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Cladistic analysis and osteological descriptions of the frog species in the *Leptodactylus fuscus* species group (Anura, Leptodactylidae)

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Abstract

The genus *Leptodactylus* is predominantly Neotropical (a few species have colonized the southern Nearctic region) and is distributed from Texas to Argentina and on certain Caribbean islands. *Leptodactylus* was divided into five groups of species: *Leptodactylus melanonotus*, *Leptodactylus ocellatus*, *Leptodactylus fuscus*, *Leptodactylus pentadactylus* and *Leptodactylus marmoratus*. Among these, the *L. fuscus* group is the one with most species, with 27 taxa. Characters unverified in most of the species are used to define the *L. fuscus* group. However, the monophyly of the group has never been tested rigorously in a quantitative phylogenetic context. Thus, the main goal of this study was to test such monophyly and to construct a phylogeny of the *L. fuscus* group. A matrix of 114 characters scored across 43 taxa was constructed, with 31 characters taken from external morphology, 58 from adult skeletons, 16 from larval chondrocranium, 5 from ethology and 4 from morphometric data were included. Out of all the species examined, 23 belonged to the ingroup and 20 to the outgroup. The data set was analysed with implied weights, by using TNT software. The monophyly of the group was strongly supported in the fittest cladogram obtained. The optimizations of some characters on this hypothesis support traditional evolutionary hypotheses. The optimizations also suggest the presence of pedomorphic character states in some species, which is also discussed.

Key words: phylogeny – *Leptodactylus fuscus* group – osteology – morphology

Introduction

The genus *Leptodactylus* (Fitzinger, 1826), composed of 81 species (Frost 2006), is predominantly Neotropical (a few species have colonized the southern portion of the Nearctic region) and it is distributed from Texas to Argentina and on certain Caribbean islands. Based on morphology and behaviour, Heyer (1969) divided the genus *Leptodactylus* into five species groups (*Leptodactylus fuscus*, *Leptodactylus melanonotus*, *Leptodactylus ocellatus*, *Leptodactylus pentadactylus* and *Leptodactylus marmoratus* groups), which were later redefined by Maxson and Heyer (1988). Among these, the *L. fuscus* group has the most species, with 27 taxa. The taxonomy of almost all the species groups was revised in various works (Heyer 1970, 1978, 1979, 1994, 2005; Angulo et al. 2003). Nevertheless, since those publications, many new species have been described, and several of these new species have not been assigned to any traditional species groups. Some phylogenetic hypotheses indicated the necessity to test the monophyly of the genus *Leptodactylus* and to corroborate the recognized species groups. In some analyses, the genus *Leptodactylus* is paraphyletic in relation to the genus *Adenomera*, *Lithodytes* and *Vanzolinius* (Heyer 1998). Recently, Frost et al. (2006) corroborated the monophyly of the genus when they considered *Adenomera* (Steindachner, 1867) as a synonym of *Lithodytes* (Fitzinger, 1843), and placed *Lithodytes* as a subgenus of *Leptodactylus*. In addition, they recognize *Vanzolinius* (Heyer, 1974) as synonym of *Leptodactylus* (De Sá et al. 2005b). In previous analyses of the genus, the species groups were poorly represented with only a few species. Furthermore, the different sources of characters were analysed independently, with the result that a robust phylogeny of the genus remained non-existent. Maxson and Heyer (1988) determined that the previously defined species groups were incongruent with the groups obtained from micro-complement fixation data on albumins. When using several species of each group, Maxson and Heyer (1988) did

not find any close relation between the three species of the *L. fuscus* group that they had analysed.

When using larval characters to distinguish between species of the *L. fuscus* group, external morphology provides less information than skull morphology. For example, Fabrezi (1997) found interspecific differences only in the chondrocranium, i.e. in trabecular horns and cartilago suprarostralis. Larson and De Sá (1998) described 26 characters in 22 species of *Leptodactylus*, 9 of which belonged to the *L. fuscus* group. Their results showed *Leptodactylus* as paraphyletic, and the monophyly of the traditional species groups was not supported; they found that the species included in the *L. pentadactylus* group were nested within the *L. fuscus* group. Additionally, Wassersug and Heyer (1988), investigating the internal oral characters of three species of *L. fuscus* group tadpoles (*L. gracilis*, *L. fuscus* and *L. mystacinus*) suggested that these species form a natural group because of morphological homogeneity, although these authors did not conduct a cladistic analysis. They also determined that the differences between the three species fell within the range for intraspecific variation.

As regards morphological and behavioural characters, a cladistics analysis showed that the genus *Leptodactylus* was found to be paraphyletic (Heyer 1998). He found that previously defined species group had little support although few characters and taxa were used; the *L. fuscus* group was represented by only two species.

The *L. fuscus* group was revised by Heyer (1978), nevertheless the phylogenetic relationships were not analysed with cladistic methodology, but members of the others species groups of *Leptodactylus* and members of the genera *Adenomera*, *Lithodytes* and *Vanzolinius* were used for comparative purposes to determine the primitive states of the characters. Since that paper, new taxa have been added to the group (i.e. Sazima and Bokermann 1978; Heyer 1983; Heyer et al. 1996; Heyer and Acuña Juncá 2003), but in all these cases, published

information for phylogenetic studies is limited to morphological characters and incomplete data regarding reproduction. In fact, character states are unconfirmed for most of the species of the *L. fuscus* group, which was historically characterized by five putative synapomorphies: (1) no fringes on toes; (2) males without thumb or chest spines; (3) dorsolateral folds usually present; (4) eggs laid in foamy mass in an underground terrestrial incubating chamber; (5) mottled larvae, with a divided labial tooth row anterior to beak (Maxson and Heyer 1988). In addition, Barrio (1965) determined that characteristics of advertisement calls also united the Argentinean species of the *L. fuscus* group. He did not, however refer to 'monophyly' in the group but merely implied a certain affinity among the species involved. Gallardo (1964) also described a series of 'unique' characters in the *L. fuscus* group such as: males have sharply defined borders in maxillae and snout, only one internal vocal sac, absence of corneous spines on fingers; arms equally developed in both sexes; white-yellowish ovules; and they build underground chambers, where foam nests are formed to raise the larvae, in one period previous to aquatic life. Although this author did not state explicitly that he considered the group monophyletic. As mentioned, past analyses exemplify the need to test the monophyly of the genus *Leptodactylus*, and to corroborate the recognized species groups. In these previous analyses, the most speciose group (*L. fuscus* group) was poorly represented, and as a consequence, the phylogeny of this group has not been resolved. The objective of this paper is to test the monophyly and to propose a phylogeny of the *L. fuscus* group, by integrating new characters (external morphology and osteology) with previous data of external morphology, larval morphology and behaviour. In addition, a description of the common osteological characters for the species of the *L. fuscus* group is presented and the skeletons of adults and juveniles of some species of the *L. fuscus* group are compared.

Materials and Methods

Morphological analysis

Details of the taxa included and voucher specimens examined for this study are provided in Appendix S1. The skeletons were studied in cleared and stained specimens prepared according to Wassersug's (1976) protocol. Dry skeletons of *Leptodactylus albilabris*, *L. pentadactylus*, *Leptodactylus knudseni* and *Leptodactylus labyrinthicus* were also examined. The osteology of adults and Gosner (1960) stage 46 juveniles of some species were compared.

For the osteological description of adults, 23 species of the *L. fuscus* group were included (see the following section on 'Cladistic analysis: Taxon selection'). The terminology for cranial and postcranial osteology follows Trueb (1973; 1993); the numbering system for the fingers and carpal osteology follows Fabrezi (1992); tarsal osteology follows Fabrezi (1993); and the laryngeal morphology follows Trewavas (1933).

Cladistic analysis

Taxa selection

Forty-three taxa were examined; 23 are species of the *Leptodactylus fuscus* group: *L. albilabris*, *L. bufonius*, *L. camaquara*, *L. cunicularis*, *L. didymus*, *L. elenae*, *L. fragilis*, *L. furnarius*, *L. fuscus*, *L. gracilis*, *L. jolyi*, *L. labrosus*, *L. latinasus*, *L. longirostris*, *L. marambaie*, *L. mystaceus*, *L. mystacinus*, *L. notoakites*, *L. plaumanni*, *L. poecilochilus*, *L. spixi*, *L. troglodytes* and *L. ventrimaculatus*.

Twenty species of the closest groups to the *L. fuscus* group were included as outgroups, according to previous systematic analyses

(Heyer 1969, 1975, 1994, 1998; Lynch 1971; Maxson and Heyer 1988; Darst and Cannatella 2003). The species of the *Leptodactylus pentadactylus* group would be the closest of them all, according to Larson and De Sá (1998), wherein the species of this group were nested inside the *L. fuscus* group. In the present study the following species of the *L. pentadactylus* group were examined: *L. pentadactylus*, *L. labyrinthicus*, *L. rhodomystax*, *L. knudseni* and *L. syphax*. Additionally, species from the other groups of the genus were included: *Leptodactylus ocellatus* group (*L. insularum* and *L. chaquensis*), *Leptodactylus melanonotus* group (*L. podicipinus*) and *Leptodactylus discodactylus* (De Sá et al. 2005b; placed *Vanzolinus discodactylus* in the synonymy of *Leptodactylus*). Frost et al. (2006) re-evaluated phylogenetic relationships of amphibians based primarily on molecular data. These authors proposed a new taxonomy for the genus *Adenomera*, placing it, together with *Lithodytes* and *Vanzolinus*, in the synonymy of *Leptodactylus*, and recognizing the clade *Adenomera* + *Lithodytes* as the subgenus *Lithodytes*. The species of the subgenus *Lithodytes* included are: *L. marmoratus*, *L. andreae* and *L. lineatus*. Regarding leptodactylids, Frost et al. (2006) partitioned the former family into several reformulated families: Batrachophryniidae, Leptodactylidae, Ceratophryidae and Cycloramphidae. Subsequently, Grant et al. (2006) analysed the family Dendrobatidae and proposed a new taxonomy for the family Leptodactylidae, which would include only the genus *Hydrotaetare* (Gallardo, 1963), *Leptodactylus*, *Paratelmatobius* (Lutz and Carvalho, 1958) and *Scythrophrys* (Lynch 1971). The other chosen outgroup taxa in this study were representatives of the subfamilies of the former family Leptodactylidae although the recently proposed changes place them in other families (Grant et al. 2006): *Engystomops pustulosus*, *Pleurodema borellii* and *Pseudopaludicola boliviana* (family Leiuperidae), *Ceratophrys cranwelli*, *Telmatobius pisanoi* and *Telmatobius scrocchii* (family Ceratophryidae), *Hylodes nasus* and *Crossodactylus gaudichaudii* (family Hylodidae).

Character selection

The states of all the characters were assigned according to the standard methodology of cladistic analysis (Hennig 1966). Osteological and external morphology characters were examined, because these characters are traditionally used in morphological and systematic studies of amphibians. Morphometric, larval and ethological characters from the literature were also included in the matrix. Morphometric characters were taken from Heyer (1978), with some modifications as indicated. Larval skull characters are some of those considered in Larson and De Sá (1998), with modifications as indicated. Ethological characters were taken from different authors: *L. marmoratus* and *L. andreae*: De la Riva (1995, 1996), Heyer (1974) and Heyer and Silverstone (1969); *L. gracilis* and *L. plaumanni*: Barrio (1973) and Solano (1987); *L. latinasus*: Cei (1949, 1980), Gallardo (1958), Heyer (1978) and Solano (1987); *L. bufonius*: Cei (1949, 1980), Heyer (1978) and Philibosian et al. (1974); *L. fuscus*: Heyer (1978), Lescure (1972), Lopes de Freitas et al. (2001), Martins (1988) and Solano (1987); *L. mystaceus*: Caldwell and Lopez (1989) and Heyer and Bellin (1973); *L. fragilis*: Heyer (1978) and Solano (1987).

Search strategies

A parsimony analysis was performed with the TNT (Tree analysis using New Technology) software (Goloboff et al. 2003) by using a traditional search. This method performs heuristic searches by using multiple random addition sequences and/or branch swapping. Shortest trees were found by submitting 2000 different addition sequences to the tree bisection-reconnection branch-swapping method (TBR), retaining 100 trees per replication. Internal branches were considered unsupported and collapsed during searches if any possible states were shared between ancestor and descendent nodes (minimum length = 0 option).

The characters were analysed by using different weights. Although some authors (Kluge 1997; Grant and Kluge 2003) assume that all the characters should be considered under equal weight, Goloboff (1993) and Fontal-Cazalla et al. (2002) believe that the trees obtained under equal weights could be selected only with the claim that all the characters provide equally strong evidence. This asseveration is rejected by every published cladistic analysis, where some characters

show significant amounts of homoplasy, while others are perfectly hierarchical. Goloboff (1993) concludes that if the data are properly weighted, those results will always be preferable to those obtained under equal weights. The weighting method applied herein was the one of implied weighting (Goloboff 1993, 1995), an improvement over the successive weighting method (Farris 1969) implemented in Hennig 86 (Farris 1988). This approach considered that when conflicts between characters exist, the degree of homoplasy of the characters in conflict can be taken into account to solve the conflict in favour of one or another character. The more homoplastic characters are considered as lower weight characters (i.e. the more homoplastic, the less fit) and the total fit of the tree will be the sum of the fits of all the characters. The favourite tree(s) is (are) that (those) which maximize the total fit. The aim of these procedures is to find one phylogeny that maximizes the influence of the less homoplastic characters at the expense of the more homoplastic ones. The fit for each character (i) is calculated as: $F_i t_i = K/K + ES_i$, where ES_i refers to the extra steps (homoplasy) that the character i requires in the evaluated tree, and K is a constant that defines the function's concavity. The function decreases as ES_i increases, hence, if there is more homoplasy there is less weight. The concave shape makes the decrease in weight from 0 to 1 extra step greater than from 1 to 2, and so on. As implemented in TNT, K varies between 1 and 100. Searches using allowed K values were run.

With regard to polymorphism, all the characters with discernible states were included. Some authors have coded polymorphic characters as missing, the so-called 'X-coding' of Doyle and Donoghue (1986). Nixon and Davis (1991, cited in Schuh 2000) criticized that approach because it does not consider variation in the terminals during phylogenetic analysis; consequently, the resulting cladograms are often not computed correctly because coding as missing implies that any state is possible (Wiens 2000).

Morphological characters that allow inference of a logical order for their states were considered as additive. The additivity in this case only reflects degrees of similarity and is independent of any consideration on the sequence in which these characters evolved (Goloboff 1997). The non-additive analysis ignores observed information about similarity among characters, which is the same as ignoring evidence (Lipscomb 1992). In addition, in an attempt to investigate the effect of character ordering on the resulting topologies, data were analysed with unordered characters also.

The support of the clades was measured with symmetric resampling (1000 replicates, with 10 addition sequences, saving up to 10 trees each) expressed as the value of GC (groups present/contradicted), with a change probability of 0.33 (Goloboff et al. 2003), and relative Bremer support (Goloboff and Farris 2001). Searches were performed retaining up to 6000 trees, suboptimal in fit values between 1 and 4 with

swap of type TBR starting from the optimal tree. Cladogram edition was done with Winclada computer program, of Nixon (1999, 2000), version 0.9.99m24 (Beta).

Results

Morphological analysis

In this section, a description of the skeletons of the species of the *L. fuscus* group is given.

Adult cranial osteology (Figs 1 and 2)

Mandible (Fig. 3c): The mentomeckelian bone is L-shaped, with the shorter ramus perpendicular to the skull's horizontal plane. The articulate middle side of this bone is subcircular-concave shaped. The dentary is narrow, anteriorly curved and not fused with the mentomeckelian bone. In cross-section it is a thin plate, not excavated; it externally covers 50% or less of Meckel's cartilage. The angular bone is long and it is the main bony component of the mandible. It is separated from the mentomeckelian bone by a space shorter than the length of the latter, covering the inner side of Meckel's cartilage. The articular region of the mandible is mineralized. The coronoid process is trapezoidal and normally developed.

Nasal capsule: The septum nasi (common middle wall of nasal capsules) and sphenethmoid originate from a single ossified structure. The septum nasi, tectum nasi (roof) and solum nasi (floor) are ossified, except in *L. labrosus* and *L. ventrimaculatus*, where they may be completely cartilaginous or partially mineralized. Anteriorly, the tectum nasi reaches or almost reaches the premaxillae.

Neurocranial braincase: The neurocranial braincase ossifications include pairs of cartilage replacement bones – sphenethmoid, prootics and exoccipitals – which are partially covered by the frontoparietals dorsally and the parasphenoid ventrally.

The sphenethmoid forms the floor, edges of the roof and the anterolateral wall of each side of the braincase. It may be in contact with the optic foramen. It is dorsally visible in the space between the nasals and frontoparietals. The latero-posterior margins are concave, convex or straight. Its ventral

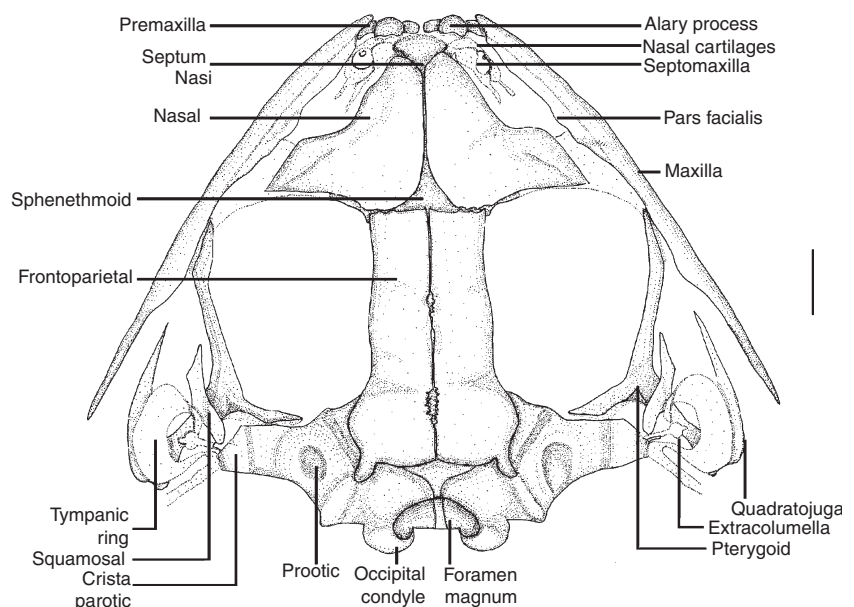


Fig. 1. Skull of *Leptodactylus mystacinus* (dorsal view). Scale line: 2 mm

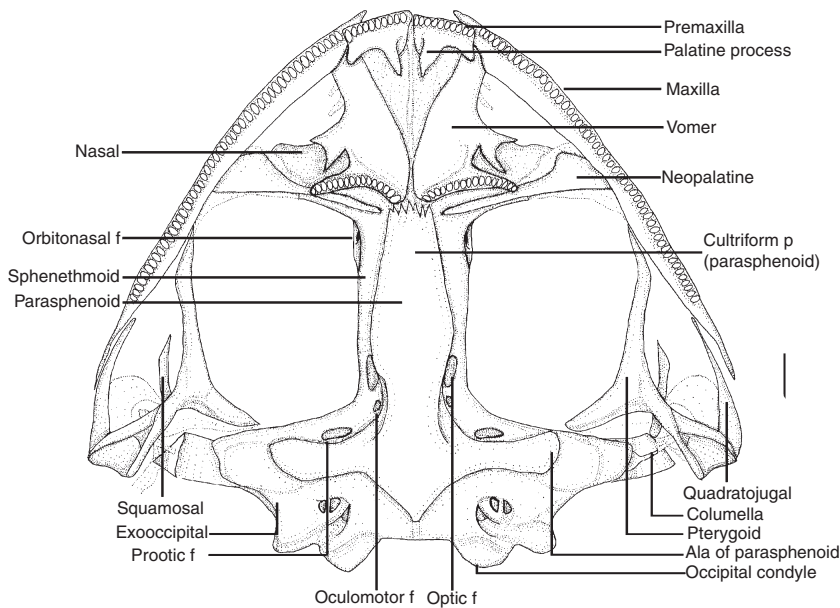


Fig. 2. Skull of *Leptodactylus mystacinus* (ventral view). Scale line: 2 mm

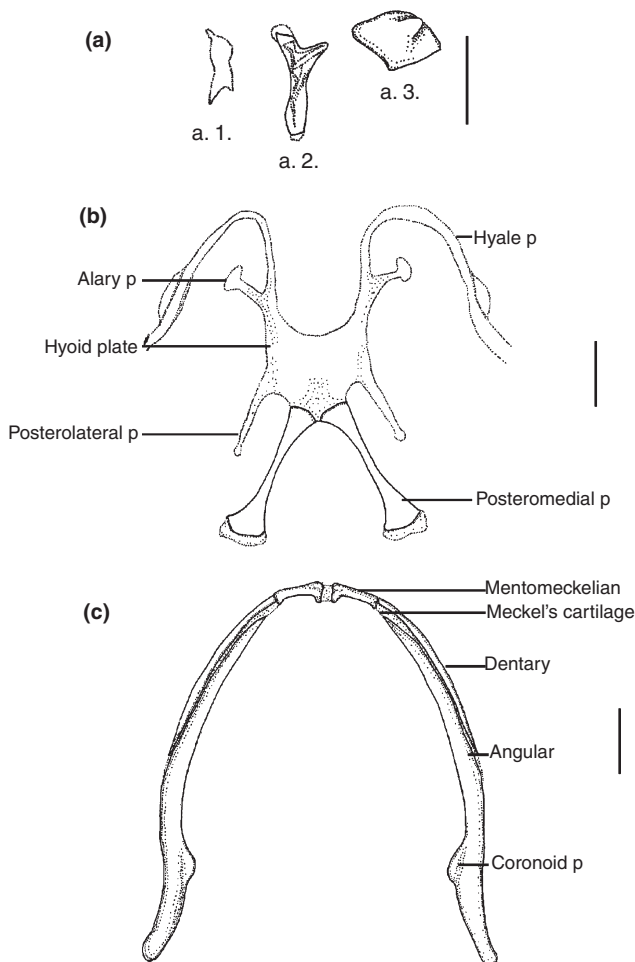


Fig. 3. (a) Pletral apparatus of *Leptodactylus mystacinus*: 1, extracolumella; 2, plectro; 3, operculum. (b) Hyoid of *L. mystacinus*. (c) Mandible of *L. mystacinus*. Scale line: 2 mm

face overlaps with the inner half or third of the neopalatines. It is not differentiated from the septum nasi, except in *L. ventrimaculatus* and *L. labrosus*. Only in these two species

is it possible to distinguish the posterior half of the nasals and the anterior half or two-thirds of the frontoparietals overlapping the sphenethmoid. Additionally, vomers overlap the sphenethmoid.

An orbitonasal foramen anterodorsal and enclosed by the sphenethmoid can be seen. The optic foramen is bordered by the parasphenoid, sphenethmoid, prootic and orbital cartilage, or only by the sphenethmoid and orbital cartilage, or only by the orbital cartilage. The orbital cartilage is sometimes mineralized. In some specimens the limits of the sphenethmoid and prootic are distinct, but these elements can fuse to form a single lateral wall along the posterior braincase.

The prootics are fused with the exoccipitals, forming the posterior region of the braincase and the otic capsules. They are not fused with the frontoparietals. They lack dorsal ornamentation, although they have protuberant crests. The prootic foramens are ovoid, pyriform or triangular in shape. The oculomotor foramens are enclosed by the prootics and orbitosphenethmoids, or only by the prootics; the prootic foramens are embedded in the prootics.

The exoccipital bones, which are covered ventrally by the parasphenoid, form the posteromedial walls of the otic capsules, the margins of foramen magnum and the occipital condyle, with mineralized cartilage between them. The occipital condyles are widely separated, diverging dorsally, and in a cross-section they appear behind the level of the posterior borders of the quadrates.

Pletral apparatus (Fig. 3a): The pars interna plectri of the plectrum is expanded (Fig. 3a. 2), with rounded edges, and it is almost completely ossified. It lies adjacent to the operculum. The pseudoperculum angle is prominent in lateral view. The apophysis is short, conical and curved dorsally. The pars media plectri is a slightly curved, ossified stylus. The operculum is mineralized, elliptical and convex externally (Fig. 3a. 3). It is adjacent to the dorsal posterior edge of the pars interna plectri, and covers the oval window almost completely.

Dermatocranium: The frontoparietals are paired bones. Their anterior extremes are straight or pointed. The lateral edges are either parallel to each other or slightly convergent. The frontoparietals are expanded and rounded posteriorly and

they overlap the prootics but do not reach the foramen magnum. The posterolateral projections may sometimes be absent; when they are present, they are distinctive, but relatively short. The frontoparietals do not reach the squamosal and they cover half the length of the sphenethmoid, but in almost all the species it is difficult to determine the percentage of overlap because the boundary between the sphenethmoid and septum nasi is indistinct. The frontoparietal fenestra extends forward from the middle third of the skull.

The nasals are paired subtriangular bones, transversally oriented, which are superimposed on the sphenethmoid. A small space separates the nasals from the oblique cartilage of the nasal capsule.

The parasphenoid is an unpaired, T-shaped bone, which is not fused with the subjacent bones. The cultriform process, which rests on the sphenethmoid, is long, not keeled, with straight or curved edges that are anteriorly convergent and its shape may be rhomboidal, rectangular or oval, and anteriorly serrated. The base of the afore mentioned process is wider than its anterior edge, which reaches the level of the neopalatines, extending between them and the vomers. The posteromedial process of the parasphenoid is acuminate and ends well forward of the foramen magnum margin. The alae deflect posteriorly and they gradually expand to the extreme edge.

The neopalatines are curved bones, concave posteriorly. The planum antorbitale cartilage covers dorsally the lateral portion of the neopalatines, whose edges are sharp without ornamentation or odontoids, and have an inferior ridge. One-third of its inner length overlaps with the sphenethmoid. The outer edge is expanded and either expands gradually or has a concave 'step' in the middle part. The outer edge reaches the pars palatina of the maxilla. The vomers cover the inner projections, which are separate from each other.

The vomers are paired bones that overlap the sphenethmoid. Each vomer comprises a dentigerous process with a transverse row of 8–19 teeth, and three alae that constitute the anterior and medial margins and, sometimes, half of the posterior margin of the choana. The anterior ramus is longer or equal in length to the middle ramus and the latter is longer or equal in length to the posterior ramus.

The maxillary arcade is complete. The upper jaw is composed of the premaxillae, maxillae and quadratojugal bones. The premaxillae are fused neither to each other nor to the maxillae. Each premaxilla bears 8–12 teeth. The lateral side articulates with the maxilla. The alary process is subrectangular, oriented dorsally, curved backwards and parallel to each other; it has a narrow base; and it does not reach the nasals. The pars palatina is subrectangular, with the posterior side concave, and the palatine process is bifid, with the inner ramus shorter than the external ramus.

The anterior tip of the maxilla bears a lateral projection that overlies the premaxilla. The narrow posterior end articulates by juxtaposition with the quadratojugal bone. The teeth are conical, curved and bicuspid. The teeth are present from the middle of the space between the extreme of the quadratojugal and the extreme of the anterior rami of the pterygoid. The pars palatina and pars facialis of the maxilla are plates. The quadratojugal is a completely ossified, rod-shaped bone. It is in dorsal contact with the squamosal and overlaps the maxilla.

Suspensorium: Each pterygoid has well-developed anterior, medial and posterior rami. The anterior ramus is expanded anteriorly, articulating with the inner side of the maxilla. A cartilaginous line between the pterygoid and the maxilla

continues through the middle line of the anterior and posterior ramus of the pterygoid. The medial ramus does not reach or cover the alae of the parasphenoid, but it rests on the prootic through the cartilage. The posterior ramus, laminar and joining the quadratojugal-squamosal complex, together with the anterior ramus forms an S-figure.

The zygomatic ramus of the squamosal is straight or slightly curved, subrectangular or subtriangular in shape. Anteriorly, it does not reach the maxilla and it can be expanded or acute; when it is expanded it can have an anterior prolongation. In dorsal view, the zygomatic rami of each squamosal bone can be parallel to each other or oriented slightly laterally. The otic ramus of the squamosal is subrectangular or subtriangular and it does not reach the frontoparietal. It is usually shorter than, although sometimes equal to or longer than, the zygomatic ramus. The end is expanded, acute or unvaryingly broad. The crista parotic is mineralized. The descendent ramus of the squamosal has a medial canal, sometimes with the distal half of it being cartilaginous. The angle between the squamosal and maxilla is less than 45°. The annulus tympanic is opened dorsally, cartilaginous and joined to the zygomatic ramus.

Hyoid apparatus (Fig. 3b)

The hyoid plate is cartilaginous, mineralized at the base of the alary, hyale, posterolateral and posteromedial processes. The margins are parallel, sometimes slightly divergent, with a deep hyoglossal sinus. The boundaries between the hyoid plate and the hyoid processes are distinct. The alary processes are thin, perpendicular to the axial axis of the hyoid plate, slightly oriented forward and with a distal expansion. The hyales are thin, curved and generally expanded at the level or near the level of the alary processes. The distal extremes are joined by cartilage with the otic capsule in the anterior part and under the columella.

The posterolateral processes are thin. Their bases arise on the posterolateral side of the hyoid plate and they are oriented postero-laterally. Their posterior ends extend well beyond the level of the posterior edge of the hyoid plate, and may or may not possess a terminal expansion. The posterior ends of the posteromedial processes are cartilaginous. Both anterior and posterior ends of the posteromedial processes are expanded.

Laryngeal cartilages

The arytenoids consist of a pair of valve-shaped cartilages, which are subtriangular in the lateral view. In some specimens there is a medial prominence on the inferior edge. The shape of the cricoid goes from a pentagon to circular ring. The oesophageal process present may be triangular, trapezoidal or quadrangular in shape, with or without a superior and an inferior slope. The bronchial process is differentiated, but thin. In females, the cricoids are thinner and the arytenoids are smaller than in males.

Postcranial osteology

Axial skeleton (Fig. 4a): Vertebrae I–V are imbricate. The relative length of the transverse processes is variable, but most specimens respond to the pattern: III > IV > V > VI > VII > VIII > II. Ventrally, the relative lengths of the vertebral centra are: 2 < 3 < 4 equal to the rest of the vertebrae. The neural arch of each vertebra has a well-developed dorsal ridge, and a pair of parasagittal processes which extend laterally.

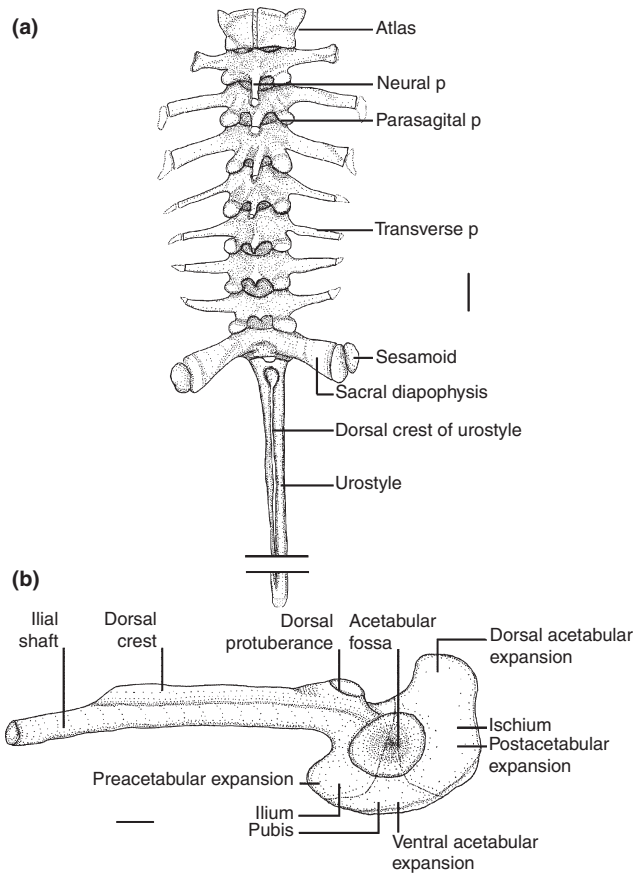


Fig. 4. (a) Vertebral column of *Leptodactylus mystacinus*. (b) Pelvic girdle of *L. mystacinus*. Scale line: 2 mm

Although the atlas is not fused to the second vertebra, both are in contact with each other dorsally through a neural process of the atlas' posterior edge. The anterior edge is convex, sometimes with a median slope and sometimes with an anterior neural prolongation. The centrum (in ventral view) is wider than the other vertebrae.

The cotylar arrangement of the atlas resembles Lynch's (1971) type I. The cervical cotyles are oriented anteriorly or lateroanteriorly and are separated from each other and are half-moon in shape, the intercotylar region being concave. The transverse processes of the second vertebra generally lack a prolongation, although some specimens have a short anterior

one. The moderately dilated sacral diapophyses are oriented backwards, and are ovoid in cross-section.

The ilio-sacral articulation is Emerson's (1982) type IIB, with a mineralized sesamoid element between them; the sacral-coccygeal articulation is bicondylar. The urostyle, which is not fused to the sacrum, is cylindrical, with a well-developed dorsal crest, and an anterior protuberance. The dorsal ridge is anteriorly highest and diminishes in height posteriorly.

Pectoral girdle (Fig. 5): The girdle is arciferous. The omosternum is cartilaginous with an expanded distal end. The anterior expansion is shorter than the xiphisternum, generally fan-shaped, with or without posterior projections and either semiovoid or triangular in shape. The episternum is slightly expanded posteriorly cartilaginous and stick-like. The mesosternum is ossified, with the anterior end being cartilaginous. Generally, it is undivided anteriorly, but in some specimens it is divided or has an incipient division. The edges converge posteriorly towards the middle part and from this point they continue parallel to each other or slightly divergent.

The xiphisternum is cartilaginous, expanded and mineralized anteriorly. Depending on the specimen, it is either longer than wide, wider than long or of almost equal proportions. It is semicircular or trapezoidal with curved posterior edges. The procoracoid is present and extends to the level of the internal extremes of the clavicles, such that the clavicles do not touch each other. A prolongation of the procoracoid extends between the clavicle and scapula.

The epicoracoid is cartilaginous with mineralized anterior and inner lateral edges. In ventral view, the right overlaps the left, but in some specimens the left overlaps the right. The pectoral fenestra, whose inner margin is concave, is wider than long and is rectangular-shaped with the principal axis transverse to the vertebral column. Each of these holes is anteriorly bordered by the procoracoid cartilage, medially by the epicoracoid cartilage, and posteriorly by the coracoid. Epicoracoidal horns are present.

Each clavicle is curved and bow-shaped, with a concave anterior side. The glenoid end of the clavicle is expanded dorsolaterally into a wedge-shaped process that articulates with the pars acromialis of the scapula. The clavicles do not reach the glenoid fossa.

The scapula is rectangular in shape and is almost equal in length to the coracoid, but the scapular width is twice that of the coracoid's width. The scapula is composed of two plates: the pars acromialis, which is posteriorly convex and represents the anterodorsal portion, and the pars glenoidalis, which is

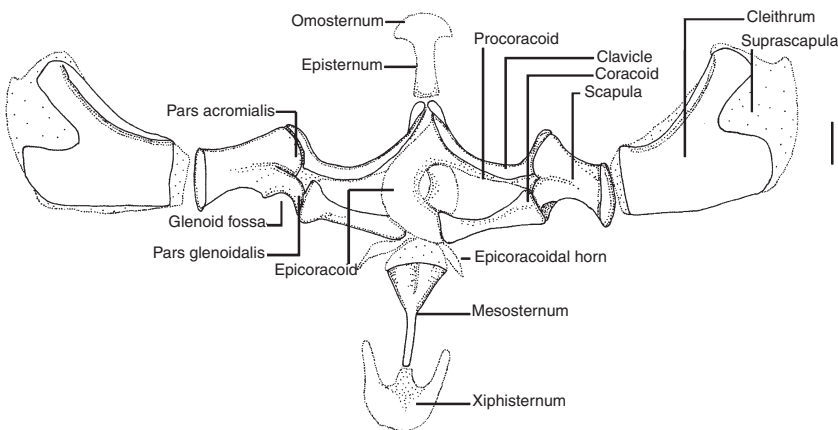


Fig. 5. Pectoral girdle of *Leptodactylus mystacinus*. Scale line: 2 mm

posteriorly concave and represents the posterodorsal part. The contact area with the suprascapula is cartilaginous and mineralized. A prolongation of procoracoid separates the pars acromialis from the clavicle. The coracoid is subrectangular and the ends are distally expanded. The glenoid cavity is limited by the scapula, pars glenoidalis and coracoid. A mineralized element is present between the scapula and the coracoid, visible internally and externally. Generally, the glenoid cavity is not bordered by cartilage.

The outer edge of the suprascapular cartilage is cartilaginous and mineralized. It has an obvious triangular projection on the outer anterior portion, just forward of the superior spur of the cleithrum, which consists of an ossified, thin and bifid lamina, with the posterior ramus shorter or equal to the anterior ramus. On the upper side, this bone has a ledge forming a longitudinal crest.

Forelimb and manus (Fig. 6a,b): The anterior end of the humerus has two humeral crests, which are separated by a groove, one more developed than the other and with no differences between the sexes. The distal head, eminentia capitata, is broadly expanded and partially ossified. The glenoid head, caput humeri, is rounded and slightly compressed and is inserted like a wedge in the glenoid cavity. The caput humeri is larger or equal in size to the eminentia capitata. Five carpal elements are present, representing Fabrezi's (1992) type E morphology: ulnare, distal carpal 5–4–3, element y + distal carpal 2, radial and proximal element of the prepollex. Conical sesamoids are present on the ventral face of the distal epiphysis of metacarpus, as well as at the

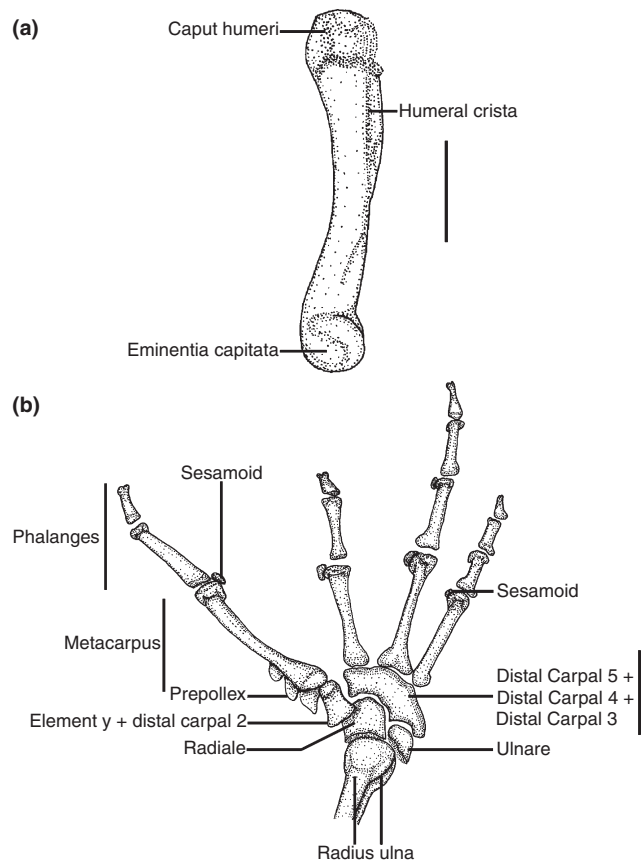


Fig. 6. (a) Humerus of *Leptodactylus mystacinus*. (b) Manus of *L. mystacinus*. Scale line: 2 mm.

distal epiphysis of the proximal phalanges of digits IV to V, the sesamoids are either separated or medially fused. The epiphyses of the metacarpus and some phalanges have small lateral projections. Metacarpal calcifications are sub-quadrangular or sub-circular. The inner metacarpus lacks nuptial spines.

The phalangeal formula is 2–2–3–3, the relative digit length being $III \cong V \ll II \cong IV$. Terminal phalanges (digit IV) have a split that defines two lobules, but sometimes the other digits are terminally knobbed. The prepollex is present, lacks nuptial spines and consists of three segments in addition to the basal segment. The mineralization of the segments diminishes from the base to the distal ends.

Pelvic girdle (Fig. 4b): The pelvic plate is semicircular. The angle between the ilial shaft and preacetabular expansion is less than 90° , but in some specimens of *L. fuscus* the angle is almost 90° . The ventral edge of the ischium reaches the level of the ilium. The internal margins of the ilia form a U. The ilial shaft is round in cross-section. The pubis is mineralized, localized as a wedge between the ilium and ischium. The ilia are firmly united to each other medially and the ischia, posteriorly; sometimes, the medial sutures are not distinct because of fusion between the elements.

The dorsal protuberance is tear-drop shaped, normally developed (height equal to the dorsal acetabular expansion) or short (lower than the dorsal acetabular expansion). The dorsal crest gradually diminishes in height from the point where it articulates with the sacral diapophysis. The ilial crest can be the same height, lower or taller than the ilial height; sometimes the crest is taller anteriorly.

Hind limb and pes: The femur is sigmoid-shaped. Both the caput femoris and the distal head of the femur are partially calcified. The tibiofibula is longer than the femur. A distinct sulcus intermedius marks the medial union of the tibia and fibula on both sides of the bone. The proximal head of the tibiofibula is almost equal in size to the distal head. The tibiale and fibulare are shorter than the femur, and widely separated from each other at their midpoint, but both the proximal and distal heads of the tibiale and fibulare are fused. Mineralized, spherical sesamoids are present at the femur-tibiofibular and tibiofibular-tibiale/fibulare articulations.

Three tarsal elements are present: element y, distal tarsal 1 and distal tarsal 2–3. The y element articulates with the base of the prehallux, distal tarsal 1, tibiale and tarsal 1. Distal tarsal 1 is the smallest of them and articulates with: element y, distal tarsal 2–3 and metatarsals 1 and 2. Distal tarsal 2–3 articulates mainly with metatarsal 3, also with metatarsals 2 and 4 and with distal tarsal 1. There are also two or three small mineralized sesamoids present under the tarsals.

The digital phalangeal formula of the pes is: 2–2–3–4–3. The terminal phalanges (digit IV) have a split that defines two lobules, whereas sometimes the other digits end in knobs. Subarticular mineralized sesamoids are present. The prehallux has three segments, with ossification decreasing from the base to the external segment.

The comparative analysis between juvenile (stage 46 – Gosner 1960) and adult skeletons indicates that there are characters that have the same state both in juveniles and adults in some species, but are found only in juveniles of other related species, and the adults from this related species have completed the development of these characters (Tables 1–3).

Table 1. Characters that appear in adults of some species in the same state as in juveniles of related species

| Character | Stage 46 (Gosner 1960) | Adult |
|---------------------------------------|--|------------------------------------|
| Frontoparietal fenestra | Not totally covered by the frontoparietals | Covered totally by frontoparietals |
| Vomerine teeth | On a straight line | In an arched series |
| Pars facialis of maxilla | Ends before the level of neopalatines | Ends at the level of neopalatines |
| The neural processes of vertebrae I–V | Not developed | Imbricate with the next vertebra |

Cladistic analysis

A matrix with 114 characters and 43 taxa was constructed (Appendix S3). The characters are taken from the following sources: 0–30, external morphology; 31–88, osteology; 89–104, larval skull; 105–109 ethological; 110–113, morphometric (Appendix S2). A single most parsimonious tree of 440 steps with a fit of 81.73 was obtained with each of the different values of the concavity constant (K) ranging from 2 to 7 (Fig. 7). The variations in the concavity constant (K) did not change the result unless very high K values (more than 7) or very low values (less than 2) were used. Therefore, in this case,

there is no difference due to the strength of the weighting against homoplasy (what K value has to be used for the search of the final result). The distribution of synapomorphies is shown in Fig. 8. The results of the ordered characters were almost identical to the analysis of unordered characters, whereas the analysis of unordered data differed in the clade containing *L. fuscus*, *L. poecilochilus*, *L. longirostris* and *L. spixi*. *Leptodactylus fuscus* and *L. spixi*, in the unordered analysis, are closest to *mystaceus* complex, while *L. poecilochilus* and *L. longirostris* have a basal position to node 9. Other difference is that in the unordered analysis, *L. syphax* results in the sister-species of *L. insularum*. Because of the reason exposed in the ‘Materials and Methods’ section, the discussion has been focused on the topology presented in Fig. 7 (resulting from the parsimony analysis of ordered characters).

The *L. fuscus* species group resulted monophyletic. The monophyly of the *L. fuscus* group is supported by three synapomorphies: tectum nasi at the same level as the alary process of premaxilla (character 41:1); posterior margin of frontoparietal: convex (character 48:2); and cultriform process of parasphenoid between neopalatines (character 58:0). The first dichotomy within the *L. fuscus* group appears to be the *L. labrosus* + *L. ventrimaculatus* clade, defined by four

Table 2. Distribution of two characters, that have the same state, both in juveniles and adults of some species and are found only in juveniles of other related species

| Adult specimens | Frontoparietal fenestra (Fig. S11) | | Vomerine teeth (Fig. S14) | |
|---|--|------------------------------------|---------------------------|---------------------|
| | Not totally covered by frontoparietals | Covered totally by frontoparietals | On a straight line | In an arched series |
| <i>Leptodactylus latinasus</i> (n = 14) | 14 | – | 13 ² | 3 ² |
| <i>Leptodactylus camaquara</i> (n = 3) | 3 | – | – | 3 |
| <i>Leptodactylus bufonius</i> (n = 10) | – | 10 | – | 10 |
| <i>Leptodactylus cunicularis</i> (n = 3) | 2 | 1 | 2 ² | 3 ² |
| <i>Leptodactylus elenae</i> (n = 6) | – | 6 | – | 6 |
| <i>Leptodactylus fragilis</i> (n = 10) | 7 | 3 ¹ | 2 | 8 |
| <i>Leptodactylus fuscus</i> (n = 10) | – | 10 | – | 10 |
| <i>Leptodactylus furnarius</i> (n = 2) | – | 2 | – | 2 |
| <i>Leptodactylus longirostris</i> (n = 2) | – | 2 | – | 2 |
| <i>Leptodactylus mystaceus</i> (n = 8) | – | 8 | – | 8 |
| <i>Leptodactylus spixi</i> (n = 2) | – | 2 | – | 2 |

¹These three specimens have the frontoparietal fenestra almost completely closed.

²Two specimens have one vomer with teeth in a straight line and the other vomer with teeth in an arched line.

Table 3. Distribution of two characters that have the same state, both in juveniles and adults of some species and are found only in juveniles of other related species

| Adult specimens | Pars facialis of maxilla (Fig. S8) | | Neural spine of vertebrae I–V (Fig. S18) | |
|---|---|-------------------------------|---|-----------------------------------|
| | Ends before the level of the neopalatines | Ends at level of neopalatines | Does not imbricate with the next vertebra | Imbricates with the next vertebra |
| <i>Leptodactylus latinasus</i> (n = 14) | 14 | – | 12 | 2 |
| <i>Leptodactylus camaquara</i> (n = 3) | 1 | 2 | 3 | – |
| <i>Leptodactylus bufonius</i> (n = 10) | – | 10 | – | 10 |
| <i>Leptodactylus cunicularis</i> (n = 3) | 1 | 2 | 1 | 2 |
| <i>Leptodactylus elenae</i> (n = 6) | 2 | 4 | – | 6 |
| <i>Leptodactylus fragilis</i> (n = 10) | 1 | 9 | 2 | 8 |
| <i>Leptodactylus fuscus</i> (n = 10) | – | 10 | – | 10 |
| <i>Leptodactylus furnarius</i> (n = 2) | – | 2 | – | 2 |
| <i>Leptodactylus longirostris</i> (n = 2) | – | 2 | – | 2 |
| <i>Leptodactylus mystaceus</i> (n = 8) | 1 | 7 | – | 8 |
| <i>Leptodactylus spixi</i> (n = 2) | – | 2 | – | 2 |

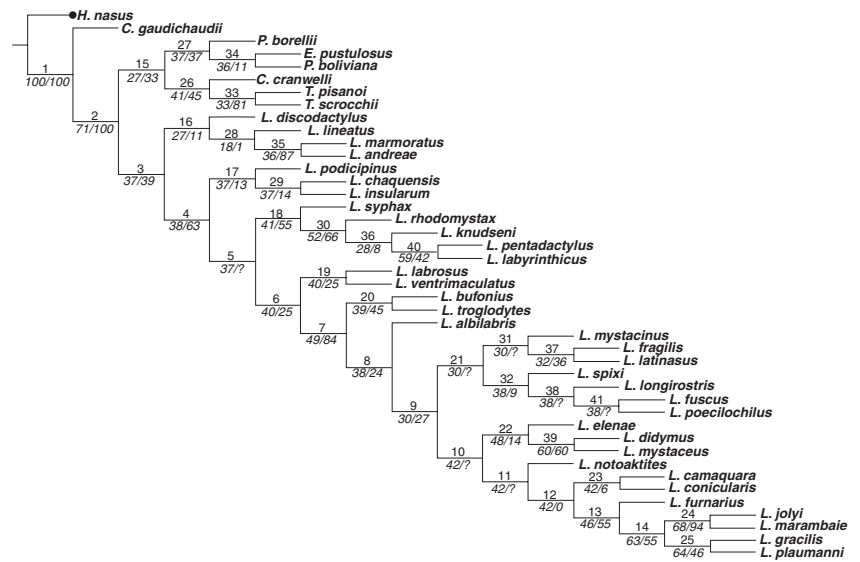


Fig. 7. Topology of the most parsimonious tree calculated under concavities $K = 2-7$. The number above and right of node are the node numbers, and the numbers below and right of nodes are the relative bremer support/groups present/contradicted (GC) values calculated under $K = 3$. Question marks in GC values represent negatives values, which are an artefact of the method, assigned to weakly supported nodes

synapomorphies: pars facialis of maxilla ends at the level of neopalatines (character 38:1); nasals close to each other or in contact along its inner border (character 52:2), posterointernal angle of nasals close to each other or in contact with frontoparietals (character 55:1) and vomers close to each other or in contact (character 64:1). The sister group of the *L. labrosus*–*L. ventrimaculatus* clade is supported by five synapomorphies (Fig. 8); within this group the pair *L. bufonius* + *L. troglodytes* stand out as basal clade – defined by two synapomorphies: tectum nasi anterior to alary processes of premaxilla (character 41:2); and anterior border of tectum nasi with a well-differentiated projection (character 42:2). *Leptodactylus albilabris* appears as intermediate between the four more basal species and the rest of the *L. fuscus* group.

With regard to the relationships in the outgroup, the species of the family Ceratophryidae examined, i.e. *Ceratophrys cranwelli* (Ceratophryinae), *T. scroccii* and *T. pisanoi* (Telmatobiinae) form a monophyletic group. The sister group of the clade Ceratophryidae is the group constituted by the species of Leiuperidae considered (*Pleurodema borellii*, *Pseudopaludicola boliviana* and *Engystomops pustulosus*). The monophyletic clade Ceratophryidae + Leiuperidae was the sister group of *Leptodactylus* clade in this study.

All the species of the genus *Leptodactylus* examined form a monophyletic group, which is supported by five synapomorphies: posterior margin of frontoparietal: straight (character 48:1); from 8 to 13 vomerine teeth (character 63:2); overlapping of vomers and neopalatines (character 66:1); otic ramus of squamosal as broad as zygomatic ramus (character 68:1); xiphisternum trapezoidal or semicircular (character 80:0). Inside the *Leptodactylus* clade, the species of the subgenus *Lithodytes* (*L. andreae*, *L. marmoratus* and *L. lineatus*) and *L. discodactylus* form a monophyletic group. This clade is defined by three synapomorphies – toe tips with an undivided expansion (character 18:1); tectum nasi at the same level as the alary processes of premaxilla (character 41:1); anteromedial process of hyoid present (character 73:1) – and constitutes the sister group of the clade that includes the rest of the species of the genus *Leptodactylus*. Within this last group, species of the *L. melanotus* group (*L. podicipinus*) together with the species of the *L. ocellatus* group (*L. chaquensis* and *L. insularum*) form a monophyletic group defined on the basis of five

synapomorphies: planum trabecular anticum is narrow (character 91:1); posterolateral extension of the palatoquadrate reaches a third of the length of the otic capsule (character 94:4); pars articularis quadrati is indistinct from processus muscularis (character 97:1); processus branchialis is closed: with a distinctive cartilaginous bridge between Ceratobranchials II and III (character 102:1); hyoquadrate process large and rounded in lateral view (character 104:1). This clade is the sister group to the clade *pentadactylus* + *fuscus*, which is supported by the unreversed synapomorphies: anterior border of nasals deeply concave (character 53:0) and attachment of the processus ascendens intermediate (character 95:1). The *L. pentadactylus* group is supported by two synapomorphies: alary processes of premaxilla directed posterodorsally (character 33:0) and males with crista humeri well developed (character 85:0).

Discussion

The genus *Leptodactylus* was found to be monophyletic, even considering the taxa that recently have been considered as synonym of the genus (*L. andreae*, *L. marmoratus*, *L. lineatus* and *L. vanzolinus*). According to the evidence presented by Heyer (1998), De Sá et al. (2005b), Frost et al. (2006) and Grant et al. (2006), *Leptodactylus* was paraphyletic with respect to *V. discodactylus* (*L. discodactylus*). Actually, De Sá et al. (2005b) placed *Vanzolinus* as a subjective junior synonym of *Leptodactylus*. The data presented here do not match the previous results, since *L. discodactylus* did not fall inside the former genus *Leptodactylus*, although it does form a monophyletic group with the subgenus *Lithodytes*. The position of the recently recognized subgenus *Lithodytes* (Frost et al. 2006), as sister clade of the rest of the species of the genus *Leptodactylus*, coincides with the classification schemes proposed by Frost et al. (2006) and Grant et al. (2006). Previous works of Heyer (1998) and Faivovich et al. (2005) showed the genus *Leptodactylus* as paraphyletic. Based on advertisement call and morphological evidence, Heyer (1998) obtained *L. lineatus* as basal in the genus, and *L. marmoratus* as sister-species of *L. bufonius*. The results of Faivovich et al. (2005), using primarily molecular characters, indicated that *Leptodactylus hylaedactylus* is sister-species of *L. ocellatus*, and

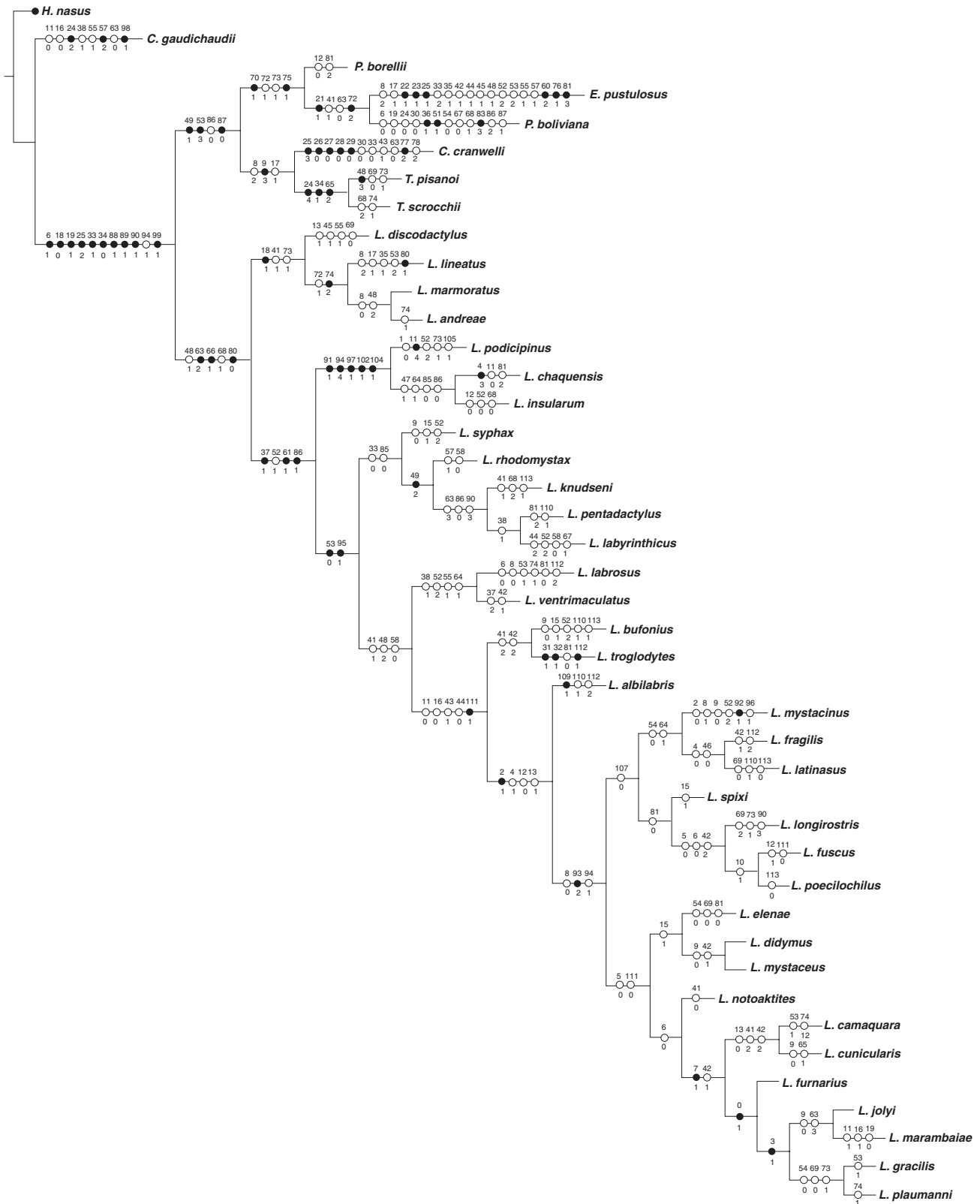


Fig. 8. Cladogram showing the synapomorphies for each node. Numbers above nodes are character numbers (see Appendix S3). Numbers below nodes are the states of each character. Empty and filled hashmarks indicate homoplasy and non-homoplasy, respectively

L. lineatus is basal to both species. The relationships of the species from the subgenus *Lithodytes* would still require confirmation, with the inclusion of a larger number of species

from the genus *Leptodactylus* and including other sources of characters. Biological data suggest a close relationship between the species of the subgenus *Lithodytes* and those from the

L. fuscus group. De la Riva (1995, 1996) described a reproductive mode in specimens of the subgenus *Lithodytes* from Amboró, Bolivia, that was equivalent to the one observed in the *L. fuscus* group. *Leptodactylus lineatus*, in a similarly opportunistic way, place the foam nest in incubating chambers (Lamar and Wild 1995). The foam-making behaviour of tadpoles also links the species of the subgenus *Lithodytes* to the species of the *L. fuscus* and the *L. pentadactylus* group (see below), as it was described for one unidentified species of this taxon (de Kokubum and Giaretta 2005).

On the other hand, the results obtained here do not coincide with those of Larson and De Sá (1998), whose larval characters were incorporated to the matrix analysed herein, because *Leptodactylus* was paraphyletic with regard to *Engystomops*. Heyer (1975) analysed the intergeneric relationships of the family Leptodactylidae by using methodology different from cladistic parsimony analysis, and because of this, the topology that he preferred cannot be compared with the tree obtained in this study. Even so, it is worth mentioning that in the relationships that Heyer prefers (Heyer 1975: fig. 9), *Leptodactylus* is closer to the recently synonymized *L. disco-dactylus* and the species of the subgenus *Lithodytes*, which is equivalent to the cladogram obtained here. Some of the characters considered by Heyer (1975), as shared by the species of the genus *Leptodactylus*, deserve further comments. First, male thumb lacking either nuptial pad or spines (character 24:0), our study here found that the members of the *L. pentadactylus*, *L. melanotus* and *L. ocellatus* groups possess nuptial spines. Second, frontoparietals meet medially, not exposing fontanelle (character 46:1), which is a widely distributed character state, although some species (i.e. *L. disco-dactylus*, *L. camaquara*, *L. fragilis*, *L. latinus* and some specimens of *L. gracilis*, *L. furnarius* and *L. cunicularis*) possessed the state frontoparietals separated medially, exposing fontanelle (character 46:0). In coincidence with other phylogenetic proposals, the former Leptodactylinae subfamily turned out to be paraphyletic. The monophyly of this subfamily has been supported by the presence of a bony sternum (... 'as compared with the cartilaginous sterna of the other leptodactylids'; Lynch 1971) as according to some authors on its foam nest habits. Incidentally, this behaviour is absent in *Pseudopaludicola* (Miranda-Ribeiro, 1926), *Limnomedusa* (Fitzinger, 1843) (Langone 1994) and some species of *Pleurodema* (Lynch 1971; Duellman and Veloso 1977; Frost et al. 2006). Darst and Cannatella (2003), based on sequences of ribosomal mtDNA, made an analysis with parsimony and obtained the Leptodactylinae as paraphyletic while an analysis with maximum likelihood turned out to be in the monophyly of the subfamily. Haas (2003), based on larval characters, found that the three species from the subfamily that he examined (*L. latinus*, *Engystomops pustulosus* and *Pleurodema kriegii*) did not happen to be a monophyletic group. On the contrary, Faivovich et al. (2005) obtained Leptodactylinae as monophyletic (except for *Limnomedusa*). However, the authors did not include *Paratelmatoobius* or *Scythrophrys* (Lynch 1971) in their analysis. In Frost et al. (2006), Leptodactylinae was para- or polyphyletic with regard to *Paratelmatoobius* and *Scythrophrys*, and to *Limnomedusa*. Grant et al. (2006) also obtained the subfamily as para- or polyphyletic. According to the phylogenies proposed up to the present, there is considerable evidence suggesting the paraphyly of the traditional Leptodactylinae. Nevertheless, the taxonomic changes proposed recently by Frost et al. (2006) were based

on a limited taxon sampling in lower taxonomical ranks, and mostly, based on molecular characters only. Given these limitations, a more complete taxon sampling is required where different sources of characters (morphological + molecular + natural history) are considered simultaneously.

The close relationship between the *L. fuscus* and *L. pentadactylus* groups is not surprising. The results obtained by Heyer (1998), when the species from the subgenus *Lithodytes* were excluded from his analysis, supported the monophyly of the *L. fuscus* group and its close relationship with the *L. pentadactylus* group. Larson and De Sá (1998), on the other hand, obtained the *L. fuscus* group as paraphyletic with the *L. pentadactylus* group nested inside it. In the present work two characters relate the species of both groups: deeply concave anterior border of nasals (character 53:0) and intermediate attachment of the processus ascendens (character 95:1) (see fig. 3a,c in Larson and De Sá 1998). The deeply concave anterior border of nasals is independently lost in some species of the *L. fuscus* group. The 'intermediate' condition of the latter mentioned character was defined by Sokol (1981). Besides the *L. fuscus* and *L. pentadactylus* group, this condition is found in larvae of the leptodactylids *L. chaquensis*, *L. ocellatus* and also in *Pleurodema briboni* and *Rana* (Sokol 1981; Larson and De Sá 1998). Biological data corroborate the close relationship between the species of *L. fuscus* and *L. pentadactylus* groups. The species in the *L. fuscus* group produce foam nests that are placed on land in subterranean chambers constructed by males; subsequent to flooding, exotrophic tadpoles enter lentic or lotic water. The *L. pentadactylus* group has representatives with a step prior to the *L. fuscus* group in the transition to terrestrial life, with deposition of foam nests in open depressions, subsequent to flooding, with exotrophic tadpole development in water (*L. knudseni*, *L. labyrinthicus*, *L. savagei*). Other members of the *L. pentadactylus* group (*L. fallax* and *L. pentadactylus*), however, demonstrate a transitional step beyond the *L. fuscus* group that is, foam nests inside burrows in the ground and development of larvae inside the nests (Prado et al. 2002). Besides indicating a close relationship with the *L. fuscus* group, the reproduction modes described in species of the *L. pentadactylus* group could be an indication of the polyphilia of this latter group. Another interesting biological character shared by species of the *L. fuscus* group and *L. labyrinthicus* (a member of the *L. pentadactylus* group), is the foam-making behaviour of tadpoles (Caldwell and Lopez 1989; Downie 1989; Giaretta and Kokubum 2004; Kokubum and Giaretta 2005; Ponssa and Barrionuevo, submitted).

Inside the monophyletic *fuscus* clade, the basal species (*L. labrosus*–*L. ventrimaculatus*) form a monophyletic group, defined by four synapomorphies, which constitute a particular cranial pattern that involves a larger development (in size and/or ossification) of some structures (e.g. nasals) to the detriment of others (e.g. septum nasi). This is an interesting pattern if the fossorial mode of life that defines the *L. fuscus* group is taken into account. Because of this, these characters are analysed below. A close relationship between both species was also found using immunological distances of serum albumins (Maxson and Heyer 1988). Heyer (1978), who did not present a phylogenetic analysis using a rigorous methodology such as cladistics, considered both species as probably 'similar to the *L. fuscus* group ancestor'. This author thought that the presence of tubercles on the tibia-tarsus and foot linked these species with *L. bufonius* and *L. troglodytes*, and

speculated that the ancestor would probably have had tubercles as well. In the tree obtained here, both species show a basal position, and the presence of tubercles is a plesiomorphy for the genus, and is therefore not a synapomorphy that defines a putative group formed by these four species. The *L. bufonius*–*L. troglodytes* pair occupies an intermediate position between the more basal *L. ventrimaculatus*–*L. labrosus* group and the rest of the species of the *L. fuscus* group. Heyer (1978) suggested a second assemblage formed by the remaining species, excluding *L. mystacinus* and *L. poecilochilus*, which would be intermediate between the two assemblages. In the phylogenetic hypothesis presented here, *L. albilabris* holds an intermediate position. However, in concordance with the proposal made by Heyer (1978), the species that belong to this second assemblage, share the 'derived' states (according to Heyer's concept) in the lip and thigh stripe characters. The presence of the thigh stripe (character 2:1) turned out to be one of the synapomorphies of the clade defined from node 8.

The traditionally considered sibling species (Heyer et al. 1996): *L. didymus*–*L. mystaceus*, *L. gracilis*–*L. plaumanni* and *L. fragilis*–*L. latinasus* demonstrate sister-species relationships in this study. The 'sibling species' concept, originally introduced by Mayr (1942), refers to species which are morphologically identical or nearly identical. From the point of view of cladistic methodology, the 'sibling species' term is restricted to two taxa that share a more recently common ancestor (De Sá et al. 2005a). In the tree, *L. didymus* and *L. mystaceus* form a monophyletic group defined by two synapomorphies: toe webbing without web or fringe (character 9:0) and anterior border of tectum nasi with a slightly developed projection (character 42:1) (Fig. 8). Morphological characters that differentiate both species were not found. Both species have been associated with *L. spixi*, *L. mystaceus* and *L. notoaktites* under the name '*L. mystaceus* species complex', because of their morphological similarity (Heyer et al. 1996). Moreover, the immunological distance data also support the close relationship between these species (Maxson and Heyer 1988). In the obtained tree, only *L. elenae* forms a monophyletic group with *L. didymus* and *L. mystaceus*. The clade is defined by the presence of the postympanic gland: pigmented in males (character 15:1). De Sá et al. (2005a) analysed the relationship between the five species of the *L. mystaceus* complex, based on molecular data. However, their results do not coincide with the present study where *L. didymus* and *L. mystaceus* did not cluster as sister-species. Based on their results, De Sá et al. (2005a) concluded that in spite of the morphological similarity, these species are not sibling species. These authors proposed two alternative hypotheses to explain the morphological similarity: (1) convergence (2) retention of one ancestral morphological pattern. Obviously, the phylogenetic hypothesis obtained by these authors does not support the second option. On the contrary, in the tree obtained here using morphological characters, both species show a sister-species relationship. As a consequence of this relationship, they would be 'sibling species' because they share a more recently common ancestor. Thus, to make more conclusive generalizations to explain this case, where the species exhibit ethological (i.e. advertisement call) and genetic differences, but do not differ in their morphology we must make a simultaneous analysis with all the evidence (morphological + genetic + ethological).

As regards *L. gracilis* and *L. plaumanni*, only the mating call has been useful to distinguish them in the wild (Kwet et al. 2001). In the cladogram, an osteological character differenti-

ates these species: the end of the posterolateral process of the hyoid is a rounded expansion (character 74:1) in *L. plaumanni* and acute (character 74:0) in *L. gracilis*. The *L. fragilis*–*L. latinasus* pair was also considered as sibling-species (Heyer 1978) and the former differs from the latter in five characters (Fig. 8); hence, these species are morphologically distinct.

Among the characters previously used to diagnose the *L. fuscus* group (Maxson and Heyer 1988), the present study considers four states of the character fringes on toes (character 9). The species from the *L. fuscus* group presented the following states: no web or fringes (character 9:0) and weak basal fringes and/or web (character 9:1). Both states could be considered as only one: absence of web or fringes, which would be the interpretation given to this character in the group diagnosis (Maxson and Heyer 1988). Even so, it would not be a useful character to define the *L. fuscus* group. In fact, the presence of a weak basal fringe is shared (although it is not a synapomorphy) by the species of the *L. fuscus* group and those of the *L. pentadactylus* group and other species such as *L. marmoratus*, *L. andreae*, *L. discodactylus* and *L. lineatus*, excluding only the species of the *ocellatus* and *melanonotus* groups. Another character from adult morphology, considered in the group diagnosis, is the presence of two to four dorsolateral folds (character 4:1), which results in a synapomorphy of the species of the *L. fuscus* group from node 7. The presence of folds is lost in the clades *L. fragilis*–*L. latinasus* and *L. cunicularis*–*L. camaquara*. Finally, with regard to the absence of thumb spines in males (character 24:0), this character is present in the species of the traditional *L. fuscus* group and in the species of the subgenus *Lithodytes* (*L. marmoratus*, *L. andreae*, *L. lineatus*) and *L. discodactylus*, whereas the presence of thumb spines (character 24:1) appears in the *L. pentadactylus* clade. Neither of these states of characters turned out to be synapomorphies.

Analysis of some characters previously thought to have evolutionary significance

The genus *Leptodactylus* has been considered a clear example of transition from a life history closely tied to water to a more terrestrial ecology (Heyer 1969). In this transition, the *L. fuscus* group is placed as one of the last steps, because it is partially independent of the aquatic environment by placing the foam nest on land in a burrow (Heyer 1969). This behavioural character has been traditionally associated with morphological characters, which may have a tendency towards more terrestrial life and burrowing habits. Some of these characters imply a strengthening of bones of the nasal region (Heyer 1969). Some features associated with this life history strategy have been considered in this study: pars facialis of maxilla (character 38), position of tectum nasi relative to alary processes of premaxilla (character 41), anterior border of tectum nasi (character 42), mineralization of tectum nasi and solum nasi (character 43) and posterointernal angle of nasals (character 55).

The optimization of these characters shows a tendency towards more ossification of these bones, whereas the derived states include more highly developed (in size or ossification) structures of the skull's anterior region. The hypothesis of correlation between morphology and burrowing habitats would be supported in the context of the phylogeny presented here; indeed the character tectum nasi at the same level of alary

processes of premaxillae (character 41:1) is a synapomorphy of the *L. fuscus* group.

There is no evidence of a certain degree of independence from the aquatic environment in all the species of the *L. fuscus* group. The group has been thought to be in one of the last steps between an aquatic to more terrestrial mode of life (Heyer 1969; Maxson and Heyer 1988), but bio-ecological data is scanty for most species. It would be necessary to include this information to determine what 'the terrestriality' of the *L. fuscus* group suggests: construction of incubating chambers, site of amplexus, placing of eggs and early larval development. In addition, in order to make a general hypothesis about the tendency of hyperossification of the cranial characters and their relationship with shift towards a terrestrial life style, it would be useful to include more species of the other groups of the genus in the cladistic analysis.

The relationship between a more rigid nasal region and fossorial habits was proposed by Heyer (1969) and supported later by Philibosian et al. (1974), Prado et al. (2002), Reading and Jofré (2003) and de Kokubum and Giarretta (2005) but is contradicted by the observations of Pisano et al. (1993) in *L. bufonius*, who observed males of these species creating incubating chamber with their limbs. Nevertheless, until more information is available, these observations could be interpreted as complementary and not contradictory, because possibly the head and limbs could be used in different stages during the construction of the chamber (Heyer, personal communications). Indeed, Emerson (1976) stated that the anurans use both their heads and arms when burrowing.

Additionally, the observed cranial pattern coincides with that proposed by Trueb et al. (2000), who noticed that when ossification occurs in the septum nasi, tectum nasi and solum nasi, it is usually contiguous to the sphenethmoid. Also, the ossification extension is usually inversely correlated with the dermal bone sizes that forms the olfactory capsule (i.e. nasals, vomers, frontoparietals and partes facialis of maxillae). In fact, *L. ventrimaculatus* and *L. labrosus*, which do not show the ossification of the septum nasi, have a large development of nasals, which are contiguous to each other (character 52:2); they reach the frontoparietals posteriorly (character 55:1) and they are contiguous to the pars facialis of maxilla (character 38:1).

Heterochronic patterns in some characters

The phenomena that are traditionally grouped under the name of heterochrony imply permutations in the timing of event differentiation and changes in development rates, through which morphological changes and novelties originate during phyletic evolution (Alberch and Alberch 1981). These perturbations result in two possible patterns of heterochrony. One is paedomorphosis, which refers to a phenomenon where a descendant passes through fewer stages of ontogenetic development than its ancestor; hence, the adult form of the descendant will have morphological characteristics that occurred in juveniles of the ancestor (McNamara 1986). The other is peramorphosis, when the ontogeny of some trait in the descendant species can be extended relative to the ancestral species (Reilly et al. 1997).

In the present study, some of the included characters occur in adults of certain species in the same state as in juveniles (stage 46 – Gosner 1960) of closely related taxa. The species that show these characters grow from small (i.e. *L. latinasus*, snout-vent length: 31.2 ± 1.7 mm) to middle size (i.e. *L. ele-*

nae, snout-vent length: 42.7 ± 2.5 mm) relative to the dimension of the remainder of the species of the *L. fuscus* group. These characters are: frontoparietal fontanelle not totally covered by frontoparietals (character 46:0), vomerine teeth in a straight line (character 61:0), pars facialis of maxilla decreases its height in front of neopalatine level (character 37:0) and neural processes of vertebrae I–IV non-imbricate with the posterior vertebrae (character 78:0). These character states suggest patterns of heterochrony. Fink (1982, 1988) emphasized the necessity of phylogenies to polarize directions of heterochronic change between species. The optimizations on a phylogenetic tree allow distinguishing whether the characters have passed through paedomorphosis or peramorphosis. When the apomorphic state implies truncated development of the trait, relative to the development of the closely related species, the character state is paedomorphic. The opposite condition, that is, extended development in an apomorphic character state, implies that the character is peramorphic (Fink 1982). In this study, the optimizations of the characters in the cladogram showed that the condition of frontoparietal fontanelle not totally covered by frontoparietals (character 46:0), vomerine teeth in a straight line (character 61:0) and neural processes of vertebrae I–IV that are not imbricate with the posterior vertebrae (character 78:0) are derived states. The optimization of the character that describes the development degree of the pars facialis of the maxilla reveals that the state that involves less development, that is, pars facialis of maxilla ending in front of the level of neopalatines (character 37:0), is ancestral in the clade *Leptodactylus*. Although on a lower level of analysis (from node 4), the state 'pars facialis of maxilla ends at level of neopalatines' (character 37:1) is plesiomorphic and the state that involves less development appears independently in several species. In consequence, it can be deduced that when the analysis only includes the species of the genus *Leptodactylus*, three of the analysed characters are apomorphies in the state that implies truncated development, a fact which allows the hypothesis that they are paedomorphic characters.

Among the analysed species of the genus *Leptodactylus*, seven of them have individuals with the frontoparietal fontanelle not totally covered by the frontoparietals. In the evolution of the skull of other species of amphibians, an open frontoparietal fontanelle was considered to be due to paedomorphosis, for instance, in salamanders of the genus *Thorius* (Cope, 1869) (Hanken 1984). In adults of *Bolitoglossa occidentalis* (Taylor, 1941), the rate of closure towards the centre of the skull of the frontals and parietals is similar to that observed in subadults from other species of the genus (Alberch and Alberch 1981). *Idiocranium russeli* (Parker, 1936) (*Gymnophiona*) has a large nasofrontal fontanelle (Wake 1986). The open frontoparietal fontanelle gives limited dorsal protection to the brain and this lack of protection could be compensated by an extended ossification of the sphenethmoid and septum nasi, a trait which is typical of almost all the species of the *L. fuscus* group. Nevertheless, without a functional analysis, it is not possible to include mechanical explanations for the morphology of the paedomorphic characters.

The four characters show intraspecific variation. Heterochrony has been traditionally used for phylogenetic patterns or interspecific comparisons. Reilly et al. (1997) stated that heterochrony also occurs on an intraspecific level. These authors suggest a new terminology to describe heterochronic patterns in individuals within species. 'Paedotypic' is proposed

for individual variants within species that exhibit truncated development in relation to the ancestral ontogeny. Reilly et al. (1997) restricted the term 'paedogenesis' to refer to the processes that produce intraspecific heterochrony. These terms should be used in the characters considered here, if an analysis of the individual species were made, due to the intraspecific variability. The open frontoparietal fontanelle is a variable feature within *L. cunicularis*, *L. furnarius* and *L. gracilis*. The disposition of vomerine teeth varies within *L. fragilis*, *L. latinasus* and *L. cunicularis*, *L. furnarius*, *L. gracilis* and *L. plaumanni*. The extension of the neural process is variable within *L. fragilis* and *L. cunicularis*. Within a species, a variety in heterochronies produces developmental differences among individuals. These differences may or may not be heritable. Heritable heterochronies, like any other heritable character, can interact in microevolutionary processes, and in consequence, they can be lost, fixed or varied (Reilly et al. 1997). The point is, as Reilly et al. (1997) mentioned, that intraspecific heterochrony is the source of variation, resulting in interspecific heterochrony.

The major conclusions resulting from this study are that the following. (1) The *L. fuscus* group is demonstrated to be monophyletic, being more closely related to the *L. pentadactylus* group than to the others groups of species in the genus. (2) The polarizations of some characters in the cladogram support the hypothesis that the morphology of an organism reflects its particular way of life. This is because of the character states that imply strengthening of bones of the nasal region derived in the *L. fuscus* group. This is characterized by more terrestrial mode of life that implies the deposition of a foam nest in a burrow, which would be excavated with the snout. (3) The observed morphology in some species is a consequence of heterochronic patterns in some characters. The polarization of these characters in the phylogenetic hypothesis would indicate that they are paedomorphic characters.

The patterns of heterochrony together with more traditional hypotheses regarding evolutionary tendencies in the genus should be subjected to further testing, with the inclusion of the other species groups of the genus *Leptodactylus*. This will allow hypotheses testing of more general conclusions.

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Resumen

Análisis cladístico y descripción osteológica de las especies del grupo Leptodactylus fuscus (Anura, Leptodactylidae)

El género *Leptodactylus* es predominantemente Neotropical (pocas especies han colonizado el sur de la región Neártica) y está distribuido desde Texas a la Argentina y en ciertas islas del Caribe. Actualmente *Leptodactylus* está dividido en cuatro grupos de especies (los grupos *L. fuscus*, *L. melanonotus*, *L. ocellatus* y *L. pentadactylus*). Entre ellos, el grupo *L. fuscus* es el más abundante, con 27 taxa. Caracteres no verificados para la mayoría de las especies definen al grupo *L. fuscus*. La monofilia del grupo nunca ha sido testada rigurosamente en un contexto filogenético. El principal objetivo de este trabajo fue testar la monofilia y proponer una filogenia para el grupo *fuscus*. Además se realizó una descripción osteológica del grupo *fuscus*. Se construyó una matriz con 114 caracteres y 43 taxones. Se incluyeron 31 caracteres de morfología externa, 58 de osteología, 16 de condrocáneo, cinco etológicos y cuatro morfométricos. Entre las especies estudiadas 23 pertenecen al grupo interno y 20 al grupo externo. El set de datos fue analizado considerando a los caracteres bajo pesos implicados, usando el programa TNT. La monofilia del grupo es soportada en el cladograma de mayor ajuste obtenido. La optimización de algunos caracteres soporta hipótesis evolutivas tradicionales. Las optimizaciones también permitieron detectar caracteres pedomórficos en algunas especies.

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Supplementary Material

The following supplementary material is available for this article online:

Fig. S1. A. *Leptodactylus elenae*, C0:0 (longitudinal mid-dorsal stripe absent), C4:1 (2–4 dorsolateral folds). B. *Leptodactylus fuscus*, C0:2 (longitudinal mid-dorsal stripe present, to the extreme of snout), C4:2 (6 dorsolateral folds).

Fig. S2. A. *Leptodactylus elenae*: C1:0 (distinct lateral cephalic stripe), C10:0 (dark stripe on the upper lip well defined); C12:0 (dark-coloured canthal and supratympanic stripe from nostril to shoulder present), C15:0 (postympanic gland not pigmented). B. *Leptodactylus bufonius*: C1:1 (indistinct lateral cephalic stripe), C10:1 (dark stripe on the upper lip absent), C12:1 (dark-coloured canthal and supratympanic stripe from nostril to shoulder not well delimited or absent), C15:1 (postympanic gland pigmented in males).

Fig. S3. A. *Leptodactylus bufonius*: C2:0 (light-coloured stripe in the posterior surfaces of the thigh indistinct). B. *Leptodactylus elenae*: C2:1 (light-coloured stripe in the posterior surfaces of the thigh distinctive).

Fig. S4. A. *Telmatobius pisanoi*, C20:1 (fingers without fringes). B. *Hylodes nasus*, C20:0 (fingers with fringes).

Fig. S5. A. *Leptodactylus latinasus*, C64:0 (vomeres separated). B. *Leptodactylus mystacinus*, C64:1 (vomeres contiguous or in contact). C. *Leptodactylus didymus*, C31:0 (hypertrophy of the mandibular symphysis absent), C32:0 (serrations of dentary absent). D. *Leptodactylus troglodytes*, C31:1 (hyper-

trophy of the mandibular symphysis present), C32:1 (Odonotoids of dentary present). Scale line: 2 mm.

Fig. S6. (A) *Leptodactylus didymus*, C33:1 (alary processes of premaxilla dorsally directed), C40:1 (anterior extreme of maxilla with a lateral prolongation). (B) *Ceratophrys cranwelli*, C33:0 (alary processes of premaxilla posterodorsally directed). (C) *Engystomops pustulosus*, C33:2 (alary processes of premaxilla anterodorsally directed), C40:0 (anterior extreme of maxilla straight). (D) *Leptodactylus didymus*, C35:0 (base of alary processes of premaxilla sub-equal or narrower than the extreme). (E) *Engystomops pustulosus*, C35:1 (base of alary processes of premaxilla broader than the extreme). Scale line: 2 mm.

Fig. S7. (A) *Hylodes nasus*, C59:0 (cultriform process of parasphenoid with keel), C60:0 (cultriform process of parasphenoid anteriorly expanded). (B) *Engystomops pustulosus*, C60:2 (cultriform process of parasphenoid posteriorly expanded). (C) *Leptodactylus mystacinus*, C59:1 (cultriform process of parasphenoid without keel), C60:1 (cultriform process of parasphenoid expanded in the middle area). (D) *Leptodactylus didymus*, C34:0 (upper half of the alary processes of premaxillae aligned with the main axis of alary process). (E) *Telmatobius pisanoi*, C34:1 (upper half of the alary process of premaxilla slightly directed outwards and not divided, although the extremity can be divided). (F) *Hylodes nasus*, C34:2 (upper half of the alary process of premaxilla is directed outwards at an angle of approximately 45°; it ends in two acute processes). Scale line: 2 mm.

Fig. S8. (A) *Leptodactylus latinasus*, C37:0 (pars facialis of maxilla ends before level of neopalatines). (B) *Leptodactylus didymus*, C37:1 (pars facialis of maxilla ends at level of neopalatines). (C) *Leptodactylus chaquensis*, C37:2 (pars facialis of maxilla ends behind level of neopalatines). (D) *Leptodactylus elenae*, 52:0 (nasals broadly separated), C54:0 (maxillary process of nasals slightly differentiated of nasal body). (E) *Leptodactylus gracilis*, C52:1 (nasals contiguous or in contact in the middle or anterior zone), C53:0 (anterior border of nasals with deep concavity). (F) *Leptodactylus labrosus*, C52:2 (nasals contiguous or in contact along its inner border), C53:1 (anterior border of nasals slightly concave), C54:1 (maxillary process of nasals well differentiated from the nasal body). Scale line: 2 mm.

Fig. S9. (A) *Leptodactylus mystacinus*, C41:0 (tectum nasi posterior to the alary processes of premaxillae), C 42:0 (anterior border of tectum nasi straight). (B) *Leptodactylus gracilis*, C41:1 (tectum nasi at the same level of the alary processes of premaxillae), C42:1 (anterior border of tectum nasi with a slightly developed projection). (C) *Leptodactylus bufonius*, C41:2 (tectum nasi anterior to the alary processes of premaxillae), C42:2 (anterior border of tectum nasi with a well differentiated projection). Scale line: 2 mm.

Fig. S10. *Leptodactylus elenae*, C47:0 (without posterolateral projection of frontoparietals or minimal, such as a swelling). (B) *Leptodactylus chaquensis*, C47:1 (posterolateral projection of frontoparietals distinctive, relatively short). Scale line: 2 mm.

Fig. S11. (A) *Leptodactylus mystacinus*, C77:1 (intercotylar region straight). (B) *Ceratophrys cranwelli*, C77:2 (intercotylar region convex). (C) *Leptodactylus mystaceus*, C77:0 (intercotylar region concave). (D) *Leptodactylus labialis*, C46:0 (frontoparietal fontanelle not totally covered by frontoparietals), C48:0 (posterior margin of frontoparietal concave), C50:0 (frontoparietals paired). (E) *Leptodactylus elenae*, C46:1

(frontoparietal fontanelle completely covered by frontoparietals), C48:1 (posterior margin of frontoparietal straight), C49:0 (anterior portion of frontoparietals of uniform width). (F) *Leptodactylus latinasus*, C48:2 (posterior margin of frontoparietal convex). (G) *Telmatobius pisanoi*, C48:3 (posterior margin of frontoparietal protuberant), C50:1 (frontoparietals single, without space or suture that divided the frontoparietals in the posterior half). (H) *Engystomops pustulosus*, C49:1 (anterior portion of frontoparietals gradually expanding towards posterior plane).

Fig. S12. (A) *Leptodactylus mystaceus*, C72:0 (anterodorsal process of hyoid: narrow, stalk-like), C73:0 (anteromedial process absent), C74:1 (extreme of posterolateral process of hyoid: rounded expansion). (B) *Leptodactylus lineatus*, C72:1 (anterodorsal process of hyoid: broad base), C73:1 (anteromedial process present), C74:2 (extreme of posterolateral process of hyoid: expanded, posterior border concave), C76:0 (shape of posteromedial process of hyoid: distal end expanded). (C) *Engystomops pustulosus*, C72:2 (anterodorsal process of hyoid: wing-like), C74:0 (extreme of posterolateral process of hyoid: acute), C76:1 (posteromedial process of hyoid of uniform width). (D) *Leptodactylus labrosus*, C53:1 (anterior border of nasals slightly concave), C56:0 (shape of nasals: triangular). (E) *Engystomops pustulosus*, C53:2 (anterior border of nasals straight), C56:1 (shape of nasals: rhomboidal). (F) *Telmatobius pisanoi*, C53:3 (anterior border of nasals slightly convex or irregular), C56:2 (shape of nasals: claw-shape). Scale line: 2 mm.

Fig. S13. (A) *Leptodactylus labrosus*, C55:1 (postero-internal angle of nasals close to each other or in contact with frontoparietals). (B) *Leptodactylus bufonius*, C55:0 (postero-internal angle of nasals broadly separated from frontoparietals). Scale line: 2 mm.

Fig. S14. (A) *Leptodactylus latinasus*, C61:0 (vomerine teeth in a straight line), C62:1 (dentigerous process: horizontal). (B) *Leptodactylus mystacinus*, C61:1 (vomerine teeth in an arched series). (C) *Leptodactylus andreae*, C62:0 (dentigerous process of vomer: diagonal). Scale line: 2 mm.

Fig. S15. (A) *Leptodactylus gracilis*, C68:1 (otic ramus of squamosal wider than zygomatic ramus), C69:1 (otic ramus of squamosal reaches the border of parotic crista). (B) *Leptodactylus longirostris*, C68:2 (otic ramus of squamosal narrower than zygomatic ramus), C69:2 (otic ramus of squamosal overlap to the parotic crista). (C) *Leptodactylus bufonius*, C68:1 (otic ramus of squamosal as broad as zygomatic ramus), C69:0 (otic ramus of squamosal does not contact with the parotic crista). Scale line: 2 mm.

Fig. S16. (A) *Leptodactylus chaquensis*, C85:0 (humeral crista in males present). (B) *Leptodactylus didymus*, C85:1 (humeral crista in males absent). Scale line: 2 mm.

Fig. S17. (A) *Leptodactylus chaquensis*, C86:0 (terminal phalanges: rounded or knobbed). (B) *Leptodactylus mystaceus*, C86:1 (terminal phalanges: rounded and bifurcate). (C) *Leptodactylus lineatus*, C86:2 (terminal phalanges: T-shape). Scale line: 2 mm.

Fig. S18. (A) *Telmatobius pisanoi*, C87:0 (angle between anterior acetabular expansion and ilial shaft: 90°). (B) *Leptodactylus mystacinus*, C87:1 (angle between anterior acetabular expansion and ilial shaft: less than 90°). (C) *Leptodactylus latinasus*, C78:1 (neural spine of vertebrae I–V: not imbricates). (D) *Leptodactylus mystacinus*, C78:2 (neural spine of vertebrae I–V: imbricates). Scale line: 2 mm.

Fig. S19. (A) *Leptodactylus didymus*, C80:0 (xiphisternum: trapezoidal or semicircle). (B) *Leptodactylus lineatus*, C80:1 (xiphisternum: V-shape). (C) *Engystomops pustulosus*, C80:2 (xiphisternum: double). (D) *Hylodes nasus*, C80:4 (xiphisternum: sub-rectangle). (E) *Crossodactylus gaudichaudii*, C80:5 (xiphisternum: mineralized and quadrangular anterior region, with two posterior cartilaginous prolongations).

Fig. S20. (A) *Leptodactylus mystacinus*, C58:0 cultriform process of parasphenoid between neopalatines). (B) *Leptodactylus andreae*, C58:1 (cultriform process of parasphenoid does not reach neopalatines). (C) *Leptodactylus jolyi*, C81:0 (posterior half of mesosternum of uniform width). (D) *Leptodactylus didymus*, C81:1 (expanded, but markedly narrower than the anterior extreme). (E) *Leptodactylus lineatus*, C81:2 (almost as expanded as the anterior extreme). (F) *Engystomops pustulosus*, C81:3 (posterior half of mesosternum: bifid). Scale line: 2 mm.

Appendix S1. Species and material examined for morphological data collection.

Appendix S2. External morphology characters: osteological characters, characters of larval chondrocranium (Larson and De Sá 1998), ethological characters, morphometric characters (Heyer 1978).

Appendix S3. Data matrix.

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