the monophyly of the group whose relationships are being studied, *i.e.*, the ingroup (Farris, 1972; Nixon & Carpenter, 1993). To achieve that, researchers usually make use, somewhat instinctively, of groups believed to be closely related to the ingroup by either such criteria as morphological or molecular data or by previously tested phylogenetic hypotheses, usually giving special emphasis to the studied group's sister-group, as such phylogenetic proximity.

Considering the magnitude of Anura, as well as the restrictions imposed by the aforementioned criteria, outgroup selection was based on the phylogenies presented by Frost *et al.* (2006), Grant *et al.* (2006), and Pyron & Wiens (2011), and inclusion of outgroup taxa took place under the same limiting conditions as for ingroup taxa (see previous section). As such, I included samples from specimens of genera *Hylodes* and *Megaelosia* (Hylodidae), as well as specimens from the following taxonomic families: Aromobatidae, Bufonidae, Centrolenidae, Ceratophryidae, Cycloramphidae, Dendrobatidae, Hylidae, Leiuperidae and Leptodactylidae. Figures 6A-B and Figure 7 show approximate place of collection for samples of *Hylodes* (localities were split in two maps, due to overlap of type- or collection localities; unidentified specimens are shown in red in both maps) and *Megaelosia*, respectively. A total of 72 outgroup taxa were included, of which 51 (1 specimen of each sampled species) belong to families other than Hylodidae, 5 (13 specimens) belong to *Megaelosia*, and 16 (48 specimens) belong to *Hylodes*. *Bokermannohyla* sp. (Hylidae) was used to root the tree. The complete list of outgroup taxa obtained from GenBank with respective accession numbers is given in Table 1.

CHARACTER SAMPLING

Molecular character coding was performed following the procedure described by Grant *et al.* (2006). As that study represents the most rigorous context in which *Crossodactylus* was analyzed, and as the present study intended to find additional evidence by expanding inclusion of ingroup and outgroup taxa relevant to the analysis of this genus, I used the same primers and loci used by Grant *et al*. (2006), with the exception of *seventh in absentia* (SIA), which could not be included for logistical reasons. Also, as noted by Grant *et al.* (2006), these genes show different degrees of variability, which allows for testing hypotheses of relationships at differing levels. Thereby I amplified and sequenced DNA samples for genes of different degrees of variability, namely: the mitochondrial genes H-strand transcription unite 1 (H1)—which includes 12S ribosomal, $tRNA^{val}$ and 16S ribosomal sequence—cytochrome *b* (*cytb*) and cytochrome oxidase *c* subunit I (COI), and the nuclear protein coding genes histone H3, rhodopsin (rhod), tyrosinase (tyr), recombination activating gene I (RAG1), and the nuclear 28S ribosomal gene. All primers used in this study are listed in Table 2.

For generating new sequences, whole cellular DNA was extracted from ethanol-

preserved tissues using the DNeasy kit (Qiagen) following the manufacturer's guidelines, followed by PCR amplification using PCR Master Mix (2X) K0171 kit (Fermentas) in 96-well plates for 25 µl reactions. The standard PCR program was the same employed by Grant *et al.* (2006: 55), which consisted of an initial denaturation step of 180 s at 94 ºC, 35–40 cycles of 60 s at 94 ºC, 60 s at 45–62 ºC, and 60 s at 72 ºC, followed by a final extension step of 360 s at 72 ºC. PCR-amplified products were purified with Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific) enzymes following protocol provided by the manufacturer. Cycle-sequencing was run in 10 µl reactions using BigDye Terminators 3.1 (Applied Byosystems), and products were cleaned and desalted by sodium acetate-ethanol precipitation. Reading of sequencing reactions was performed by Macrogen Inc. (Seoul, Korea) and Genomic Engenharia Molecular (São Paulo, Brazil). Sets of overlapping sequences from each sample and for each gene were assembled into contigs using Sequencher 4.9 (Gene Codes). All sequences were cross-checked and compared with GenBank sequences using NCBI's (National Center for Biotechnology Information) BLAST tool in order to identify possible sequencing and identification errors, as well as cross-contamination. One contaminated fragment was identified and excluded prior to the analyses.

Although there is some morphological evidence available for hylodids, that evidence is very restricted. While Grant *et al*. (2006) coded a considerable variety of morphological characters, only three specimens of Hylodidae were sampled: *C. schmidti*, *H. phyllodes* and *M. goeldii*. Nuin & do Val (2005), in turn, while having a broader taxon sampling of the family (13 species; see "Taxonomic History"), had a much narrower, and perhaps less informative (given that the character list was adapted from works on leptodactylids, and the lack of resolution obtained in their results) character sampling. Due to time limitations, I decided to invest on generating larger amounts of data by applying my efforts in obtaining molecular data, instead of expanding morphological character sampling of hylodids, which would mean a lesser cost-benefit relationship in terms of time spent and evidence obtained. This should not be taken to mean that I disregard the importance of morphological characters as a source of evidence, or of the morphological evidence already available. Instead, it should be simply taken as what it is: a practical decision, made while bearing practical limitations in mind.

PHYLOGENETIC METHOD

Character Treatment

Sequences were initially aligned using default parameters and examined on BioEdit v. 7.1.5 (Ibis Biosciences). This preliminary alignment was used to identify highly conserved regions in all sequences, which were then used to divide the sequences into few homologous fragments following the method described in Grant et al. (2006: 56). By dividing sequences into smaller fragments through the insertion of pound signs (#) at the se regions, search speed is optimized by restricting comparisons between fragments of, instead of complete sequences, which greatly reduces the number of comparisons made and hence the memory requirements for the analyses. This procedure also allows for the inclusion of incomplete sequences (*e.g.,* multiple fragments of H1) and the removal of long strands of nucleotides of unknown identity, making it particularly relevant for the inclusion of sequences obtained from GenBank. It is important to note that although the use of highly conserved regions for breaking sequences into fragments generally avoids arbitrary assumptions of homology, as these conserved regions provide evidence for the homology of those fragments, this procedure was done sparingly so as not risk overly constraining the analysis and to minimize any assumptions made, however well-grounded they might seem. This procedure was only employed when necessary to accommodate incomplete sequences or to have fragments no longer than around 500 bases in order to accelerate searches under dynamic homology (see below). Once all highly conserved regions were identified and all necessary pound signs inserted, all gaps were removed.

Longer fragments downloaded from GenBank, after preliminary alignment, had exceeding nucleotides removed to match the length of the generated sequences, as these were a minority and, if kept longer, would result in such great amount of missing data for the remaining terminals that the quality of the analysis could be compromised. Although aware that this procedure might eliminate informative variation, I “clipped” those sequences right before the start of the primer region used for generating the new sequences, and, as these primers correspond to highly conserved regions, the same principle for breaking long sequences explained above applies.

Optimality Criterion

All molecular data obtained were submitted to POY 4 (Varón *et al.*, 2010) for a total evidence analysis[2] under the criterion of maximum parsimony, with equal weights attributed to character-states transformation events. The choice of maximum parsimony as an optimality criterion, the use of equal weights for transformation events and of the software POY 4 for the analyses was done so as to maximize explanatory power (*sensu* Kluge & Grant, 2006).

Kluge & Grant (2006) follow the Popperian logic in asserting that the simplest hypotheses have greater explanatory power as they are more restrictive, less probable and therefore more easily refuted. They employ this assertion in operationalizing Baker's (2003) anti-superfluity principle (ASP) as a justification for maximum parsimony. According to the ASP, the simplest hypothesis (*i.e.*, the one that requires the least transformation events) to explain the character-states observed in the terminal taxa is the most refutable one and hence has greater explanatory power. A less parsimonious hypothesis involves additional explanation (transformations) and/or require auxiliary claims so as to match the explanatory power of a most parsimonious hypothesis. These transformations and auxiliary claims, however, are superfluous and restrict one's ability to refute an hypothesis, and therefore actually decrease explanatory power and should be disregarded (Kluge & Grant, 2006; Grant & Kluge, 2008a). As such, the simultaneous analysis of all available evidence, equally weighted, maximizes explanatory power in that it characterizes a more severe test by maximizing precision, and minimizing incongruence among independent data by minimizing the total number of hypotheses of transformation events (Grant & Kluge, 2003; Kluge & Grant, 2006). The choice of POY 4 as the software to run the analyses was also done so as to maximizes explanatory power by taking advantage of the analytical framework of dynamic homology. Through dynamic homology, the most parsimonious solution(s) is sought by generating different nucleotide alignments for each topology obtained in order to minimize transformation events in DNA sequences, and,

[2] In the sense that all available molecular data is analyzed simultaneously. Although the term may be confused with the simultaneous analysis of morphological and molecular data, it actually refers to a simultaneous analysis of all available evidence, without making any distinctions as to what type of evidence that is, but treating everything as equally important parts of a whole, the individual from whence the evidence came. I refer to "individual" to mean "species" or "specimen"; not to be confused with "historical individuals" of Grant & Kluge (2004).

thus, minimize the length of most parsimonious trees[3] (Wheeler, 2001). Additionally, employing dynamic homology guarantees an explicit optimality criterion (the maximum parsimony criterion) in generating sequence alignments while also eliminating the subjectivity of “manual corrections”, most often applied to multiple sequence alignments, and the loss of optimality that comes with them (T. Grant, unpubl. data).

Phylogenetic Analyses

Phylogenetic analyses were performed under dynamic homology in POY 4.1.2.1 (Varón et al., 2010) using equal weights for all transformations and the parsimony optimality criterion (see previous section). Gaps were treated as a fifth character-state, as treating them as missing data would result in their erroneous interpretation as a transformation from one nucleotide into another, and not as the transformation events they actually reflect: the loss or gain of a nucleotide.

Analyses were performed using the command “search,” which implements a driven search composed of random addition sequence Wagner builds (RAS), Subtree Pruning and Regrafting (SPR) and Tree Bisection and Reconnection (TBR) branch swapping, Parsimony Ratcheting (Nixon, 1999), and Tree Fusing (Goloboff, 1999), storing the shortest trees of each independent run and performing a final round of Tree Fusing on the pooled trees. Four independent runs, two consisting of three 8-hour driven searches and two consisting of six 8-hour driven searches, were implemented in parallel on a dual hexacore server at the Museu de Zoologia da Universidade de São Paulo, with the best trees from all searches saved for subsequent refinement. As a heuristic to accelerate searches, fragments that presented no length variation (*viz.* COI, cytochrome *b*, RAG1 and H3a) were initially analyzed as prealigned sequences; this constraint was removed (*i.e.*, insertion and deletion events were permitted) in a final search composed of 200 generations of Tree Fusing and TBR of all trees saved during the searches. Goodman–Bremer support values (Goodman *et al.*, 1982; Bremer, 1988; see Grant and Kluge, 2008b) were estimated using inverse constraints to search for next-most-optimal trees with 10 RAS + TBR analyses of the implied alignment; the values obtained from this search are

[3] Some may argue that this procedure is tautological, as a topology found through an alignment is used to generate a new alignment, which in turn can be used to produce a new topology. Dynamic homology testing, however, does not constitute tautology, as it is not a matter of hypothesis built on hypothesis, but is merely Hennig’s (1966) reciprocal illumination principle at its best. For a detailed discussion see Grant & Kluge (2003; 2009, and references therein).

upper bounds and are likely to overestimate support in many cases. To provide additional insights into the amount of evidence that delimits each clade, branch lengths, calculated as the number of unambiguously optimized transformations on a given node, were obtained using WinClada (Nixon, 2002).

Species Limits

In light of the many outstanding problems in *Crossodactylus* species taxonomy, I evaluated the (1) cladistic distance, (2) total evidence patristic distance (branch length), and unweighted pairwise distance of the cytochrome *b* sequences between potentially conspecific terminals, following Grant et al. (2006: 60–62). Cytochrome *b* was used for pairwise comparisons because that locus was sequenced for all but 4 terminals (see Table 4) and has been used for for this purpose previously (*e.g.*, Grant et al. 2006).

RESULTS

MOLECULAR RESULTS

I generated the following number of sequences (ingroup/outgroup numbers are given in parenthesis): 146 (88 ingroup/58 outgroup) sequences for H1; 141 (84/57) sequences for cytochrome *b*; 80 (52/28) sequences for COI; 83 (44/39) sequences for histone H3; 97 (72/25) sequences for RAG1; 118 (73/45) sequences for rhodopsin; 42 (29/13) sequences for tyrosinase; and 35 (31/4) sequences for 28S, for an approximate total of 742 (473/269) sequences generated, and 610,876 (383,704/227,172) basepairs analyzed (Table 3; these numbers do not include sequences downloaded from GenBank). A complete list of sequences generated for ingroup and outgroup terminals as well as loci they were sequenced for is given in Table 4 and Table 5 (names of undetermined samples corrected to reflect results; see below).

Four identification errors were found, the most prominent of which lead to the inclusion of *Bokermannohyla* sp. (sample 11-056, collected from a tadpole and originally identified only as “Hylodidae”) in the analyses. Originally, I intended to use *Hypsiboas boans*—used by Grant *et al.* (2006) and for which sequences are available on GenBank—as the root, but the discovery of the true identity of sample 11-056, though early on (thanks to the molecular pipeline described in Materials & Methods), meant possible confusion and chance for errors should I choose to discontinue its sequencing. To avoid an increasing probability of making mistakes by swapping samples whenever amplifying a new locus, I chose to continue sequencing sample 11-056 and to use it for rooting the resulting topology(ies) instead. In addition to this sample, sample 11-069, *Hylodes meridionalis*, was originally identified as *H. perplicatus* (see southernmost collection locality for that species in Figure 6A), while samples 11-030 (from Ilha Grande, Rio de Janeiro; see Figure 5) and 11-100 (from Paranapiacaba, at the municipality of Santo André, São Paulo; see Figure 6A-B), *C.* aff. *gaudichaudii* and *H. phyllodes*, were identified in the opposite genera as *Hylodes* sp. and *C. gaudichaudii*. For those samples, I am unaware of the life stage of each specimen. These terminals are figured in my topology as originally identified (Figure 16-17).

ANALYSIS RESULTS

Following preliminary runs to detect data formatting errors, a total of 727 random addition SPR/TBR+Ratchet searches and 2461 generations of Tree Fusing were performed, resulting in five optimal trees of 25,510 steps. Removal of the prealigned constraint and Tree Fusing and swapping all trees saved during all searches further decreased the length to 25,508 steps found in 14 trees, the consensus of which is given in Figures 15–17.

CLADISTIC RELATIONSHIPS

Outgroup Relationships

Relationships Outside Hylodidae

The relationships among outgroup taxa differ—in some cases greatly—from the expected based on previous phylogenetic studies. It is important to bear in mind, however, that this study was not designed to test the limits of clades outside Hylodidae, and as such, taxon sampling was too restricted to be provide a severe test of outgroup hypotheses. Outgroup relationships, as recovered by these analyses, are shown in Figure 15.

The first clade to mention is naturally Dendrobatoidea, first for its close relationship with Hylodidae in previous phylogenetic hypotheses, second for its odd position in the current hypothesis, as the sister-group to a clade containing all remaining taxa (with the obvious exception of the root). Though recovered as a monophyletic group with a Goodman-Bremer support (GB) of 171, its placement and intergeneric relationships differ from the hypothesis of Grant *et al.* (2006), with Aromobatidae being found nested in Dendrobatidae, and Aromobatinae nested in Anomaloglossinae. It is important to notice, however, that this study sampled only seven species of dendrobatoids (see Table 1) corresponding to seven out of 17 genera, unlike the extensive taxon and character sampling done by Grant *et al.* (2006). Furthermore, an additional analysis constraining the sister-group relationship of Dendrobatoidea and Hylodidae found one most parsimonious tree of 25,543 steps, only 35 steps longer than the fourteen optimal trees found during my analyses, which suggests that increased taxon sampling of the superfamily could bring my results closer to those of Grant *et al*. (2006).

The next outgroup clade consisted of Leiuperidae, which was recovered as a monophyletic group with a GB value of 47, and as sister-group to Centrolenidae + remaining taxa. Centrolenidae had a GB support of 105, and was sister-group to a Leptodactylidae, which was paraphyletic with respect to all remaining taxa. The first Leptodactylidae clade contained the five species of *Leptodactylus* sampled (see Table 1), with GB of 75, and was sister to the second Leptodactylidae clade + remaining taxa. This second clade had a GB of 79, while its sister-group had a GB of 19.

Next up the tree, I recovered Ceratophryidae as paraphyletic with respect to Cycloramphidae and Bufonidae, the latter of which was nested in one of three clades of Alsodinae genera (*sensu* Grant *et al.*, 2006). The first clade of Ceratophryidae comprised Telmatobiinae, and Ceratophryinae, and was supported by a GB of 38. The first clade of Cycloramphidae recovered Cycloramphinae embedded in Alsodinae (*Thoropa* was sister to *Rhinoderma*, and *Cycloramphus* was sister to the remainder of that clade; see Figure 15), and was supported by a GB of 59. The second Ceratophryidae clade had a GB of 79, consisted of Batrachylinae, and was sister to the second Cycloramphidae (third Alsodinae) clade, which had a GB of 16. This second Cycloramphidae clade recovered *Eupsophus* + *Alsodes* as sister to *Limnomedusa* + Bufonidae, and was recovered as the sister-group to Hylodidae. Although consistent with the results of Pyron & Wiens (2011) in terms of the paraphyly of Ceratophryidae and Cycloramphidae, my results are much different when it comes to intergeneric relationships, and specially to the placement of Bufonidae. The conclusion that can be drawn from this is that there is indeed a body of evidence for the close relationship of the Ceratophryidae and the Cycloramphidae, and there is still much to be learned about these groups, should they be studied in detail.

Outgroup Relationships Within Hylodidae

Hylodidae was recovered as a monophyletic group, supported by a GB of 38, and showing a branch length (BL) of 98 molecular transformations. Basally, it was divided into two clades, one containing *Megaelosia* and *Hylodes*, the other containing *Crossodactylus* (Figure 16). Within the first clade, which has a GB = 38, and BL = 77, *Megaelosia* was found to be paraphyletic with respect to *Hylodes*. Most terminals of *Megaelosia* were recovered in a clade (GB = 25, BL = 110) sister to another comprised of *M. goeldii* + *Hylodes* (GB = 20, BL = 46). The first *Megaelosia* clade contains *M. apuana*, *M. massarti*, *M. boticariana*, and unidentified terminals from Ubatuba and Boracéia, both municipalities

in the state of São Paulo. The second contains three terminals, *M. goeldii* from Cachoeiras de Macacu, and one unidentified terminal from Parque Nacional Serra dos Órgãos, located in the municipality of Teresópolis, both in the state of Rio de Janeiro; the third terminal is that of Frost *et al.* (2006) and Grant *et al.* (2006), and is also from Teresópolis.

The monophyly of *Hylodes* was corroborated, despite its placement inside *Megaelosia*. The clade was supported by a GB of 54, and showed a BL of 69. *Hylodes* was basally divided into two large clades, the first of which (GB =24, BL = 39) contained *H.* cf. *charadranaetes*, *H. nasus*, *H. dactylocinus*, and *H. asper*; the second (GB = 38, BL = 34) contained *H. fredi*, *H. pipilans*, *H. phyllodes*, *H. glaber*, *H. sazimai*, *H. magalhaesi*, *H. otavioi*, *H. lateristrigatus*, *H. babax*, *H. meridionalis*, *H. perplicatus*, and *H. heyeri*.

Ingroup Relationships

Crossodactylus was recovered as the well supported (GB = 43) sister clade of the *Megaelosia* + *Hylodes* clade (see above). In light of the non-monophyly of currently recognized species groups and the many species-level taxonomic problems underscored by the optimal tree, below I describe the ingroup relationships in terms of species complexes composed of closely related terminals that were unidentified prior to analysis or were originally considered to be conspecific.

Crossodactylus was divided basally into two large clades, A and B (Figure 17). Clade A has GB of 17, BL of 34, and includes terminals identified as *C. gaudichaudii* and *C. aeneus* prior to analysis, referred to here as the *C. gaudichaudii* species complex, and all terminals from localities in the Brazilian states of Espírito Santo and Bahia, which I refer to as the ES/BA species complex. Clade B has GB of 11, BL of 28, and includes terminals identified as *C. bokermanni*, *C. schmidti*, *C. caramaschii*, and all unidentified terminals from southern Brazil and from the state of São Paulo. Those I refer to as the *C. bokermanni*, *C. schmidti*, and *C. caramaschii* complexes, and unidentified terminals are divided among those. In order to clarify the complexity of these relationships, percent uncorrected pairwise distances (UPD) between sequences of cytochrome *b* of each terminal (when available) for each species complex were calculated and are given in each section.

The *C. gaudichaudii* Complex

The *C. gaudichaudii* complex is paraphyletic with respect to both *C. aeneus* and the ES/BA complex, both of which are monophyletic. A clade with a GB support of 118, and BL of 137 is the sister to all other terminals of Clade A. This clade is composed of five terminals from Casimiro de Abreu, Maricá and Saquarema (all in the state of Rio de Janeiro). Localities were not monophyletic: sample 11-156, from Maricá, was recovered as sister to all remaining four species, with sample 11-154, from that same locality, being more closely related to sample 11-138, from Casimiro de Abreu, while samples 11-143 and 11-152, from Maricá, were more closely related to each other. Though the next clade comprising has a high GB of 84, the branch length is very low, with only three transformations, which suggests an inflated support value due to a superficial GB search. The short branch lengths within this clade, combined with low UPD values of 0.3–2.4% (Table 6) between terminals indicate that these constitute one single species, which is probably not *C. gaudichaudii* based on collection locality (see below).

The remainder of the *C. gaudichaudii* complex is composed of a clade of specimens from the city of Rio de Janeiro, which is sister to a clade composed of *C. gaudichaudii* (plus sample 11-030; see Molecular Results) from Ilha Grande, and of *C. aeneus*. This more inclusive clade is supported by GB of 69, and shows a BL of 56. The first of the less inclusive clades is composed of four terminals, all from localities in the Parque Nacional Floresta da Tijuca, and shows GB = 38, and BL = 20. The sample from Bom Retiro (11-150) is sister to the other three, and localities are again non-monophyletic, with one of the samples from Estrada Dona Castorina (11-130) being more closely related to the sample from Córrego Mayrink (11-152) than to another sample from that same locality (11-134). All samples in this clade show very low UPD values between one another, which range from 0.3% to 1.3% (Table 6), suggesting a single species. As all these samples derive from the city of Rio de Janeiro, they most likely represent *C. gaudichaudii sensu stricto.*

The last three samples originally identified as *C. gaudichaudii* (with the exception of sample 11-100, and inclusion of sample 11-030; see Molecular Results), from Ilha Grande, form a clade sister to *C. aeneus*. This more inclusive clade shows GB = 33 and BL = 13, while the Ilha Grande clade shows GB = 25 and BL = 20, and the *C. aeneus* clade shows GB = 15 and BL = 12. UPDs between all terminals from Ilha Grande are 0%, while UPDs between terminals of *C. aeneus* vary from 0% to 5.2%; UPDs between terminals of these two clades range from 4.7% to 5.8% (Table 6). Given the high variation within the *C.*

aeneus clade, it is unclear whether or not the clade from Ilha Grande represents a separate species.

The ES/BA Complex

The ES/BA complex is formed by a clade containing all terminals from Muniz Freire and the terminal from Cariacica (collected at the Reserva Biológica Duas Bocas; both from the state of Espírito Santo), sister to a clade containing terminals from Santa Teresa (collected at the Reserva Biológica Augusto Ruschi; also in Espírito Santo) and all terminals from Bahia. This more inclusive clade has a GB of 20, and BL of 22. The first clade in the complex, with GB = 87 and BL = 78, recovered the terminal from Cariacica (sample 11-123) as sister to the terminals from Muniz Freire; these, in turn, formed an unresolved clade with GB = 56 and BL = 21. The number of transformations separating the terminal from Cariacica and the clade from Muniz Freire, taken with the UPDs between the former and latter terminals, which ranged from 9.7% to 9.9% (Table 7), suggests that the terminal from Cariacica represents a separate species from those from Muniz Freire.

The remainder of the ES/BA complex is formed by a clade of samples from Santa Teresa, which is sister to a clade containing all samples from Bahia. This more inclusive clade shows GB support of 27, and BL of 36. The Santa Teresa clade has GB = 103, and BL = 84, and brings sample 11-092 as sister to samples 11-097 + TG-11-011. UPDs between terminals in this clade are short, however UPDs between these terminals and those in its sister clade are quite long at 14.3–15.1% (Table 7), indicating that the Santa Teresa clade constitutes a separate species from the Bahia clade. The Bahia clade is supported by a GB of 67, and shows BL of 44. Localities in this clade were not recovered as monophyletic, with one terminal from Jussari (collected at RPPN Serra do Teimoso), sample 11-136, being recovered as sister to all other terminals in that clade. The terminal in question shows a BL of 44, and UPDs between itself and remaining terminals from that region of 8.9–9.1% (Table 7), suggesting that the Bahia clade comprises more than one species. The remainder of terminals from Bahia are grouped in a clade supported by a GB of 60, with BL of 43. Values of UPD between terminals in this clade are very low, 0–0.6% (Table 7), suggesting they all represent a single species, though samples 11-095 and 11-098, from Camacan (RPPN Serra Bonita), seem more closely to each other than to the remainder of that clade, including two other samples from the same mountain range (11-008 and 11-014). That relationship, however, is supported by GB of 1 (BL = 1), while

the relationship of the remainder terminals is supported by a GB of only 1, but a BL of 43.

The *C. bokermanni* Complex

The *C. bokermanni* complex is sister to all remaining terminals in Clade B. This clade has GB = 101, and BL = 114, and is composed of one clade containing all terminals from Catas Altas, and another with all remaining terminals identified as *C. bokermanni*. This latter clade has a GB support of 103, and BL of 64, and is composed of three samples from the municipality of Santana do Riacho and two from Serra do Cipó (Cipó Mountain Range), both in the state of Minas Gerais. Localities were not recovered as monophyletic, with one sample from Serra do Cipó (11-159) and one sample from Santana do Riacho (11-132) being more closely related to each other than to samples from the same localities. UPDs between terminals in this clade are very low, ranging from 0% to 0.3% (Table 8), indicating that these terminals are conspecific.

The other more inclusive clade within the *C. bokermanni* complex is comprised of five samples from the municipality of Catas Altas, one of them initially identified as *C. bokermanni*, and the other four, collected at the RPPN Serra do Caraça, undetermined. This clade has a GB of 52, and a BL of 54, with relationships within it unresolved. One terminal, sample 11-124, has a BL of 18, however UPDs between all terminals in this clade were calculated at 0% (Table 8), indicating they belong to the same species, and a possible undetected error in one of the sequences for that terminal. This only comes to highlight the importance of consistently employing a rigorous, detailed screening process for errors, and the benefits of sequencing multiple terminals for any given locality.

Values of UPD between terminals from the two more inclusive clades varied from 8.1% to 8.4% (Table 8), indicating that each clade corresponds to a different species. All these terminals were collected relatively close to the type-locality of *C. bokermanni* (see Figure 5), yet it is unclear which clade represents *C. bokermanni sensu stricto*, as the type-locality of *C. trachystomus* is also in close proximity. It is possible, in fact, that each one of these clades represent *C. bokermanni* and *C. trachystomus*, but it is not possible to determine which clade corresponds to which species, if not to a third, without examining the voucher specimens.

The *C. schmidti* Complex

The *C. schmidti* complex is the sister to the *C. caramaschii* complex (GB = 26, BL = 34). It is supported by a GB of 9, and has a BL of 36. This clade is comprised of *C. schmidti* from Misiones (Argentina; this is the same terminal analyzed by Frost *et al.* [2006], and Grant *et al.* [2006]), one terminal originally identified as *C. caramaschii*, from São Bento do Sul (state of Santa Catarina), most samples (eight out of 12) from the state of Paraná—namely those from the municipalities of Apucaraninha, Ortigueira, Pinhalão, and Wenceslau Brás—and one sample from Ourinhos, a city at the border of that state and the state of São Paulo (where it is situated). The terminal from Misiones was found to be the sister to the terminal from São Bento do Sul, with a GB of 9, (BL = 16), but the very high UPD value of 15.6% (Table 9) between these terminals indicates that they are not conspecific, and that the species found in Santa Catarina does not correspond to *C. schmidti*—at least not exclusively. The remaining terminals in the *C. schmidti* complex form a clade with GB = 45, and BL = 66. Not all localities in this clade are monophyletic, with one terminal from Ortigueira (11-021) being more closely related to those from Apucaraninha (11-001, and 11-007; which were monophyletic) than to another terminal (11-099) from that same locality. These three terminals formed a clade with GB = 25, and BL = 13, sister to a clade containing the remainder of this complex, with GB = 26, and BL = 17. Terminals from Pinhalão and Wenceslau Brás formed monophyletic groups (GB = 8, BL = 2, and GB = 9 BL = 7, respectively), and were more closely related to each other (GB = 9, BL = 6). Notwithstanding, UPD values between terminals from Paraná within the *C. schmidti* complex are very low, ranging from 0.3% to 3.7% (Table 9), which suggests that, despite their structuring in the tree, all these terminals belong to a single species, different from *C. schmidti* and from that found in Santa Catarina.

The *C. caramaschii* Complex

The *C. caramaschii* complex has a GB of 10, and BL of 19, and appears to comprise the highest number of putative species: UPDs between terminals in this clade range from 0% to 15.1%. The terminals in this complex are basically divided in two large clades, supported by a GB of 15 (BL = 16) and a GB of 21 (BL = 45), respectively. Localities were largely monophyletic, with the exception of terminals from Ribeirão Grande, one of which (11-017) was recovered as sister to one terminal from Piedade (11-043; GB/BL = 1) in a

large polytomy in the first large clade, while the other two (11-020 and 11-028) were recovered as monophyletic (GB = 11, BL = 8) in the second.

The first major clade in the *C. caramaschii* complex comprises terminals exclusively from the state of São Paulo, collected at the municipalities of Sete Barras (at Parque Estadual Carlos Botelho), Itanhaém, Juquiá, Piedade, Ribeirão Grande, and Caucaia do Alto (at Quilombo). The terminal from Sete Barras (sample 11-110) was recovered as sister to all remaining terminals. The UPD between this terminal and others in the clade ranged from 8.6% to 10.4% (Table 10-A), suggesting it may represent a separate species. The terminals from Itanhaém were recovered in a monophyletic group (GB = 47, BL = 37), sister to the remaining terminals; such relationship was supported by a GB of 59, with a BL of 12. This next clade (GB = 17, BL = 46) brings one terminal from Juquiá (11-048) as the sister to all others, whose relationship are mostly unresolved (with the exception of samples 11-017 and 11-043, mentioned above). UPDs between terminals from Itanhaém and its sister-group ranged from 5.8% to 7.1% (Table 10-A), indicating that these might not be conspecific. UPDs between sample 11-048 (from Juquiá, see above) and the terminals in the polytomy were very low, ranging from 0% to 1.1%, indicating that in spite of the lack of structure in this part of the tree, these terminals are all conspecific.

The second major clade in the *C. caramaschii* complex comprises terminals from São Paulo, collected at the municipalities of Eldorado Paulista (at Parque Estadual de Jacupiranga), Ribeirão Grande, Capão Bonito (at Fazenda Intervales), and Iporanga (at Parque Estadual Turístico do Alto Ribeira), and four terminals from Paraná, collected at the municipalities of Campo Magro, Ponta Grossa, Piraquara, and Balsa Nova. The terminals from Paraná were monophyletic (GB = 69, BL = 63), and the sister-group to the terminals from São Paulo. In this Paraná clade, the terminals from Campo Magro and Ponta Grossa were recovered as more closely related to each other (GB = 3, BL = 2), while the terminals from Piraquara and Balsa Nova were more closely related to one another (GB/BL = 2). Values of UPD between terminals in this clade were low, *i.e.*, 0.3–1.9,% (Table 10-B), however values between these terminals and those in its sister-group were high, ranging from 9.9% to 12.3% (Table 10-B), suggesting that there is yet another species in the state of Paraná. The clade from São Paulo was supported by a GB of 32, with BL of 22, and all localities in this clade were found to be monophyletic. The terminals from Eldorado Paulista showed GB = 14, BL = 12, and UPDs of 0–0.8% (Table 10-B). These terminals were collected closest to the type-locality of *C. caramaschii* (see Table 5), and so probably represent that species. The sister to that clade has a GB of 2, and BL of 4; the terminals from Ribeirão Grande were recovered as sister-group to the remainder of

the complex, with GB = 11, and BL = 8. The terminals from Capão Bonito were recovered as sister to the terminals from Iporanga, in a clade supported by a GB of 4, with a BL of 3. UPDs between terminals from these three localities and from Eldorado Paulista ranged from 0% to 3.9% (Table 10-B), which indicates these might all represent *C. caramaschii sensu stricto*.

DISCUSSION

This study yet again supports the monophyly of Hylodidae, although the identity of its sister group remains somewhat unclear. Both Dendrobatoidea and Cycloramphidae have been repeatedly recovered as sister to Hylodidae, and both putative sister groups show phenotypic and ecological similarities with that family, leaving the issue as an open question, and inviting additional research.

Megaelosia was recovered as a paraphyletic group, with *M. goeldii* being more closely related to *Hylodes* than to the other species of *Megaelosia*. Insofar as *Megaelosia* was described on the basis of specimens of *M. goeldii* (see below), this creates a taxonomic problem that could be solved in one of two ways: (1) transfer *M. goeldii* to *Hylodes* and name a new genus for the remaining species of *Megaelosia*, or (2) place *Megaelosia* in synonymy of *Hylodes*. The first solution, though appealing in that it communicates cladistic information is difficult to implement because not all recognized species of *Megaelosia* were analyzed in the present study. As such, *M. bocainensis*, *M. jordanensis* and *M. lutzae* would be *incertae sedis*. Branch lengths and GB within the *M. goeldii* clade recovered by this study (see Figure 16) suggest that there is actually more than one species conflated under this epithet, which further demonstrates the gaps in our current understanding of *Megaelosia* and complicates working with the first option. Furthermore, *Megaelosia* was described by Miranda-Ribeiro (1923) with *Elosia bufonium* Girard, 1853—which is a junior synonym of *Hylodes nasus* (Liechtenstein, 1823)—as type-species, as noted by Lutz (1930) and Giaretta *et al.* (1993), while the specimens described and figured by the author were actually *Hylodes goeldii* Baumann, 1912. Consequently to fully resolve the situation of *Megaelosia*, firstly the type-species must be fixed as *Hylodes goeldii,* in accordance to Article 70.3 of the International Code of Zoological Nomenclature (ICZN, 1999), and secondly all species currently referred to as *Megaelosia* must be transferred to *Hylodes*. Further clarification of the relationships among those species is necessary before attributing species to a new genus.

The monophyly of *Crossodactylus* was corroborated, despite the lack of morphological evidence supporting this arrangement in literature. The monophyly of the species groups proposed by Caramaschi & Sazima (1985), on the contrary, was refuted, with the *C. gaudichaudii* group being paraphyletic with respect to *C. schmidti* and a high likelihood that the *C. trachystomus* group is also embedded in the *C. gaudichaudii* group. Given the meager evidence used to create these groups—snout shape and length, and

shape of *canthus rostralis*—this is hardly surprising. Unfortunately, not all recognized species could be sampled for this study, so that the placement of *C. cyclospinus*, *C. dantei*, *C. dispar*, *C. grandis*, and *C. lutzorum* is still unknown. For that reason, the currently defined species groups in *Crossodactylus* should be abandoned, until the placement of such species is known, and the intrageneric relationships of the genus are better understood.

What this study has certainly revealed is that the current taxonomy of *Crossodactylus* grossly under-represents actual species diversity. Considering how many species were not sampled, it is quite likely that, even after taking these results into account, the number of species is still underestimated. The total number of species suggested by the molecular evidence is approximately 14 (see The *C. gaudichaudii* Complex and The *C. bokermanni* Complex; Table 11), which more than doubles the currently known diversity of this group. This not only highlights how little is known about Hylodidae, but also has potential implications for biogeography and conservation (see Pimenta *et al.*, 2005, 2008), as species currently believed to be widespread (*e.g., C. caramaschii* and *C. schmidti*, which are believed to range from the state of São Paulo to Santa Catarina, and from Misiones to Paraná, respectively; B. Pimenta, personal commun.; Frost, 2011) appear to be complexes of several narrowly distributed species.

An important priority for future studies of the systematics of *Crossodactylus* is the inclusion of the five species that were omitted from the present study, namely *C. cyclospinus, C. dantei*, *C. dispar*, *C. grandis*, *C. lutzorum*, and *C. trachystomus* (but see Results). Most of these species were last collected in the 1970s and/or 1980s and appear to have undergone massive declines, and possibly extinction, especially *C. dispar* and *C. grandis* (B. Pimenta; personal obs.). DNA quality tissues samples of these species were not and are unlikely to become available—although additional field work is always warranted. As such, the phylogenetic placement of these species will require the analysis of evidence from morphology.

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Table 1: Data for DNA sequences obtained from Genbank.

Identification	Accession number	Locus	Length	Reference
Adenomera hylaedactyla	DQ283063	12S, $tRNA^{val}$, 16S	2419	Frost *et al.*, 2006
Adenomera hylaedactyla	DQ284093	histone H3	328	Frost *et al.*, 2006
Adenomera hylaedactyla	DQ283790	rhodopsin	316	Frost *et al.*, 2006
Allobates femoralis	DQ502092	12S, $tRNA^{val}$, 16S	2408	Grant *et al.*, 2006
Allobates femoralis	DQ502811	COI	658	Grant *et al.*, 2006
Allobates femoralis	DQ502325	histone H3	328	Grant *et al.*, 2006
Allobates femoralis	DQ503327	RAG1	435	Grant *et al.*, 2006
Allobates femoralis	DQ503215	rhodopsin	316	Grant *et al.*, 2006
Allobates femoralis	DQ503156	tyrosinase	532	Grant *et al.*, 2006
Allobates femoralis	DQ502524	cytochrome *b*	385	Grant *et al.*, 2006
Alsodes gargola	AY843565	12S, $tRNA^{val}$, 16S	2410	Faivovich *et al.*, 2005
Alsodes gargola	AY844197	28S	757	Faivovich *et al.*, 2005
Alsodes gargola	DQ284118	histone H3	328	Frost *et al.*, 2006
Alsodes gargola	AY844362	RAG1	428	Faivovich *et al.*, 2005
Alsodes gargola	AY844539	rhodopsin	316	Faivovich *et al.*, 2005
Alsodes gargola	AY843787	cytochrome *b*	385	Faivovich *et al.*, 2005
Anomaloglossus sp. “Ayanganna”	DQ502129	12S, $tRNA^{val}$, 16S	2411	Grant *et al.*, 2006
Anomaloglossus sp. “Ayanganna”	DQ502993	28S	767	Grant *et al.*, 2006
Anomaloglossus sp. “Ayanganna”	DQ502836	COI	658	Grant *et al.*, 2006
Anomaloglossus sp. “Ayanganna”	DQ502345	histone H3	328	Grant *et al.*, 2006
Anomaloglossus sp. “Ayanganna”	DQ503344	RAG1	435	Grant *et al.*, 2006
Anomaloglossus sp. “Ayanganna”	DQ503235	rhodopsin	316	Grant *et al.*, 2006
Anomaloglossus sp. “Ayanganna”	DQ503163	tyrosinase	532	Grant *et al.*, 2006
Anomaloglossus sp. “Ayanganna”	DQ502560	cytochrome *b*	385	Grant *et al.*, 2006
Aromobates nocturnus	DQ502590	cytochrome *b*	385	Grant *et al.*, 2006
Aromobates nocturnus	DQ502154	12S, $tRNA^{val}$, 16S	2415	Grant *et al.*, 2006
Aromobates nocturnus	DQ502859	COI	658	Grant *et al.*, 2006
Aromobates nocturnus	DQ503243	rhodopsin	316	Grant *et al.*, 2006
Aromobates nocturnus	DQ502357	histone H3	328	Grant *et al.*, 2006
Aromobates nocturnus	DQ502996	28S	767	Grant *et al.*, 2006
Atelognathus patagonicus	AY843571	12S, $tRNA^{val}$, 16S	2428	Faivovich *et al.*, 2005
Atelognathus patagonicus	AY844203	28S	732	Faivovich *et al.*, 2005
Atelognathus patagonicus	AY844368	RAG1	428	Faivovich *et al.*, 2005
Atelognathus patagonicus	AY844545	rhodopsin	316	Faivovich *et al.*, 2005
Atelognathus patagonicus	AY844027	tyrosinase	532	Faivovich *et al.*, 2005
Atelognathus patagonicus	AY843793	cytochrome *b*	385	Faivovich *et al.*, 2005
Atelopus flavescens	AY995987	cytochrome *b*	375	Noonan & Gaucher, 2005
Atelopus flavescens	DQ283259	12S, $tRNA^{val}$, 16S	2421	Frost *et al.*, 2006
Atelopus flavescens	DQ284282	histone H3	328	Frost *et al.*, 2006
Atelopus flavescens	DQ283928	rhodopsin	316	Frost *et al.*, 2006
Atelopus flavescens	DQ068411	tyrosinase	1473	Noonan & Gaucher, 2005
Atelopus spumarius	AY995954	cytochrome *b*	375	Noonan & Gaucher, 2005
Atelopus spumarius	DQ283260	12S, $tRNA^{val}$, 16S	2422	Frost *et al.*, 2006
Atelopus spumarius	DQ284283	histone H3	328	Frost *et al.*, 2006
Atelopus spumarius	DQ283929	rhodopsin	316	Frost *et al.*, 2006

Atelopus spumarius	DQ068447	tyrosinase	965	Noonan & Gaucher, 2005
Atelopus spurrelli	DQ502200	12S, $tRNA^{val}$, 16S	2415	Grant *et al.*, 2006
Atelopus spurrelli	DQ502895	COI	658	Grant *et al.*, 2006
Atelopus spurrelli	DQ503380	RAG1	435	Grant *et al.*, 2006
Atelopus zeteki	DQ283252	16S	1518	Frost *et al.*, 2006
Atelopus zeteki	DQ502857	COI	658	Grant *et al.*, 2006
Batrachyla leptopus	AY843572	12S, $tRNA^{val}$, 16S	2416	Faivovich *et al.*, 2005
Batrachyla leptopus	AY844204	28S	732	Faivovich *et al.*, 2005
Batrachyla leptopus	DQ284119	histone H3	328	Frost *et al.*, 2006
Batrachyla leptopus	AY844369	RAG1	428	Faivovich *et al.*, 2005
Batrachyla leptopus	AY844546	rhodopsin	316	Faivovich *et al.*, 2005
Batrachyla leptopus	AY844028	tyrosinase	532	Faivovich *et al.*, 2005
Ceratophrys cranwelli	AY843575	12S, $tRNA^{val}$, 16S	2422	Faivovich *et al.*, 2005
Ceratophrys cranwelli	AY844207	28S	728	Faivovich *et al.*, 2005
Ceratophrys cranwelli	AY843797	cytochrome *b*	385	Faivovich *et al.*, 2005
Ceratophrys ornata	AY326013	12S, $tRNA^{val}$, 16S	2368	Darst & Cannatella, 2004
Ceratophrys ornata	AY364218	RAG1	559	Biju & Bossuyt, 2003
Ceratophrys ornata	AY364399	rhodopsin	316	Biju & Bossuyt, 2003
Ceratophrys ornata	DQ347168	tyrosinase	532	Bossuyt *et al.*, 2006
Ceratophrys ornata	L10983	cytochrome *b*	429	Graybeal 1993
Colostethus fraterdanieli	DQ502615	cytochrome *b*	385	Grant *et al.*, 2006
Colostethus fraterdanieli	DQ502179	12S, $tRNA^{val}$, 16S	2417	Grant *et al.*, 2006
Colostethus fraterdanieli	DQ502882	COI	658	Grant *et al.*, 2006
Colostethus fraterdanieli	DQ503259	rhodopsin	316	Grant *et al.*, 2006
Colostethus fraterdanieli	DQ502375	histone H3	328	Grant *et al.*, 2006
Colostethus fraterdanieli	DQ503375	RAG1	435	Grant *et al.*, 2006
Colostethus fraterdanieli	DQ503017	28S	764	Grant *et al.*, 2006
Crossodactylus schmidti	AY843579	12S, $tRNA^{val}$, 16S	2413	Faivovich *et al.*, 2005
Crossodactylus schmidti	AY843801	cytochrome *b*	385	Faivovich *et al.*, 2005
Crossodactylus schmidti	AY844031	tyrosinase	532	Faivovich *et al.*, 2005
Crossodactylus schmidti	AY844210	28S	767	Faivovich *et al.*, 2005
Crossodactylus schmidti	AY844375	RAG1	428	Faivovich *et al.*, 2005
Crossodactylus schmidti	AY844552	rhodopsin	316	Faivovich *et al.*, 2005
Crossodactylus schmidti	DQ284050	histone H3	328	Frost *et al.*, 2006
Crossodactylus schmidti	DQ502738	COI	658	Grant *et al.*, 2006
Crossodactylus schmidti	DQ503298	RAG1	435	Grant *et al.*, 2006
Cycloramphus boraceiensis	DQ502588	cytochrome *b*	385	Grant *et al.*, 2006
Cycloramphus boraceiensis	DQ283097	12S, $tRNA^{val}$, 16S	2425	Frost *et al.*, 2006
Cycloramphus boraceiensis	DQ283498	28S	742	Frost *et al.*, 2006
Cycloramphus boraceiensis	DQ502856	COI	658	Grant *et al.*, 2006
Cycloramphus boraceiensis	DQ284147	histone H3	328	Frost *et al.*, 2006
Cycloramphus boraceiensis	DQ503357	RAG1	435	Grant *et al.*, 2006
Cycloramphus boraceiensis	DQ283813	rhodopsin	316	Frost *et al.*, 2006
Cycloramphus boraceiensis	DQ282924	tyrosinase	532	Frost *et al.*, 2006
Dendrophryniscus minutus	AY843804	cytochrome *b*	385	Faivovich *et al.*, 2005
Dendrophryniscus minutus	DQ502120	12S, $tRNA^{val}$, 16S	2412	Grant *et al.*, 2006
Dendrophryniscus minutus	DQ502828	COI	658	Grant *et al.*, 2006
Dendrophryniscus minutus	DQ284096	histone H3	328	Frost *et al.*, 2006

Dendrophryniscus minutus	DQ158346	RAG1	790	Pramuk, 2006
Dendrophryniscus minutus	AY844555	rhodopsin	316	Faivovich *et al.*, 2005
Dendrophryniscus minutus	EF364362	tyrosinase	518	Fouquet *et al.*, 2007
Edalorhina perezi	AY843807	cytochrome *b*	385	Faivovich *et al.*, 2005
Edalorhina perezi	AY843585	12S, $tRNA^{val}$, 16S	2400	Faivovich *et al.*, 2005
Edalorhina perezi	DQ283474	28S	756	Frost *et al.*, 2006
Edalorhina perezi	DQ284095	histone H3	328	Frost *et al.*, 2006
Edalorhina perezi	AY844558	rhodopsin	316	Faivovich *et al.*, 2005
Engystomops petersi	FJ668193	12S, $tRNA^{val}$, 16S	2376	Targueta *et al.*, 2010
Engystomops petersi	GQ375544	RAG1	429	Targueta *et al.*, 2010
Engystomops petersi	FJ668241	rhodopsin	316	Targueta *et al.*, 2010
Espadarana prosoblepon	AY843796	cytochrome *b*	385	Faivovich *et al.*, 2005
Espadarana prosoblepon	AY843574	12S, $tRNA^{val}$, 16S	2424	Faivovich *et al.*, 2005
Espadarana prosoblepon	AY844206	28S	732	Faivovich *et al.*, 2005
Espadarana prosoblepon	AY844548	rhodopsin	316	Faivovich *et al.*, 2005
Espadarana prosoblepon	AY844371	RAG1	428	Faivovich *et al.*, 2005
Espadarana prosoblepon	FJ766593	COI	648	Crawford *et al.*, 2010
Eupemphix nattereri	AY326020	12S, $tRNA^{val}$, 16S	2444	Darst & Cannatella, 2004
Eupsophus calcaratus	AY843808	cytochrome *b*	385	Faivovich *et al.*, 2005
Eupsophus calcaratus	AY843587	12S, $tRNA^{val}$, 16S	2416	Faivovich *et al.*, 2005
Eupsophus calcaratus	AY844214	28S	757	Faivovich *et al.*, 2005
Eupsophus calcaratus	DQ502852	COI	658	Grant *et al.*, 2006
Eupsophus calcaratus	DQ284120	histone H3	328	Frost *et al.*, 2006
Eupsophus calcaratus	AY844560	rhodopsin	316	Faivovich *et al.*, 2005
Eupsophus calcaratus	AY844036	tyrosinase	532	Faivovich *et al.*, 2005
Hylodes phyllodes	DQ282923	tyrosinase	532	Frost *et al.*, 2006
Hylodes phyllodes	DQ283096	12S, $tRNA^{val}$, 16S	2416	Frost *et al.*, 2006
Hylodes phyllodes	DQ283812	rhodopsin	316	Frost *et al.*, 2006
Hylodes phyllodes	DQ284146	histone H3	328	Frost *et al.*, 2006
Hylodes phyllodes	DQ502171	12S, $tRNA^{val}$, 16S	2411	Grant *et al.*, 2006
Hylodes phyllodes	DQ502368	histone H3	328	Grant *et al.*, 2006
Hylodes phyllodes	DQ502587	cytochrome *b*	385	Grant *et al.*, 2006
Hylodes phyllodes	DQ502606	cytochrome *b*	385	Grant *et al.*, 2006
Hylodes phyllodes	DQ502873	COI	658	Grant *et al.*, 2006
Hylodes phyllodes	DQ503009	28S	791	Grant *et al.*, 2006
Hylodes phyllodes	DQ503253	rhodopsin	316	Grant *et al.*, 2006
Hylodes phyllodes	DQ503367	RAG1	435	Grant *et al.*, 2006
Hyloxalus bocagei	DQ502469	cytochrome *b*	385	Grant *et al.*, 2006
Hyloxalus bocagei	DQ502038	12S, $tRNA^{val}$, 16S	2417	Grant *et al.*, 2006
Hyloxalus bocagei	DQ502764	COI	658	Grant *et al.*, 2006
Hyloxalus bocagei	DQ503199	rhodopsin	316	Grant *et al.*, 2006
Hyloxalus bocagei	DQ502308	histone H3	328	Grant *et al.*, 2006
Hyloxalus bocagei	DQ503314	RAG1	435	Grant *et al.*, 2006
Hyloxalus bocagei	DQ502961	28S	760	Grant *et al.*, 2006
Lepidobatrachus laevis	DQ283152	12S, $tRNA^{val}$, 16S	2423	Frost *et al.*, 2006
Lepidobatrachus laevis	DQ283543	28S	729	Frost *et al.*, 2006
Lepidobatrachus laevis	DQ284191	histone H3	328	Frost *et al.*, 2006
Lepidobatrachus laevis	DQ283851	rhodopsin	316	Frost *et al.*, 2006

Leptodactylus discodactylus	DQ283433	12S, tRNA^{val}, 16S	2419	Frost *et al.*, 2006
Leptodactylus discodactylus	DQ283742	28S	744	Frost *et al.*, 2006
Leptodactylus discodactylus	DQ284410	histone H3	328	Frost *et al.*, 2006
Leptodactylus discodactylus	DQ284033	rhodopsin	316	Frost *et al.*, 2006
Leptodactylus fuscus	DQ283404	12S, tRNA^{val}, 16S	2423	Frost *et al.*, 2006
Leptodactylus fuscus	DQ283716	28S	748	Frost *et al.*, 2006
Leptodactylus fuscus	DQ284385	histone H3	328	Frost *et al.*, 2006
Leptodactylus fuscus	AY323770	RAG1	1504	Hoegg *et al.*, 2004
Leptodactylus fuscus	DQ284015	rhodopsin	316	Frost *et al.*, 2006
Leptodactylus fuscus	AY341760	tyrosinase	579	Vences *et al.*, 2003
Leptodactylus ocellatus	AY843934	cytochrome *b*	385	Faivovich *et al.*, 2005
Leptodactylus ocellatus	AY843688	12S, tRNA^{val}, 16S	2420	Faivovich *et al.*, 2005
Leptodactylus ocellatus	AY844302	28S	742	Faivovich *et al.*, 2005
Leptodactylus ocellatus	DQ284104	histone H3	328	Frost *et al.*, 2006
Leptodactylus ocellatus	DQ158343	RAG1	790	Pramuk, 2006
Leptodactylus ocellatus	AY844681	rhodopsin	316	Faivovich *et al.*, 2005
Limnomedusa macroglossa	AY843935	cytochrome *b*	385	Faivovich *et al.*, 2005
Limnomedusa macroglossa	AY843689	12S, tRNA^{val}, 16S	2415	Faivovich *et al.*, 2005
Limnomedusa macroglossa	DQ284127	histone H3	328	Frost *et al.*, 2006
Limnomedusa macroglossa	AY844471	RAG1	428	Faivovich *et al.*, 2005
Limnomedusa macroglossa	AY844682	rhodopsin	316	Faivovich *et al.*, 2005
Limnomedusa macroglossa	AY844128	tyrosinase	532	Faivovich *et al.*, 2005
Lithodytes lineatus	AY843936	cytochrome *b*	385	Faivovich *et al.*, 2005
Lithodytes lineatus	AY843690	12S, tRNA^{val}, 16S	2420	Faivovich *et al.*, 2005
Lithodytes lineatus	AY844303	28S	746	Faivovich *et al.*, 2005
Lithodytes lineatus	DQ284112	histone H3	328	Frost *et al.*, 2006
Lithodytes lineatus	AY844472	RAG1	428	Faivovich *et al.*, 2005
Lithodytes lineatus	AY844683	rhodopsin	316	Faivovich *et al.*, 2005
Lithodytes lineatus	AY844129	tyrosinase	532	Faivovich *et al.*, 2005
Macrogenioglottus alipioi	FJ685684	16S	547	Amaro *et al.*, 2009
Macrogenioglottus alipioi	FJ685704	RAG1	428	Amaro *et al.*, 2009
Macrogenioglottus alipioi	FJ685664	cytochrome *b*	594	Amaro *et al.*, 2009
Megaelosia goeldii	DQ283072	12S, tRNA^{val}, 16S	2414	Frost *et al.*, 2006
Megaelosia goeldii	DQ283797	rhodopsin	316	Frost *et al.*, 2006
Megaelosia goeldii	DQ284109	histone H3	328	Frost *et al.*, 2006
Megaelosia goeldii	DQ502563	cytochrome *b*	385	Grant *et al.*, 2006
Megaelosia goeldii	DQ502839	COI	658	Grant *et al.*, 2006
Megaelosia goeldii	DQ503346	RAG1	435	Grant *et al.*, 2006
Megaelosia goeldii	DQ282911	tyrosinase	532	Frost *et al.*, 2006
Melanophryniscus klappenbachi	AY843944	cytochrome *b*	385	Faivovich *et al.*, 2005
Melanophryniscus klappenbachi	AY843699	12S, tRNA^{val}, 16S	2417	Faivovich *et al.*, 2005
Melanophryniscus klappenbachi	AY844306	28S	740	Faivovich *et al.*, 2005
Melanophryniscus klappenbachi	DQ502739	COI	658	Grant *et al.*, 2006
Melanophryniscus klappenbachi	DQ284060	histone H3	328	Frost *et al.*, 2006
Melanophryniscus klappenbachi	DQ503299	RAG1	421	Grant *et al.*, 2006
Melanophryniscus klappenbachi	DQ283765	rhodopsin	316	Frost *et al.*, 2006
Nymphargus bejaranoi	AY843798	cytochrome *b*	385	Faivovich *et al.*, 2005
Nymphargus bejaranoi	AY844372	RAG1	428	Faivovich *et al.*, 2005

Nymphargus bejaranoi	DQ284066	histone H3	328	Frost *et al.*, 2006
Nymphargus bejaranoi	AY844549	rhodopsin	316	Faivovich *et al.*, 2005
Nymphargus bejaranoi	AY844208	28S	732	Faivovich *et al.*, 2005
Nymphargus bejaranoi	AY844029	tyrosinase	532	Faivovich *et al.*, 2005
Nymphargus bejaranoi	AY843576	12S, $tRNA^{val}$, 16S	2422	Faivovich *et al.*, 2005
Odontophrynus achalensis	DQ283248	12S, $tRNA^{val}$, 16S	2422	Frost *et al.*, 2006
Odontophrynus achalensis	DQ283611	28S	780	Frost *et al.*, 2006
Odontophrynus achalensis	DQ284273	histone H3	328	Frost *et al.*, 2006
Odontophrynus achalensis	DQ283918	rhodopsin	316	Frost *et al.*, 2006
Odontophrynus americanus	AY843949	cytochrome *b*	385	Faivovich *et al.*, 2005
Odontophrynus americanus	AY843704	12S, $tRNA^{val}$, 16S	2419	Faivovich *et al.*, 2005
Odontophrynus americanus	AY844309	28S	778	Faivovich *et al.*, 2005
Odontophrynus americanus	AY844480	RAG1	428	Faivovich *et al.*, 2005
Odontophrynus americanus	AY844695	rhodopsin	316	Faivovich *et al.*, 2005
Odontophrynus americanus	FJ685666	cytochrome *b*	594	Amaro *et al.*, 2009
Paratelmatobius sp. CFBH-T 240	DQ283098	12S, $tRNA^{val}$, 16S	2423	Frost *et al.*, 2006
Paratelmatobius sp. CFBH-T 240	DQ283499	28S	730	Frost *et al.*, 2006
Paratelmatobius sp. CFBH-T 240	DQ284148	histone H3	328	Frost *et al.*, 2006
Paratelmatobius sp. CFBH-T 240	DQ283814	rhodopsin	316	Frost *et al.*, 2006
Paratelmatobius sp. CFBH-T 240	DQ282925	tyrosinase	532	Frost *et al.*, 2006
Physalaemus cuvieri	AY843975	cytochrome *b*	385	Faivovich *et al.*, 2005
Physalaemus cuvieri	AY843729	12S, $tRNA^{val}$, 16S	2412	Faivovich *et al.*, 2005
Physalaemus cuvieri	AY844330	28S	758	Faivovich *et al.*, 2005
Physalaemus cuvieri	AY844499	RAG1	428	Faivovich *et al.*, 2005
Physalaemus cuvieri	AY844717	rhodopsin	316	Faivovich *et al.*, 2005
Physalaemus gracilis	DQ283417	12S, $tRNA^{val}$, 16S	2413	Frost *et al.*, 2006
Physalaemus gracilis	DQ283728	28S	759	Frost *et al.*, 2006
Physalaemus gracilis	DQ284022	rhodopsin	316	Frost *et al.*, 2006
Pleurodema brachyops	AY843979	cytochrome *b*	385	Faivovich *et al.*, 2005
Pleurodema brachyops	AY843733	12S, $tRNA^{val}$, 16S	2422	Faivovich *et al.*, 2005
Pleurodema brachyops	DQ284111	histone H3	328	Frost *et al.*, 2006
Pleurodema brachyops	AY844503	RAG1	428	Faivovich *et al.*, 2005
Pleurodema brachyops	AY844721	rhodopsin	316	Faivovich *et al.*, 2005
Pleurodema thaul	DQ864536	12S	346	Correa & Mendez, unpub.
Pleurodema thaul	DQ864560	12S, $tRNA^{val}$, 16S	1719	Correa & Mendez, unpub.
Proceratophrys avelinoi	DQ283038	12S, $tRNA^{val}$, 16S	1524	Frost *et al.*, 2006
Proceratophrys avelinoi	DQ283039	16S	587	Frost *et al.*, 2006
Proceratophrys avelinoi	DQ284065	histone H3	328	Frost *et al.*, 2006
Proceratophrys avelinoi	FJ685711	RAG1	428	Amaro *et al.*, 2009
Proceratophrys avelinoi	DQ283769	rhodopsin	316	Frost *et al.*, 2006
Proceratophrys avelinoi	DQ282903	tyrosinase	532	Frost *et al.*, 2006
Proceratophrys avelinoi	FJ685671	cytochrome *b*	611	Amaro *et al.*, 2009
Proceratophrys boiei	FJ685713	RAG1	428	Amaro *et al.*, 2009
Proceratophrys boiei	FJ685673	cytochrome *b*	611	Amaro *et al.*, 2009
Pseudopaludicola falcipes	AY843987	cytochrome *b*	385	Faivovich *et al.*, 2005
Pseudopaludicola falcipes	AY843741	12S, $tRNA^{val}$, 16S	2413	Faivovich *et al.*, 2005
Pseudopaludicola falcipes	DQ284117	histone H3	328	Frost *et al.*, 2006
Pseudopaludicola falcipes	AY844507	RAG1	428	Faivovich *et al.*, 2005

Pseudopaludicola falcipes	AY844728	rhodopsin	316	Faivovich *et al.*, 2005
Pseudopaludicola falcipes	AY844168	tyrosinase	532	Faivovich *et al.*, 2005
Rhaebo guttatus	DQ283375	12S, $tRNA^{val}$, 16S	2427	Frost *et al.*, 2006
Rhaebo guttatus	DQ283994	rhodopsin	316	Frost *et al.*, 2006
Rhaebo guttatus	DQ284361	histone H3	328	Frost *et al.*, 2006
Rhaebo guttatus	DQ283693	28S	752	Frost *et al.*, 2006
Rhaebo guttatus	DQ158381	RAG1	790	Pramuk, 2006
Rhaebo guttatus	EF364361	tyrosinase	414	Fouquet *et al.*, 2007
Rheobates palmatus	DQ502694	cytochrome *b*	385	Grant *et al.*, 2006
Rheobates palmatus	EU342508	12S, $tRNA^{val}$, 16S	2399	Santos *et al.*, 2009
Rheobates palmatus	DQ502925	COI	658	Grant *et al.*, 2006
Rheobates palmatus	DQ503271	rhodopsin	316	Grant *et al.*, 2006
Rheobates palmatus	DQ503172	tyrosinase	532	Grant *et al.*, 2006
Rhinoderma darwinii	DQ502589	cytochrome *b*	385	Grant *et al.*, 2006
Rhinoderma darwinii	DQ283324	12S, $tRNA^{val}$, 16S	2417	Frost *et al.*, 2006
Rhinoderma darwinii	DQ283654	28S	744	Frost *et al.*, 2006
Rhinoderma darwinii	DQ502858	COI	658	Grant *et al.*, 2006
Rhinoderma darwinii	DQ284320	histone H3	328	Frost *et al.*, 2006
Rhinoderma darwinii	AY364222	RAG1	559	Biju & Bossuyt, 2003
Rhinoderma darwinii	DQ283963	rhodopsin	316	Frost *et al.*, 2006
Scythrophrys sawayae	DQ283099	12S, $tRNA^{val}$, 16S	2430	Frost *et al.*, 2006
Scythrophrys sawayae	DQ283500	28S	728	Frost *et al.*, 2006
Scythrophrys sawayae	DQ284149	histone H3	328	Frost *et al.*, 2006
Scythrophrys sawayae	DQ283815	rhodopsin	316	Frost *et al.*, 2006
Scythrophrys sawayae	DQ282926	tyrosinase	532	Frost *et al.*, 2006
Silverstoneia nubicola	DQ502596	cytochrome *b*	385	Grant *et al.*, 2006
Silverstoneia nubicola	DQ502161	12S, $tRNA^{val}$, 16S	2419	Grant *et al.*, 2006
Silverstoneia nubicola	DQ502863	COI	658	Grant *et al.*, 2006
Silverstoneia nubicola	DQ503245	rhodopsin	316	Grant *et al.*, 2006
Silverstoneia nubicola	DQ503359	RAG1	435	Grant *et al.*, 2006
Silverstoneia nubicola	DQ503000	28S	776	Grant *et al.*, 2006
Telmatobius jahuira	DQ502448	cytochrome *b*	385	Grant *et al.*, 2006
Telmatobius jahuira	DQ283040	12S, $tRNA^{val}$, 16S	2424	Frost *et al.*, 2006
Telmatobius jahuira	DQ502743	COI	658	Grant *et al.*, 2006
Telmatobius jahuira	DQ283770	rhodopsin	316	Frost *et al.*, 2006
Telmatobius marmoratus	DQ284068	histone H3	328	Frost *et al.*, 2006
Telmatobius sibiricus	AY844355	28S	718	Faivovich *et al.*, 2005
Telmatobius sibiricus	AY844529	RAG1	428	Faivovich *et al.*, 2005
Telmatobius sibiricus	AY844757	rhodopsin	316	Faivovich *et al.*, 2005
Telmatobius sp. AMNH-A 165130	DQ283041	12S, $tRNA^{val}$, 16S	2422	Frost *et al.*, 2006
Telmatobius sp. AMNH-A 165130	DQ284067	histone H3	328	Frost *et al.*, 2006
Telmatobius sp. AMNH-A 165130	DQ283771	rhodopsin	316	Frost *et al.*, 2006
Telmatobius sp. AMNH-A 165114	AY844014	cytochrome *b*	385	Faivovich *et al.*, 2005
Thoropa miliaris	DQ502607	cytochrome *b*	385	Grant *et al.*, 2006
Thoropa miliaris	DQ283331	12S, $tRNA^{val}$, 16S	2424	Frost *et al.*, 2006
Thoropa miliaris	DQ502874	COI	658	Grant *et al.*, 2006
Thoropa miliaris	DQ502369	histone H3	328	Grant *et al.*, 2006
Thoropa miliaris	FJ685702	RAG1	406	Amaro *et al.*, 2009

Table 2: Primers used in this study (adapted from Grant *et al.*, 2006).[1]

Gene Region	Primer Name	Direction	Primer Sequence (5' to 3')	Source
12S rDNA, tRNA^{val}, 16S rDNA	MVZ59	*Forward*	ATAGCACTGAAAAYGCTDAGATG	Graybeal, 1997
	MVZ50	*Reverse*	TYTCGGTGTAAGYGARAKGCTT	Graybeal, 1997
	L13	*Forward*	TTAGAAGAGGCAAGTCGTAACATGGTA	Feller & Hedges, 1998
	Titus I	*Reverse*	GGTGGCTGCTTTTAGGCC	Titus & Larson, 1996
	L2A	*Forward*	CCAAACGAGCCTAGTGATAGCTGGTT	Hedges, 1994
	H10	*Reverse*	TGATTACGCTACCTTTGCACGGT	Hedges, 1994
	AR	*Forward*	CGCCTGTTTATCAAAAACAT	Palumbi *et al.*, 1991
	BR	*Reverse*	CCGGTCTGAACTCAGATCACGT	Palumbi *et al.*, 1991
cytochrome oxidase *c* subunit I	LCO1490	*Forward*	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*, 1994
	HCO2198	*Reverse*	TAAACTTCAGGGACCAAAAAATCA	Folmer *et al.*, 1994
cytochrome *b*	MVZ 15-L	*Forward*	GAACTAATGGCCCACACWWTACGNAA	Moritz *et al.*, 1992
	H15149	*Reverse*	AAACTGCAGCCCCTCAGAAATGATATT TGTCCTCA	Kocher *et al.*, 1989
rhodopsin exon 1	Rhod1A	*Forward*	ACCATGAACGGAACAGAAGGYCC	Bossuyt & Milinkovitch, 2000
	Rhod1C	*Reverse*	CCAAGGGTAGCGAAGAARCCTTC	Bossuyt & Milinkovitch, 2000
tyrosinase exon 1	TyrC	*Forward*	GGCAGAGGAWCRTGCCAAGATGT	Bossuyt & Milinkovitch, 2000
	TyrG	*Reverse*	TGCTGGCRTCTCTCCARTCCCA	Bossuyt & Milinkovitch, 2000
histone H3	H3F	*Forward*	ATGGCTCGTACCAAGCAGACVGC	Colgan *et al.*, 1999
	H3R	*Reverse*	ATATCCTTRGGCATRATRGTGAC	Colgan *et al.*, 1999
28S rDNA	28SV	*Forward*	AAGGTAGCCAAATGCCTCATC	Hillis & Dixon, 1991
	28SJJ	*Reverse*	AGTAGGGTAAAACTAACCT	Hillis & Dixon, 1991
recombi-nation activating gene 1	RAG1-TG1F	*Forward*	CCAGCTGGAAATAGGAGAAGTCTA	Grant *et al.*, 2006
	RAG1-TG1R	*Reverse*	CTGAACAGTTTATTACCGGACTCG	Grant *et al.*, 2006

[1] The gray line separates mitochondrial (above) and nuclear (below) loci.

Table 3: Summary of DNA sequence data.[1]

Sequence	Approx. no. basepairs	No. terminals	Basepairs per locus
Mitochondrial H-strand transcription unit 1	2400	146	350400
Cytochrome *b*	385	141	54285
Cytochrome *c* oxidase I	658	81	52640
Histone H3	328	129	27224
Recombination activating gene 1	435	97	42195
Rhodopsin	316	90	37288
Tyrosinase	532	42	22344
28S	700	35	24500
Total	5754	761	610876

[1] Approximate number of base pairs refers to complete sequences.

Table 4: Ingroup sequences generated in this study. Numbering of undetermined specimens based on optimal topology.[1, 2]

Species	Sample ID	Source	Locality	Abbreviation	H1	Cyt *b*	COI	H3	RAG1	Rhod	Tyr
C. aeneus	11-059	CFBH 4476	Barreiras/RS (sic [RJ])	*C_aeneus_Bar1*	X	X		X	X	X	
C. aeneus	11-135	MNRJ 44585	Sítio Dona Ana, Barreira, Guapimirim/RJ	*C_aeneus_Bar2*	X	X	X				
C. aeneus	11-147	MNRJ 47763	PE Três Picos, Cachoeiras de Macacu/RJ	*C_aeneus_Mac*	X	X	X				X
C. aeneus	11-115	MNRJ 37311	Riacho próx. Rio Soberbo, PARNA Serra dos Órgãos, RJ	*C_aeneus_PARNASO1*	X	X	X		X	X	X
C. aeneus	11-118	MNRJ 37312	Riacho próx. Rio Soberbo, PARNA Serra dos Órgãos, RJ	*C_aeneus_PARNASO2*	X	X	X		X		X
C. aff. *gaudichaudii*	11-030	MTR 15541	Ilha Grande/RJ	*Hylodes_sp_IlhaGrande*	X	X		X	X	X	
C. bokermanni	11-112	UFMG-T 9346	Catas Altas/MG	*C_bokermanni_CAl*	X	X			X	X	
C. bokermanni	11-159	MTR 20327	Serra do Cipó/MG	*C_bokermanni_Cipo1*	X	X	X		X	X	X
C. bokermanni	11-160	MTR 20345	Serra do Cipó/MG	*C_bokermanni_Cipo2*	X	X	X		X	X	X
C. bokermanni	11-119	MNRJ 38465	Riacho na trilha atrás IBAMA, Alto do Palácio, Serra do Cipó, Santana do Riacho/MG	*C_bokermanni_SRiacho1*	X	X	X		X	X	X
C. bokermanni	11-126	MNRJ 39982	Riacho na trilha atrás IBAMA, Alto do Palácio, Serra do Cipó, Santana do Riacho/MG	*C_bokermanni_SRiacho2*	X	X	X		X	X	X
C. bokermanni	11-132	MNRJ 41459	Riacho na trilha atrás IBAMA, Alto do Palácio, Serra do Cipó, Santana do Riacho/MG	*C_bokermanni_SRiacho3*	X	X	X		X	X	X
C. caramaschii	11-145	MNRJ 73989	Balsa Nova/PR	*C_caramaschii_BNova*	X	X			X	X	X
C. caramaschii	11-048	H0154	Juquitiba/SP	*C_caramaschii_Juq1*	X	X	X	X	X	X	
C. caramaschii	11-052	H0184	Juquitiba/SP	*C_caramaschii_Juq2*	X	X	X	X	X	X	
C. caramaschii	11-110	CTMZ - 04569	Parque Estadual de Carlos Botelho, Sete Barras/SP	*C_caramaschii_PECB*	X	X			X	X	
C. caramaschii	11-102	CTMZ - 02130	Parque Estadual de Jacupiranga, Núcleo Caverna do Diabo, Eldorado Paulista/SP	*C_caramaschii_PEJ1*	X	X	X		X	X	
C. caramaschii	11-103	CTMZ - 02131	Parque Estadual de Jacupiranga, Núcleo Caverna do Diabo, Eldorado Paulista/SP	*C_caramaschii_PEJ2*	X	X			X	X	
C. caramaschii	11-106	CTMZ - 02255	Parque Estadual de Jacupiranga, Núcleo Caverna do Diabo, Eldorado Paulista/SP	*C_caramaschii_PEJ3*	X	X			X	X	
C. caramaschii	11-107	CTMZ - 02079	Parque Estadual de Jacupiranga, Núcleo Caverna do Diabo, Eldorado Paulista/SP	*C_caramaschii_PEJ4*	X	X			X	X	
C. caramaschii	11-109	CTMZ - 02640	Parque Estadual de Jacupiranga, Núcleo Caverna do Diabo, Eldorado Paulista/SP	*C_caramaschii_PEJ5*	X	X			X	X	

C. caramaschii	11-088	CFBH 3093	PET Alto Ribeira/SP	*C_caramaschii_PETAR*	X	X		X	X	X		
C. caramaschii	11-043	H-532	Piedade/SP	*C_caramaschii_Pie*	X	X	X	X	X			
C. caramaschii	11-017	Alc 86-79	Ribeirão Grande/SP	*C_caramaschii_RibGr1*	X			X				
C. caramaschii	11-020	AF 520	Ribeirão Grande/SP	*C_caramaschii_RibGr2*	X	X		X	X	X		
C. caramaschii	11-028	AF 521	Ribeirão Grande/SP	*C_caramaschii_RibGr3*	X	X		X	X	X		
C. caramaschii	11-111	UMFG-T 15956	São Bento do Sul/SC	*C_caramaschii_SBS*	X	X			X	X		
C. cf. caramaschii	11-089	CFBH 5302	Itanhaém/SP	*C_caramaschii_Ita1*	X	X	X	X	X	X		
C. cf. caramaschii	11-091	CFBH 5303	Itanhaém/SP	*C_caramaschii_Ita2*	X	X	X	X	X	X		
C. cf. caramaschii	11-094	CFBH 7925	Itanhaém/SP	*C_caramaschii_Ita3*	X	X		X	X	X	X	
C. cf. caramaschii	11-005	AF 374	Fazenda Intervales, Capão Bonito/SP	*C_cf_caramaschii_Int1*	X	X						
C. cf. caramaschii	11-015	AF 373	Fazenda Intervales, Capão Bonito/SP	*C_cf_caramaschii_Int2*	X	X		X	X			
C. gaudichaudii	11-150	MNRJ 74089	Bom Retiro, PARNA Floresta da Tijuca, RJ	*C_gaudichaudii_BRet*	X	X			X	X	X	
C. gaudichaudii	11-130	MNRJ 40552	Estrada Dona Castorina, PARNA Tijuca, RJ	*C_gaudichaudii_DCast1*	X	X			X	X	X	
C. gaudichaudii	11-134	MNRJ 40553	Estrada Dona Castorina, PARNA Tijuca, RJ	*C_gaudichaudii_DCast2*	X	X			X		X	
C. gaudichaudii	11-121	MNRJ 38750	Riacho na trilha Praia do Caxadaço, PE Ilha Grande, Angra dos Reis/RJ	*C_gaudichaudii_IlhaGrande1*	X	X			X	X	X	
C. gaudichaudii	11-125	MNRJ 38752	Riacho na trilha Praia do Caxadaço, PE Ilha Grande, Angra dos Reis/RJ	*C_gaudichaudii_IlhaGrande2*	X	X	X		X	X	X	
C. gaudichaudii	11-143	MNRJ 73068	Espraiado, Maricá/RJ	*C_gaudichaudii_Mar1*	X	X	X		X	X	X	
C. gaudichaudii	11-152	MNRJ 73527	Espraiado, Maricá/RJ	*C_gaudichaudii_Mar2*	X	X	X		X		X	
C. gaudichaudii	11-146	MNRJ 74088	Córrego Mayrink, PARNA Floresta da Tijuca, RJ	*C_gaudichaudii_May*	X	X	X		X	X	X	
C. gaudichaudii	11-154	MNRJ 76761	Saquarema/RJ	*C_gaudichaudii_Saq1*	X	X	X		X	X	X	
C. gaudichaudii	11-156	MNRJ 76774	Saquarema/RJ	*C_gaudichaudii_Saq2*	X	X	X		X	X	X	
Crossodactylus sp.	11-138	MNRJ 40701	Morro de São João, Casimiro de Abreu/RJ	*Crossodactylus_sp_CAbreu*	X	X			X			
Crossodactylus sp. 1	11-123	MNRJ 39465	REBIO Duas Bocas, Cariacica/ES	*Crossodactylus_sp_RBDB*	X	X			X	X	X	
Crossodactylus sp. 2	11-058	CFBH 10799	Sítio Recanto da Mata, Muniz Freire/ES	*Crossodactylus_sp_Mun1*	X			X	X	X		
Crossodactylus sp. 2	11-063	CFBH 10800	Sítio Recanto da Mata, Muniz Freire/ES	*Crossodactylus_sp_Mun2*	X	X		X		X		
Crossodactylus sp. 2	11-068	CFBH 10801	Sítio Recanto da Mata, Muniz Freire/ES	*Crossodactylus_sp_Mun3*	X	X	X	X	X	X		
Crossodactylus sp. 2	11-093	CFBH 11960	Muniz Freire/ES	*Crossodactylus_sp_Mun4*	X	X		X		X		
Crossodactylus sp. 2	11-096	CFBH 11961	Muniz Freire/ES	*Crossodactylus_sp_Mun5*	X	X				X		
Crossodactylus sp. 3	11-092	CFBH 12401	REBIO Augusto Ruschi, Santa Teresa/ES	*Crossodactylus_sp_RBAR1*	X			X	X	X		

Crossodactylus sp. 3	11-097	CFBH 12367	REBIO Augusto Ruschi, Santa Teresa/ES	*Crossodactylus_sp_RBAR2*	X	X	X		X	X	
Crossodactylus sp. 3	TG-11-011	MCP 11572	Córrego Lombardia, REBIO Augusto Ruschi, Santa Teresa/ES	*Crossodactylus_sp_RBAR3*	X	X			X	X	
Crossodactylus sp. 4	11-136	MNRJ 44952	RPPN Serra do Teimoso, Jussari/BA	*Crossodactylus_sp_STei2*	X	X			X	X	X
Crossodactylus sp. 5	11-008	MTR 16259	Serra Bonita, Camacan/BA	*Crossodactylus_sp_SBon1*	X	X	X	X		X	
Crossodactylus sp. 5	11-014	MTR 16243	Serra Bonita, Camacan/BA	*Crossodactylus_sp_SBon2*	X	X	X	X	X	X	
Crossodactylus sp. 5	11-095	CFBH 9400	RPPN Serra Bonita, Camacan/BA	*Crossodactylus_sp_SBon3*	X	X		X	X	X	
Crossodactylus sp. 5	11-098	CFBH 9401	RPPN Serra Bonita, Camacan/BA	*Crossodactylus_sp_SBon4*	X	X	X			X	
Crossodactylus sp. 5	11-003	MTR 16321	Serra das Lontras, Arataca/BA	*Crossodactylus_sp_SLon1*	X		X	X	X	X	
Crossodactylus sp. 5	11-006	MTR 16320	Serra das Lontras, Arataca/BA	*Crossodactylus_sp_SLon2*	X	X	X	X	X	X	
Crossodactylus sp. 5	11-016	MTR 16654	Serra da Onça, Santa Luzia/BA	*Crossodactylus_sp_SOnc1*	X	X		X	X		
Crossodactylus sp. 5	11-019	MTR 16655	Serra da Onça, Santa Luzia/BA	*Crossodactylus_sp_SOnc2*	X	X	X	X	X	X	
Crossodactylus sp. 5	11-012	MTR 6021	Serra do Teimoso, Jussari/BA	*Crossodactylus_sp_STei1*	X	X	X	X	X	X	
Crossodactylus sp. 5	11-035	AF 916	Fazenda Unacau/BA	*Crossodactylus_sp_Unac*	X	X	X	X	X	X	
Crossodactylus sp. 6	11-116	MNRJ 38316	Caraça, Catas Altas/MG	*Crossodactylus_sp_CAl1*	X	X	X		X	X	X
Crossodactylus sp. 6	11-120	MNRJ 38474	Banho do Belchior, RPPN Serra do Caraça, Catas Altas/MG	*Crossodactylus_sp_CAl2*	X	X	X				X
Crossodactylus sp. 6	11-124	MNRJ 38476	Riacho Cascudos, RPPN Serra do Caraça, Catas Altas/MG	*Crossodactylus_sp_CAl3*	X	X	X		X	X	X
Crossodactylus sp. 6	11-128	MNRJ 38477	Córrego cont. Banho do Belchior, RPPN Serra do Caraça, Catas Altas/MG	*Crossodactylus_sp_CAl4*	X	X	X		X		
Crossodactylus sp. 7	11-001	AF 436	Apucaraninha/PR	*Crossodactylus_sp_Apu1*	X	X	X	X		X	
Crossodactylus sp. 7	11-007	AF 437	Apucaraninha/PR	*Crossodactylus_sp_Apu2*	X	X	X	X	X	X	
Crossodactylus sp. 7	11-021	II-H128	Ortigueira/PR	*Crossodactylus_sp_Ort1*	X	X		X		X	
Crossodactylus sp. 7	11-099	CFBH 11181	Reserva Indígena de Mococa, Ortigueira/PR	*Crossodactylus_sp_Ort2*	X	X	X			X	
Crossodactylus sp. 7	11-046	UF 76-31	Ourinhos/SP	*Crossodactylus_sp_Our*	X	X	X	X	X	X	
Crossodactylus sp. 7	11-037	II-H010	Pinhalão/PR	*Crossodactylus_sp_Pin1*	X	X	X	X		X	
Crossodactylus sp. 7	11-041	AF 1334	Pinhalão/PR	*Crossodactylus_sp_Pin2*	X	X	X	X	X	X	
Crossodactylus sp. 7	11-042	IIH-212	Wenceslau Brás/PR	*Crossodactylus_sp_WBras1*	X	X	X	X	X	X	
Crossodactylus sp. 7	11-045	H017	Wenceslau Brás/PR	*Crossodactylus_sp_WBras2*	X	X	X	X	X	X	
Crossodactylus sp. 8	11-026	AF 1332	Juquitiba/SP	*Crossodactylus_sp_Juq1*	X	X	X	X	X	X	
Crossodactylus sp. 8	11-040	AF 1320	Juquitiba/SP	*Crossodactylus_sp_Juq2*	X	X	X	X	X	X	

Crossodactylus sp. 8	11-024	IT-H0276	Piedade/SP	*Crossodactylus_sp_Pie1*	X	X	X	X	X	X		
Crossodactylus sp. 8	11-029	IT-H0330	Piedade/SP	*Crossodactylus_sp_Pie2*	X	X	X	X	X	X		
Crossodactylus sp. 8	11-047	H0072	Piedade/SP	*Crossodactylus_sp_Pie3*	X	X	X	X	X	X		
Crossodactylus sp. 8	11-050	UF 84-50	Quilombo, Caucaia do Alto/SP	*Crossodactylus_sp_Qui1*	X	X	X	X	X	X		
Crossodactylus sp. 8	11-053	AF 1603	Quilombo, Caucaia do Alto/SP	*Crossodactylus_sp_Qui2*	X	X	X	X	X	X		
Crossodactylus sp. 9	11-129	MNRJ 40199	Cascata da Professorinha, Campo Magro/PR	*Crossodactylus_sp_CM*	X	X	X		X	X	X	
Crossodactylus sp. 9	11-137	MNRJ 40207	Fazenda Morro Alto, Ponta Grossa/PR	*Crossodactylus_sp_PG*	X	X			X	X	X	
Crossodactylus sp. 9	11-133	MNRJ 40200	Mananciais da Serra, Piraquara/PR	*Crossodactylus_sp_Pir*	X	X	X		X	X	X	
Crossodactylus sp. 10	11-018	AF 71	PET Alto Ribeira/SP	*Crossodactylus_sp_PETAR1*	X	X		X				
Crossodactylus sp. 10	11-057	CFBH 430	PET Alto Ribeira (Núcleo Santana), Iporanga/SP	*Crossodactylus_sp_PETAR2*	X	X		X				
Crossodactylus sp. 10	11-067	CFBH 431	PET Alto Ribeira (Núcleo Santana), Iporanga/SP	*Crossodactylus_sp_PETAR3*	X	X		X	X	X	X	

[1] Numbering of undetermined specimens reflects optimal topology.
[2] For loci abbreviations, refer to text.

Table 5: Outgroup sequences generated in this study.[1]

Species	Sample ID	Source	Locality	Abbreviation	H1	Cyt *b*	COI	H3	RAG 1	Rhod	Tyr	28S
Bokermannohyla sp.	11-056	CFBH 10828	PARNA Serra da Bocaina, Campo de Fruticultura, São José do Barreiro/SP	*Bokermannohyla_sp*	X	X		X	X	X		X
H. aff. *lateristrigatus*	11-117	MNRJ 38413	Santa Lúcia, Santa Teresa/ES	*H_aff_lateristrigatus_STer*	X	X	X		X	X	X	
H. asper	11-044	IIH-211	Bertioga/SP	*H_asper_Bert*	X	X	X	X	X	X		
H. asper	11-148	MNRJ 64834	PARNA Serra da Bocaina, Parati/RJ	*H_asper_Boc*	X	X	X		X	X	X	
H. asper	11-036	AF 768	Barra do Una/SP	*H_asper_BUna*	X	X		X		X		
H. asper	11-073	CFBH 2658	Ilha Bela (São Sebastião)/SP	*H_asper_IlhaBela*	X	X	X	X		X	X	
H. asper	11-158	MNRJ 60170	Reserva Ecológica de Guapiaçu, Cachoeiras de Macacu/RJ	*H_asper_Mac*	X	X			X	X	X	
H. asper	11-140	MNRJ 51026	PARNA Serra dos Órgãos (Sede Guapimirim), Teresópolis/RJ	*H_asper_PARNASO*	X	X			X	X	X	
H. asper	11-076	CFBH 4445	Teresópolis/RJ	*H_asper_Ter*	X	X		X	X	X	X	
H. babax	11-031	MTR 15803	PARNA Caparaó/ES	*H_babax*	X	X	X	X		X		

H. cf. *charadranaetes*	11-155	MNRJ 59065	Reserva Ecológica de Guapiaçu, Cachoeiras de Macacu/RJ	*H_cf_charadranaetes_Mac*	X	X	X				X	
H. dactylocinus	11-074	CFBH 857	Estação Ecológica Juréia (Itatins), Peruíbe/SP	*H_dactylocinus_Jur1*	X	X				X		
H. dactylocinus	11-077	CFBH 858	Estação Ecológica Juréia (Itatins), Peruíbe/SP	*H_dactylocinus_Jur2*	X	X				X		
H. fredi	11-114	MNRJ 36077	Trilha Dois Rios, Cachadaço, Ilha Grande, Angra dos Reis/RJ	*H_fredi*	X	X			X	X	X	X
H. glaber	11-049	MTR 10993	Campos do Jordão/SP	*H_glaber_Camp*	X	X	X	X	X	X		
H. gr. *asper*	11-013	AF 379	Fazenda Intervales/SP	*H_gr_asper_Jur*	X	X		X				
H. gr. *lateristrigatus*	11-002	AF 378	Fazenda Intervales/SP	*H_gr_lateristrigatus_Int1*	X	X		X				
H. gr. *lateristrigatus*	11-009	AF 377	Fazenda Intervales/SP	*H_gr_lateristrigatus_Int2*	X	X		X		X		
H. heyeri	11-075	CFBH 1598	PET Alto Ribeira (Núcleo Caboclos), Iporanga/SP	*H_heyeri_Gua1*	X	X				X		
H. heyeri	11-079	CFBH 10259	Guaratuba (Fazenda Creminácio, Serra do Araraquara)/PR	*H_heyeri_Gua2*	X	X		X				
H. heyeri	11-082	CFBH 10260	Guaratuba (Fazenda Creminácio, Serra do Araraquara)/PR	*H_heyeri_PETAR*	X	X		X		X		
H. lateristrigatus	11-141	MNRJ 56074	Reserva São Lourenço, Santa Teresa/ES	*H_lateristrigatus*	X	X	X			X		
H. magalhaesi	11-060	CFBH 2293	Campos do Jordão/SP	*H_magalhaesi_Camp1*	X	X	X	X		X		
H. magalhaesi	11-062	CFBH 2294	Campos do Jordão/SP	*H_magalhaesi_Camp2*	X	X	X	X	X	X		
H. magalhaesi	11-064	CFBH 2295	Campos do Jordão/SP	*H_magalhaesi_Camp3*	X	X				X		
H. magalhaesi	11-066	CFBH 5117	Campos do Jordão/SP	*H_magalhaesi_Camp4*	X	X	X	X		X		
H. meridionalis	TG-11-050	TG 2262	São Francisco de Paula/RS	*H_meridionalis*		X				X		
H. nasus	11-113	MNRJ 35434	Estrada Dona Castorina, Floresta da Tijuca, RJ	*H_nasus_DCast*	X	X	X		X	X		
H. nasus	11-034	AF 440	Rio de Janeiro (Horto Florestal)/RJ	*H_nasus_RJHorto*	X	X		X	X			
H. otavioi	11-131	MNRJ 41456	Estrada Real entre Morro do Pilar e Conceição do Mato Dentro, riacho afluente do Rio Mafa Cavalo, Morro do Pilar/MG	*H_otavioi*	X	X			X	X	X	
H. perplicatus	11-061	CFBH 12614	Estrada em Rio Vermelho para Corupá, próx. pesque-e-pague, São Bento do Sul/SC	*H_perplicatus_SBS1*	X	X		X		X		
H. perplicatus	11-069	CFBH 11683	Barragem do Rio São Bento, Siderópolis/SC	*H_perplicatus_SBS2*	X	X						
H. perplicatus	11-071	CFBH 3243	São Bento do Sul/SC	*H_perplicatus_SBS3*	X	X		X	X		X	
H. phyllodes	11-100	CTMZ - 07228	Parque Natural Municipal Nascentes de Paranapiacaba, Santo André/SP	*C_gaudichaudii_SAndre*	X	X			X	X		X
H. phyllodes	11-054	Alc 102-79	Bertioga/SP	*H_phyllodes_Bert*	X	X	X	X	X	X		X

H. phyllodes	11-142	MNRJ 64822	PARNA Serra da Bocaina, Parati/RJ	*H_phyllodes_Boc*	X	X	X		X	X	X		
H. phyllodes	11-033	AF 767	Barra do Una/SP	*H_phyllodes_BUna*	X	X	X	X	X	X			X
H. phyllodes	11-081	CFBH 3150	Itanhaém/SP	*H_phyllodes_Ita1*	X	X	X	X		X			
H. phyllodes	11-083	CFBH 3873	Itanhaém/SP	*H_phyllodes_Ita2*	X	X	X	X	X	X			
H. phyllodes	11-085	CFBH 3878	Itanhaém/SP	*H_phyllodes_Ita3*	X	X	X	X	X	X			
H. pipilans	11-144	MNRJ 47760	PE Três Picos, Cachoeiras de Macacu/RJ	*H_pipilans_Mac*	X	X							
H. pipilans	11-122	MNRJ 39371	1º riacho cruzando estrada interna PARNA Serra dos Órgãos, Sede Guapimirim, RJ	*H_pipilans_PARNASO*	X	X					X		
H. sazimai	11-078	CFBH 10786	PARNA Itatiaia, Itatiaia/RJ	*H_sazimai_PARNAI1*	X	X		X		X			
H. sazimai	11-080	CFBH 10787	PARNA Itatiaia, Itatiaia/RJ	*H_sazimai_PARNAI2*	X	X							
Hylodes	11-011	AF 343	Fazenda Intervales/SP	*Hylodes_sp_Int*	X	X		X		X			
Hylodes sp.	11-022	3449[3]	Cunha/SP	*Hylodes_sp_Cunha*	X	X	X	X	X	X			
Hylodes sp.	11-025	3339[3]	Ilha Bela/SP	*Hylodes_sp_IlhaBela*	X	X		X					
Hylodes sp.	11-038	H0157	Juquitiba/SP	*Hylodes_sp_Juq*	X	X		X	X	X			
M. apuana	11-084	CFBH 6667	Domingos Martins/ES	*M_apuana_Dom1*	X	X	X	X	X	X			
M. apuana	11-090	CFBH 9118	Domingos Martins (Pedra Azul)/ES	*M_apuana_Dom2*	X	X	X	X		X			
M. apuana	11-023	MTR 12614	PARNA Caparaó/ES	*M_apuana_PCap*	X	X	X	X		X			
M. boticariana	11-065	CFBH 425	Caçapava (Serra da Mantiqueira)/SP	*M_boticariana_Cac1*	X	X	X	X		X			
M. boticariana	11-070	CFBH 426	Caçapava (Serra da Mantiqueira)/SP	*M_boticariana_Cac2*	X	X	X	X		X	X		
M. goeldii	11-139	MNRJ 44620	PE Três Picos, Cachoeiras de Macacu/RJ	*M_goeldii_Mac*	X	X	X						
M. massarti	11-086	CFBH 6933	PESM, Núcleo Curucutú, Itanhaém/SP	*M_massarti*	X		X	X		X			
Megaelosia sp.	11-027	AF 1745	Estação Biológica de Boracéia/SP	*Megaelosia_sp_Bora1*	X	X	X	X	X	X			
Megaelosia sp.	11-039	AF 1744	Estação Biológica de Boracéia/SP	*Megaelosia_sp_Bora2*	X	X		X	X	X			
Megaelosia sp.	11-087	CFBH 9330	PARNA Serra dos Órgãos (Sede Teresópolis), Teresópolis/RJ	*Megaelosia_sp_PARNASO*	X	X	X	X		X			
Megaelosia sp.	TG-11-049	MCP 11575	Córrego do Convento no bairro Ribeirão Grande, próximo à Fazenda Nova Gokula, Pindamonhangaba/SP	*Megaelosia_sp_Pind*		X				X			
Megaelosia sp.	11-032	AF 766	Ubatuba/SP	*Megaelosia_sp_Uba*	X	X		X					

[1] For loci abbreviations, refer to text.
[3] Sequences obtained from M.T. Rodrigues's private collection. No acronyms available.

Table 6: Percent uncorrected pairwise distances between cytochrome *b* sequences for terminals in the *C. gaudichaudii* complex.

	Terminal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	C_gaudichaudii_Mar1	—																
2	C_gaudichaudii_Mar2	0.3	—															
3	C_gaudichaudii_Saq1	0.8	0.6	—														
4	C_gaudichaudii_Saq2	1.1	0.8	1.3	—													
5	Crossodactylus_sp_CAbreu	1.9	1.6	1.1	2.4	—												
6	C_gaudichaudii_BRet	16.9	17.2	16.7	16.7	17.5	—											
7	C_gaudichaudii_DCast1	17.2	17.5	16.9	16.9	17.7	0.8	—										
8	C_gaudichaudii_DCast2	16.9	17.2	16.7	16.7	17.5	0.6	0.3	—									
9	C_gaudichaudii_May	17.2	17.5	16.9	16.9	17.7	1.3	0.6	0.8	—								
10	C_gaudichaudii_IlhaGrande1	16.2	16.4	16.4	15.9	17.2	6.3	6	5.8	6	—							
11	C_gaudichaudii_IlhaGrande2	16.2	16.4	16.4	15.9	17.2	6.3	6	5.8	6	0	—						
12	Hylodes_sp_IlhaGrande	16.2	16.4	16.4	15.9	17.2	6.3	6	5.8	6	0	0	—					
13	C_aeneus_Bar1	16.2	16.4	16.4	15.9	17.2	4.7	4.5	4.2	4.5	4.7	4.7	4.7	—				
14	C_aeneus_Bar2	16.2	16.4	16.4	15.9	17.2	4.7	4.5	4.2	4.5	4.7	4.7	4.7	0	—			
15	C_aeneus_Mac	15.6	15.9	15.9	15.4	16.7	6	5.8	5.5	5.8	5.5	5.5	5.5	5	5	—		
16	C_aeneus_PARNASO1	16.4	16.7	16.7	16.2	17.5	6	5.8	5.5	5.8	5.8	5.8	5.8	5	5	2.1	—	
17	C_aeneus_PARNASO2	16.4	16.7	16.7	16.2	17.5	4.5	4.7	4.5	4.7	5	5	5	0.3	0.3	5.2	5.2	—

Table 7: Percent uncorrected pairwise distances between cytochrome *b* sequences for terminals in the ES/BA complex.

	Terminal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Crossodactylus_sp_RBDB	—																
2	Crossodactylus_sp_Mun2	9.7	—															
3	Crossodactylus_sp_Mun3	9.7	0	—														
4	Crossodactylus_sp_Mun4	9.7	0	0	—													
5	Crossodactylus_sp_Mun5	9.9	0.3	0.3	0.3	—												
6	Crossodactylus_sp_RBAR2	17.5	15.9	15.9	15.9	16.2	—											
7	Crossodactylus_sp_RBAR3	17.7	16.2	16.2	16.2	16.4	0.3	—										
8	Crossodactylus_sp_STei2	16.9	16.7	16.7	16.7	16.9	14.3	14.6	—									
9	Crossodactylus_sp_SBon1	18	15.6	15.6	15.6	15.9	14.6	14.9	9.1	—								
10	Crossodactylus_sp_SBon2	17.7	15.4	15.4	15.4	15.6	14.9	15.1	8.9	0.3	—							
11	Crossodactylus_sp_SBon3	17.7	15.4	15.4	15.4	15.6	14.9	15.1	8.9	0.3	0	—						
12	Crossodactylus_sp_SBon4	17.7	15.4	15.4	15.4	15.6	14.9	15.1	8.9	0.3	0	0	—					
13	Crossodactylus_sp_SLon2	18	15.6	15.6	15.6	15.9	14.6	14.9	9.1	0	0.3	0.3	0.3	—				
14	Crossodactylus_sp_SOnc1	18	15.6	15.6	15.6	15.9	14.6	14.9	9.1	0	0.3	0.3	0.3	0	—			
15	Crossodactylus_sp_SOnc2	18	15.6	15.6	15.6	15.9	14.6	14.9	9.1	0	0.3	0.3	0.3	0	0	—		
16	Crossodactylus_sp_STei1	17.5	15.1	15.1	15.1	15.4	14.6	14.9	9.1	0.6	0.3	0.3	0.3	0.6	0.6	0.6	—	
17	Crossodactylus_sp_Unac	18	15.6	15.6	15.6	15.9	14.6	14.9	9.1	0	0.3	0.3	0.3	0	0	0	0.6	—

Table 8: Percent uncorrected pairwise distances between cytochrome *b* sequences for terminals in the *C. bokermanni* complex.

	Terminal	1	2	3	4	5	6	7	8	9	10
1	C_bokermanni_Cipo1	—									
2	C_bokermanni_Cipo2	0	—								
3	C_bokermanni_SRiacho1	0	0	—							
4	C_bokermanni_SRiacho2	0.3	0.3	0.3	—						
5	C_bokermanni_SRiacho3	0.3	0.3	0.3	0	—					
6	C_bokermanni_CAl	8.4	8.4	8.4	8.1	8.1	—				
7	Crossodactylus_sp_CAl1	8.4	8.4	8.4	8.1	8.1	0	—			
8	Crossodactylus_sp_CAl2	8.4	8.4	8.4	8.1	8.1	0	0	—		
9	Crossodactylus_sp_CAl3	8.4	8.4	8.4	8.1	8.1	0	0	0	—	
10	Crossodactylus_sp_CAl4	8.4	8.4	8.4	8.1	8.1	0	0	0	0	—

Table 9: Percent uncorrected pairwise distances between cytochrome *b* sequences for terminals in the *C. schmidti* complex.

	Terminal	1	2	3	4	5	6	7	8	9	10	11
1	Crossodactylus_schmidti	—										
2	C_caramaschii_SBS	15.6	—									
3	Crossodactylus_sp_Apu1	18.2	14.1	—								
4	Crossodactylus_sp_Apu2	18	13.8	0.3	—							
5	Crossodactylus_sp_Ort1	18.2	14.1	0.3	0.6	—						
6	Crossodactylus_sp_Our	17.5	14.3	3.4	3.7	3.7	—					
7	Crossodactylus_sp_Ort2	16.9	13.8	3.4	3.7	3.7	0.6	—				
8	Crossodactylus_sp_Pin1	17.5	13.8	2.9	3.2	3.2	1.1	1.1	—			
9	Crossodactylus_sp_Pin2	17.5	13.8	2.9	3.2	3.2	1.1	1.1	0	—		
10	Crossodactylus_sp_WBras1	17.2	13.6	3.2	3.4	3.4	1.3	1.3	0.3	0.3	—	
11	Crossodactylus_sp_WBras2	17.2	13.6	3.2	3.4	3.4	1.3	1.3	0.3	0.3	0	—

Table 10-A: Percent uncorrected pairwise distances between cytochrome *b* sequences for terminals in the *C. caramaschii* complex.[1]

	Terminal	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	C_caramaschii_PECB	—													
2	C_caramaschii_Ita1	9.9	—												
3	C_caramaschii_Ita2	8.6	1.9	—											
4	C_caramaschii_Ita3	9.7	0.3	1.6	—										
5	C_caramaschii_Juq1	10.4	6.8	6	6.5	—									
6	C_caramaschii_Juq2	10.2	6.5	5.8	6.3	0.3	—								
7	C_caramaschii_Pie	9.9	6.8	6	6.5	0.6	0.3	—							
8	Crossodactylus_sp_Juq1	10.2	6.5	5.8	6.3	0.3	0	0.3	—						
9	Crossodactylus_sp_Juq2	10.2	6.5	5.8	6.3	0.3	0	0.3	0	—					
10	Crossodactylus_sp_Pie1	10.2	6.5	5.8	6.3	0.3	0	0.3	0	0	—				
11	Crossodactylus_sp_Pie2	10.2	7.1	6.3	6.8	0.8	0.6	0.8	0.6	0.6	0.6	—			
12	Crossodactylus_sp_Pie3	10.2	6.5	5.8	6.3	0.3	0	0.3	0	0	0	0.6	—		
13	Crossodactylus_sp_Qui1	10.2	6.5	5.8	6.3	0.3	0	0.3	0	0	0	0.6	0	—	
14	Crossodactylus_sp_Qui2	10.2	7.1	6.3	6.8	0.8	0.6	0.3	0.6	0.6	0.6	1.1	0.6	0.6	—
15	Crossodactylus_sp_CM	15.1	12.8	12.5	12.5	13.6	13.3	13	13.3	13.3	13.3	13.8	13.3	13.3	13.3
16	Crossodactylus_sp_PG	14.9	12.5	12.3	12.3	13.3	13	12.8	13	13	13	13.6	13	13	13
17	Crossodactylus_sp_Pir	14.9	13	12.3	12.8	13.3	13	12.8	13	13	13	13	13	13	13
18	C_caramaschii_BNova	15.1	12.8	12.5	12.5	13.6	13.3	13	13.3	13.3	13.3	13.3	13.3	13.3	13.3
19	C_caramaschii_PEJ1	12.8	10.4	9.9	10.2	13	13.3	13.6	13.3	13.3	13.3	13.6	13.3	13.3	13.8
20	C_caramaschii_PEJ2	12.8	10.4	9.9	10.2	13	13.3	13.6	13.3	13.3	13.3	13.6	13.3	13.3	13.8
21	C_caramaschii_PEJ3	13.6	10.7	10.2	10.4	13.3	13.6	13.8	13.6	13.6	13.6	13.8	13.6	13.6	14.1
22	C_caramaschii_PEJ4	13.6	10.7	10.2	10.4	12.8	13	13.3	13	13	13	13.3	13	13	13.6
23	C_caramaschii_PEJ5	13.3	10.4	9.9	10.2	13	13.3	13.6	13.3	13.3	13.3	13.6	13.3	13.3	13.8
24	Crossodactylus_sp_RibGr2	14.1	10.2	9.4	9.9	12.5	12.3	12.5	12.3	12.3	12.3	12	12.3	12.3	12.8
25	Crossodactylus_sp_RibGr3	13.8	10.4	9.7	10.2	12.8	12.5	12.8	12.5	12.5	12.5	12.3	12.5	12.5	13
26	C_cf_caramaschii_Int1	14.3	11.2	10.7	11	14.1	13.8	14.1	13.8	13.8	13.8	13.6	13.8	13.8	14.3

27	C_cf_caramaschii_Int2	14.3	11.2	10.7	11	14.1	13.8	14.1	13.8	13.8	13.8	13.6	13.8	13.8	14.3
28	C_caramaschii_PETAR	14.3	11	10.4	10.7	12.8	12.5	12.8	12.5	12.5	12.5	12.3	12.5	12.5	13
29	Crossodactylus_sp_PETAR1	14.3	11	10.4	10.7	12.8	12.5	12.8	12.5	12.5	12.5	12.3	12.5	12.5	13
30	Crossodactylus_sp_PETAR2	14.1	10.7	10.2	10.4	12.5	12.3	12.5	12.3	12.3	12.3	12	12.3	12.3	12.8
31	Crossodactylus_sp_PETAR3	14.1	10.7	10.2	10.4	12.5	12.3	12.5	12.3	12.3	12.3	12	12.3	12.3	12.8

[1] The gray line separates the two major clades in this complex.

Table 10-B: Percent uncorrected pairwise distances between cytochrome *b* sequences for terminals in the *C. caramaschii* complex.

	Terminal	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
15	Crossodactylus_sp_CM	—																
16	Crossodactylus_sp_PG	0.3	—															
17	Crossodactylus_sp_Pir	1.9	1.6	—														
18	C_caramaschii_BNova	1.1	0.8	1.3	—													
19	C_caramaschii_PEJ1	11.2	11	11.7	11.7	—												
20	C_caramaschii_PEJ2	11.2	11	11.7	11.7	0	—											
21	C_caramaschii_PEJ3	11.5	11.2	12	12	0.8	0.8	—										
22	C_caramaschii_PEJ4	11.5	11.2	12	12	0.8	0.8	0.6	—									
23	C_caramaschii_PEJ5	11.2	11	11.7	11.7	0.6	0.6	0.3	0.3	—								
24	Crossodactylus_sp_RibGr2	10.7	10.4	10.2	10.2	3.4	3.4	3.7	3.2	3.4	—							
25	Crossodactylus_sp_RibGr3	11	10.7	10.4	10.4	3.2	3.2	3.4	2.9	3.2	0.3	—						
26	C_cf_caramaschii_Int1	12.3	12	11.2	11.7	3.7	3.7	3.9	3.4	3.7	2.6	2.4	—					
27	C_cf_caramaschii_Int2	12.3	12	11.2	11.7	3.7	3.7	3.9	3.4	3.7	2.6	2.4	0	—				
28	C_caramaschii_PETAR	10.7	10.4	10.2	10.2	3.2	3.2	3.4	2.9	3.2	2.1	1.9	1.6	1.6	—			
29	Crossodactylus_sp_PETAR1	10.7	10.4	10.2	10.2	3.2	3.2	3.4	2.9	3.2	2.1	1.9	1.6	1.6	0	—		
30	Crossodactylus_sp_PETAR2	10.4	10.2	9.9	9.9	3.2	3.2	3.4	2.9	3.2	2.6	2.4	2.1	2.1	0.6	0.6	—	
31	Crossodactylus_sp_PETAR3	10.4	10.2	9.9	9.9	3.2	3.2	3.4	2.9	3.2	2.6	2.4	2.1	2.1	0.6	0.6	0	—

Table 11: Putative undescribed species within species complexes.

Complex	Putative Species	Occurrence (Municipality — State)
C. gaudichaudii	"*C. gaudichaudii*"	Casimiro de Abreu, Maricá, Saquarema — Rio de Janeiro
	Crossodactylus sp.	
	"*C. gaudichaudii*"	Ilha Grande — Rio de Janeiro
ES/BA	*Crossodactylus* sp. 1	Cariacica — Espírito Santo
	Crossodactylus sp. 2	Muniz Freire — Espírito Santo
	Crossodactylus sp. 3	Santa Teresa — Espírito Santo
	Crossodactylus sp. 4	Jussari — Bahia
	Crossodactylus sp. 5	Arataca, Camacan, Fazenda Unacau, Santa Luzia — Bahia
C. bokermanni	*Crossodactylus* sp. 6	Catas Altas — Minas Gerais
C. schmidti	"*C. caramaschii*"	São Bento do Sul — Santa Catarina
	Crossodactylus sp. 7	Apucaraninha, Ortigueira, Ourinhos, Pinhalão, Wenceslau Brás — Paraná
C. caramaschii	"*C. caramaschii*"	Sete Barras — São Paulo
	"*C. caramaschii*"	Itanhaém — São Paulo
	"*C. caramaschii*"	Caucaia do Alto, Juquitiba, Piedade, Ribeirão Grande — São Paulo
	Crossodactylus sp. 8	
	"*C. caramaschii*"	Balsa Nova, Campo Magro, Piraquara, Ponta Grossa — Paraná
	Crossodactylus sp. 9	

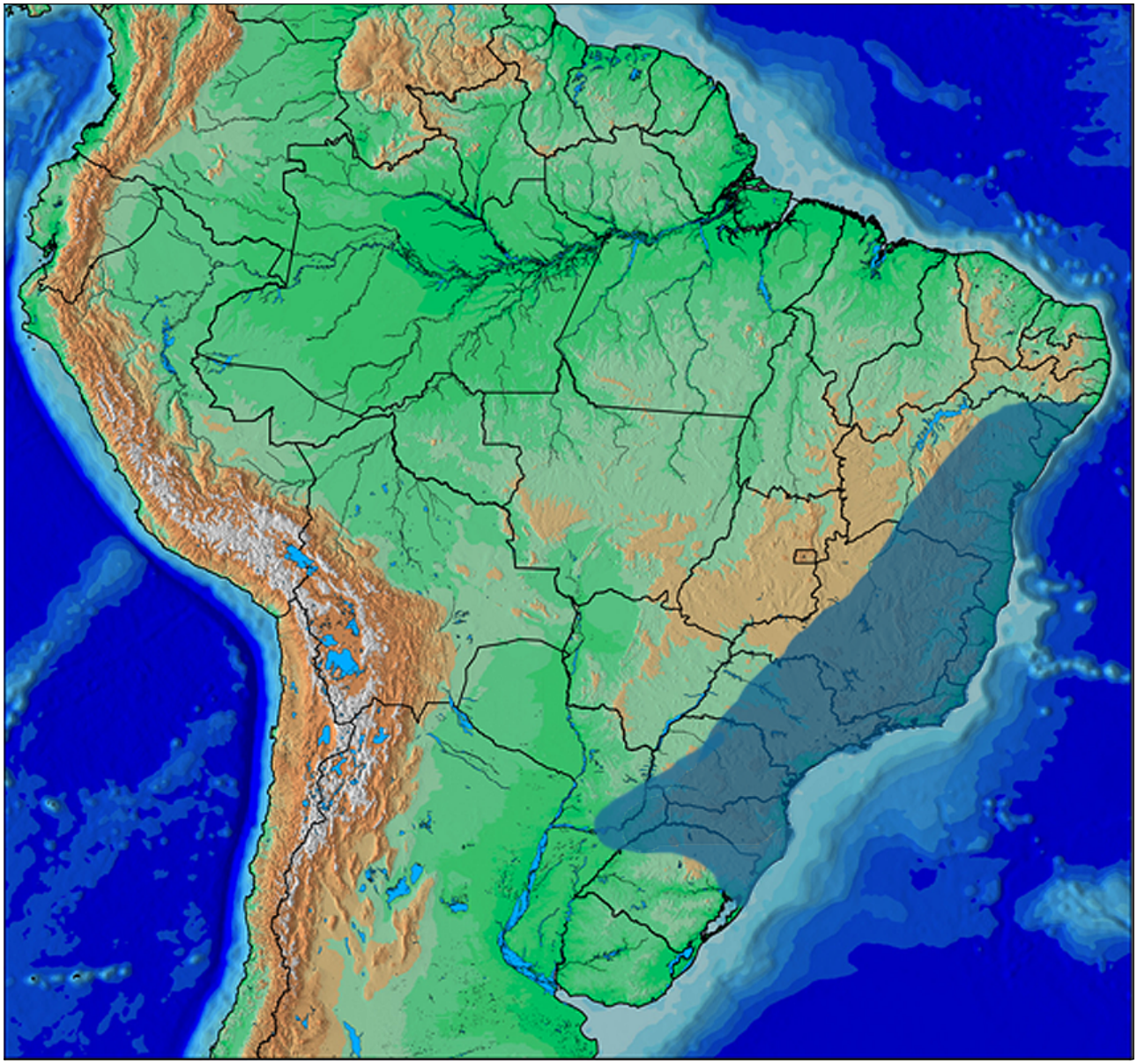


Figure 1: Distribution map for Hylodidae, from northern Argentina, through southern Paraguay and Brazil, in Rio Grande do Sul, to northeastern Brazil in Alagoas.

Figure 2: *Crossodactylus* sp. Photo by A. Giaretta.

Figure 3: *Hylodes meridionalis*, metamorphosing individual. Photo by T. Grant.

Figure 4: *Megaelosia goeldii*. Photo by M. Teixeira Jr.

Figure 5: Map of collection localities for *Crossodactylus* specimens analyzed in this study. Dots mark localities where samples were collected, stars mark type-localities. Species listed next to a star were not sampled.

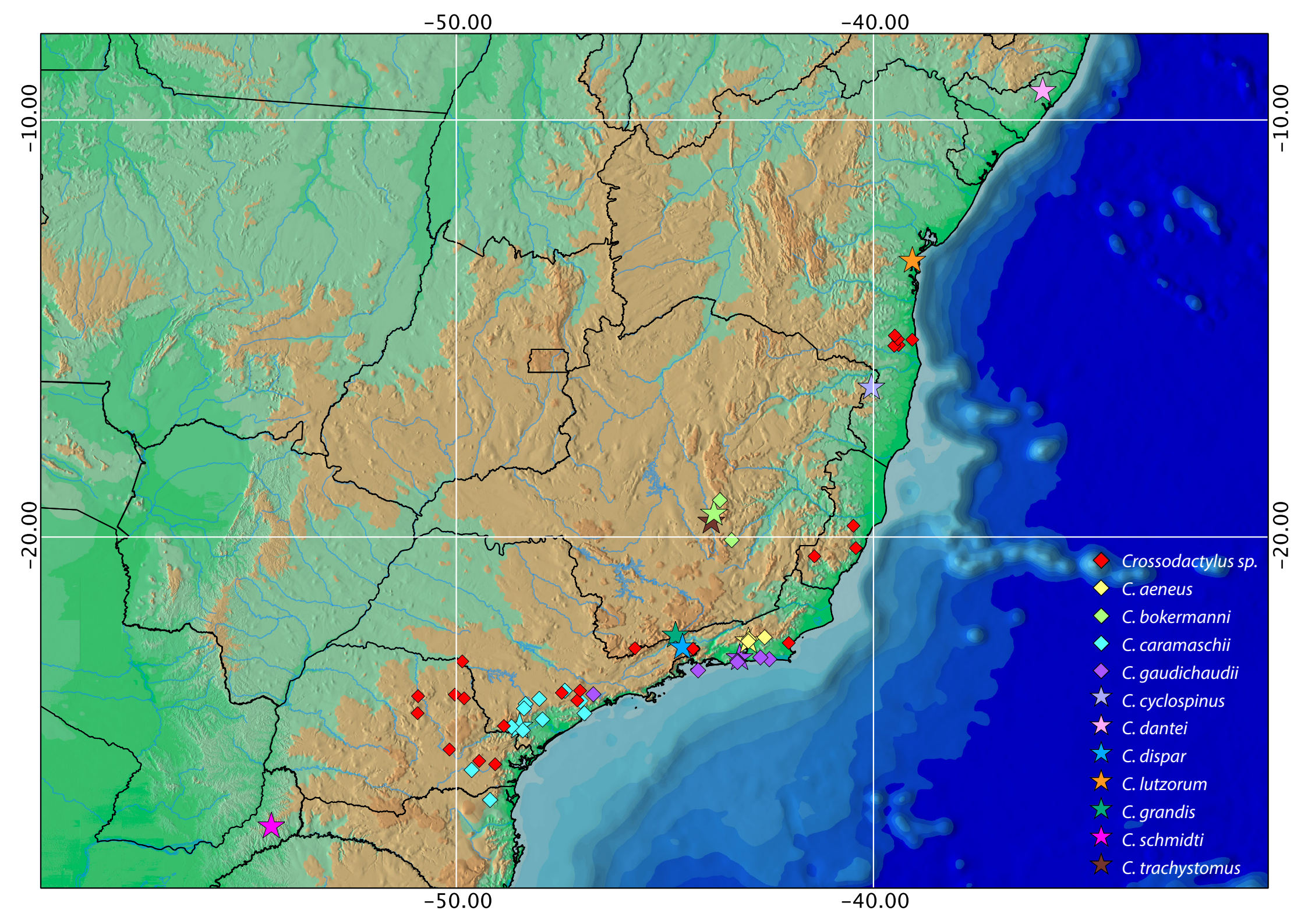

-50.00
-40.00
-10.00
-20.00
Crossodactylus sp.
C. aeneus
C. bokermanni
C. caramaschii
C. gaudichaudii
C. cyclospinus
C. dantei
C. dispar
C. lutzorum
C. grandis
C. schmidti
C. trachystomus

Figure 6-A: Map of collection localities for *Hylodes* specimens analyzed in this study. Dots mark localities where samples were collected, stars mark type-localities. Species listed next to a star were not sampled.

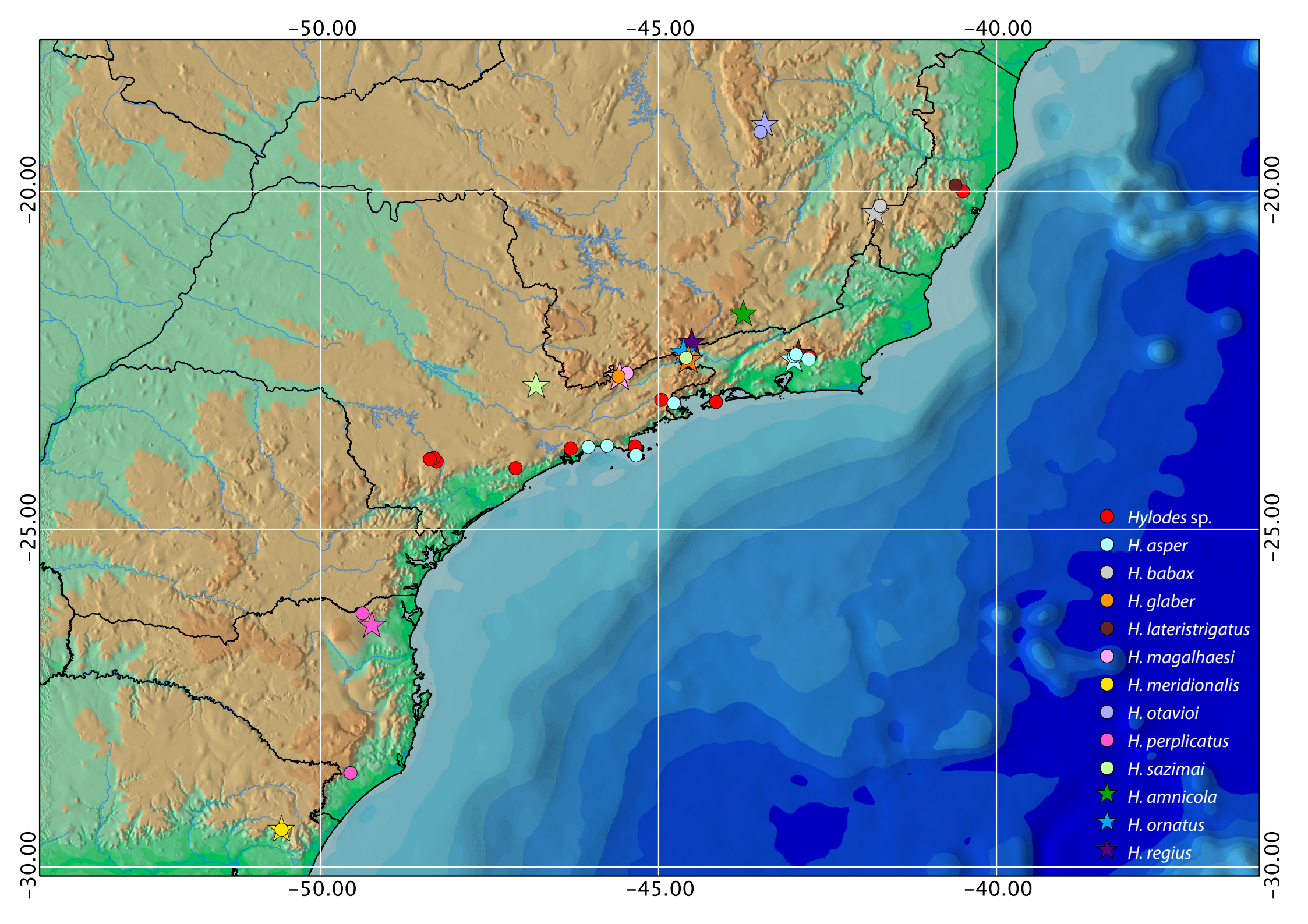

-50.00
-45.00
-40.00
-20.00
-25.00
-30.00
Hylodes sp.
H. asper
H. babax
H. glaber
H. lateristrigatus
H. magalhaesi
H. meridionalis
H. otavioi
H. perplicatus
H. sazimai
H. amnicola
H. ornatus
H. regius

Figure 6-B: Map of collection localities for *Hylodes* specimens analyzed in this study. Dots mark localities where samples were collected, stars mark type-localities. Species listed next to a star were not sampled.

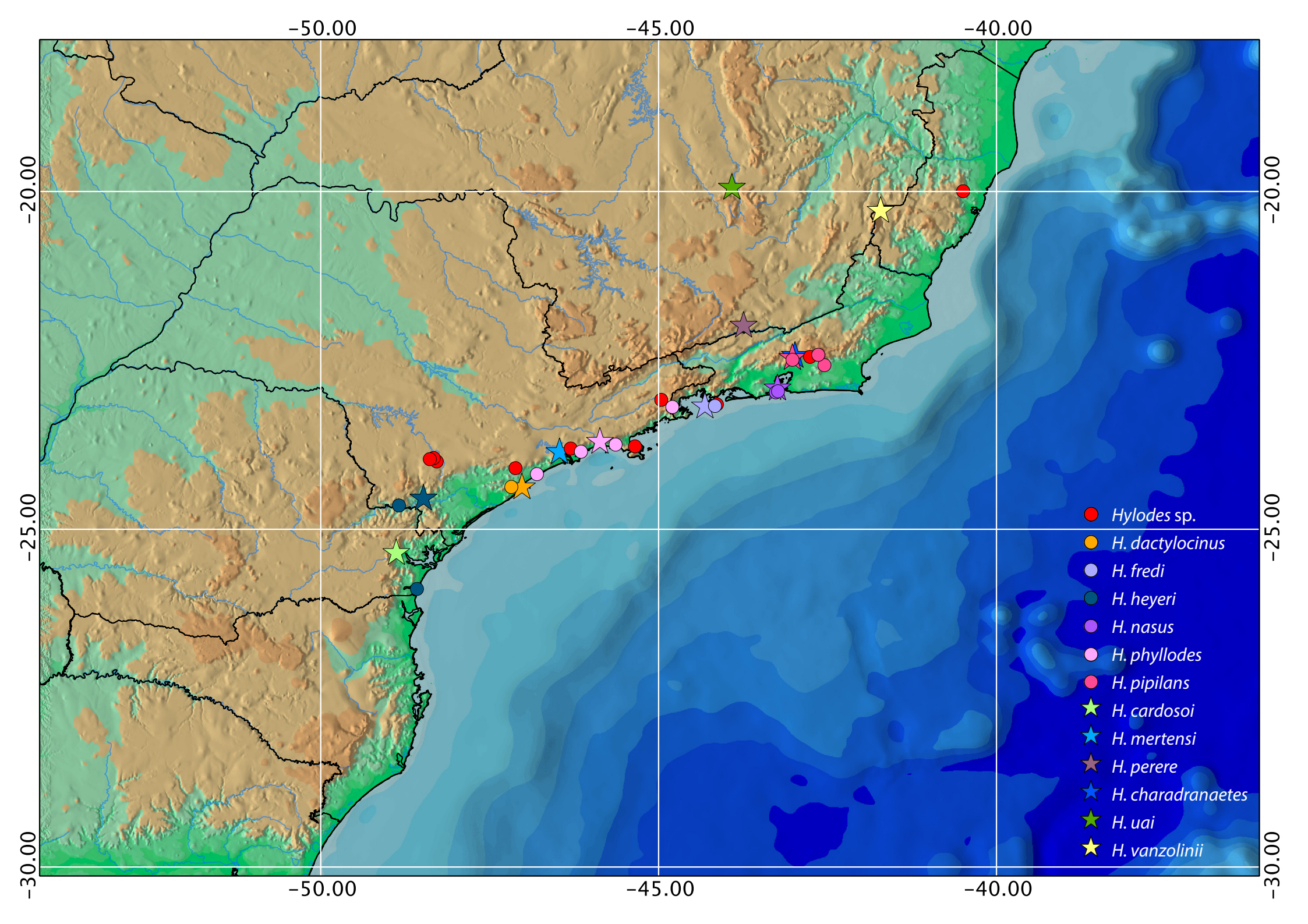
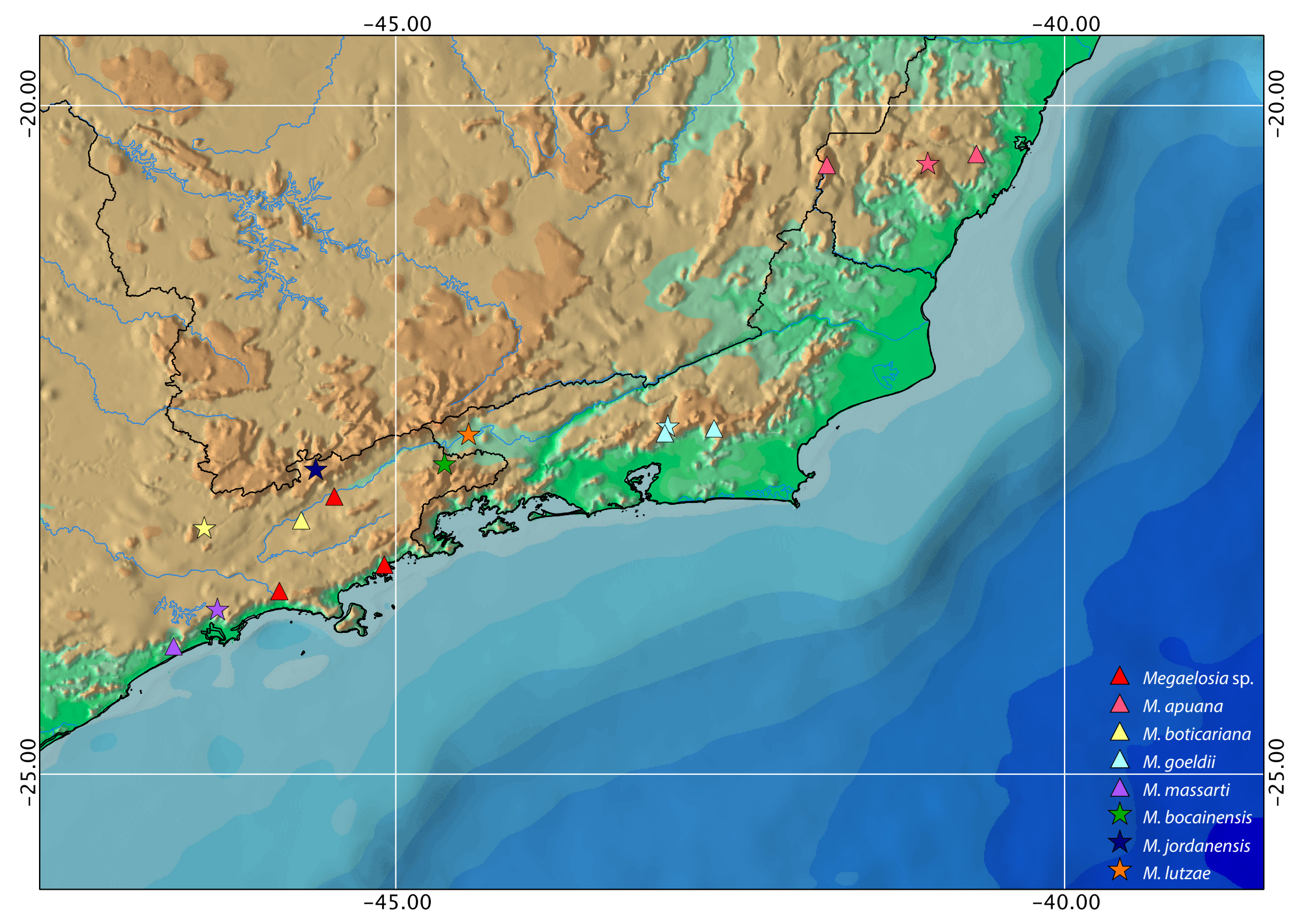

-50.00
-45.00
-40.00
-20.00
-25.00
-30.00
Hylodes sp.
H. dactylocinus
H. fredi
H. heyeri
H. nasus
H. phyllodes
H. pipilans
H. cardosoi
H. mertensi
H. perere
H. charadranaetes
H. uai
H. vanzolinii

Figure 7: Map of collection localities for *Megaelosia* specimens analyzed in this study. Dots mark localities where samples were collected, stars mark type-localities. Species listed next to a star were not sampled.


-45.00
-40.00
-20.00
-25.00
Megaelosia sp.
M. apuana
M. boticariana
M. goeldii
M. massarti
M. bocainensis
M. jordanensis
M. lutzae

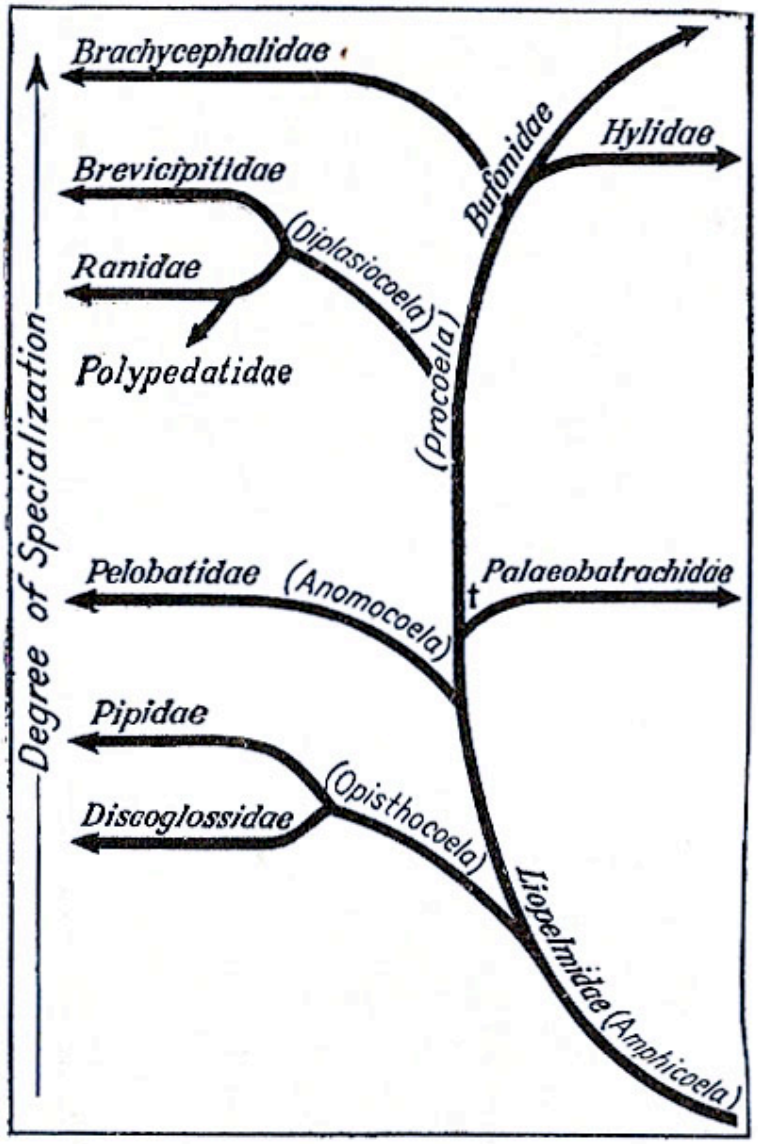


Figure 8: Phylogeny of Salientia according to Noble (1931, Figure 153). Elosiinae were included in Brachycephalidae.

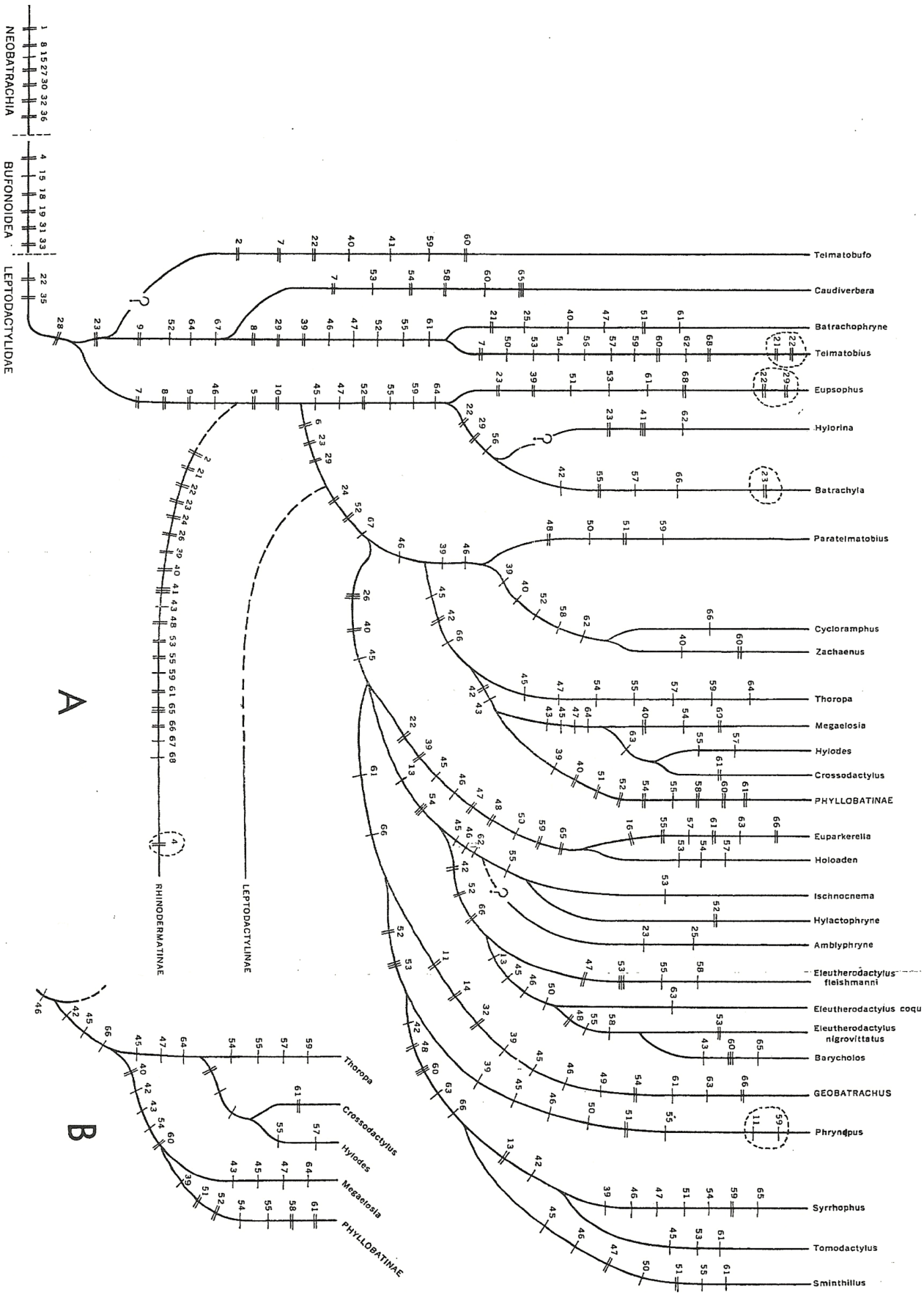


Figure 9: Ardila-Robayo's (1979) two most parsimonious trees (the second simplified in B) , showing (A) *Megaelosia* + (*Crossodactylus* + *Hylodes*) as sister-group to Phyllobatinae and *Thoropa* as sister-group to that clade, and (B) *Thoropa* + (*Crossodactylus* + *Hylodes*) as sister-group to *Megaelosia* + Phyllobatinae.

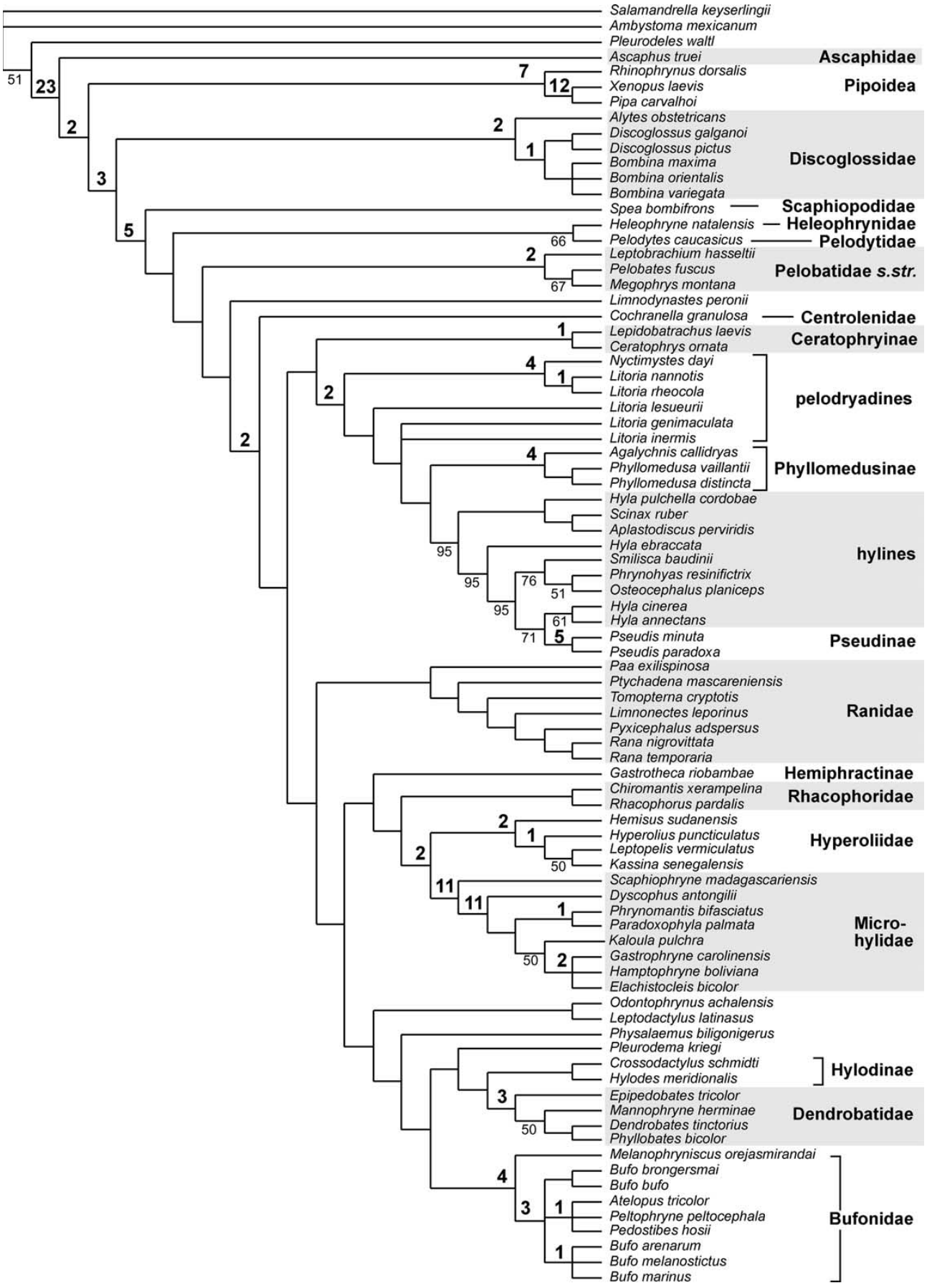


Figure 10: Majority rule consensus tree of Haas (2003), showing *Crossodactylus schmidti* and *Hylodes meridionalis* to form a clade, the sister of which consisted of Dendrobatidae.

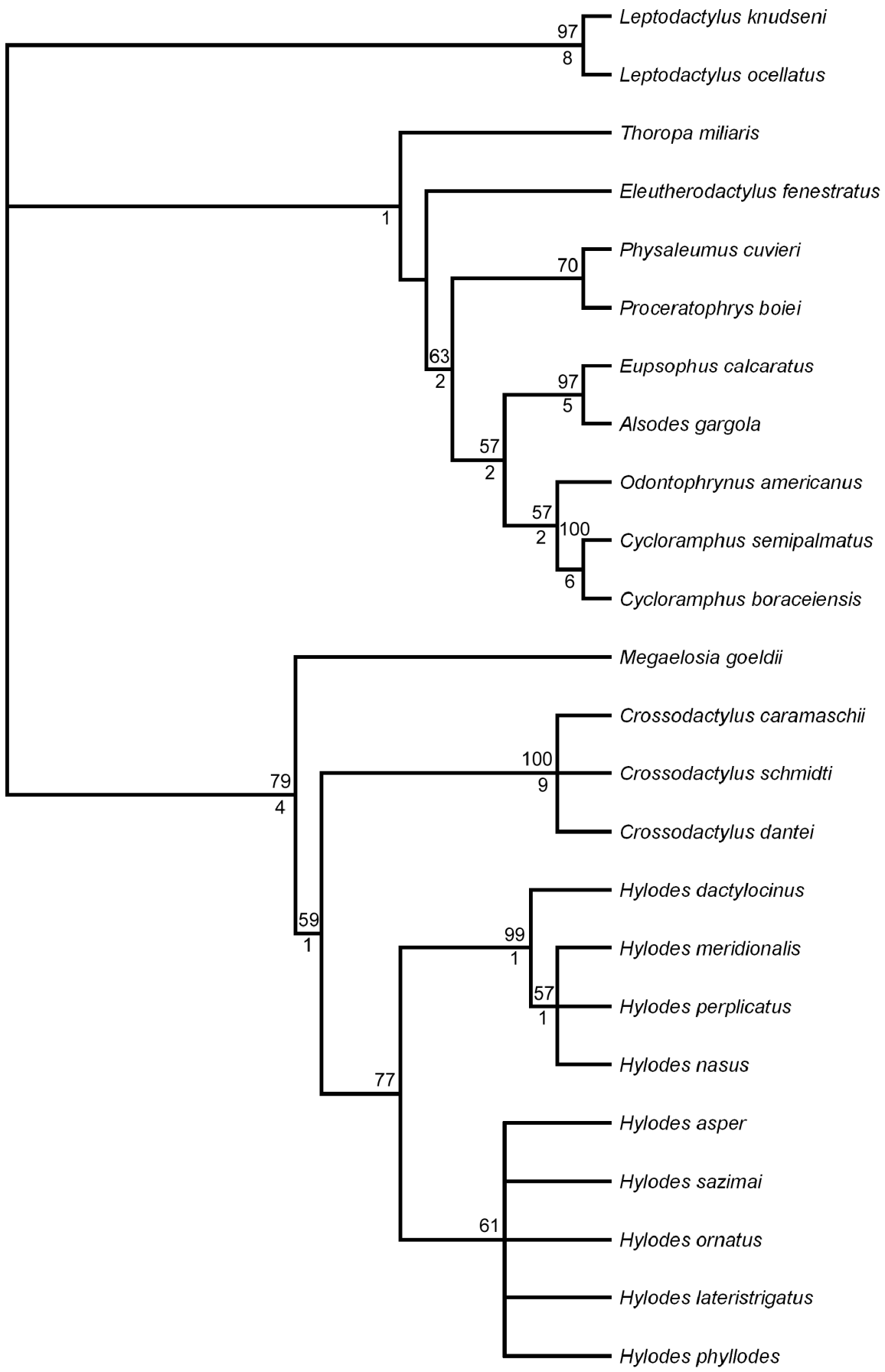


Figure 11: Strict consensus tree of Nuin & do Val (2005), showing *Megaelosia* as sistergroup to *Crossodactylus* + *Hylodes*, and unresolved relationships of the outgroup taxa.

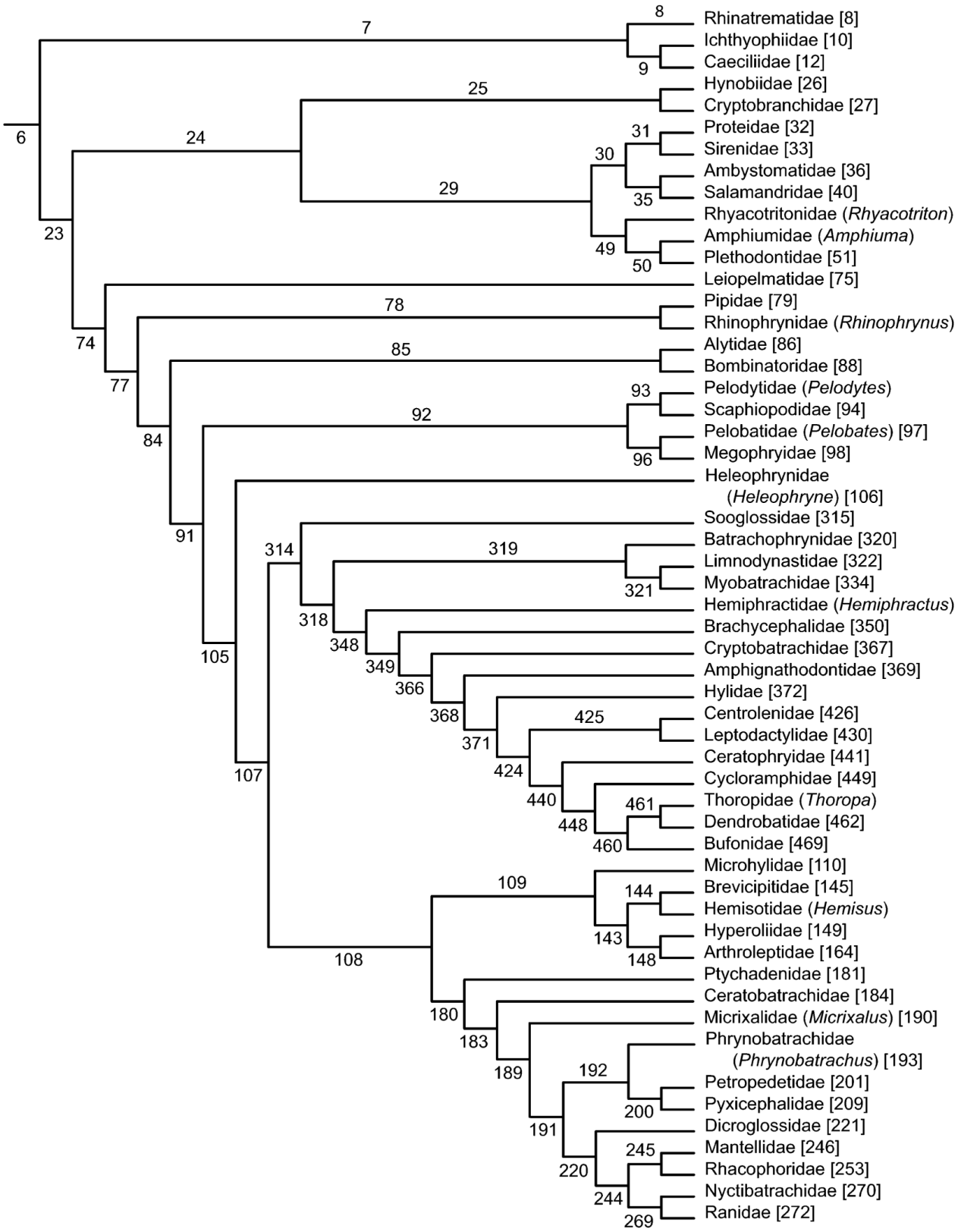


Figure 12: Simplified tree showing only families of Frost *et al.* (2006). Hylodids were recovered as a subfamily in Cycloramphidae, which was sister-group to Bufonidae + (Dendrobatidae + *Thoropa*).

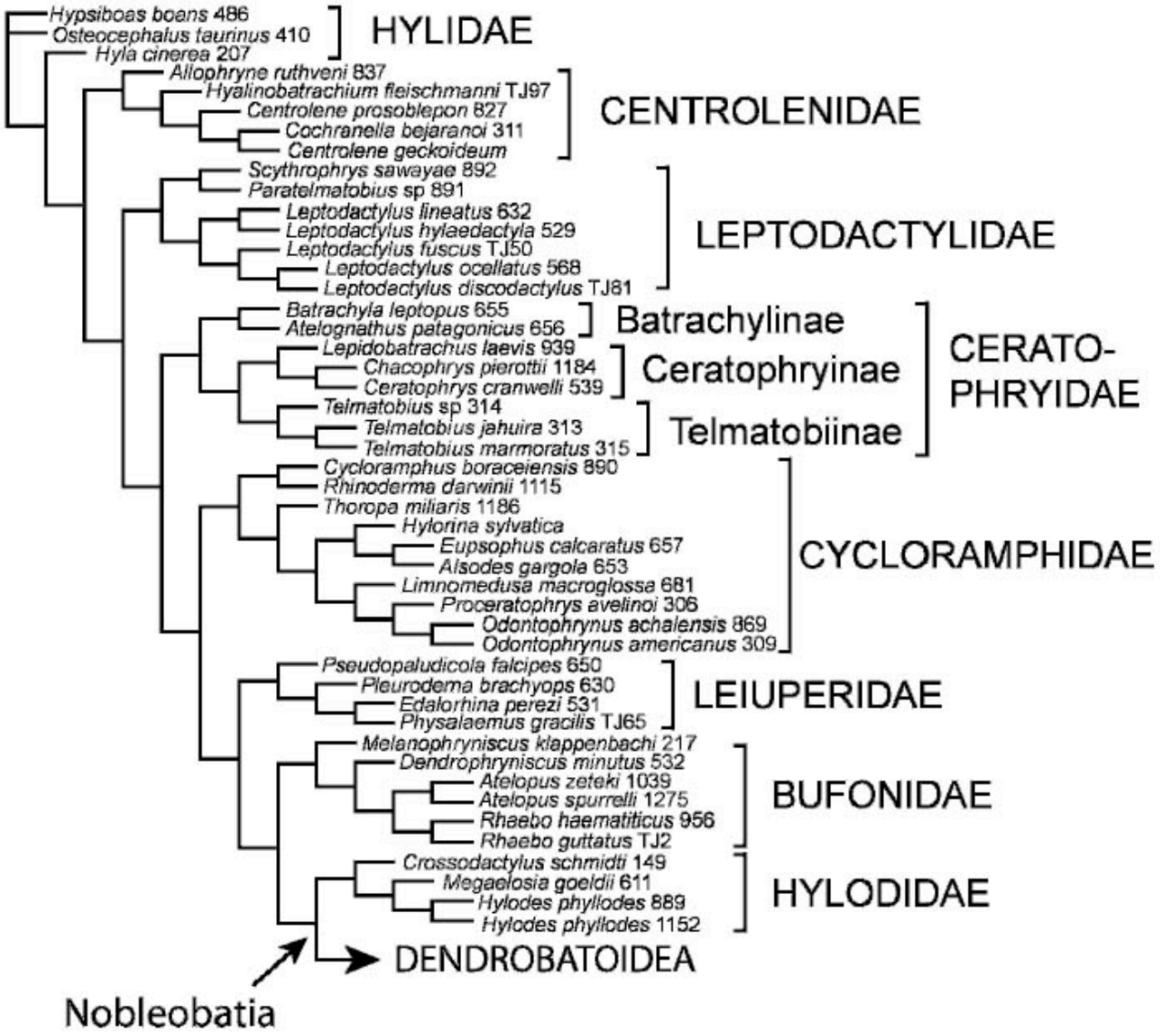


Figure 13: Strict consensus tree of Grant *et al.* (2006), recovering Hylodidae as a monophyletic group, sister to Dendrobatoidea.

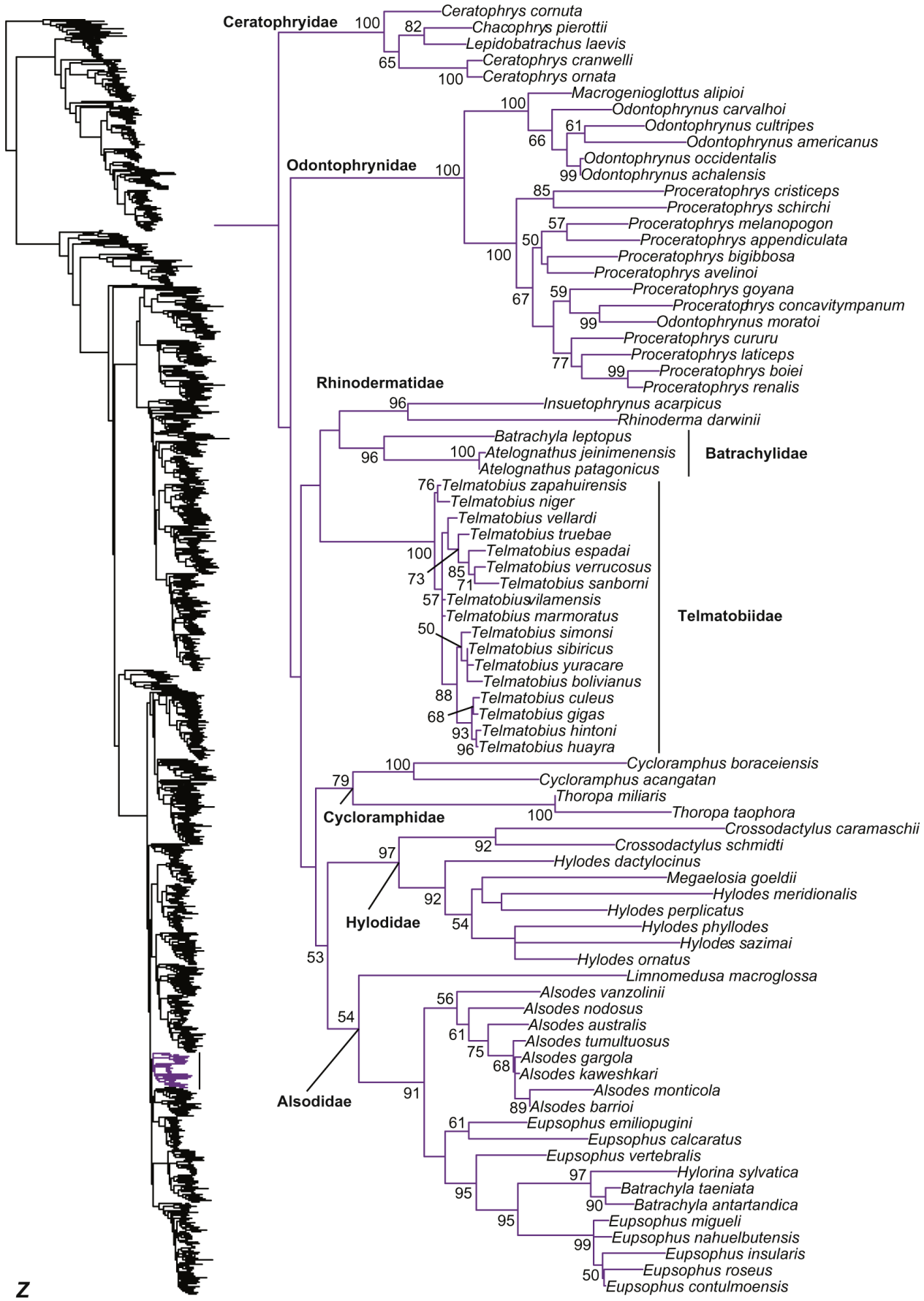


Figure 14: Maximum-likelihood tree of Pyron & Wiens (2011), showing a monophyletic Hylodidae embedded in paraphyletic Ceratophryidae and Cycloramphidae.

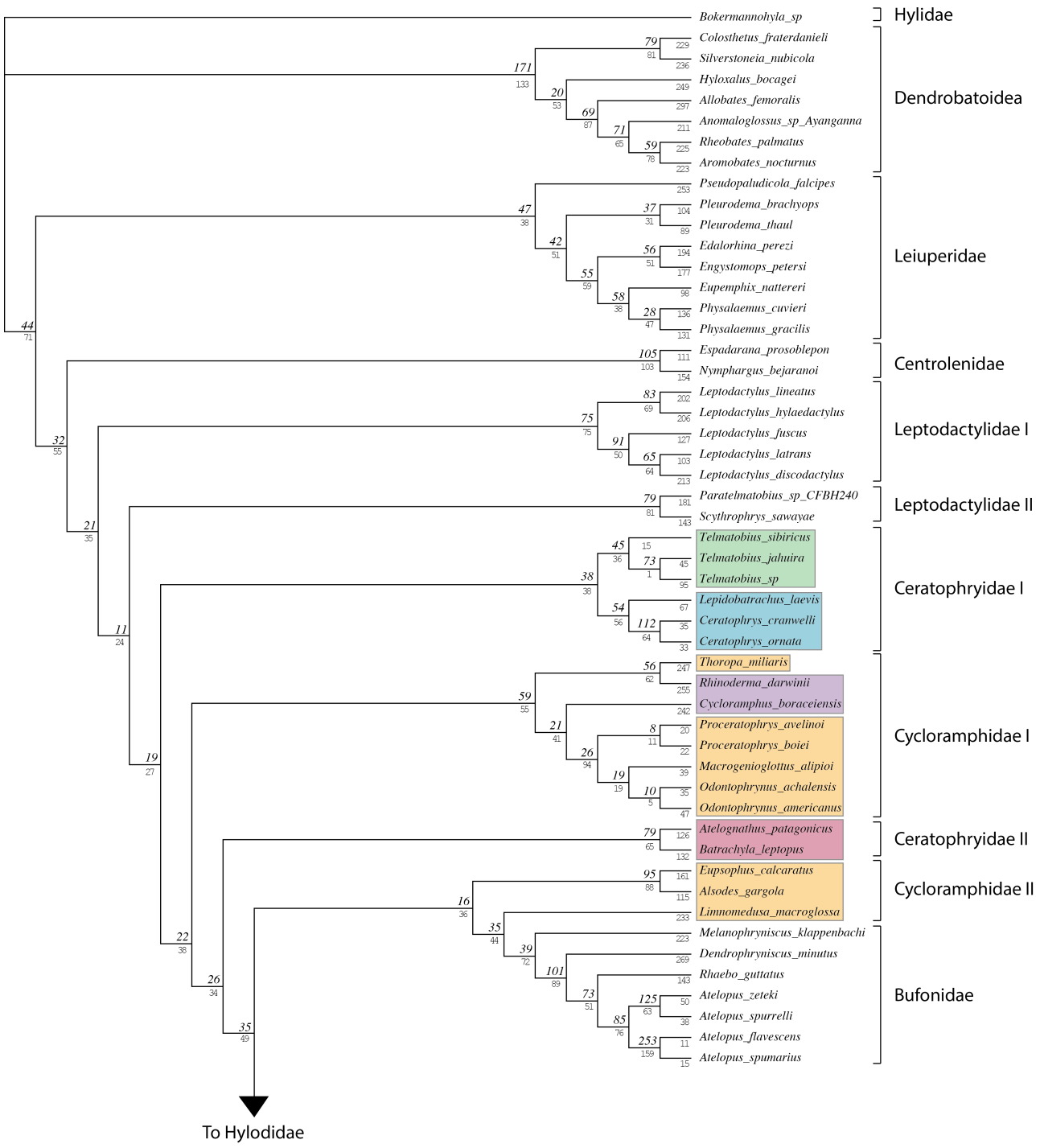


Figure 15: Strict consensus tree of 14 equally most parsimonious trees of 25,508 steps showing outgroup relationships outside Hylodidae. Values above nodes denote Goodman-Bremer support, values below nodes denote branch lengths. Color coding as follows: Green = Telmatobiinae, Blue = Ceratophryinae, Orange = Alsodinae, Purple = Cycloramphinae, Red = Batrachylinae.

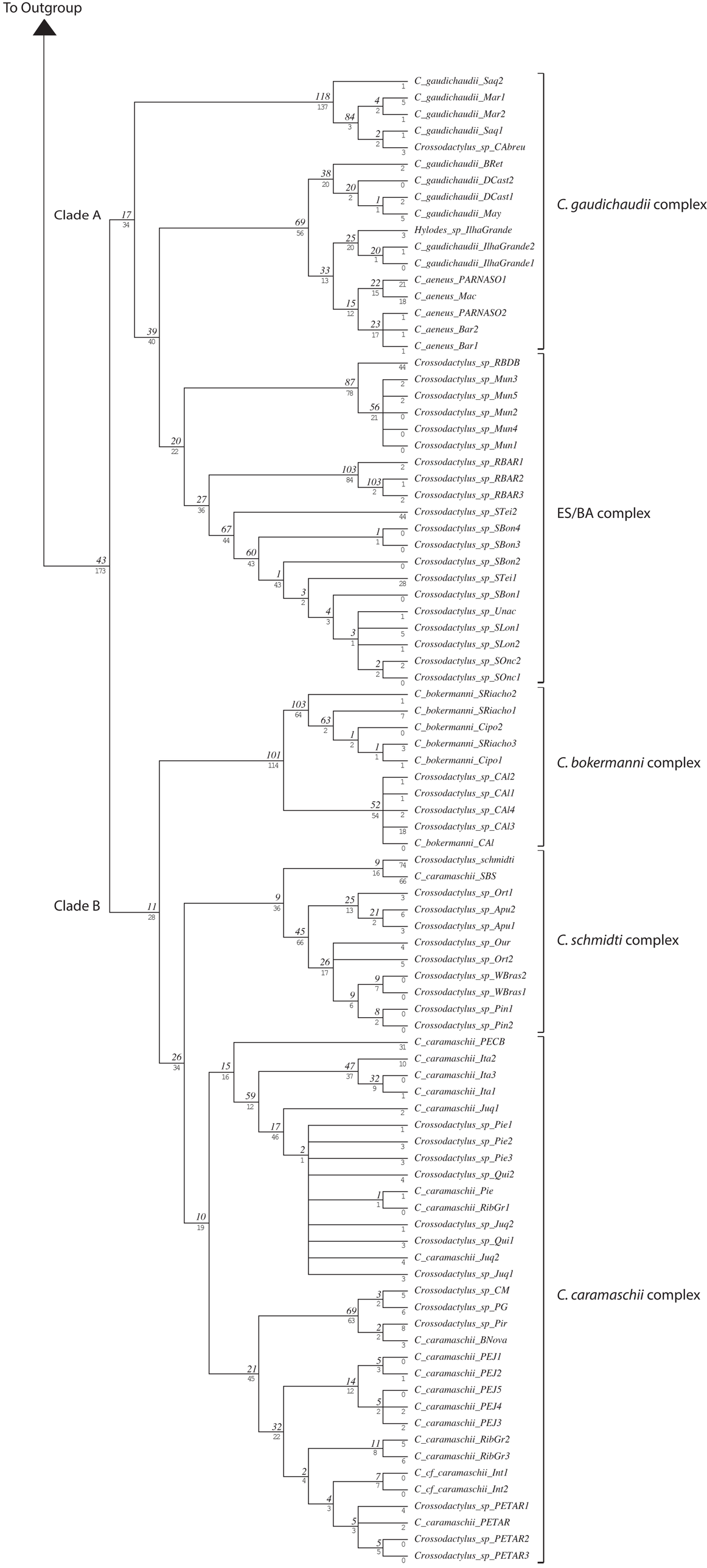
Figure 16: Strict consensus tree of 14 equally most parsimonious trees of 25,508 steps showing outgroup relationships within Hylodidae. Values above nodes denote Goodman-Bremer support, values below nodes denote branch lengths. *Megaelosia* was recovered as paraphyletic with respect to *Hylodes*, which is monophyletic.

To Outgroup

M_apuana_Dom1
M_apuana_Dom2
M_apuana_PCap
Megaelosia_sp_Pind
M_massarti
M_boticariana_Cac1
M_boticariana_Cac2
Megaelosia_sp_Uba
Megaelosia_sp_Bora1
Megaelosia_sp_Bora2
M_goeldii_Mac
Megaelosia_sp_PARNASO
Megaelosia_goeldii
H_cf_charadranaetes_Mac
Hylodes_sp_Juq
H_nasus_DCast
H_nasus_RJHorto
H_gr_asper_Jur
H_dactylocinus_Jur1
H_dactylocinus_Jur2
H_asper_Mac
H_asper_Ter
H_asper_PARNASO
Hylodes_sp_Cunha
H_asper_Boc
H_asper_IlhaBela
Hylodes_sp_IlhaBela
H_asper_Bert
H_asper_BUna
H_fredi
H_pipilans_Mac
H_pipilans_PARNASO
H_phyllodes_Ita1
H_phyllodes_Ita2
H_phyllodes_Ita3
C_gaudichaudii_SAndre
H_phyllodes_Boc
Hylodes_phyllodes2
H_phyllodes_BUna
H_phyllodes_Bert
Hylodes_phyllodes
H_glaber_Camp
H_sazimai_PARNAI2
H_sazimai_PARNAI1
H_magalhaesi_Camp4
H_magalhaesi_Camp3
H_magalhaesi_Camp2
H_magalhaesi_Camp1
H_otavioi
H_aff_lateristrigatus_STer
H_lateristrigatus
H_babax
H_perplicatus_SBS2
Hylodes_meridionalis
H_perplicatus_SBS3
H_perplicatus_SBS1
H_heyeri_PETAR
H_heyeri_Gua2
H_heyeri_Gua1
H_gr_lateristrigatus_Int1
Hylodes_sp_Int
H_gr_lateristrigatus_Int2

To *Crossodactylus*

Figure 17: Strict consensus tree of 14 equally most parsimonious trees of 25,508 steps showing ingroup relationships. Values above nodes denote Goodman-Bremer support, values below nodes denote branch lengths. *Crossodactylus* was recovered as a monophyletic group and was basally divided in two large clades, A and B.


To Outgroup
Clade A
Clade B
C_gaudichaudii_Saq2
C_gaudichaudii_Mar1
C_gaudichaudii_Mar2
C_gaudichaudii_Saq1
Crossodactylus_sp_CAbreu
C_gaudichaudii_BRet
C_gaudichaudii_DCast2
C_gaudichaudii_DCast1
C_gaudichaudii_May
Hylodes_sp_IlhaGrande
C_gaudichaudii_IlhaGrande2
C_gaudichaudii_IlhaGrande1
C_aeneus_PARNASO1
C_aeneus_Mac
C_aeneus_PARNASO2
C_aeneus_Bar2
C_aeneus_Bar1
C. gaudichaudii complex
Crossodactylus_sp_RBDB
Crossodactylus_sp_Mun3
Crossodactylus_sp_Mun5
Crossodactylus_sp_Mun2
Crossodactylus_sp_Mun4
Crossodactylus_sp_Mun1
Crossodactylus_sp_RBAR1
Crossodactylus_sp_RBAR2
Crossodactylus_sp_RBAR3
Crossodactylus_sp_STei2
Crossodactylus_sp_SBon4
Crossodactylus_sp_SBon3
Crossodactylus_sp_SBon2
Crossodactylus_sp_STei1
Crossodactylus_sp_SBon1
Crossodactylus_sp_Unac
Crossodactylus_sp_SLon1
Crossodactylus_sp_SLon2
Crossodactylus_sp_SOnc2
Crossodactylus_sp_SOnc1
ES/BA complex
C_bokermanni_SRiacho2
C_bokermanni_SRiacho1
C_bokermanni_Cipo2
C_bokermanni_SRiacho3
C_bokermanni_Cipo1
Crossodactylus_sp_CAl2
Crossodactylus_sp_CAl1
Crossodactylus_sp_CAl4
Crossodactylus_sp_CAl3
C_bokermanni_CAl
C. bokermanni complex
Crossodactylus_schmidti
C_caramaschii_SBS
Crossodactylus_sp_Ort1
Crossodactylus_sp_Apu2
Crossodactylus_sp_Apu1
Crossodactylus_sp_Our
Crossodactylus_sp_Ort2
Crossodactylus_sp_WBras2
Crossodactylus_sp_WBras1
Crossodactylus_sp_Pin1
Crossodactylus_sp_Pin2
C. schmidti complex
C_caramaschii_PECB
C_caramaschii_Ita2
C_caramaschii_Ita3
C_caramaschii_Ita1
C_caramaschii_Juq1
Crossodactylus_sp_Pie1
Crossodactylus_sp_Pie2
Crossodactylus_sp_Pie3
Crossodactylus_sp_Qui2
C_caramaschii_Pie
C_caramaschii_RibGr1
Crossodactylus_sp_Juq2
Crossodactylus_sp_Qui1
C_caramaschii_Juq2
Crossodactylus_sp_Juq1
Crossodactylus_sp_CM
Crossodactylus_sp_PG
Crossodactylus_sp_Pir
C_caramaschii_BNova
C_caramaschii_PEJ1
C_caramaschii_PEJ2
C_caramaschii_PEJ5
C_caramaschii_PEJ4
C_caramaschii_PEJ3
C_caramaschii_RibGr2
C_caramaschii_RibGr3
C_cf_caramaschii_Int1
C_cf_caramaschii_Int2
Crossodactylus_sp_PETAR1
C_caramaschii_PETAR
Crossodactylus_sp_PETAR2
Crossodactylus_sp_PETAR3
C. caramaschii complex