

# Phylogenetic analysis of the subfamily Hylodinae (Anura, Leptodactylidae) based on morphological characters

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**Abstract.** The systematics and phylogenetic relationships of the family Leptodactylidae are controversial as is the intrafamilial phylogeny of the leptodactylids. Here we analyze the relationships of the leptodactylid subfamily Hylodinae. This subfamily has been considered to be monophyletic and composed of three genera, *Hylodes*, *Crossodactylus* and *Megaelosia*. In the present study 49 characters were used, based on different studies on Leptodactylidae phylogeny. Maximum parsimony methods with unweighted and successively weighted characters were used to estimate the phylogeny of the Hylodinae. Upon analysis, the data provided further evidence of the monophyletic status of the three genera, with *Megaelosia* being the basal genus and the other two genera being sister taxa. The analysis with successive weighting results in a more resolved topology of the species subgroups of the genus *Hylodes* and separates this genus from *Crossodactylus* and confirms that the hylodines are monophyletic.

## Introduction

According to Lynch (1971), the subfamily Hylodinae Günther 1858 (Anura, Leptodactylidae) is composed of three genera of small and medium-sized [3-15 cm Snout-Vent Length, SVL] Neotropical frogs: *Hylodes*, *Crossodactylus* and *Megaelosia*. At present these genera contain 19, 10 and 6 species, respectively (Frost, 2002; Pombal et al., 2003). Frogs in all three genera are morphologically homogeneous, with *Megaelosia* being larger and having a distinct cranial morphology. The 35 described species of this group are diurnal, commonly associated with mountain streams, and their distribution is restricted primarily to the Brazilian Atlantic Rain Forest. The one exception is *Crossodactylus schmidti* which inhabits the forests of the Northeast of Argentina.

Originally, Noble (1922, 1931) considered these three genera as bufonids, a conclusion that was changed later with the inclusion of these species in the Leptodactylidae (Davis, 1936). Due to a large number of morphological similarities, the subfamily Hylodinae was consid-

ered ancestral to the Dendrobatidae, but this arrangement was later refuted by Ford (1993). Lynch (1971), in his extensive work on leptodactylids, considered *Hylodes*, *Crossodactylus* and *Megaelosia* to belong to a separate monophyletic subfamily, based mainly on results obtained from osteological and myological characters. In the most recent re-classification Frost (2002), based on data from Laurent (1986), included these genera in the subfamily Cycloramphinae, which includes six other genera.

*Hylodes* Fitzinger 1826 includes 20 described species that are mainly distinguished by their morphological characteristics. They can be classified into four different species groups: *lateristrigatus*, *mertensi*, *glaber* and *nasus* (Heyer, 1982; Heyer and Cocroft, 1986; Frost, 1985; Duellman, 1993; Glaw et al., 1998; Frost, 2002). Due to their morphological similarity, there are still many taxonomic issues to be resolved with regards to their intrageneric classification. Two groups, *mertensi* and *glaber*, include single species which are now thought to be extinct, as collection efforts have been unsuccessful for over 20 years (Heyer, pers. comm.). The species included in the *lateristrigatus* group vary in body size between small to medium SVL, have a slender body, and have a distinct white dor-

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solateral stripe. Species from the *nasus* group are slightly larger and lack the lateral stripes. All members of this genus have a distribution ranging from the North-East to the South of Brazil.

The frogs of the genus *Crossodactylus* have a distribution that is similar to that of the genus *Hylodes*, with one species in the North-East of Argentina (*Crossodactylus schmidti*, Misiones Province). In general these frogs are smaller than *Hylodes* species. These species can be divided into three different groups: *gaudichaudii*, *trachystomus* and *schmidti* (Caramaschi and Sazima, 1985) based mainly on differences in snout and *canthus rostralis* morphology. According to Lynch (1971), *Crossodactylus* is also characterised by several primitive characters (larval morphology, secondary sexual characters) and some derived ones (loss of quadratojugal).

In number of species, *Megaelosia* is the smallest genus in the group, composed of only 6 large (10-15 cm SVL) frog species endemic to the Atlantic Rain Forest of the states of São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo, South-Eastern Brazil (Giarretta et al., 1993; Pombal et al., 2003). *Megaelosia* has been considered to be the most primitive genus of the subfamily Hylodinae, as it has distinct cranial structures, increased maxilla and quadratojugal and occipital condyles (Lynch, 1971). The squamosal architecture is distinct from *Hylodes* and *Crossodactylus*, but based on external morphology of adults, *Megaelosia* has been assigned to the Hylodinae.

All previous studies treating relationships of the genera *Crossodactylus*, *Hylodes*, and *Megaelosia* have been based on very limited taxon sampling within these genera. For example, Lynch (1971) examined 3 species of *Crossodactylus*, 5 species of *Hylodes*, and 1 species of *Megaelosia*, while Heyer (1975) examined 1, 2, and 1 species respectively of these genera (of the same species Lynch examined). There are no rigorous analyses of interspecific relationships for these genera. All previous rigorous analyses

have used the genera, not the species as units of analysis. The purposes of this study are to re-evaluate the relationships among the genera based on more intensive species and character sampling and to determine whether these three genera comprise a monophyletic clade based on this expanded data set.

## Material and methods

A total of 13 hylodine and 11 other leptodactylid species were selected to be part of the analysis (table 1). Despite scarce museum collections and difficulties in collecting individuals of *Hylodes*, *Crossodactylus* and *Megaelosia*, we attempted to select species representing the diversity found in each of the Hylodinae genera. In *Hylodes*, species representing each of the subgroups were obtained, except for the *mertensi* and *glaber* groups, which appear to be extinct. *Crossodactylus* species from distant geographical regions and two distinct subgroups were also selected. A single *Megaelosia* (*M. goeldii*) was included because of difficulties in obtaining specimens. The outgroup was composed of species from the three other subfamilies of Leptodactylidae, including representatives from distinct geographic regions.

From the 49 characters analyzed (Appendix), 35 (1-35) were defined in the study of Heyer (1975). Heyer used genera as the Operational Taxonomic Units (OTU) whereas we use species as the OTUs as indicated previously. Character numbers 36 to 40 were defined in a study of *Physalaemus* osteology by Lobo (1994). The other nine characters (41-49) were adapted from Heyer (1973) in his study of the genus *Leptodactylus* (*L. marmoratus* group).

The analysis of external characters was performed, when possible, with 10 specimens of each gender. Osteology and myology were assessed from one specimen of each species. After the myological data were taken, the specimens were cleared and stained (Taylor and Van Dyke, 1985).

We carried out Maximum Parsimony (MP) analyses using PAUP\* (Swofford, 2002) and performing branch and bound searches and tree bisection reconnection branch-swapping heuristic searches. All characters were considered ordered according to Wilkinson (1992) and Campbell and Frost (1993). Two analyses were carried out, one with unweighted and one with successive weighting (Farris, 1969) with weights being applied according to the Retention Index (RI) until achieving stability in the number of steps. When more than one tree resulted from the analysis, a strict consensus tree was calculated.

Branch stability was accessed by the Bremer index (Bremer, 1988; Källersjö et al., 1992; Bremer, 1994) calculated using AutoDecay PPC version 4.01 (Eriksson, 2002). Bootstrap (Felsenstein, 1985) and jackknife (only in the unweighted analysis) (Farris et al., 1996) analyses were also performed, each with 1000 pseudoreplications.

**Table 1.** List of the leptodactylid species analyzed to obtain phylogenetic relationships.

Subfamily	Genera	species	subgroup	
Hylodinae	<i>Hylodes</i>	<i>lateristrigatus</i>	<i>lateristrigatus</i>	
		<i>asper</i>	<i>nasus</i>	
		<i>phyllodes</i>	<i>lateristrigatus</i>	
		<i>ornatus</i>	<i>lateristrigatus</i>	
		<i>sazimai</i>	<i>lateristrigatus</i>	
		<i>nasus</i>	<i>nasus</i>	
		<i>meridionalis</i>	<i>nasus</i>	
		<i>perplicatus</i>	<i>nasus</i>	
		<i>dactylocinus</i>	<i>nasus</i>	
		<i>Crossodactylus</i>	<i>caramaschii</i>	<i>gaudichaudii</i>
			<i>dantei</i>	<i>gaudichaudii</i>
			<i>schmidti</i>	<i>schmidti</i>
		<i>Megaelosia</i>	<i>goeldii</i>	–
<i>Leptodactylus</i>	<i>knudseni</i>		–	
	<i>ocellatus</i>		–	
	<i>Physalaemus</i>	<i>cuvieri</i>	–	
Telmatobiinae	<i>Alsodes</i>	<i>gargola</i>	–	
	<i>Eupsophus</i>	<i>calcaratus</i>	–	
	<i>Thoropa</i>	<i>miliaris</i>	–	
	<i>Cycloramphus</i>	<i>boraceiensis</i>	–	
	<i>Cycloramphus</i>	<i>semipalmatus</i>	–	
	Ceratothrynae	<i>Eleutherodactylus</i>	<i>fenestratus</i>	–
		<i>Proceratophrys</i>	<i>boiei</i>	–
<i>Odontophrynus</i>		<i>americanus</i>	–	

## Results

The unweighted phylogenetic analysis resulted in five equally parsimonious trees, each with 139 steps, consistency index (CI) of 0.48 (Kluge and Farris, 1969) and retention index (RI) of 0.74 (Farris, 1989) (results not shown). With application of successive weighting, two most parsimonious trees were obtained, each one with 46.2 steps and CI = 0.59 and RI = 0.82. The resulting strict consensus tree is shown in figure 1. From the 49 analyzed characters, 38 were informative under the parsimony criterion, four were constants and seven uninformative.

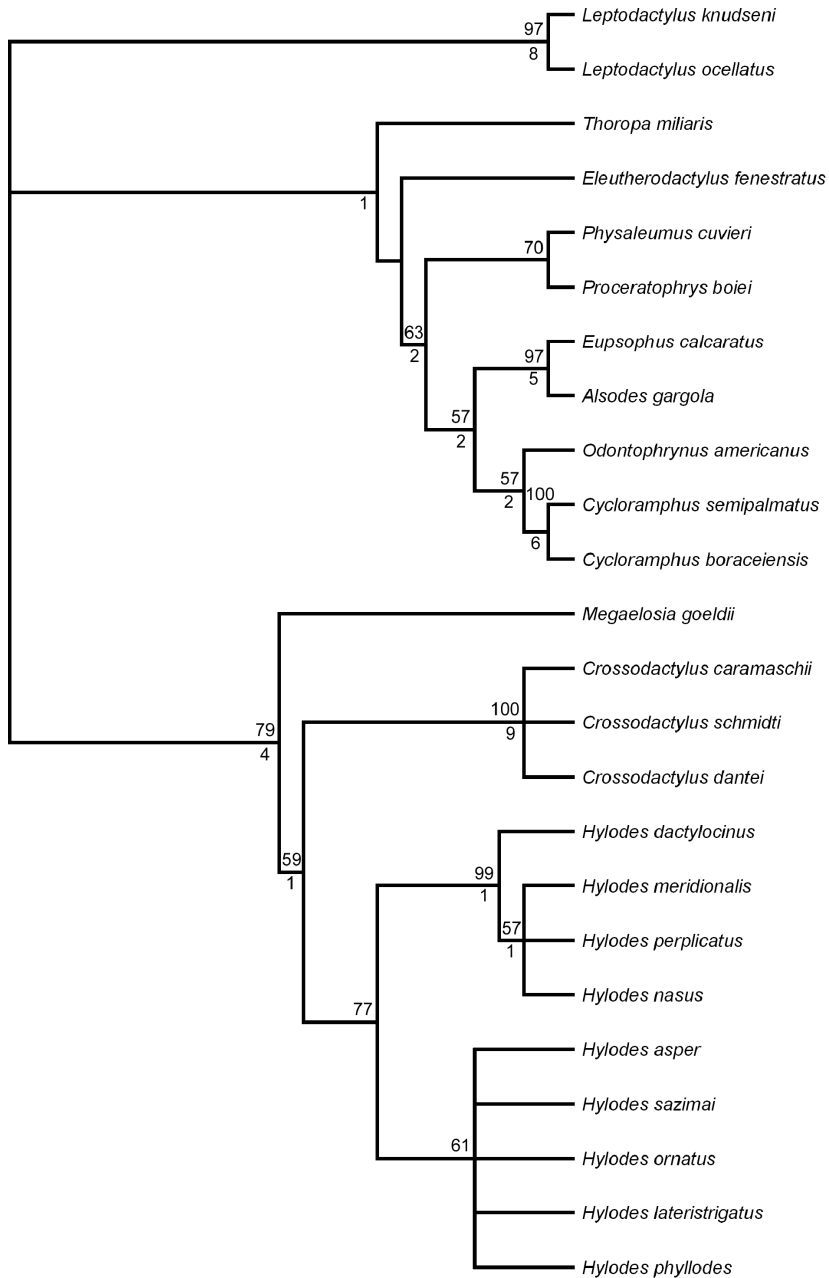
The main difference between the two consensus trees for the unweighted and weighted analyses is the better resolution of the ingroup (the three Hylodinae genera) obtained using the successive weighting method. The successive weighting consensus tree (fig. 1) shows a division among *Hylodes* subgroups (*lateristrigatus* and *nasus*) and an unresolved clade of *Crossodactylus* species on one branch. In both cases, *Megaelosia* appeared as sister-group of

the two other Hylodinae genera. Bootstrap and Bremer values obtained for both methods were similar.

The outgroup species had the same tree topology in both analyses. Species from different subfamilies were placed in the same branch. There is high bootstrap support for clades composed of the two species of *Leptodactylus* and the two species of *Cycloramphus*.

## Discussion

*Phylogeny of hylodines.* Although this study did not include all species from the subfamily Hylodinae, the results obtained are congruent with the monophyly of the three genera. This corroborates the opinion of Lynch (1971) and Heyer (1975) regarding the relationships of *Hylodes*, *Crossodactylus* and *Megaelosia*. Lynch (1971) considered this subfamily as monophyletic, having evolved from an Alsodinae stock, but this could not be substantiated in our study because of resolution problems among the out-



**Figure 1.** Consensus topology from two most parsimonious trees, obtained after successive weighting, with 46.2 steps and CI = 0.59 and RI = 0.82. Bootstrap values are shown over the branches. Bremer decay index values under the branches.

group species. This problem also made it impossible to determine the sister-group of the hylodines.

In this study, *Megaelosia* appears as a sister group of the other two genera of the ingroup, indicating that *Crossodactylus* and *Hylodes* could

have evolved from a common ancestor. Lynch (1971) suggested that *Megaelosia* was the primitive genus of the group and that it was difficult to separate from *Hylodes*. In the same study, he indicated that *Crossodactylus* was the basal genus of the subfamily Hylodinae from which

the other two genera have evolved as well as some groups of the family Dendrobatidae.

Both bootstrap and jackknife analysis resulted in good support values for the Hylodinae branch, varying from 79% to 69% with and without successive weighting respectively. *Crossodactylus* had the highest bootstrap and jackknife values with 100% support for both analyses. The *Hylodes/Crossodactylus* clade had 50% and 77% of support in both analyses and under successive weighting both genus were in separated groups.

*Characters used and evolution.* Of the 49 characters analyzed, four were constant in all taxa: pupil shape (1), musculus adductor mandibulae (10), musculus genihyoideus medialis (12) and anterior process of the hyalus (29). Another seven characters were non-informative: body glands (4), outer metatarsal tubercle (7), musculus depressor mandibulae (11), musculus petrohyoideus anterior (13), insertion of the sternohyoid (14), relation of the transverse process of the last presacral vertebrae with the sacral vertebrae (32) and dorsal crest of ilium (35).

There are only a few characters that support the interspecific relationships of the subfamily Hylodinae and the relationships of this group with other subfamilies. Our results show 32 characters with homoplasy, which might explain the problems with relationship resolutions. Only two characters (less than 5%) had unique synapomorphies for the three ingroup genera: adhesive disks with a dorsal scute (5) and tarsum with extensible fold (6). The genus *Crossodactylus* had only one autapomorphy, namely quadratojugal absent (20), and *Megaelosia* also had only one autapomorphic character, related to the squamosal (24). On the other hand, *Hylodes* showed two autapomorphies with fusion of the prootic with the frontoparietals (27) and alary process of the hyoid rudimental (30).

The low number of autapomorphies and synapomorphies reflect the difficulties of a morphological analysis of the family Leptodactylidae. The morphological homogeneity of some

groups and the lack of valid and informative morphological characters are the main obstacles in obtaining a supported phylogeny for the family and its subdivisions. A greater number of species and characters should be included in order to clarify the topologies.

Meyer's (1975) phylogenetic study was the only one to include genera from the Hylodinae, but in his work genera were used as OTUs. He suggested that the Hylodinae would be monophyletic with the genus *Thoropa* being their sister group. In this study the monophyly of the group formed by *Hylodes*, *Crossodactylus* and *Megaelosia* is supported by two synapomorphies, similar to the results of Heyer (1975).

The main difference between our study and the study by Heyer is that we used a larger number of species from the Hylodinae, but with a smaller diversity of outgroup genera. The monophyletic state of the subfamily Hylodinae seems to be well supported. Also, the use of other character sources, such as DNA and protein sequences, should help to obtain better support for their relationships.

## Conclusions

Cladistic analysis of 49 characters for 24 species strongly supports monophyly of the Subfamily Hylodinae. Thus, future studies determining relationships of the Hylodinae with other subfamilies of Leptodactylidae and the Family Dendrobatidae (Lynch, 1971 had the Dendrobatidae as a clade within the Family Leptodactylidae) will not require intensive species-level sampling of the Subfamily Hylodinae. Our results are consistent with maintaining monophyletic genera for *Crossodactylus*, *Hylodes*, and *Megaelosia*, although additional taxon sampling could overturn this conclusion. Due to the problems of gathering additional morphological data for several of the named species of the Subfamily Hylodinae, focusing on molecular data would seem to be a better approach to resolve relationships within the Hylodinae.

## Specimens analyzed

### External morphology

*Hylodes asper*: MZUSP 10148, 10151, 10153-54, 20462-64, 69848-49, 23850, 8852-54, 10012, 70471-72, 9975-82; *Hylodes phyllodes*: MZUSP 3529, 37701-03, 1708-10, 1704-06, 37573-85; *Hylodes meridionalis*: MZUSP 89938-40; *Hylodes lateristrigatus*: MZUSP 23861-64, 475, 2329, 2332, 2340, 14002, 10946, 23847-49, 53259-61, 7932; *Hylodes dactylocinus*: MZUSP 96263, 89904 (Holotype), 89912-37 (Paratype); *Hylodes nasus*: MZUSP 60965, 301, 21884-88, 13472, 23549, 23551-55, 23557; *Crossodactylus caramaschii*: MZUSP 30628 (Paratype), 21894-96; *Crossodactylus dantei*: MZUSP 76865-69; *Crossodactylus schmidtii*: MACN 37523-25; *Megaelosia goeldii*: MZUSP 27717, 1009, 2149, 895, 1479; *Thoropa miliaris*: MZUSP 53262-67, 9852-59, 9861-62, 9864, 9868, 9870-71, 57955-63; *Physalaemus cuvieri*: MZUSP 7152-64, 4643-47; *Eupsophus calcaratus*: MZUSP 94378-79; *Alsodes gargola*: Still not included in the collection, private collection number 165-66; *Cycloramphus semipalmatus*: MZUSP 23766-779, 9001-4, 37766, 37563; *Cycloramphus boraceiensis*: MZUSP 95878; *Leptodactylus knudseni*: MZUSP 16658-668; *Leptodactylus ocellatus*: MZUSP 96266; *Proceratophrys boiei*: MZUSP 23381, 60955-56, 60953, 9587, 26858, 23384, 31353, 35649-653, 14231; *Eleutherodactylus fenestratus*: MZUSP 61961-61997.

### Internal morphology

*Hylodes lateristrigatus* MZUSP 10230; *Hylodes asper* MZUSP 37664; *Hylodes phyllodes* MZUSP 94381; *Hylodes ornatus* MZUSP 96195; *Hylodes sazimai* MZUSP 96264; *Hylodes nasus* MZUSP 20869; *Hylodes meridionalis* MZUSP 89940; *Hylodes perplicatus* MZUSP 96196; *Hylodes dactylocinus* MZUSP 96263; *Crossodactylus caramaschii* MZUSP 88001; *Crossodactylus dantei* MZUSP 76867; *Crossodactylus schmidtii* MACN 37524; *Megaelosia goeldii* MZUSP 53333; *Leptodactylus knudseni* MZUSP 16660; *Leptodactylus ocellatus* MZUSP 96266; *Physalaemus cuvieri* MZUSP 71577; *Alsodes gargola* \* PASN I65; *Eupsophus calcaratus* MZUSP 94379; *Thoropa miliaris* MZUSP 53623; *Cycloramphus boraceiensis* MZUSP 95878; *Cycloramphus semipalmatus* MZUSP 23767; *Eleutherodactylus fenestratus* MZUSP 61980; *Proceratophrys boiei* MZUSP 35031; *Odontophrynus americanus* MZUSP 96198; \*still not included in MZUSP collection.

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## References

- Bremer, K. (1988): The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795-803.
- Bremer, K. (1994): Branch support and tree stability. *Cladistics* **10**: 295-304.
- Campbell, J.A., Frost, D.R. (1993): Anguid lizards of the genus *Abronia*: revisionary notes, descriptions of four new species, a phylogenetics analysis, and key. *Bull. Amer. Mus. Nat. Hist.* **216**: 1-121.
- Caramaschi, U., Sazima, I. (1985): Uma nova espécie de *Crossodactylus* da Serra do Cipó, Minas Gerais, Brasil (Amphibia: Leptodactylidae). *Rev. Bras. Zool* **3**: 43-49.
- Davis, D.D. (1936): The distribution of bidder's organ in the Bufonidae. *Zool. Series Fields Mus. Nat. Hist.* **20**(15): 115-125.
- Duellman, W.E. (1993): Amphibian species of the world: additions and corrections. *Univ. Kansas Mus. Nat. Hist. Sp. Publ.* **21**: 1-372.
- Eriksson, T. (2002): AutoDecay 5.03. Available at <http://www.bergianska.se/personal/TorstenE>
- Farris, J.S. (1969): A successive approximations approach to character weighting. *Syst. Zool.* **18**: 374-385.
- Farris, J.S. (1989): The retention index and the rescaled consistency index. *Cladistics* **5**: 417-419.
- Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G. (1996): Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12**: 99-124.
- Felsenstein, J. (1985): Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783-791.
- Ford, L.S. (1993): The phylogenetic position of the dart-poison frogs (Dendrobatidae) among anurans: an examination of the competing hypotheses and their characters. *Ethol. Ecol. Evol.* **5**: 219-231.
- Frost, D.R. (1985): Amphibian species of the world. Lawrence: Allen Press, 732 pp.
- Frost, D.R. (2002): Amphibian Species of the World: an online reference. V2.21. Electronic database available at <http://research.amnh.org/herpetology/amphibia/index.html>.
- Giaretta, A.A., Bokermann, W.C.A., Haddad, C.F.B. (1993): A review of the genus *Megaelosia* (Anura, Leptodactylidae) with a description of a new species. *J. Herpet.* **27**: 276-285.
- Glaw, F., Köhler, J., Lötters, S., Vences, M. (1998): Vorläufige Liste und Bibliographie neubeschriebener Amphibienarten und -unterarten von 1993 bis 1997. *Elaphe* **6**: 41-72.
- Heyer, W.R. (1973): Relationships of the *marmoratus* species group (Amphibia, Leptodactylidae) within the subfamily Leptodactylinae. *Contr. Sci. Nat. Hist. Mus. LA County* **253**: 1-45.
- Heyer, W.R. (1975): Preliminary analysis of the intergeneric relationships of the frog family Leptodactylidae. *Smithsonian Contrib. Zool.* **199**: 1-55.
- Heyer, W.R. (1982): Two new species of the frog genus *Hylodes* from Caparaó, Minas Gerais, Brasil (Amphibia: Leptodactylidae). *Proc. Biol. Soc. Washington* **95**: 377-385.

- Heyer, W.R., Cocroft, R.B. (1986): Descriptions of two new species of *Hylodes* from the Atlantic Rain Forests of Brazil (Amphibia, Leptodactylidae). *Proc. Biol. Soc. Washington* **99**: 100-109.
- Källersjö, M., Farris, J.S., Kluge, A.G., Bult, C. (1992): Skewness and permutation. *Cladistics* **8**: 275-287.
- Kluge, A.G., Farris, J.S. (1969): Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**: 1-32.
- Laurent, R.F. (1986): Sous classe des lissamphibiens. *Systématique*. In: *Traité de Zoologie*, Tome 14, 1B, pp. 594-797. Grassé, P.-P., Delsol, M., Eds, Masson, Paris.
- Lobo, F. (1994): El esqueleto de *Physalaemus* (Anura: Leptodactylidae): descripción de nuevos caracteres y análisis comparativo en diez especies estudiadas. *Bol. Asoc. Herpet. Argent.* **10**: 25-26.
- Lynch, J.D. (1971): Evolutionary relationships, osteology, and zoogeography of leptodactyloid frogs. *Misc. Publ. Mus. Nat. Hist. Univ. Kansas* **53**: 1-238.
- Noble, G.K. (1922): The phylogeny of Salientia. In: *The osteology and thigh musculature; their bearing on classification and phylogeny*. *Bull. Amer. Mus. Nat. Hist.* **46**: 1-87.
- Noble, G.K. (1931): *The Biology of Amphibia*. New York: McGraw-Hill Book Co., 577 pp.
- Pombal, Jr., J.P., Prado, G.M., Canedo, C. (2003): A new species of giant torrent frog, genus *Megaelosia*, from the Atlantic Rain Forest of Espírito Santo, Brazil (Amphibia: Leptodactylidae). *J. Herpet.* **37**(3): 453-466.
- Starrett, P.H. The phylogenetic significance of the jaw musculature in anuran amphibians. PhD thesis. Univ. Michigan.
- Swofford, D.L. (2000): PAUP: Phylogenetic Analysis Using Parsimony, version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Taylor, W.R., Van Dyke, G.C. (1985): Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybiurn* **9**: 107-119.
- Wilkinson, M. (1992): Ordered versus unordered characters. *Cladistics* **8**: 375-385.
- 6 — Tarsal decoration. 0: tarsum with fold or not modified, 1: tarsum with tarsal tubercle, 2: tarsum with extensible fold.
- 7 — Outer metatarsal tubercle. 0: not present, 1: present.
- 8 — Inner metatarsal tubercle. 0: regular or acute; 1: cornified spade.
- 9 — Interdigital membrane. 0: membrane present, 1: membrane not present, 2: lateral fringe present.
- 10 — Musculus adductor mandibulae. 0: posterior subexternal adductor mandibular and superficial external adductor mandibular muscles present [“s + e” (Starret, 1968)], 1: only posterior subexternal adductor mandibular present [“e” (Starret, 1968)].
- 11 — Musculus depressor mandibulae. 0: originating from the dorsal fascia, the squamosal and the otic region, and from the tympanic ring, or from the squamosal, otic region and tympanic ring, 1: originating from the dorsal fascia, and the squamosal and the otic region, 2: originating from the squamosal and otic region.
- 12 — Musculus geniohyoideus medialis. 0: muscle contiguous in the medial line, 1: muscle divided at the medial line.
- 13 — Musculus anterior petrohyoideus. 0: muscle insertion on the lateral border of the hyoid plate, 1: muscle insertion on the ventral body of hyoid.
- 14 — Insertion of the musculus sternohyoideus. 0: muscle insertion close to the border of the hyoid body; 1: part of the muscle fibers insertion close to the medial line of the hyoid and part of the fibers insertion close to the lateral of the hyoid body.
- 15 — Musculus homohyoideus. 0: present, 1: not present.
- 16 — Musculus iliacus externus. 0: muscle extending up to half of anterior portion of the ilium, 1: muscle extending from half to three quarters of the anterior portion of the ilium, 2: muscle extending from three quarters to the total of the anterior portion of the ilium.
- 17 — Musculus tensor fascia latae. 0: muscle insertion distally to the anterior extension of the iliacus on the ilium, 1: muscle insertion at the same level of the iliacus extension on the ilium, 2: muscles insertion anteriorly to the distal extension of the iliacus on the ilium, 3: muscle insertion on the anterior region of the ilium immediately anterior to the external iliacus, both muscles are contiguous in part of their extension [for detailed drawings please refer to Heyer (1975)].
- 18 — Musculus semitendinosus. 0: inner and outer portions equal size, outer portion connected by a tendon to the inner, both portions dislocated, 1: outer portion smaller and connected to the inner by a tendon, 2: inner and outer portions of same volume or outer portion with larger volume, 3: outer portion rudimental, connected to the inner portion by a tendon or outer portion not present.
- 19 — Musculus adductor longus. 0: muscle well developed, insertion close or on the knee, muscle generally visible on the surface, 1: muscle poorly developed, inserting totally on the adductor magnus, covered in its totality by the muscle sartorius, 2: muscle not present.
- 20 — Quadratojugal. 0: quadratojugal present, in contact with the maxilla, 1: quadratojugal not present.
- 21 — Nasal-maxillary contact. 0: nasal bone is in contact with the maxilla or both are near, 1: nasal fused with the maxilla.

## Appendix 1

### Character list

- 1 — Pupil shape. 0: pupil round, 1: pupil horizontal.
- 2 — Tympanum visibility. 0: tympanum well developed, visible externally, 1: tympanum partially covered, still visible externally, 2: tympanum fully covered or not present.
- 3 — Male thumb. 0: male thumb with no nuptial pad or spines, 1: some nuptial modification present, nuptial pad present.
- 4 — Body glands. 0: parotoid, inguinal and dorsolateral glands not defined, 1: parotoid glands present and well defined, 2: inguinal glands present and well defined, 3: dorsolateral glands present and well defined.
- 5 — Adhesive disks. 0: disks not defined, 1: disks present, 2: disks present with a circular ridge, 3: disks present with a dorsal scute.

- 22 — Nasal-frontoparietal contact. 0: nasal not connected to the frontoparietal, 1: nasal in contact with the frontoparietal, 2: nasal fused to the frontoparietal.
- 23 — Extension of the fontanelle covered by the frontoparietals. 0: frontoparietal approaching each other medially, but with no exposure of the fontanella (any dubious state was placed in this state), 1: frontoparietals not meeting medially, exposing the fontanella.
- 24 — Squamosal. 0: zygomatic ramus slightly bigger, slightly smaller or of the same size than the otic ramus, no ramus modified with an expanded bony plate, 1: similar to state 1, with a possible contact of zygomatic ramus with maxilla, 2: zygomatic ramus bigger than the otic ramus, with possible contact with maxilla and/or otic plate, 3: otic ramus much bigger than the zygomatic ramus.
- 25 — Vomerine teeth. 0: teeth present, 1: teeth not present.
- 26 — Medial contact of the vomers. 0: vomers with no medial contact, 1: vomers in medial contact.
- 27 — Fusion of the prootic and the frontoparietal. 0: bones not fused, 1: bones fused.
- 28 — Occipital condyles. 0: condyles confluent or close, 1: condyles separated.
- 29 — Anterior process of the hyale. 0: anterior process present (only well defined structures were included), 1: anterior process not present.
- 30 — Alary process of the hyoid. 0: alary process narrow, stem like, 1: alary process rudimental, 2: alary process not present, 3: alary process not stem-like, generally broad wing-shape.
- 31 — Posterior sternum. 0: posterior sternum like a cartilaginous plate, both sides of this plate broad posterior to the connection with the pectoral girdle, a posterior bifurcation can be present or not, 1: posterior sternum cartilaginous, both sides are parallel or get slender posterior to the connection with the pectoral girdle, 2: similar to state 1, with mineral deposits in the mesosternum, 3: posterior sternum differentiated in a bony mesosternum and a xiphisternum.
- 32 — Relation of the transverse process of the last presacral vertebrae with the sacral vertebrae. 0: last presacral vertebrae the same width as the sacral vertebrae, 1: last presacral vertebrae much smaller than the sacral vertebrae.
- 33 — Sacral diapophysis. 0: sacral diapophysis expanded, 1: sacral diapophysis rounded.
- 34 — Terminal phalanges. 0: terminal phalanges simple, round or claw shaped, 1: terminal phalanges T-shaped.
- 35 — Ilium dorsal crest. 0: no dorsal crest, 1: dorsal crest well defined.
- 36 — Frontoparietals and exoccipitals. 0: frontoparietals superimposed on the exoccipitals, 1: bones not superimposed.
- 37 — Prevomer shape. 0: dentary process flexed to the front in relation to the bone axis, 1: dentary process follows the same line of the bone axis.
- 38 — Joint position of the quadratum. 0: maxillary arch reaches the quadratum joint in an anterior position to the parasphenoid wings, 1: maxillary arch reaches the quadratum joint posteriorly to parasphenoid wings.
- 39 — Position of the ilium in relation to the sacral diapophysis. 0: ilium anterior extreme considerably overpasses the level of the sacral diapophysis, reaching half of the presacral vertebra VIII, 1: ilium anterior extreme never overpasses the diapophysis level.
- 40 — Inclination of the sacral diapophysis. 0: sacral diapophysis with a 90 degree angle to the body axis, 1: sacral diapophysis flexed back.
- 41 — Maxillary teeth. 0: present, 1: not present.
- 42 — Nasal bones. 0: nasal bones fused or just separated, 1: nasals noticeably separated.
- 43 — Anterior joint of vomer. 0: vomers do not articulate with premaxilla or with maxilla, 1: vomers articulate with premaxilla or maxilla.
- 44 — Relation of the sphenethmoid with the optical foramen. 0: sphenethmoid outer portion well apart from the optical foramen, 1: outer portion of sphenethmoid closer to the optical foramen, 2: outer portion of sphenethmoid borders the optical foramen.
- 45 — Anterior extension of sphenethmoid. 0: sphenethmoid anteriorly extends up to half the length of the vomerine bones, 1: sphenethmoid anteriorly extends over half the length of the vomerine bones.
- 46 — Pterygoid parasphenoid superposition. 0: no superposition, 1: bones superimposed in antero-posterior plane but with no contact, 2: bones superimposed and in contact.
- 47 — Hyoid posterolateral process. 0: present, 1: not present.
- 48 — Omosternum. 0: present, expanded 1: not present.
- 49 — Mesosternum. 0: bony, broad, only posteriorly, 1: bony, broad, bifurcated posteriorly, 2: bony, amorphous, 3: composed by cartilaginous plaques.

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Appendix 2

Matrix of character states.

Species/Character	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49			
<i>Hyalodes asper</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	1	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	0	1	1	0	2	1	0	0	1	0	0	1		
<i>Hyalodes phyllodes</i>	0	0	0	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	1	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	0	1	0	2	1	0	0	1	0	0	1		
<i>Hyalodes lateristrigatus</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	1	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	0	1	0	2	1	0	0	1	0	0	1		
<i>Hyalodes sazimai</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	1	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	1	0	1	0	2	1	0	0	1	0	0	1	
<i>Hyalodes ornatus</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	1	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	1	0	1	0	2	1	0	0	1	0	0	1	
<i>Hyalodes nasus</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	1	0	0	0	2	1	0	0	1	0	0	1	
<i>Hyalodes meridionalis</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	1	0	0	0	2	1	0	0	1	0	0	1	
<i>Hyalodes daercylocinus</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	1	0	0	0	2	1	0	0	1	0	0	1	
<i>Hyalodes perpicatus</i>	0	0	1	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	0	1	0	0	0	0	0	1	1	1	1	2	0	1	1	1	1	1	1	1	0	0	0	2	1	0	0	1	0	0	1	
<i>Crossodactylus caramaschii</i>	0	0	0	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	1	1	0	0	1	0	0	1	1	0	1	3	2	0	1	1	1	1	1	1	0	0	0	1	0	2	1	0	0	1		
<i>Crossodactylus danietzi</i>	0	0	0	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	1	1	0	0	1	0	0	1	1	0	1	3	2	0	1	1	1	1	1	1	1	0	0	0	1	0	2	1	0	0	1	
<i>Crossodactylus schmidti</i>	0	0	0	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	1	1	0	0	1	0	0	1	1	0	1	3	2	0	1	1	1	1	1	1	1	0	0	0	1	0	2	1	0	0	1	
<i>Crossodactylus schmidti</i>	0	0	0	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	1	1	0	0	1	0	0	1	1	0	1	3	2	0	1	1	1	1	1	1	1	0	0	0	1	0	2	1	0	0	1	
<i>Megaelosta goeldii</i>	0	0	0	0	3	2	1	0	2	1	0	2	1	0	0	0	1	0	3	0	1	1	0	0	0	2	0	0	0	1	2	2	0	1	1	1	1	1	1	1	0	1	1	0	2	1	0	0	3	0	0	1
<i>Physalaeus cuvieri</i>	0	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	0	0	0	0	0	1	0	0	1	2	3	0	1	0	1	0	1	0	1	0	1	0	0	2	0	0	0	1	0	0	1	
<i>Eleutheroactylus fenestratus</i>	0	0	1	0	1	0	1	1	1	1	1	1	1	0	0	2	3	2	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	1	0	0	1	
<i>Leptodactylus knudseni</i>	0	0	0	0	1	0	1	1	1	1	1	1	0	0	0	2	0	0	0	0	0	1	2	0	0	0	1	0	1	0	3	0	1	0	1	0	0	1	0	0	1	1	0	1	2	1	0	0	2	0	0	2
<i>Leptodactylus ocellatus</i>	0	0	0	0	1	0	1	1	1	1	1	1	0	0	0	2	0	0	0	0	1	2	0	0	1	0	1	0	1	0	3	0	1	0	1	0	0	1	0	0	1	1	0	1	2	1	0	0	2	0	0	2
<i>Cycloramphus semipalmatus</i>	0	2	1	0	1	0	1	1	1	1	1	1	0	0	1	0	3	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	2	0	0	1	1	1	1	1	1	1	0	0	0	1	0	3	0	0	1	
<i>Cycloramphus boracetensis</i>	0	2	1	0	1	0	1	1	1	1	1	1	0	0	1	0	3	0	0	0	0	1	0	0	0	1	0	0	1	0	2	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	3	0	0	1
<i>Eiropsophus calcaratus</i>	0	2	1	0	0	0	1	0	1	1	1	1	0	0	1	0	3	1	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1	2	0	1	0	3	
<i>Proceratophrys boiei</i>	0	2	1	0	0	0	1	0	1	1	1	1	0	0	1	0	3	1	0	1	0	1	0	1	0	1	0	0	0	1	3	0	1	0	1	0	1	0	1	1	0	0	0	2	0	1	1	0	1	0	1	
<i>Thoropa militaris</i>	0	0	0	0	1	0	1	1	1	1	1	1	0	0	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	3	0	0	1	1	1	1	0	1	0	2	0	1	1	0	1	2		
<i>Odontophrynus americanus</i>	0	2	0	1	0	0	1	1	1	1	1	0	0	0	1	1	2	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1	1	1	1	0	1	1	0	1	0	1	0	3		
<i>Aixodes gargola</i>	0	2	1	0	0	0	1	0	1	1	1	1	0	0	0	1	0	3	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1	2	0	1	3	