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Internal larval characters in anuran systematic studies: a phylogenetic hypothesis for *Leptodactylus* (Anura, Leptodactylidae)

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Abstract

There are few systematic studies based on internal buccal and larval cranium morphology of anuran tadpoles. Here we hypothesized phylogenetic relationships of frogs of the *Leptodactylus* genus with 84 internal larval characters, where 63 of them were described for the first time. We recovered *Leptodactylus* as monophyletic with two major clades. A similar topological arrangement was found by combining the larval with 98 adult morphology characters. PBS analysis revealed that the two data sets contributed differentially to establish major clades of *Leptodactylus* in the overall tree. This result was corroborated by the IDL test, which also indicated incongruences between data sets. Together with an overview of internal larval descriptions and discussions about the performance of these characters to reconstruct the phylogeny of *Leptodactylus* (i.e. homologies and homoplasies), we also provided information regarding intraspecific and populational variation found among the morphologies of the tadpoles sampled.

Key words: *Leptodactylus* – buccal morphology – larval cranial – phylogeny

Introduction

The application of larval characters in anuran systematic studies, although incipient, is relatively old. Lataste (1879), Noble (1926) and Orton (1953, 1957) proposed the use of morphological features such as the spiracle (structure through which water is evacuated from the branchial chambers) and external mouthparts of tadpoles to complement the classification of Anura. Orton (1953) grouped the described tadpoles in four basic types and pointed out that some characters are quite variable while others are more conserved. However, some authors questioned the validity of using these characters in systematic analysis (Griffiths 1963; Griffiths and de Carvalho 1965; Sokol 1975). This controversy proceeds from the low number of characters analysed by Lataste (1879), Noble (1926) and Orton (1953), which could cause misinterpretations regarding systematic relationships among families of Anura.

Internal larval characters tend to be phylogenetically conserved (i.e. characters show low homoplasy), having a great potential for use in phylogenetic studies (Andrade et al. 2007) that could be used together with adult characters and molecular data (Wassersug 1980). Diverse systematic studies using both internal buccal and larval cranial structures have been conducted (e.g. Wassersug and Heyer 1988; Larson and de Sá 1998; Haas 2003; Pugener et al. 2003), expanding our knowledge of the morphological diversity of tadpoles and anuran systematics. Considering the family Leptodactylidae Werner, 1896, larger systematic studies with these types of characters are absent, with the exception of some studies that have described the internal buccal morphology (e.g. Wassersug and Heyer 1988; Vera Candiotti et al. 2007) and larval cranium (Larson and de Sá 1998; Vera Candiotti et al. 2007). Wassersug and Heyer (1988) analysed buccal microanatomy of eight species of *Leptodactylus* Fitzinger, 1826. Larson

and de Sá (1998) conducted an extensive description, comparison and a phylogenetic inference from the morphological features of larval cranium in 22 species of *Leptodactylus*. But studies involving the use of both sets of characters (internal buccal morphology and larval cranial) in *Leptodactylus* systematics have not been conducted.

Until recently, Leptodactylidae was a polyphyletic assemblage divided into five subfamilies (Ruvinsky and Maxson 1996; Frost et al. 2006). Frost et al. (2006) combined anatomical characters described by Haas (2003) and molecular data to establish a new system for Lissamphibia. Grant et al. (2006) analysed molecular and phenotypic characters (morphology of adults and larvae, skin secretion molecules and behavioural data) and implemented changes in the systematics proposed by Frost et al. (2006). Pyron and Wiens (2011) with a comprehensive sampling of taxa proposed a new molecular phylogenetic hypothesis for Amphibia. As a result of these studies, Leptodactylidae is currently composed of 200 species allocated in three subfamilies: Leiuperinae Bonaparte, 1850; Leptodactylinae Werner, 1896; and Paratelmatobiinae Ohler and Dubois, 2012 (Pyron and Wiens 2011; Frost 2014).

In the Leptodactylinae, *Leptodactylus* is the most diverse genus, containing 76 species distributed from North America (southern Texas) through Central and South America. Heyer (1969) described five phenetic groups in the genus based on behavioural attributes, morphology and ecology of adults. Although several systematic studies of these groups were performed by Heyer (1970, 1973, 1974, 1978, 1979, 2005), the phylogenetic relationships of members of *Leptodactylus* and the family Leptodactylidae are not fully resolved (e.g. Larson and de Sá 1998; Frost et al. 2006; Ponssa 2008).

In an early phylogenetic study of *Leptodactylus*, Heyer (1998) used characters of adult morphology and larval buccal morphology, morphological features of eggs and vocalization parameters to demonstrate that *Leptodactylus* is paraphyletic in relation to *Adenomera* Steindachner, 1867, *Lithodytes* Fitzinger, 1843, and *Vanzolinius* Heyer 1974. Frost et al. (2006) suggested that the genus *Leptodactylus* was monophyletic only if *Adenomera* and *Lithodytes* are included, and the genus *Vanzolinius* was

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a synonym of *Leptodactylus*. Nevertheless, most authors recently rejected these conclusions (e.g. Ponssa 2008) because only few species had been sampled. Pyron and Wiens (2011) and Fouquet et al. (2013) considered *Adenomera* as a monophyletic valid genus. The phylogenetic hypothesis of Larson and de Sá (1998), based on characters of the larval cranium, neither supported the monophyly of the genus *Leptodactylus* nor the relationships among the groups proposed by Heyer (1969). Ponssa (2008) published a phylogenetic hypothesis for the *L. fuscus* group, based on external morphology, osteology, larval cranial morphology and behaviour. She corroborated the monophyly of the *L. fuscus* group and hypothesized that the *L. pentadactylus* and *L. fuscus* groups were sister groups.

We report on a comparative analysis of the larval internal buccal morphology and larval cranial of species of *Leptodactylus* to: (1) describe and investigate the use of new larval characters in phylogenetic studies of *Leptodactylus*, (2) propose a phylogenetic hypothesis for the genus, (3) assess the impact of larval characters on a combined tree, generated by larval (present work) and adult (previous analysis) characters and (4) evaluate congruence between these different datasets.

Material and Methods

Sampling

Tadpoles and eggs of *Leptodactylus* species were collected in the field, and additional biological materials were obtained from scientific collections. Nineteen species of 30 different populations were sampled (Table S1). Three tadpoles, preferably at Stage 36 (Gosner 1960), of each species were dissected to examine internal buccal morphology, and three were also cleared and stained to examine the larval cranium.

Internal buccal morphology

The floor and roof of the oral cavity were exposed following the dissection procedure described by Wassersug (1976). Mouthparts were stained with methylene blue 5% and Sudan Black B + methylene blue, submerged in water and photographed with a stereomicroscope equipped with a digital photographic system. The identification and description of buccal structures are based on the terminology proposed by Wassersug (1976).

Larval cranial morphology

Tadpoles were cleared and stained following the protocols of Dingerkus and Uhler (1977) and Song and Parenti (1995), with modifications. Observations, measurements and photographs of larval cranium structures were conducted with a stereomicroscope with eyepiece micrometre and digital photographic system. The identification and description of the structures are based on terminology employed by Larson and de Sá (1998), Cannatella (1999) and Haas (2003).

Phylogenetic analyses

Characters coded based on previous studies – 12 characters of Larson and de Sá (1998) and nine characters of Prado (2006) – were used as described by the authors or adapted as needed (Tables S2 and S3). Character states of the internal buccal and larval cranium morphology for *Leptodactylus gracilis* (Duméril and Bibron, 1840), *L. notoaktites* Heyer 1978 and *L. pustulatus* (Peters, 1970) are based on published descriptions (Wassersug and Heyer 1988; Larson and de Sá 1998; de Sá et al. 2007a, b) and accounts of the buccal microanatomy of *L. labyrinthicus* (Spix, 1824), *L. petersii* (Steindachner, 1864), *L. podicipinus* (Cope, 1862) and *L. vastus* Lutz, 1930 by Vieira et al. (2007) and Miranda and Ferreira (2008, 2009).

Among the 76 species of the genus *Leptodactylus*, 19 were sampled in this study (Table S1). Five species of four genera that have been suggested to be close relatives of *Leptodactylus* (Larson and de Sá 1998;

Frost et al. 2006; Grant et al. 2006; Ponssa 2008) were used as outgroups: *Alsodes vanzolinii* (Donoso-Barros, 1974); *A. verrucosus* (Philippi, 1902) (Alsodidae); *Ceratophrys cranwelli* Barrio, 1980 (Ceratophryidae); *Telmatobius scrocchii* Laurent and Lavilla, 1986 (Telmatobiidae); and *Crossodactylus gaudichaudii* Duméril and Bibron, 1841 (Hyloidae). Character states of outgroups were obtained from the literature (Lavilla and Fabrezi 1992; Larson and de Sá 1998; Ramón Formas and Brieva 2004; Vera Candioti 2005; Weber and Caramschi 2006; Vera Candioti 2008). Characters varying between populations were treated as polymorphisms and were included in the analysis. Maximum parsimony analyses using heuristic searches were implemented in PAUP 4.0b1 (Swofford 1998) and TNT 1.1 (Goloboff et al. 2003). Treelength, indices (consistency and retention) and character changes in the clades of cladograms were analysed with MACCLADE 4.0 (Maddison and Maddison 1992) and MESQUITE 2.71 (Maddison and Maddison 2009). In heuristic searches, the most parsimonious trees were found by 2000 multiple random addition sequences, and the method of branch swapping was tree bisection and reconnection (TBR), retaining replicate trees. Support for clades was obtained by bootstrap analysis of 10 000 pseudoreplications (Felsenstein 1985; Hillis and Bull 1993; Müller 2005) and by Bremer support (Bremer 1994). Bootstrap values were calculated by PAUP 4.0b1 and Bremer support by TNT 1.1. All series of transformations were considered as unordered and unweighted.

Impact of larval characters to infer *Leptodactylus* phylogeny and homogeneity test

We also assessed the impact of larval characters to infer the relationships of species of the *Leptodactylus* genus. To evaluate this impact, we conducted the following steps: (1) we combined the larval partition generated in the present work with the data set of Ponssa 2008 (see Fig. 1 representing the schematic diagram of partitions used in the combined analysis). Note that we excluded Ponssa's larval characters because these originated from Larson and de Sá's (1998) work and were replaced by our own data. In our data matrix, characters 1–98 are derived from Ponssa (2008) and characters 99–182 were from this work (Data S1). Only species sampled in both studies were included in the combined analysis resulting in 18 terminal taxa, and (2) a phylogenetic analysis was performed (under maximum parsimony) with these two partitions combined. The most parsimonious trees were obtained by 2000 multiple random addition sequences using the method of branch swapping (TBR) (TNT 1.1), and (3) by performing a Partitioned Bremer Support (PBS) analysis (Gatesy et al. 1999). PBS examines the impact of each partition (among multiple partitions) to the overall tree, estimating support values to each node produced by each data set. PBS analysis was conducted in TNT 1.1 by using the script published by Peña et al. (2006).

A test of homogeneity (incongruence length difference ILD) (Farris et al. 1995; Dolphin et al. 2000) to verify congruence between the two partitions was implemented in PAUP, with 1000 replications, random additions of taxa and heuristic search.

Results

Overview of *Leptodactylus* internal larval morphology

Overall, the buccal morphology among *Leptodactylus* tadpoles presented two remarkable features: the diversity of structures and reduction (in size and quantity) of these structures (Fig. 2). The infralabial papillae are small and simple in most species studied, except in *L. riveroi* where those are more complex. The number of papillae delimiting of the buccal floor arena showed a large variation. Some tadpoles as *L. chaquensis* and *L. latrans* have more papillae (Fig. 2A). Also, the presence of a glandular zone is clearly noticed in the species of aquatic habits such as *L. natalensis*, *L. latrans*, *L. petersii*, *L. podicipinus* and *L. pustulatus* (Fig. 2B–D). These species are known to deposit the spawn directly in water bodies and tadpoles developed entirely in the aquatic environment (Heyer 1969). Conversely in tadpoles of *L. riveroi*, *L. rhodomystax*, *L. vastus*, *L. knudseni* and *L. labyrinthicus* (Fig. 2E–F), buccal structures are particularly reduced.

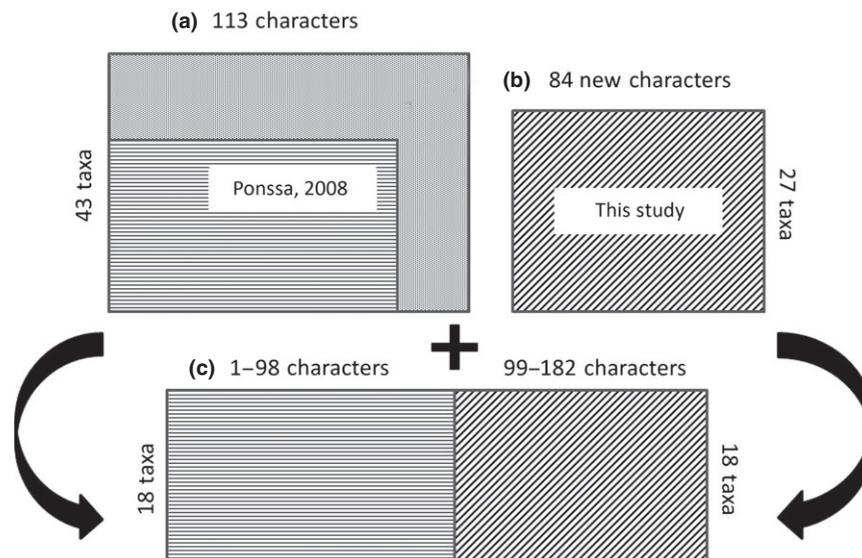


Figure 1. Schematic diagram representing how different matrices were combined to this study. One data set was taken from Ponssa (2008), originally containing 43 taxa and 113 characters (complete square at top left). We deleted Ponssa's larval matrix characters [99–113] (cross-hatched; a), eliminating 25 species and 15 characters. The second was obtained from this work, eliminating 16 species (diagonal stripes; b). The resulting matrix is then shown in c: characters 1–98 are derived from Ponssa (2008) (horizontal stripes) and characters 99–182 from this work (diagonal stripes; 84 characters). Only species sampled in both studies were included in the analysis. The total data set examined in this study therefore represented 18 taxa and 182 characters.

The reduction in the number of papillae in the buccal roof arena is a widespread feature in *Leptodactylus*, reaching the full absence in *L. riveroi* (Fig. 2G).

Among larval cranium, we found nine features common to all *Leptodactylus* species sampled: *processus posterior dorsalis* in alar of suprarrostral; crista parotica; commissura quadratoorbitalis; *processus dorsomedialis*, *p. ventromedialis* and *p. retroarticularis* present in Meckel's cartilage; ceratobranchials fused to the hypobranchial plates; lateral projections present in ceratobranchials I, II and III; *p. branchialis anterior*; palatoquadrate narrow anteriorly and broad and slightly rounded posteriorly and otic capsules representing about 30% of the total cranial length (Fig. 3).

To illustrate the structures of floor and roof of buccal cavity, larval cranium was analysed in the present work (see Figs 2 and 3). These figures help to understand how characters were delimited and coded. Full morphological descriptions of buccal anatomy and larval cranium of *Leptodactylus* species are in preparation for further publication.

Phylogeny reconstruction using larval characters

Our anatomical analysis resulted in a matrix of 42 characters of internal oral features and 42 larval cranium characters (Data S2). Characters are listed in Table S2 (internal oral morphology) and Table S3 (larval cranium morphology), where 63 of them were described for the first time (35 from internal oral and 28 of larval cranium morphology). In the 24 taxa examined, three of the 84 characters were uninformative. Heuristic searches resulted in four most parsimonious trees (Data S3) with an optimal parsimony score of 417 steps. The strict consensus cladogram (Fig. 4) has 423 steps, a consistency index of 0.371, a consistency index excluding uninformative characters of 0.366 and a retention index of 0.484.

The genus *Leptodactylus* is recovered as monophyletic, supported by the following buccal morphological characters (Character number: State; Data S2): triangular buccal floor arena (10:1); slightly prominent projections on the posterior margin of ventral velum (17:1); postnarial arena small (27:0); lateral ridge papillae

small (30:1); and glandular zone of the dorsal velum occupying half the surface (41:1). Larval cranium characters supporting the monophyly of *Leptodactylus* are as follows (Character number: State; Data S2): ventromedial fusion of the *corpus* of the supra-rostral narrower than the body (43:1); presence of ventrolateral projections in the *corpus* of supra-rostral (44:1); posterolateral extension of the palatoquadrate extends beyond the anterior margin of otic capsules (63:3); angle between the posterior margin of the *processus ascendens* and the braincase between 70° and 80° (65:1); presence of the commissura quadratoorbitalis (70:1); Meckel's cartilage long and curved (71:1); *pars reuniens* and Copula II with the same length (74:0); *processus urobranchialis* wide (77:1); lateral process of the ceratobranchial triangular (78:1); and presence of projections just in the posterior portion of ceratobranchials (84:2).

Our phylogenetic analysis of larval characters produced two major clades within *Leptodactylus* (Fig. 4). One (clade 1) is composed of species traditionally assigned to the *L. fuscus* group (*L. bufonius* Boulenger, 1894; *L. camaquara* Sazima and Bokermann, 1978; *L. latinasus* Jiménez de la Espada, 1875; *L. troglodytes* Lutz, 1926; *L. notoaktites*; *L. mystacinus* (Burmeister, 1861); *L. tapiti* Sazima and Bokermann, 1978; *L. furnarius* Sazima and Bokermann, 1978; *L. gracilis*; *L. spixi* Heyer, 1983; and *L. fuscus*), the *L. latrans* group (*L. chaquensis* Cei, 1950 and *L. latrans*) and the *L. melanonotus* group [*L. natalensis* Lutz, 1930; *L. petersii*, *L. podicipinus* and *L. pustulatus* (Peters, 1870)]. *Leptodactylus bufonius* and *L. camaquara* are successive basal branches in this clade, which is supported by the following characters of internal oral anatomy: infralabial posterolateral papillae conical or triangular (5:1); triangular median ridge (28:3); and absence of pustules and/or papillae on the posterior and/or bottom of the dorsal velum (42:0). Four larval cranium characters also support the clade 1: otic capsules representing 30% or more of the length of larval cranium (54:1); *cornua trabeculae* representing over 20% of the length of larval cranium (60:0); posterolateral extension of the palatoquadrate reaching the level of attachment of *processus ascendens* to the braincase (63:1); and hypobranchial plates separated (80:0).

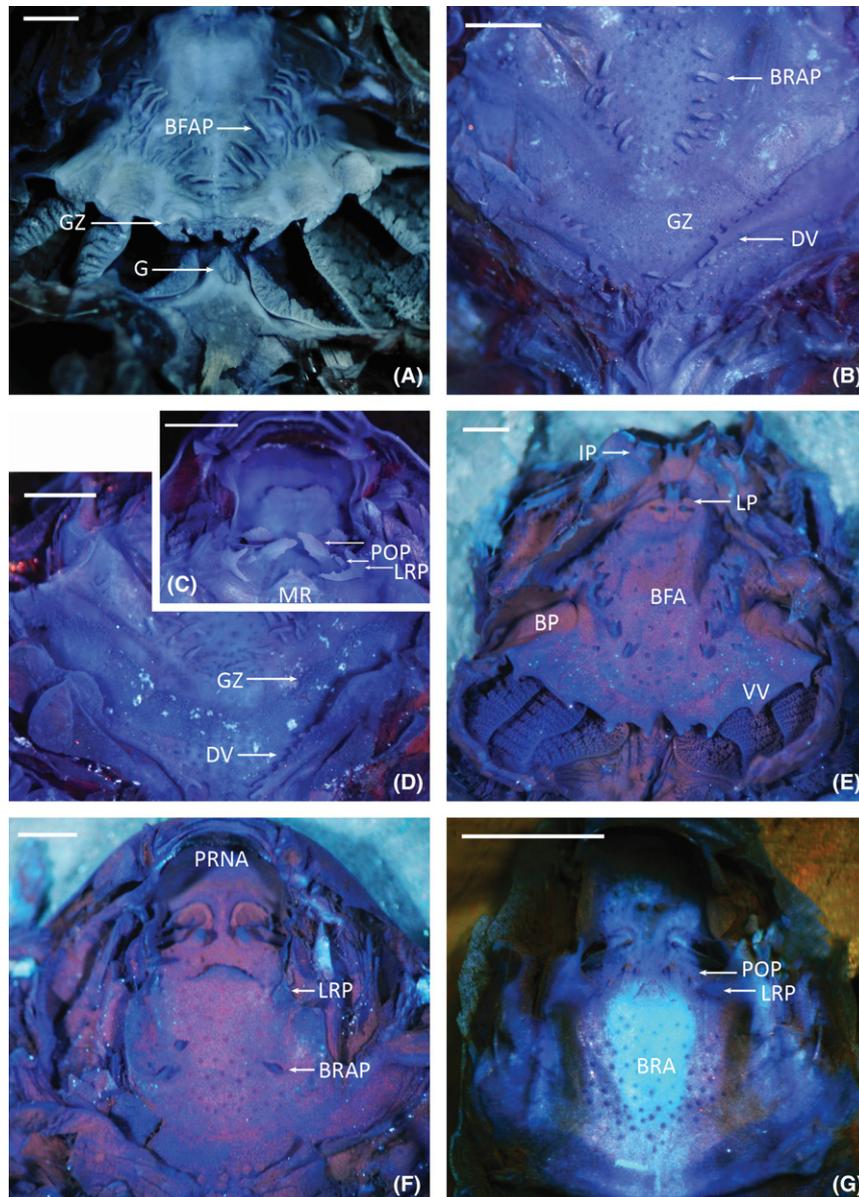


Figure 2. Overview of internal buccal morphology: (A) buccal floor arena papillae and glandular zone in buccal floor of *Leptodactylus latrans*, stage 36 (Gosner 1960); (B) glandular zone in buccal roof of *L. chaquensis*, stage 37; (C and D) prenarial and postnarial arena, postnarial papillae, lateral ridge papillae, median ridge and glandular zone of the buccal roof of *L. latrans*, stage 36; (E and F) reduction of the structures in buccal floor and roof of *L. knudseni*, stage 34; (G) absence of the buccal roof arena papillae in *L. riveroi*, stage 26. BFA, buccal floor arena; BP, buccal pocket; BFAP, buccal floor arena papillae; BRA, buccal roof arena; BRAP, buccal roof arena papillae; DV, dorsal velum; G, glottis; GZ, glandular zone; IP, infralabial papillae; LP, lingual papillae; LRP, lateral ridge papillae; MR, median ridge; POP, postnarial papillae; PRNA, prenarial arena; VV, ventral velum. Scale bars = 1.0 mm.

The other clade (clade 2; Fig. 4) comprises the species *Leptodactylus labyrinthicus*, *L. knudseni* Heyer, 1972 and *L. vastus*, which traditionally have been assigned to the *L. pentadactylus* group. This clade is defined by five character states of internal buccal anatomy: central papillae anteriorly positioned in relation to lateral papillae (9:1); buccal floor arena rectangular or trapezoidal (10:3); more than 30 pustules on the buccal floor arena (11:2); five or less papillae limiting the buccal roof arena (13:1); and posterior wall of the nostrils low (24:0). Among the larval cranium characters are suprarostrals *corpus* and *alas* of the same width (47:0); a distinct *processus posterolateralis* at the *crista parotica* (62:0); and Meckel's cartilage short and curved (71:0).

Bootstrap and Bremer supports indicate that *Leptodactylus* is a monophyletic clade well supported in the analyses with 82% and

decay index of 5, respectively. The clade (*L. vastus* + (*L. knudseni* + *L. labyrinthicus*)) has a bootstrap support of 79% and Bremer support of 5. The clade composed of species of groups *L. fuscus*, *L. melanonotus* and *L. latrans* has a Bremer value of 3. The clade ((*L. chaquensis* + *L. latrans*) + (*L. pustulatus* + (*L. podicipinus* + (*L. natalensis* + *L. petersii*)))) has a bootstrap support of 60% and a Bremer of 5.

Impact of larval characters to infer *Leptodactylus* phylogeny and homogeneity test

The analysis of 182 characters and 18 taxa (Data S1) reveals 18 constant characters, and 34 uninformative and 130 informative characters. We recovered 25 most parsimonious trees (Data S4)

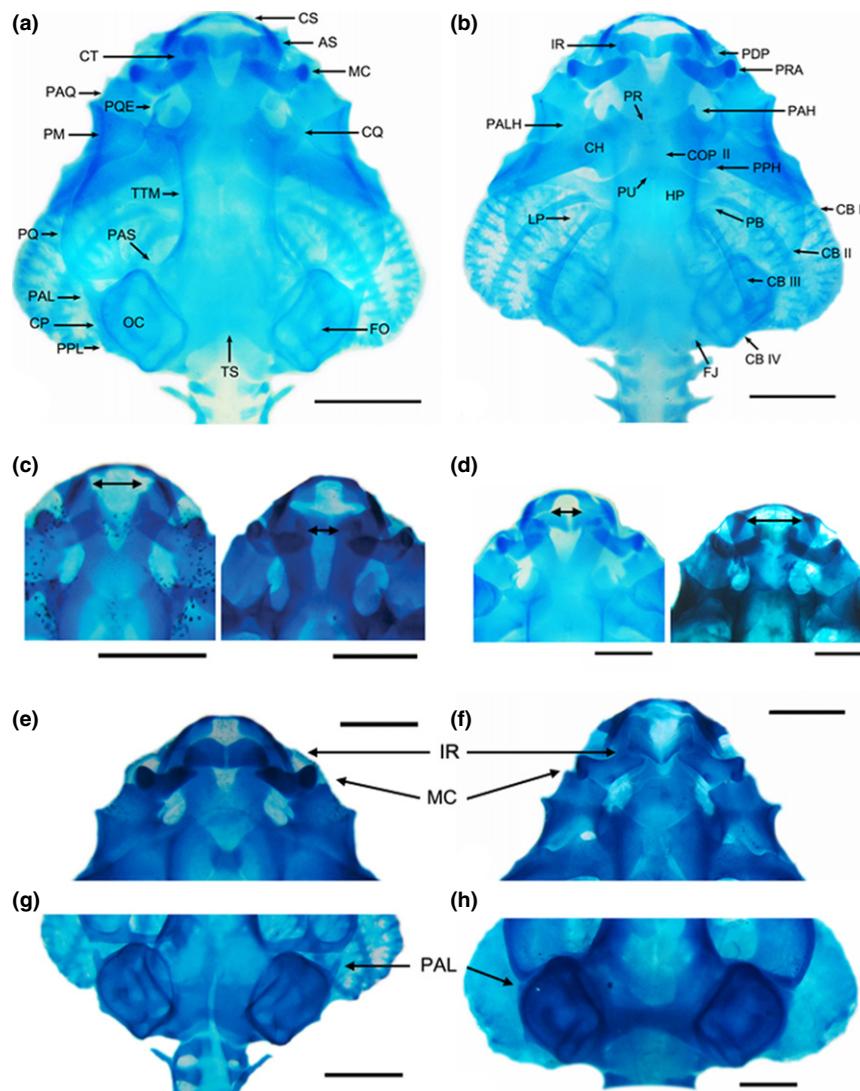


Figure 3. Representations of larval cranium morphologies of *Leptodactylus* tadpoles: (a–b) dorsal and ventral overviews of *L. fuscus*, stage 38 (Gosner 1960); (c) the arrows represent the two states of the Character 56 (angle between *cornua trabeculae* in relation to the width of larval cranium) of *L. camaquara*, stage 29 (state 1, left figure) and *L. furnarius*, stage 32 (state 0, right figure); (d) the arrows represent the two states of the Character 49 (space between the *corpus* of suprarostrals) of *L. troglodytes*, stage 35 (state 0, left figure) and *L. chaquensis*, stage 35 (state 1, right figure); (e–f) robustness of infraorbital elements and states of Meckel's cartilage, *L. knudseni*, stage 33 (more robust, state 0) and *L. tapiti*, stage 39 (less robust, state 1); (g–h) represent two states of the Character 61 (*processus anterolateralis* of crista parotica), *L. furnarius*, stage 32 (state 3) and *L. vastus*, stage 34 (state 1). AS, alar of suprarostrals; CB, ceratobranchials; CH, ceratohyal; COP, copula; CP, crista parotica; CQ, commissura quadratoorbitalis; CS, *corpus* of suprarostrals; CT, *cornua trabeculae*; FJ, *foramen jugulare*; FO, *fenestra ovalis*; IR, *infraorbital*; HP, *hypobranchial plates*; LP, *lateral projections*; MC, Meckel's cartilage; OC, *otic capsule*; PAH, *p. anterior hyalis*; PALH, *p. anterolateralis hyalis*; PAL, *processus anterolateralis*; PAQ, *pars articularis quadrati*; PAS, *p. ascendens*; PB, *p. branchialis*; PDP, *p. dorsalis posterior*; PM, *p. muscularis quadrati*; PPL, *p. posterior lateralis*; PPH, *p. posterior hyalis*; PQ, *palatoquadrate*; PQE, *p. quadratoethmoidalis*; PR, *pars reuniens*; PRA, *p. retroarticularis*; PU, *p. urobranchialis*; TS, *tectum synoticum*; TTM, *taenia tecti marginalis*. Scale bars = 1.0 mm.

with 487 steps each. The strict consensus cladogram (Fig. 5a) has 557 steps, a consistency index of 0.472, a consistency index excluding uninformative characters of 0.424 and a retention index of 0.309.

The monophyly of the genus *Leptodactylus* is supported, but there are some polytomies (Fig. 5a). However, close relationships among the species allocated to the *L. fuscus*, *L. latrans*, *L. melanotus* and the *L. pentadactylus* groups are maintained. Thirty-five characters with unambiguous changes support the *Leptodactylus* clade; among these, 12 characters were provided by Ponssa (2008).

It is expected that if the PBS results' partitions (in this case, larval and adult characters; see Fig. 1) support a relationship rep-

resented by a node in the combined tree, then the PBS value will be positive. If, conversely, a partition supports an alternative relationship, the PBS value will be negative. The magnitude of PBS values indicates the level of support for or disagreement with a node. Our results of PBS test revealed that each data set (larval and adult characters) was congruent and supports monophyly of the *Leptodactylus* clade (Fig. 5a; both positive values). Although, this contribution shows to differentially support this arrangement (2.8 for adult characters and 6.2 for larval characters). The clade formed by *Leptodactylus chaquensis* + *L. podicipinus* and the other by *L. knudseni* + *L. labyrinthicus* (Fig. 5a) contributed differentially for this arrangement (both -5.2 for adult characters and 8.2 for larval characters). Adult and larval

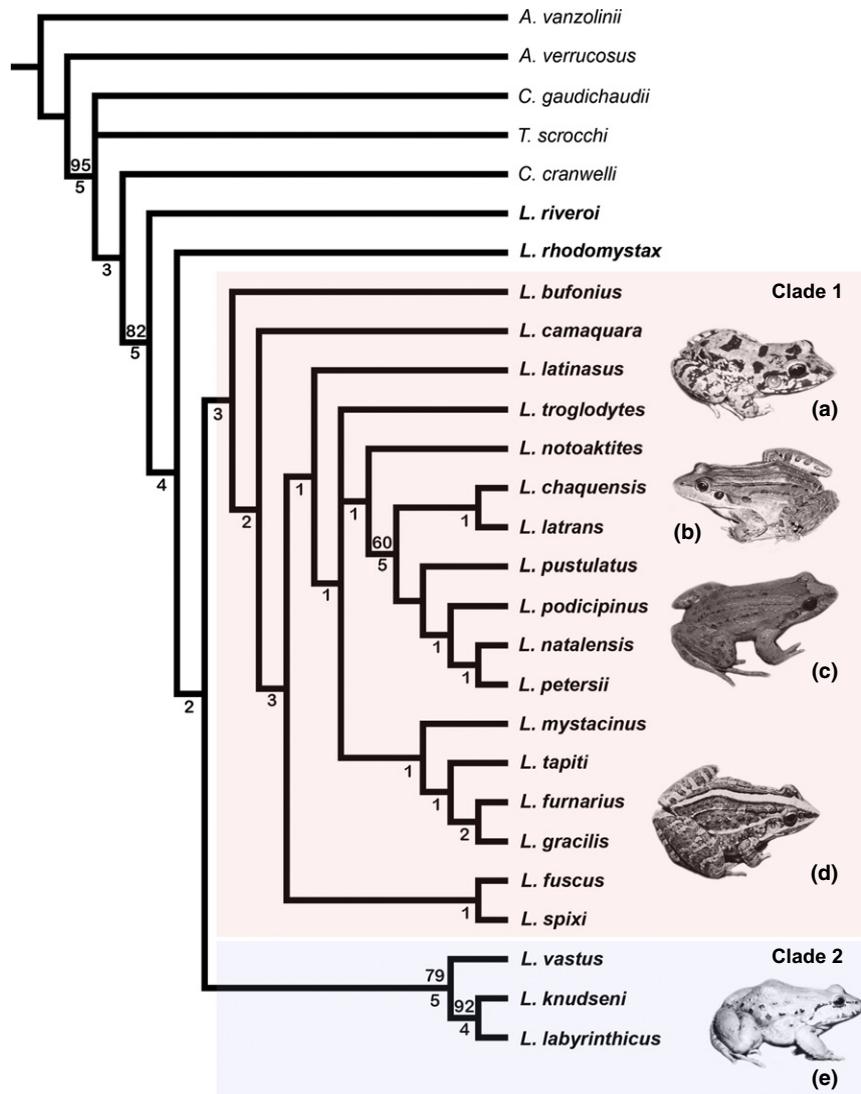


Figure 4. Strict consensus cladogram from the maximum parsimony analysis of 84 larval characters (length = 423 steps, CI = 0.366 and RI = 0.424; excluding uninformative characters). Numbers above nodes are bootstrap values and below the Bremer support values. (a) *Leptodactylus troglodytes* (*L. fuscus* group); (b) *L. latrans* (*L. latrans* group); (c) *L. podicipinus* (*L. melanonotus* group); (d) *L. fuscus* (*L. fuscus* group); and (e) *L. labyrinthicus* (*L. pentadactylus*). Photos a, b and e by Antonio Sebben. Photos c and d by Ariovaldo A. Giaretta.

characters support the clade formed by *L. knudseni* + *L. labyrinthicus* + *L. rhodomystax*; however, both contributed with small values to this representation (0.8 for adult characters and 0.2 for larval characters; Fig. 5a).

The homogeneity test showed that the compared partitions (present work and Ponssa's) are incongruous ($p < 0.001$).

The 50% majority rule consensus tree of the combined analyses (Fig. 5b) is more similar to the tree based only on larval characters. We recovered a large clade composed by species of the *L. fuscus*, *L. latrans* and *L. melanonotus* groups, and another composed by species of the *L. pentadactylus* group. The bootstrap analyses of the strict consensus tree support the *Leptodactylus* clade to a level of 96%.

Discussion

Polymorphisms found in internal larval morphologies of *Leptodactylus*

We recorded intraspecific and populational morphology variation among tadpoles sampled. Intraspecific and intrapopulational vari-

ations in buccal morphology were equally observed between the left and right sides of the same individual. The features with variations were the amount of pustules throughout arenas of floor and buccal roof and the amount of papillae rounding it, as well as the presence of pustules or papillae in the region anterior to buccal pocket. These kinds of variation (quantity) are expected and therefore should not be used to systematic of tadpoles (Wassersug and Heyer 1988). But the disproportion among variations (extensions) observed could be used for phylogenetic reconstruction purpose.

Interpopulational variations in buccal morphology could be evaluated only in cases of species sampled for more than one locality: *L. chaquensis*, *L. fuscus*, *L. mystacinus*, *L. latrans*, *L. podicipinus* and *L. troglodytes*. *Leptodactylus chaquensis* and *L. latrans* presented variation in infralabial papillae. In the first species mentioned, we found four infralabial papillae in Argentinian populations and three in the population from Corumbá municipality, Mato Grosso do Sul state, Brazil. Populations of *L. latrans* from Brazilian states of Paraná, São Paulo and Roraima presented three infralabial papillae, while population of

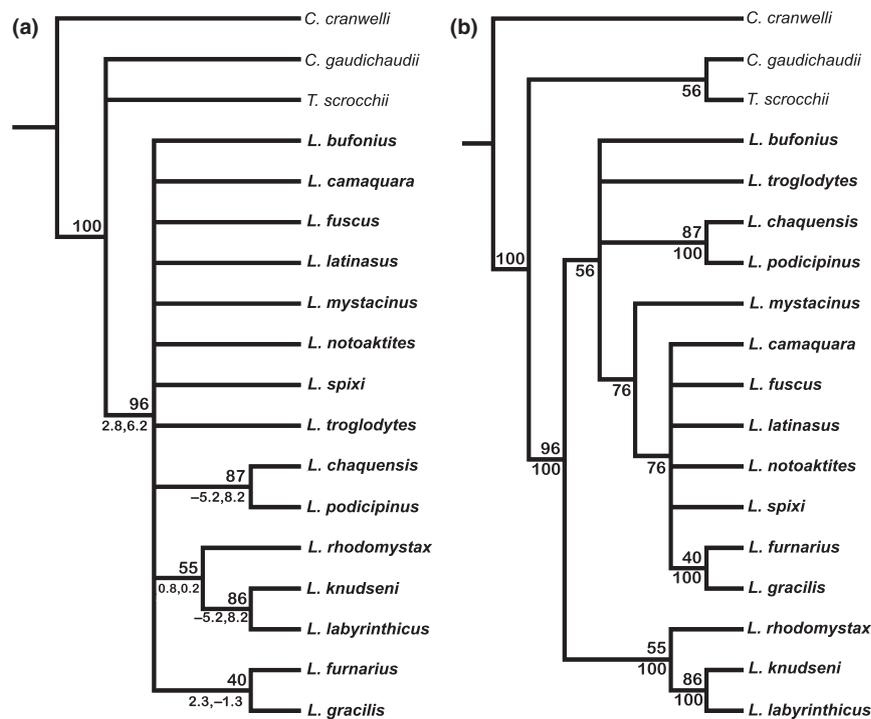


Figure 5. Combined maximum parsimony analysis of 98 characters of external morphology, osteology, ethology and morphometric data of adult specimens (Ponssa 2008) and 84 larval characters. (a) strict consensus cladogram (length = 557 steps, CI = 0.472 and RI = 0.309). Numbers above nodes are bootstrap values, and PBS values are found below nodes inside squares (adult characters left, larval characters right). (b) 50% majority rule consensus cladogram (length = 11 steps, CI = 0.580, RI = 0.550). Numbers above nodes are bootstrap values and below the frequencies of clades.

Seropédica municipality, Rio de Janeiro state, presented five. Populations of *L. latrans* also presented variations in lingual papillae. The populations from Brazilian states of Paraná, São Paulo and Roraima presented four lingual papillae, and population of Rio de Janeiro state presented three lingual papillae. The amount of dispersed pustules on the arena of buccal floor was a quite variable character, being all the species already mentioned presenting such variation. The number of papillae limiting the arena buccal floor varied in populations of *Leptodactylus fuscus*. Population from Chapada dos Guimarães National Park in Mato Grosso Brazilian state showed a greater amount of papillae limiting the arena (Miranda and Ferreira 2009). Differences in height of the nostrils posterior wall were observed in tadpoles of *L. mystacinus* and *L. podicipinus* (Miranda and Ferreira 2009). Tadpoles of *L. mystacinus* from Argentina presented four post-auricular papillae per side and the individuals collected in Brasília, Federal District, Brazil, two papillae per side. The median ridge was relatively small in species examined as already demonstrated by Wassersug and Heyer (1988), although the shape gradually varied between semicircular to triangular in *L. fuscus*, *L. latrans* and *L. troglodytes*. The shape of lateral ridge papillae varied in tadpole populations of *L. latrans*. Individuals from Paraná, São Paulo and Rio de Janeiro Brazilian states presented ramificated and complex papillae, while tadpoles from Roraima Brazilian state presented chela-shaped lateral ridge papillae. The shape of the buccal roof arena presented by *L. fuscus* and *L. podicipinus* varied between triangular and trapezoidal. Differences in amount of pustules in the buccal roof arena were observed in tadpoles of *L. podicipinus* as already verified by Miranda and Ferreira (2009).

Differences in the buccal morphology and larval cranium presented less intraspecific and populational variations. Features presented intraspecific variation were ceratobranchial process, ornamentations of etmoidal plate and fusion of infrarostral ele-

ments. Interpopulational variations also were observed. The population of *Leptodactylus fuscus* from Monte Alegre municipality, Goiás state, Brazil, showed a *processus dorsalis posterior* of alar widest than the remaining populations examined. Adostrals were observed in *L. mystacinus* tadpoles from Brasília, Federal District, Brazil, and *L. troglodytes* of Monte Alegre municipality. However, we could not observe this feature in Argentinian tadpole populations of *L. mystacinus* and *L. troglodytes* from João Pessoa municipality, Paraíba state, Brazil. Differences in length and width ratio of larval cranial were found in *L. chaquensis*, *L. mystacinus* and *L. latrans*. The frontoparietal fontanelle is subdivided in tadpoles of *L. latrans* from Paraná, São Paulo and Roraima states of Brazil, but not partitioned in individuals from Seropédica municipality, Rio de Janeiro state, Brazil. Variation in size of *p. urobranchialis* was observed in populations of *L. chaquensis*, *L. fuscus* and *L. latrans* and the fusion degree of hypobranchial plates in *L. fuscus*, *L. mystacinus* and *L. latrans*.

These findings point the demand for further interpopulational studies in tadpoles. Langone and de Sá (2005) showed variations regarding external morphology of *L. fuscus*. Besides, works dealing with internal morphological variations in tadpoles are lacking. The species for which these variations were observed to present a wide distribution could be possibly a different species. These variations could indicate the need to conduct taxonomic revisions in these species, analysing the characters of the tadpoles as well as adults and molecular data (Wynn and Heyer 2001).

Performance of internal buccal larval morphology characters

This is an innovative work that accessed internal buccal characters to test its contribution to the study of phylogenetic inference in the genus *Leptodactylus*. Wassersug and Heyer (1988) suggested that buccal larval characters should not be used in phylogenetic studies. They argued that internal buccal morphology is

strongly correlated with the larval ecology, and homoplasticity may render buccal characters phylogenetically uninformative. Nonetheless, these authors also argued that there was a phylogenetic pattern in buccal characteristics. This set of data was not exploited in previously published phylogenetic analyses that used larval characters (Larson and de Sá 1998; Maglia et al. 2001; Haas 2003; Pugener et al. 2003).

We have identified four features of buccal larval anatomy that are synapomorphies of *Leptodactylus*, as follows:

(1) Buccal floor arena triangular (Character 10: State 1). This character state also occurs in *Physalaemus* Fitzinger, 1826 (Wassersug and Heyer 1988; Miranda and Ferreira 2009), suggesting that this characteristic may have arisen earlier or more than once in the evolution of anurans.

(2) Slightly prominent projections on the posterior margin of ventral velum (17:1). The presence and morphology of these projections may vary among diverse genera, but is present in all *Leptodactylus* sampled in this study. The codification of this character needs to be refined in future studies.

(3) Small postnarial arena (27:0). This character state is also found in other genera – for example *Eupemphix* Steindachner, 1863 and *Physalaemus* (Wassersug and Heyer 1988; Miranda and Ferreira 2009).

(4) Small lateral ridge papillae (30:1). Absence of the lateral ridge papillae is an attribute found in basal families of Anura (Pipidae, Rhinophrynidae, Alytidae, Leiopelmatidae and Bombinatoridae) (Wassersug 1980). The absence of these structures was also reported in *Ceratophrys cranwelli* (Ceratophryidae; Vera Candioti 2005). These observations suggest that this character may have arisen more than once in the evolutionary history of Anura, and their presence or absence may be a result of multiple evolutionary events (Maglia et al. 2001). The presence of a pair of lateral ridge papillae was observed in all previously examined *Leptodactylus* tadpoles.

None of the larval characters analysed are synapomorphic for the phenetic species groups of *Leptodactylus*. Among the 42 buccal characters, 16 are homoplastic.

(1) Presence of spherical protuberances on the anterior portion of the infrastroral region (Character 1: State 1). This character occurs in *Leptodactylus chaquensis*, *L. latrans*, *L. podicipinus* and *L. labyrinthicus*. We expected such anatomical similarity among *L. chaquensis*, *L. latrans* and *L. podicipinus*; though, *L. labyrinthicus* is not closely related. The convergence is surprising because these species do not occupy the same type of environment, having disparate dietary habits (Agostinho et al. 2002; Prado et al. 2005; Silva et al. 2005).

(2) A pair of narrowly separated medial infralabial papillae (3:2). This feature is present in *Leptodactylus natalensis*, *L. notaktites*, *L. spixi* and *L. vastus* and shows no apparent distributional pattern.

(3) Lateral pair of infralabial papillae quadrangular (5:2). This character occurs in species that are not closely related.

(4) Lingual papillae in number of three (7:2). Lingual papillae occur in the *Leptodactylus fuscus*, *L. latrans* and *L. melanonotus* groups, and the *L. pentadactylus* clade. Associations between the larval ecology and the amount of lingual papillae are unknown.

(5) Arrangement of lingual papillae (9:1). Central papillae anteriorly positioned in relation to lateral papillae were observed in tadpoles of the *Leptodactylus pentadactylus* group and in *L. spixi*, which traditionally is placed in the *L. fuscus* group.

(6) More than 30 pustules on the buccal floor arena (11:2). This feature is found in many species of *Leptodactylus*, including those not closely related; it also occurs in the *Crossodactylus gaudichaudii*.

(7) Projections grouped laterally within the anterior limits of ventral velum, absent or with pustules (15:0 and 15:2). The absence and reduction in number and size of selection structures (infralabial papillae, lingual papillae and arena papillae) is related to macrophagous and carnivorous habits. Thus, the absence of these projections and their presence with reduced size (pustules) in the *Leptodactylus pentadactylus* group is expected. These features also occur in *L. bufonius*, *L. camaquara*, *L. furnarius* and *L. spixi*, for which there are no data on foraging habits.

(8) Prominent projections on the posterior margin of ventral velum (17:2). This character state is present in species of the *Leptodactylus fuscus* + *L. latrans* + *L. melanonotus* clade and also occurs in *L. knudseni*.

(9) More than five projections on each side of the posterior margin of ventral velum (18:1). This attribute is present in out-groups, especially in *Crossodactylus* Duméril and Bibron, 1841 and *Telmatobius* Wiegmann, 1834 (Weber and Caramschi 2006; Vera Candioti 2008), and also occurs in *Leptodactylus bufonius* and *L. vastus*.

(10) Orientation of choanae in relation to the longitudinal axis of the body (21:0 and 21:1). This character is highly homoplastic and variable and probably has slight systematic value.

(11) Posterior wall of the nostril, low (24:0). All tadpoles in the *Leptodactylus pentadactylus* clade, *L. tapiti* and *L. spixi* share this character, which has no known ecological significance.

(12) Median ridge shape (28:0 and 28:2). The median ridge is relatively small in all species of *Leptodactylus* previously analysed (Wassersug and Heyer 1988), but its shape is convergent in nine of 22 species examined.

(13) Border of median ridge (29:1 and 29:2). The shape of the median ridge margin also showed to be convergence in the species analysed. This character together with Character 28 indicates that this anatomical feature (median ridge) should be used with prudence in future studies.

(14) Buccal roof arena quadrangular or trapezoidal (33:3). This character state occurs in seven species of the *Leptodactylus fuscus* group, *L. labyrinthicus* and in one out-group (*Telmatobius scroccii*).

(15) Dorsal velum narrow in relation to buccal roof (37:1). This feature was found in all species traditionally assigned to the *Leptodactylus latrans* and *L. melanonotus* groups, and also *L. notaktites* and *L. labyrinthicus*. A narrow velum and the presence of a poorly developed glandular zone are associated with macrophagous larvae (Wassersug 1980), such as *L. latrans* and *L. labyrinthicus* (Agostinho et al. 2002; Prado et al. 2005; Silva et al. 2005). The dietary habits of the other species are unknown.

(16) Dorsal glandular zone occupying half the surface of the velum (41:1). This character occurs in *Leptodactylus labyrinthicus*; *L. natalensis*; *L. riveroi* Heyer and Pyburn 1983; and *L. rhodomystax* Boulenger, 1884. The reduction of this secretory structure in *L. labyrinthicus* and *L. rhodomystax* is correlated with dietary habits (Wassersug 1980; Wassersug and Heyer 1988; Hero and Galatti 1990; Agostinho et al. 2002; Prado et al. 2005; Silva et al. 2005).

Our results corroborate Wassersug and Heyer's (1988) observation that many features of larval internal buccal morphology are homoplastic. However, this does not refute the importance of including these characters in phylogenetic studies. Maglia et al. (2001) claimed that it is useful to know whether certain morphologies have evolved in parallel, because this information enables identification of potential functional or ecological pressures (in convergences), as well as the complexity of development processes. Recognition of parallelisms in developmental patterns in the evolutionary histories of taxa may yield useful insights into the homologies of the adult morphologies (Maglia et al. 2001).

More studies should be performed to assess the utility of such characters in phylogenetic inference in anurans.

Performance of larval cranium morphology characters

Larval cranium characters have been widely used in phylogenetic studies (Larson and de Sá 1998; Maglia et al. 2001; Haas 2003; Pugener et al. 2003) and are considered to present less homoplasy than features of internal buccal morphology. We found four larval cranial characters to be synapomorphic for *Leptodactylus*:

- (1) Ventromedial fusion of the *corpus* of the suprarostrum narrower (Character 43: State 1). Larson and de Sá (1998) also observed this trait in *Leptodactylus* species.
- (2) Ventrolateral projections in the *corpus* of suprarostrum (44:1). Variably sized projections are present in all species of *Leptodactylus* examined. In *L. fuscus* + *L. latrans* + *L. melanonotus* (except *L. bufonius*) clade, the projections are small. The projections are well developed and fused in the *L. pentadactylus* clade and *L. bufonius*.
- (3) Angle of the posterior margin of the *processus ascendens* in relation to the braincase at an angle between 70° and 80° (65:1). This feature was found in most species of the genus, except members of the *Leptodactylus fuscus* group, in which the posterior margin of *processus ascendens* is nearly perpendicular to the braincase (Larson and de Sá 1998).
- (4) Lateral process of ceratobranchials triangular (78:1). This feature is absent in *Leptodactylus riveroi* and in the *L. pentadactylus* clade. Few are known about its distribution, because the character has not been used before.

The clade composed by the species traditionally assigned to the *Leptodactylus fuscus*, *L. latrans* and *L. melanonotus* groups is supported by one synapomorphy (Character 63: State 1). The *L. pentadactylus* clade also is supported by one synapomorphy (47:0).

Among 42 larval cranium characters, 22 showed some degree of convergence, suggesting that this data partition has fewer homoplastic characters than does that of internal buccal anatomy, at least for *Leptodactylus*.

- (1) Presence of ventrolateral projections in the *corpus* of the suprarostrum (Character 44: State 2). Members of the *Leptodactylus pentadactylus* clade and *L. bufonius* share this feature. Larson and de Sá (1998) also observed the same structure in the *L. fuscus* group (*L. albilabris* and *L. longirostris*).
- (2) Broad space between the *corpora* of the suprarostrum (49:1). Usually, the space between the *corpora* of the suprarostrum is narrow, but it is wide in *Leptodactylus chaquensis* and *L. latrans*. These two species are morphologically similar and closely related. This characteristic (49:1) is present also in *L. riveroi*, found to be basal in our hypotheses (Fig. 4).
- (3) Presence of adostrals (51:1). Both presence and absence of adostrals seem not to be related to common heritage because are observed in species that are not phylogenetically closely related (i.e. in-group and out-groups).
- (4) Width larval cranium more than 90% of its length (52:2). Wide chondrocranium occurs in *Alsodes* Bell, 1843 and *Ceratophrys cranwelli*, in some species of the *Leptodactylus fuscus* + *L. latrans* + *L. melanonotus* clade and in *L. knudseni*.
- (5) Length of the otic capsule 30% or more of the length of larval cranial (54:1). This condition is widely distributed in *Leptodactylus* and is unrelated to the size of the tadpoles. It was found in small (*Leptodactylus riveroi*, *L. bufonius*, *L. camaquara*, *L. petersii*, *L. pustulatus*, *L. notoaktites* and *L. gracilis*) and large tadpoles (*L. knudseni*).

(6) Ornamentation in the ethmoid plate (Character 55). This character is polymorphic and convergent among the species analysed.

(7) Narrower angle (<10%) between *cornua trabeculae* in relation to larval cranial (56:0). This character occurs in outgroups, in the *Leptodactylus fuscus* + *L. latrans* + *L. melanonotus* clade and also in *L. labyrinthicus*.

(8) Presence of a *taenia tecti transversalis* and *taenia tecti medialis* subdividing the fontanelle into a frontal and two parietal fontanelles (57:1). This character state was observed in the out-group (tadpoles of *Alsodes verrucosus*) in the *Leptodactylus fuscus* + *L. latrans* + *L. melanonotus* clade and tadpoles of *L. labyrinthicus* and *L. rhodomystax*. It seems to be related to larval ontogeny and its heritage pattern is difficult to access.

(9) *Foramen prooticum* visible in lateral view (more than 1/3 of its opening) (58:1). This character state was observed in *Leptodactylus chaquensis*, *L. natalensis*, *L. podicipinus*, *L. spixi*, *L. knudseni* and in *Alsodes*. All other species (except *L. riveroi*) had a small *f. prooticum* in lateral view. This character seems to be unrelated to the size of the tadpole, but as the knowledge of this structure is limited, it is necessary to examine different species to understand their distribution patterns.

(10) *Foramen opticum* greater than the *f. oculomotorium* (59:0). This state character is present in some species of the *Leptodactylus fuscus* + *L. latrans* + *L. melanonotus* clade and also in the tadpole of *L. labyrinthicus*. It seems that this character is not related to the body size or other ecological similarity between species.

(11) Length of *cornua trabeculae* relative to larval cranial length (60:1). Feature observed in tadpoles of *Alsodes* and in both clades of the genus resurrected in this phylogenetic analyses. For studies involving a greater diversity of species, a new coding could be required to elucidate the evolutionary pattern of this character.

(12) *Processus anterolateralis* of crista parotica small and triangular (61:0). This character occurs in *Leptodactylus podicipinus* and *L. petersii*. Species with similar morphologies. The character state was also observed in *L. rhodomystax*. No morphological or ecological similarities are known between *L. rhodomystax* and (*L. petersii* + *L. podicipinus*). The convergence in this character could correspond to multiple evolutionary events.

(13) *Processus posterolateralis* of crista parotica distinct (62:0). As this process is slender when compared to the previously mentioned, its use must be viewed with some caution as is hard to analyse, and also there is risks of loss or damage of this structure during the process of clearing and staining.

(14) Posterolateral extension of the palatoquadrate (63:2 and 63:3). We followed the coding of Larson and de Sá (1998). Characters states 0, 1 and 4 are informative and non-convergent. States 2 and 3 are homoplastic, both in the in-group and out-groups, suggesting that this character requires better delineation.

(15) Triangular projection at anterolateral margin of the *cornua trabecula* reduced or absent (68:1). This polymorphic character is homoplastic and, thus, not phylogenetically informative.

(16) Width of *processus muscularis* (in dorsal view) two-thirds that of *pars articularis quadrati* (69:1). *Leptodactylus rhodomystax* shares this character with some species of the *L. fuscus* + *L. latrans* + *L. melanonotus* clade and members of out-groups (*Alsodes* and *Crossodactylus*). The shape of this structure is similar in all species examined.

(17) Presence of a commisure quadratoorbitalis (70:1). This character occurs in all species of *Leptodactylus* examined, as well as *Telmatobius*. This suggests that this attribute may have arisen before and/or more than once in the evolution of Leptodactylidae.

(18) Copula II larger than the *pars reuniens* (74:2). Sizes are variable in both in-group and out-group.

(19) Large *processus urobranchialis* (77:1). The size of *p. urobranchialis* proved to be slightly informative and homoplastic.

(20) *Processus anterior hyalis* and *p. posterior hyalis* equal in size (79:1). *Leptodactylus knudseni* shares this character with a few other species of the *L. fuscus* + *L. latrans* + *L. melanonotus* clade.

(21) *Processus branchialis* closed (82:1). According to Larson and de Sá (1998), all species of *Leptodactylus* belonging to the *L. fuscus* and *L. pentadactylus* groups have an open *p. branchialis*, whereas in species of *L. latrans* and *L. melanonotus* groups and in *L. riveroi* and *L. silvanimbus*, the *p. branchialis* is closed – data corroborated by our analysis. Although in the clade formed by the *L. latrans*, *L. melanonotus* and the *L. fuscus* groups, the character is homoplastic.

(22) Ceratobranchials presenting lateral projections only in the posterior portion (84:2). This character may be plesiomorphic for *Leptodactylus*, which is corroborated by presence of this character state in tadpoles of *L. riveroi* and *L. rhodomystax*. But it also may have arisen more than once during the evolutionary history of the group, as it is present in *L. petersii* (*L. fuscus* + *L. latrans* + *L. melanonotus* clade) and in *L. labyrinthicus* and *L. knudseni* (*L. pentadactylus* clade).

Additional phylogenetic remarks

The most remarkable difference between the phylogenetic hypotheses presented here and earlier ones is the fact that the monophyly of the *Leptodactylus fuscus* group could not be corroborated. Larson and de Sá (1998) identified two major clades in *Leptodactylus* (*pentadactylus* – *fuscus* and *latrans* – *melanonotus*) based on 26 characters of larval cranium morphology and suggested that the species of the *L. pentadactylus* group might be paraphyletic once two species placed in the *L. fuscus* group (*L. albilabris* and *L. labrosus*) were more closely related to the species of the *L. pentadactylus* group than any other species. We could not verify this relationship because *L. albilabris* (Günther 1859) and *L. labrosus* Jiménez de la Espada, 1875 were not sampled in this study. Ponsa (2008) corroborated the results of Larson and de Sá (1998) by showing a close relationship between those species and the *L. fuscus* group. Ponsa (2008) and Pyron and Wiens (2011) recovered *L. fuscus* group as monophyletic and most closely related to the *L. pentadactylus* group. These relationships were not corroborated in the present study. We identified two major clades – a clade formed by the *L. fuscus* + *L. latrans* + *L. melanonotus* groups and the other formed by the *L. pentadactylus* group. The *L. latrans* and *L. melanonotus* groups are closely related and embedded within the *L. fuscus* group. This major clade is closely related to the *L. pentadactylus* clade. We did not recover *L. rhodomystax* (currently considered a member of the *L. pentadactylus* group) in the *L. pentadactylus* clade; instead, *L. rhodomystax* is basal in relation to the two major clades – that is the *L. fuscus* + *L. latrans* + *L. melanonotus* clade and *L. pentadactylus* clade. This relationship was also recovered by Pyron and Wiens (2011). *Leptodactylus riveroi* is sister of all other species in the genus, suggesting that this species does not represent an evolutionary transition between the *L. latrans* and *L. melanonotus* groups (Heyer and Pyburn 1983; Larson and de Sá 1998).

Heyer (1969) suggested a close relationship between the *Leptodactylus latrans* and *L. melanonotus* groups, with the *L. pentadactylus* group being the sister group of the former two, based on behaviour, morphology and ecology of adults. The *L. fuscus* group formed the sister group of the large clade composed of species of

the three other species groups – *L. latrans*, *L. melanonotus* and *L. pentadactylus*. The *L. marmoratus* group (now *Adenomera*) is not closely related to the aforementioned groups previously. In recent phylogenetic hypotheses, Pyron and Wiens (2011) and Fouquet et al. (2013) resurrected *Adenomera* as sister group of *Leptodactylus* species. Because we were unable to sample species of *Adenomera*, we have no insights on the issue. There are a few discrepancies of data processing by the present analysis and the hypothesis presented by Ponsa (2008). Ponsa (2008) ordered some characters in her analysis and also implemented the method of character successive weighting (Goloboff 1993, 1995). This methodology has been criticized (Turner and Zandee 1995; Kluge 1997; Grant and Kluge 2003), and we prefer not to implement it in the present work.

Most amphibian systematic studies include data from adult specimens and/or molecular data. However, as mentioned by Maglia et al. (2001), the morphology of an organism, including those with a bimodal life cycle, is not restricted solely to adult; it is part of a continuous ontogenetic process that includes different forms with different attributes that can be assessed. Our phylogenetic analyses based on larval and adult morphological data sets yielded the same two clades (*fuscus* – *latrans* – *melanonotus* and *pentadactylus*) for larval characters alone and combined larval and adult datasets. The unique difference was the topological position of *Leptodactylus rhodomystax*, which was included in the *L. pentadactylus* clade in the combined analysis. This confirms that different classes of characters are important in the resolution of relationships among species of *Leptodactylus*. Unfortunately, only 15 species of *Leptodactylus* were included in the combined analysis. The great amount of missing data in Ponsa's (2008) data set may be a source of inconsistencies, affecting the results. It is necessary to sample a larger number of species in the genus *Leptodactylus* to clarify both the relationships and the behaviour of larval and adult characters. In the present work, when different partitions were combined (i.e. Ponsa 2008 and the larval characters analysed by the present work), the resulting phylogeny proved to be mostly congruent to the one obtained by using larval characters only. Hillis and Wiens (2000) suggested that subsampling characters and/or taxa may produce phylogenetic inconsistencies.

Some of the characters used in the phylogenetic analysis, both from this work and that coded by Ponsa (2008), were polymorphic. The inclusion of polymorphic characters in phylogenetic analysis does not necessarily make the matrix more informative (Wiens 2000). Some authors code polymorphic characters as missing data or chose to exclude them from analysis (Farris 1966; Kluge and Farris 1969). Although the polymorphic characters are less informative than the characters with fixed states (Wiens 1995), their inclusion is more informative than their exclusion (Wiens 2000).

PBS results suggest an incongruence produced by larval and adult characters found in many topological areas of the strict consensus tree (Fig. 4a). ILD also supports this incongruence. The considerable disagreement between two partitions on the combined trees is probably related to the nature of characters, which are subject to different evolutionary processes.

The monophyly of the *Leptodactylus* is corroborated by both larval and adult morphological characters. Nevertheless, the intra-generic relationships remain to be resolved as additional species, and different data sets (e.g. molecular characters) can be sampled.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Species, locality, acronym and collection number, and tadpoles analysed.

Table S2. List of characters and character states of the internal buccal morphology used in this study.

Table S3. List of characters and character states of the larval cranial morphology used in this study.

Data S1. Combined matrix used this study.

Data S2. Matrix representing 84 larval characters and 27 taxa.

Data S3. Four most parsimonious cladograms from the maximum parsimony analysis of 84 larval characters.

Data S4. Twenty-five most parsimonious cladograms from the maximum parsimony analysis of data of adult specimens and larval characters.