A survey of shape variation in keratinized labial teeth of anuran larvae as related to phylogeny and ecology

M. FLORENCIA VERA CANDIOTI[1]* and RONALD ALTIG[2]

[1]*Instituto de Herpetología, Fundación Miguel Lillo Miguel Lillo 251, 4000 Tucumán, Argentina*
[2]*Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762, USA*

Labial teeth of anuran tadpoles are keratinized structures derived from the activity of a single epidermal cell of the oral labia; they are not homologous with adult anuran teeth, nor with teeth of other vertebrates. The present study comprises a first approach for studying labial tooth shape variation that will be useful for future studies of comparative development and the functional mechanics of feeding structures. We examined interspecific shape variations in the labial teeth of anuran tadpoles and searched for correlations of these variations with ecomorphological guilds and phylogeny. Species ordination shows that important variations at various taxonomic levels are related mainly to the general curvature of the tooth axis, the angle between the labial tooth base and tip, head length and curvature, and sheath width. The teeth of most basal taxa are broad-based and curved, although some broad-based teeth also characterize some phthanobatrachian species. Teeth of hyloids and ranoids differ in the oral angle, overall curvature, and sheath width. A phylogenetically independent ecomorphological effect is significant only for lotic suctorial and gastromyzophorous guilds; teeth in these forms have short, thick and curved heads, wide sheaths, and generally acute oral angles. The lack of a significant correlation between labial tooth shape and trophic guilds suggests that labial tooth harvesting ability has a wide latitude that could be particularly functional only under specific circumstances. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, ••, ••–••.

ADDITIONAL KEYWORDS: basal tadpoles – canonical phylogenetic ordination – curvature – ecomorphological guilds – eigenshape analysis – head – Hyloides – Ranoides – sheath.

INTRODUCTION

Vertebrate teeth have evolved in a direct relationship with ecological aspects, particularly feeding habits. In all groups, species radiation involved a wide tooth morphological diversity related to food features such as energy content and mechanical properties. Tooth shape variation relative to diet types has been studied in extant sharks, bony fishes, crocodiles, lizards, and marsupial and placental mammals, as well as extinct taxa such as pelycosaurs, ichthyosaurs, and dinosaurs (Massare, 1987; Sumida & Murphy, 1987; Hanken & Hall, 1993; Reilly, McBrayer & White, 2001; Rüber & Adams, 2001; Briggs & Crowther, 2003; Ungar & M'Kirera, 2003; Herrel, Vanhooydonck & Van Damme,

2004; Geerinckx, De Poorter & Adriaens, 2007). In most cases, shape variation includes a strong phylogenetic component that determines similarities and differences beyond ecological convergences. In modern adult amphibians, some tooth morphological variations are suggested to be correlated with dietary specialization in several groups. For example, unlike most of anuran species, ceratophryines (Ceratophryidae), *Hemiphractus* (Hemiphractidae), and *Pyxicephalus* (Pyxicephalidae) have nonpedicellate monocuspid teeth, show aggressive biting behaviour, and eat large vertebrate prey; these species also have well-developed odontoids (i.e. fang-like outgrowths of the lower jaw; Fabrezi, 2001; Fabrezi & Emerson, 2003).

Labial teeth of anuran tadpoles differ from calcified teeth in composition, morphology, and developmental pattern. They are also called 'keratodonts' (Van Dijk, 1966; Dubois, 1995) to highlight its nonhomologous

*Corresponding author. E-mail: florivc@yahoo.com

nature regarding adult anuran true teeth and teeth of other vertebrates. Labial teeth are single keratinized structures derived from the activity of epidermal cells (Fiorito de López & Echeverría, 1984, 1989). They are arranged in rows on parallel transverse ridges on the upper and lower labia of the oral disc; the number and configuration of tooth rows is expressed as a labial tooth row formulae (LTRF). Each erupted labial tooth normally sits on top of several replacement teeth constituting a labial tooth series that extend deep into the labial tooth ridge (Héron-Royer & Van Bambeke, 1889; Altig, 2007). Most labial teeth have three regions: a strongly compressed sheath, a flattened, more or less convex head with or without cusps, and a weakly delimited body connecting them (Altig & Pace, 1974). Analogous structures from distant taxa include cestode hooks (Dujardin & Duriez, 1995), molluscan radular teeth (Padilla, 2003), and the unculi of loricariid catfishes (Geerinckx, De Poorter & Adriaens, 2007).

Labial teeth are involved in substrate anchoring and feeding mechanisms; they momentarily affix the oral disc to a substrate so that the jaw sheaths remain close to the surface, and then labial tooth rows are released in a serial fashion to lift material off the surface and generate a suspension of food particles that are sucked into the mouth (Taylor, Altig & Boyle, 1996; Wassersug & Yamashita, 2001). Labial tooth morphological variation could be expected to occur among tadpoles that inhabit different microhabitats or feed on different food types and through different mechanisms. Alternatively, labial tooth phenotypic variations could respond mainly to historical constraints and exhibit a taxonomic structure unrelated to ecological types.

The present study comprises a first approach for studying labial tooth evolution in tadpoles. We first survey morphological variation through a geometric morphometric ordination method. The first studies of the diversity of tadpole labial tooth shapes (Héron-Royer & Van Bambeke, 1889 and Gosner, 1959) involving European and North American species emphasized variations in cusp pattern. We explored other sources of variation in addition to cusp patterns and focused on features that likely have functional implications. For example, the shape of the base, as it affects the strength of the rooting of the teeth, and the overall curvature of the labial tooth, as it affects the angle of attack and allowable forces before breakage. We then use a phylogenetic comparative method in order to correlate shape variables with phylogenetic information and ecomorphological guild membership.

MATERIAL AND METHODS

We worked with labial teeth of anuran tadpoles of 108 species (54 genera and 23 families) from Herpetologi-cal Collections of the Smithsonian Institution, Fundación Miguel Lillo, and personal collections of the authors (a list of species is provided in the Supporting information, Table S1). Most tadpoles were in Gosner Stages 30–37 (Gosner, 1960), except for *Trachycephalus venulosus* (Stage 39). The phylogenetic hypothesis employed as a framework for the analyses was based on Frost *et al*. (2006; updated in Frost, 2009), Grant *et al*. (2006), Pramuk (2006), Ponssa (2008), Barrionuevo (2009), and Cei (1980). This hypothesis constitutes a meta-tree in the sense that it combines phylogenetic analyses of various degrees of robustness, by grafting phylogenies onto a fixed-base tree (Funk & Specht, 2007). Species were assigned to ecomorphological guilds *sensu* Altig & McDiarmid (1999a). Tadpole guilds were originally defined on the basis of developmental modes, microhabitats, and several external morphological features (e.g. body shape, tail shape, and oral disc features); labial teeth were not considered in that categorization so, although a fair amount of labial tooth shape variation might be expected to be related to guilds, it could be that labial tooth variation occurs independently.

The shape analysis was performed on images of each labial tooth in left, lateral view (right-oriented images were reversed with the assumption that this would not affect the results significantly). The images come from three sources: (1) scanning electron microscopy micrographs (Altig & Pace, 1974); (2) a published image (Orrico, Mongin & Carvalho-e-Silva, 2007); and (3) photographs taken via light microscopy. In the latter case, teeth were extracted from the medial section of the uppermost tooth row (A1 row) with small forceps or micropipette and air-dried on microscope slides. In those tadpoles with LTRF > 2/3 that add upper labial rows distally during oral ontogeny (e.g. *Hypsiboas curupi*), we selected the second upper row in a proximo-distal direction from the upper jaw sheath, which is suggested to be homologous to row A1 in LTRF 2/3 tadpoles (Altig & Johnston, 1989). In *Ascaphus truei*, the very unusual labial teeth from the third posterior row (row P3; Altig & Pace, 1974) were considered. Although we removed and photographed several labial teeth (1–10) per tadpole to explore intraindividual variation, only one randomly selected labial tooth was included in subsequent quantitative analyses. Images were treated with an image editing software before data acquisition, by manually digitizing an outline along inner and outer profiles. In some teeth, cusps along the head margin are very long, curved, and project into the lateral profile; in those cases, cusps were not considered part of the outline. Most teeth were also photographed in the frontal view, although shape variation could not be quantified because of methodological problems with outline acquisition. Nevertheless, the information provided
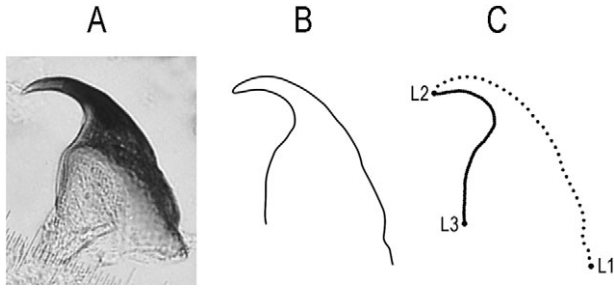
Figure 1. Schematic representation of the labial tooth outline acquisition: (A) original image, (B) open outline captured automatically, and (C) outline with interpolated coordinates (79 and 37 points for inner and outer interlandmark segments; 99% accuracy regarding the original outline) plus landmarks on comparable geometrical points (L1–3).

by the labial tooth face view was qualitatively described and taken into account in the discussion.

For the labial tooth lateral views, we applied mean-centered extended eigenshape analysis (Lohmann, 1983; MacLeod, 1999), which requires coordinates of points along an outline plus landmarks placed at comparable geometrical points. The additional landmarks on the outline constrain the sequencing of the boundary coordinate points and force them into alignment, and then the degree of shape variation generated through biological miscorrespondence of the outline can be reduced (MacLeod, 1999). The outlines were captured automatically with TPSDIG2 (Rohlf, 2008), excluding the connection between the labial tooth bases because this area can be broken or difficult to distinguish in light photographs. Each outline was thus represented through an open curve formed of 200 pairs of equidistant boundary coordinates; MacLeod (1999) recommended not artificially closing the outlines because this might inflate the interobject similarity estimates. Three additional landmarks were located at the labial tooth front and back bases and the tip. The dataset was submitted to the internet-accessible extended eigenshape MORPHO-TOOL (Krieger, 2008), which implements previously described techniques (MacLeod, 1999, 2002; Krieger, Guralnick & Smith, 2007). As a previous step, the analysis uses a recursive search for the minimum number of boundary coordinates needed to reproduce the perimeter of the original curve; these reductions increase the computational efficiency of the subsequent multivariate analyses and can affect the orientation of the eigenshape axes (MacLeod, 1999). Intralandmark boundary curves were interpolated such that a minimum of 99% accuracy in the interpolated length was achieved over the entire sample. Figure 1 shows the scheme of data preparation from the original image to the outline with the new interpolated set of points. Each set of coordinates was then converted to a phi function (Zahn & Roskies, 1972), which represents the set of angle changes required to move around the outline, removing rotation, scale, and positional information. The phi functions were employed as variables in a singular value decomposition, which calculates variation axes that define a morphospace on which the objects (teeth) are scattered; the ordination was carried out on the variance matrix (instead of the correlation matrix) because a previous normalization would increase the contribution of variables with low variance, and this can cause some very different shapes to appear similar (Rohlf, 1986; MacLeod, 1999). Finally, the analysis allowed for the modelling of shapes along the eigenshapes, which is useful for the interpretation of trends in morphological change on the axes; for each eigenshape axis, five models were generated, corresponding to the minimum, 25%, 50%, 75%, and maximum scores.

To explore the relationship between labial tooth shape, ecomorphology and phylogenetic structure, we applied a canonical phylogenetic ordination (CPO; Giannini, 2003), which consisted in this case of a variance partitioning analysis by partial redundancy analysis (Borcard, Legendre & Drapeau, 1992) involving a phylogenetic tree matrix. This allows the variance of the main labial tooth shape matrix (phi functions for each species) to be accounted for by two external matrices of predictor variables. Ecomorphological and phylogenetic matrices are constructed assigning each species 0 s and 1 s for guild/clade membership until each taxon is assigned to all the groups to which it belongs. The CPO then specifies a relevant subset of groups/clades (i.e. groups that best explain the pattern in the main morphological matrix) according to a Monte Carlo randomization test; the final model is built by a process of group selection based only on the subset of individually significant groups. An F-test is performed, and the total amount of variation explained by external matrices is calculated as a ratio of inertias. The explained variation can be then discriminated into variation explained purely by ecomorphological guilds, purely by phylogeny, and shared variation. Multivariate analyses were carried out with CANOCO 4.5 (Ter Braak & Smilauer, 1997). One methodological issue is worth noting in that we did not include a character-change model in our analysis. On one hand, as mentioned above, the phylogenetic hypothesis used comprises a meta-tree representing a combination of available hypothesis (even current classifications not necessarily based on explicit phylogenetic analysis), and thus we have no comparable branch length information for all our taxa. On the other hand, CPO does

not require (although it permits) explicit microevolutionary assumptions, and we agree with Giannini (2003) with respect to restricting the testing of phylogenetic effects on nonmolecular comparative data to tree topology alone. As explained by Giannini (2003), we understand that there is no reason to assume that the processes controlling evolutionary variation in the genes that originated the phylogeny are the same as those controlling evolution in a morphologic comparative trait. A more profound discussion on the use of branch lengths and evolutionary models in general is addressed elsewhere (Giannini, 2003; Goloboff, 2003).

Finally, we used TNT available from http://www.cladistics.com/aboutTNT.html to fit labial tooth shape data to the phylogenetic meta-tree we employed. The matrix of interpolated (calculated with extended eigenshape analysis) and aligned (with TPSRelw; Rohlf, 2005) coordinates was submitted to TNT to calculate the optimal ancestral position for each point in the outline through a generalization of Farris optimization (Catalano, Goloboff & Giannini, 2010). The locations for the ancestral points that minimize ancestor/descendant differences are found, and this results in a reconstruction of the ancestral labial tooth for each node.

Teeth are progressively smaller as one proceeds from medial to lateral within a row, and young labial tooth generations, and young and metamorphic specimens often produce teeth with few cusps (Hosoi *et al.*, 1995; Altig R. & Vera Candioti M. F., pers. observ.). To assess the effects of some of these variations and provide a calibration of how intraspecific variation relates to interspecific variation, we analyzed labial tooth samples from all rows of the sibling species *Leptodactylus latrans* and *Leptodactylus chaquensis* (LTRF 2/3; $N = 2$ per species, Stages 31–33). Nine teeth per row (left, centre, right parts of each row, and erupted, middle, and deep from each labial tooth series) for a total of 90 teeth per species were analyzed as described above.

RESULTS

LABIAL TOOTH GENERAL CONFIGURATION

Most labial teeth have three well differentiable parts: (1) a spatulate or oblong head, more or less convex with cusps that vary in number, shapes, and orientations along the head; (2) a weakly delimited body; (3) and a broad, laterally compressed sheath, with different inner (oral) and outer profiles. In a few species (e.g. *Ceratophrys cranwelli* and *Spea bombifrons*), labial teeth are noncusped (Figs 2, 3). A summary of the distinctive labial tooth features in the species we studied, as well as descriptions of labial teeth of tadpoles studied by other researchers, are provided in the Supporting information (Table S1); we tried to compile all literature referring to labial tooth shape, although we might have inadvertently missed some pertinent studies.

EXTENDED EIGENSHAPE ANALYSIS

The interpolation of coordinates to 99% of the original outline resulted in 79 and 37 points for inner (oral) and outer interlandmark segments, respectively. In the shape analysis, the first two eigenshapes accounted for approximately 46% of the total variation (Fig. 4). The first axis shows variation of the general curvature of the labial tooth axis seen primarily as the inclination of the head to the body. The taxa with higher scores (i.e. more curved labial teeth) include *L. latrans*, *Odontophrynus achalensis*, *Pseudacris ornata*, *Telmatobius ceiorum*, *Telmatobius atacamensis*, *Thoropa miliaris*, and *S. bombifrons*. The taxa with lower scores (i.e. less curved labial teeth) include *Ansonia muelleri*, *Calyptocephalella gayi*, *C. cranwelli*, *Leptopelis natalensis*, *Phyllomedusa sauvagii*, and *Polypedates leucomystax*. The second axis shows variation on the proportion between inner (oral) and outer labial tooth profile ($\cong$ angle between labial tooth base and labial tooth tip, the oral angle) and the head shape; *A. truei* represents an extreme with an oral angle $< 90\,°$ and a short, very curved head, and *S. bombifrons* and *Phyllomedusa boliviana* have oral angle $> 90\,°$ and longer heads. A third axis (10.5% of the remaining variation; not shown) shows variation in sheath width; *Leptobrachium montanum* and *S. bombifrons*, and *Rana cascadae* and *Alsodes* sp. are the widest- and narrowest-based, respectively.

CANONICAL PHYLOGENETIC ORDINATION

Monte Carlo permutation tests on ecomorphological and phylogenetic matrices reduced the number of significant groups to be included in the CPO model. In the phylogenetic matrix, six partitions were significant for labial tooth shape ordination independently of ecomorphological guilds: Sokolanura, Anomocoela, Hyloides/Ranoides, Pelodryadinae + Phyllomedusinae, Lophiohylini + Hylini, and Bufonidae excluding *Melanophryniscus* (together, these accounted for 32% of the total labial tooth shape variation; $P = 0.001$–0.026). In the ecomorphological matrix, only a small, phylogenetically independent effect of the suctorial and gastromyzophorous guilds was significant (7% of the total variance; $P \leq 0.002$). Table 1 shows individual significances of groups in both external matrices, and Table 2 summarizes the variance partitioning results of the labial tooth shape matrix; the overall variance in labial tooth shape explained by ecomorphological guilds and phylogeny is 49% and,
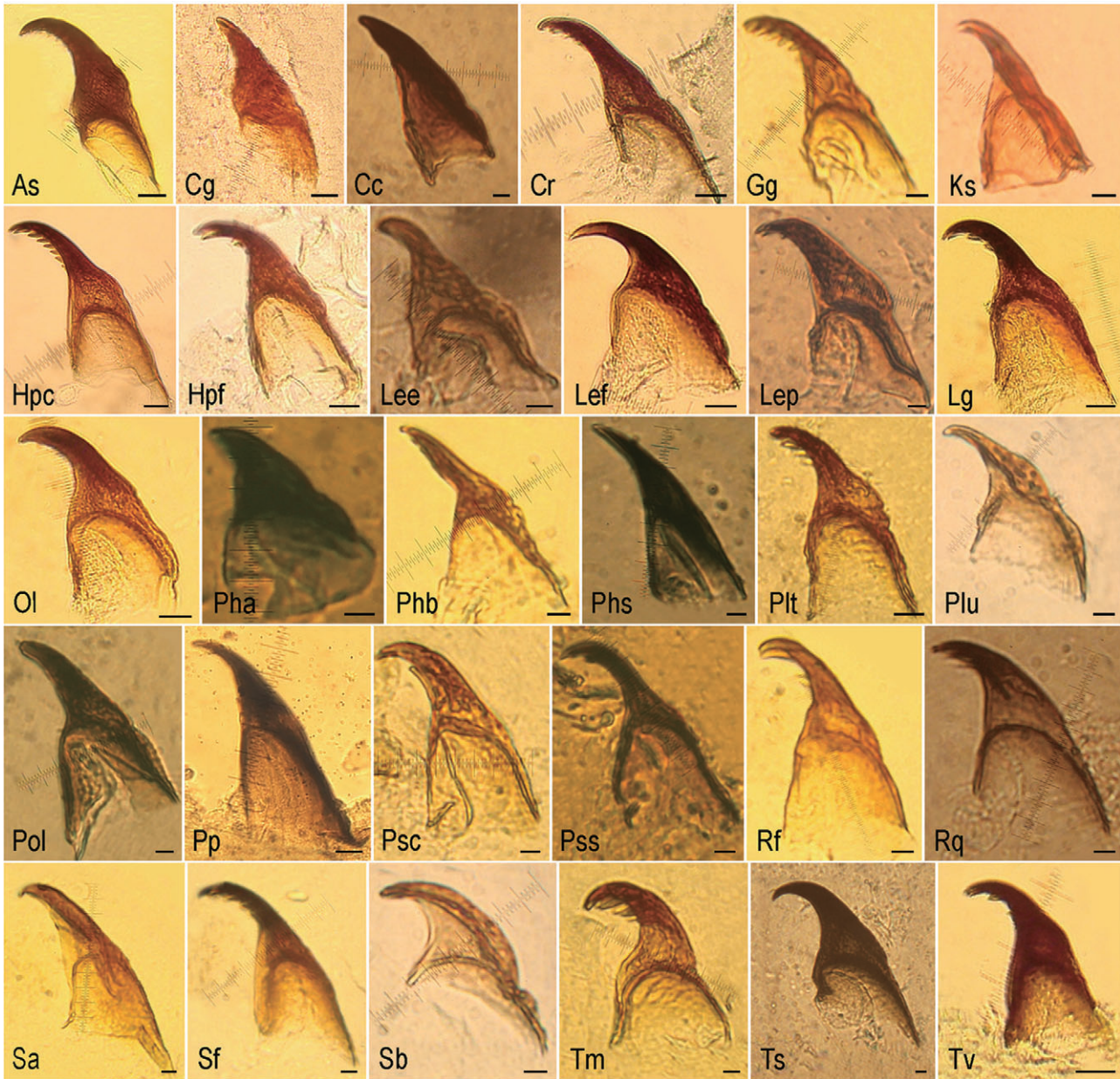
Figure 2. Lateral views of some of the labial teeth analyzed. AS, *Alsodes* sp.; CG, *Calyptocephalella gayi*; CC, *Ceratophrys cranwelli*; CR, *Crossodactylus schmidti*; GG, *Gastrotheca gracilis*; HPC, *Hypsiboas curupi*; HPF, *Hypsiboas faber*; KS, *Kassina senegalensis*; LEE, *Leptodactylus elenae*; LEF, *Leptodactylus fuscus*; LEP, *Leptodactylus* cf. *pentadactylus*; LG, *Limnomedusa macroglossa*; OL, *Odontophrynus lavillai*; PHA, *Phyllomedusa azurea*; PHB, *Phyllomedusa boliviana*; PHS, *Phyllomedusa sauvagii*; PLT, *Pleurodema thaul*; PLU, *Pleurodema tucumanum*; POL, *Polypedates leucomystax*; PP, *Pseudis platensis*; PSC, *Physalaemus cuqui*; PSS, *Physalaemus santafecinus*; RF, *Rhinella fernandezae*; RQ, *Rhinella quechua*; SA, *Scinax acuminatus*; SF, *Scinax fuscovarius*; SB, *Spea bombifrons*; TM, *Thoropa miliaris*; TS, *Telmatobius schreiteri*; TV, *Trachycephalus venulosus*. Scale bars = 0.005 mm, except for AS, CG, CR, HPC, HPF, LEF, LG, OL, and TV, where the scale bar = 0.02 mm.

after the partial CPO, this variance was partitioned into variances unique to ecomorphological guilds (7% of the labial tooth shape matrix), unique to phylogeny (35%), and the shared variance (7%).

LABIAL TOOTH SHAPE OPTIMIZATION

Figure 5 shows labial tooth outlines on the phylogenetic meta-tree we employed. The labial teeth of most basal taxa (e.g. *A. truei*, *Alytes obstetricans*, *S. bom-*
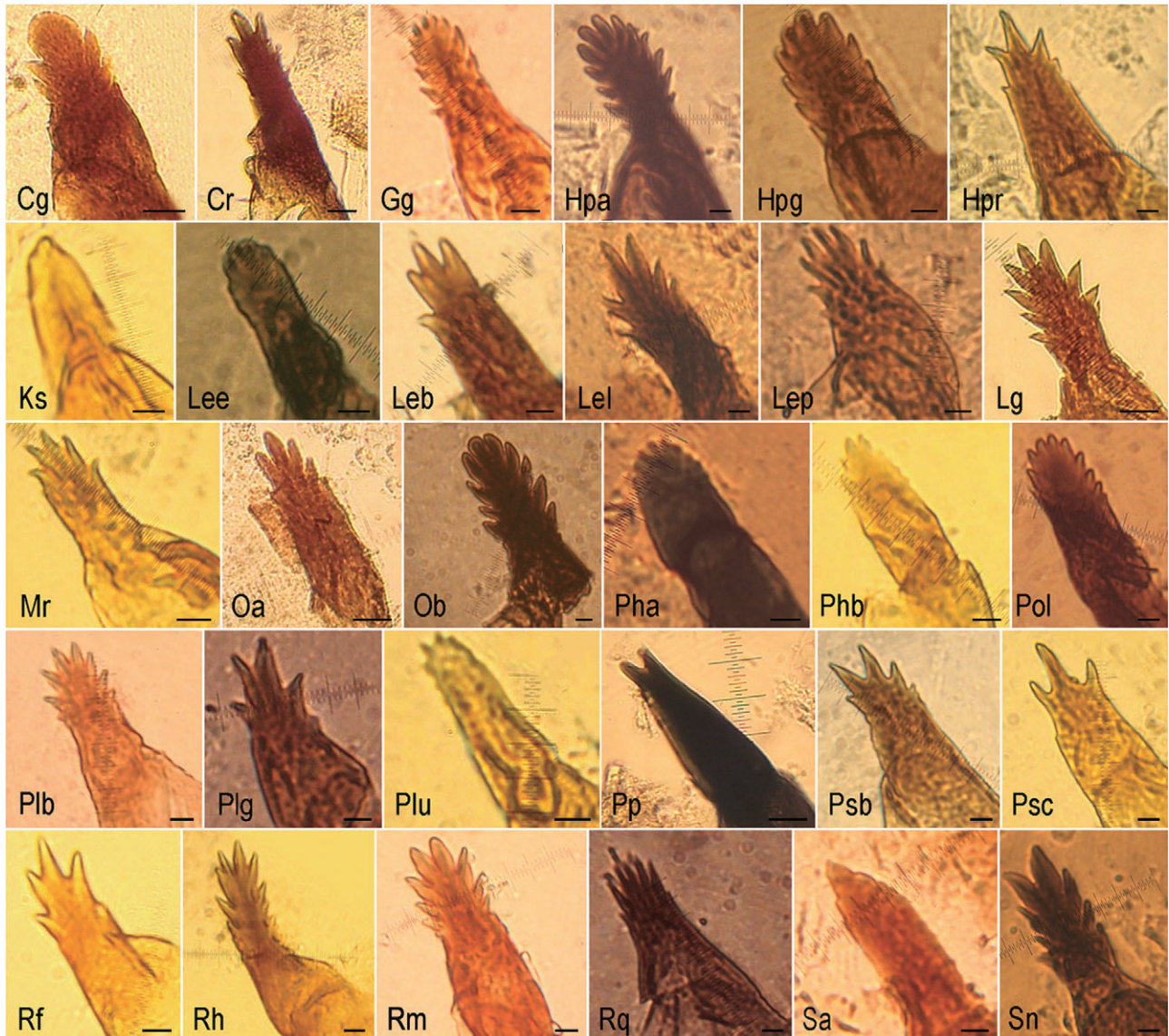
Figure 3. Frontal views of some of the labial teeth analyzed. Images are shown for illustrative purposes but were not quantitatively analyzed because of problems with outline acquisition as mentioned in the text. CG, *Calyptocephalella gayi*; CR, *Crossodactylus schmidti*; GG, *Gastrotheca gracilis*; HPA, *Hypsiboas andinus*; HPG, *Hypsiboas rosenbergi*; HPR, *Hypsiboas raniceps*; KS, *Kassina senegalensis*; LEE, *Leptodactylus elenae*; LEB, *Leptodactylus bufonius*; LEL, *Leptodactylus latrans*; LEP, *Leptodactylus* cf. *pentadactylus*; LG, *Limnomedusa macroglossa*; MR, *Melanophryniscus rubriventris*; OA, *Odontophrynus achalensis*; OB, *Odontophrynus barrioi*; PHA, *Phyllomedusa azurea*; PHB, *Phyllomedusa boliviana*; PLB, *Pleurodema borellii*; PLG, *Pleurodema* cf. *guayapae*; PLU, *Pleurodema tucumanum*; POL, *Polypedates leucomystax*; PP, *Pseudis platensis*; PSB, *Physalaemus biligonigerus*; PSC, *Physalaemus cuqui*; RF, *Rhinella fernandezae*; RH, *Rhinella schneideri*; RM, *Rhinella major*; RQ, *Rhinella quechua*; SA, *Scinax acuminatus*; SN, *Scinax nasicus*. Scale bars = 0.005 mm, except for CG, CR, HPR, LG, OA, and OB, where the scale bar = 0.02 mm.

bifrons, *L. montanum*, and *Heleophryne regis*) are broad-based and curved. The oral angle is acute in *Ascaphus*, obtuse in the ancestor of Hyloides, and straighter in the ancestor of Ranoides; these two major groups also differ in labial tooth overall curvature and sheath width. Detailed taxonomic variation is summarized and compared with published data in the Supporting information (Table S1).

INTRASPECIFIC VARIATION

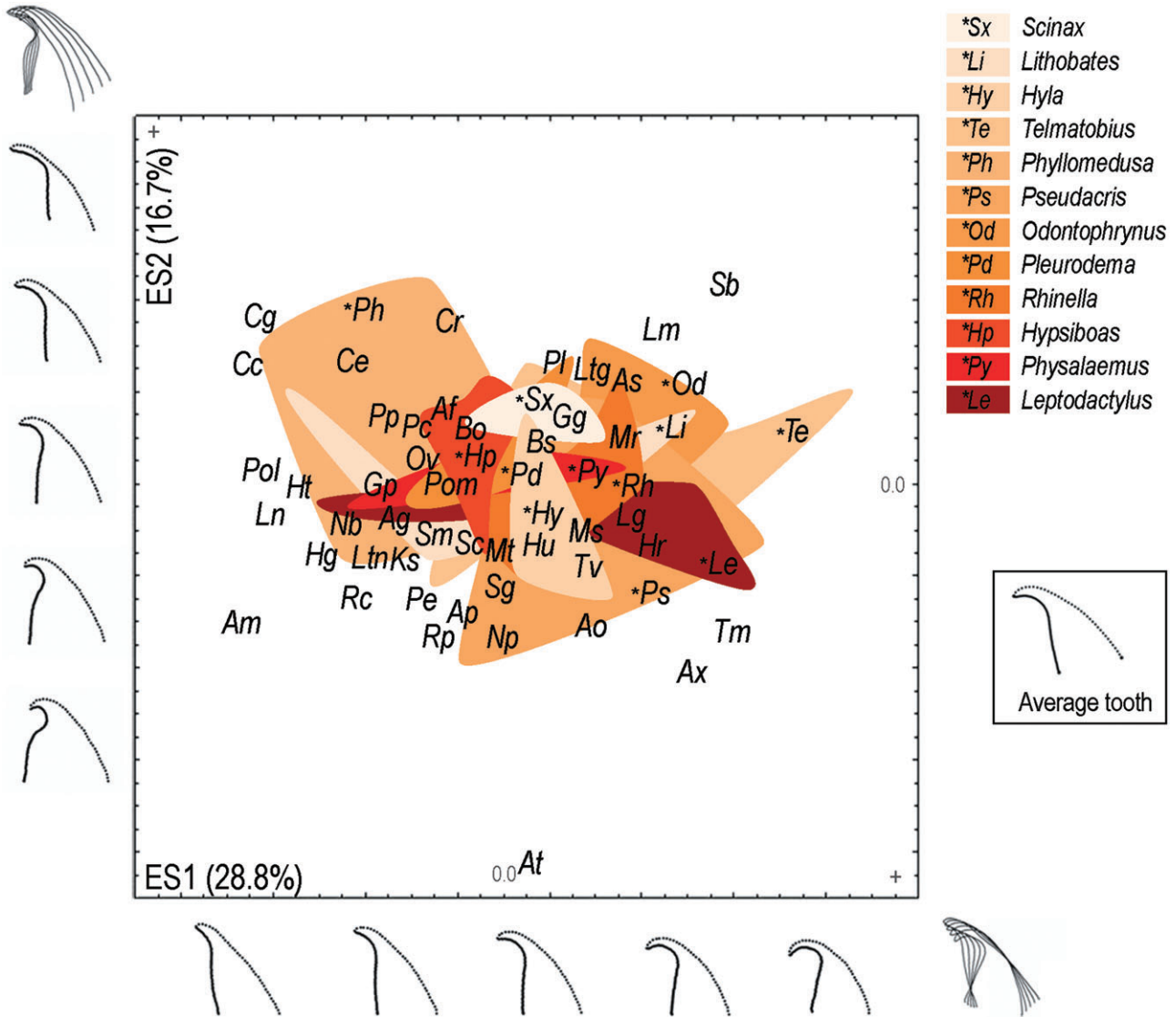The first three eigenshapes account for axes of 60% of the total variation of labial tooth shape of *L. latrans*

Figure 4. Scatterplot of species ($N = 108$) grouped by genera on the first two eigenshapes (ES1–ES2 $\cong$ 46% total variation) plus models of morphological shape change along each axis; labial teeth modelled at the minimum, 25%, 50%, 75%, and maximum scores. Morphological variation, highlighted in the overlapped outlines, relates mainly to the overall curvature of the labial tooth, oral angle, and head shape. Mean shape (at plot coordinates 0,0) is shown within the square at the right of the plot. Genera with more than two species included are shown as shaded polygons. AF, *Allobates femoralis*; AG, *Acris gryllus*; AM, *Ansonia muelleri*; AO, *Alytes obstetricans*; AP, *Atelopus* cf. *petersi*; AS, *Alsodes* sp.; AT, *Ascaphus truei*; AX, *Anaxyrus terrestris*; BO, *Boophis* sp.; BS, *Batrachyla* sp.; CC, *Ceratophrys cranwelli*; CE, Centrolenid sp.; CG, *Calyptocephalella gayi*; CR, *Crossodactylus schmidti*; GG, *Gastrotheca gracilis*; GP, *Gastrotheca pseustes*; HG, *Hemisus guttatus*; HP, *Hypsiboas* spp.; HR, *Heleophryne regis*; HT, *Hyperolius tuberilinguis*; HU, *Huia cavitympanum*; HY, *Hyla* spp.; KS, *Kassina senegalensis*; LE, *Leptodactylus* spp.; LG, *Limnomedusa macroglossa*; LI, *Lithobates* spp.; LM, *Leptobrachium montanum*; LN, *Leptopelis natalensis*; LTG, *Litoria genimaculata*; LTN, *Litoria nyakalensis*; MR, *Melanophryniscus rubriventris*; MS, *Mantidactylus* sp.; MT, *Mannophryne trinitatis*; NB, *Natalobatrachus bonebergi*; NP, *Nyctixalus pictus*; OD, *Odontophrynus* spp.; OV, *Osteocephalus verruciger*; PC, *Plectrohyla chrysopleura*; PD, *Pleurodema* spp.; PE, *Pyxicephalus edulis*; PH, *Phyllomedusa* spp.; PL, *Pseudis limellum*; POL, *Polypedates leucomystax*; POM, *Polypedates megacephalus*; PP, *Pseudis platensis*; PS, *Pseudacris* spp.; PY, *Physalaemus* spp.; RC, *Rana cascadae*; RH, *Rhinella* spp.; RP, *Rhacophorus pardalis*; SB, *Spea bombifrons*; SC, *Schismaderma carens*; SG, *Strongylopus grayii*; SM, *Smilisca baudinii*; SX, *Scinax* spp.; TE, *Telmatobius* spp.; TM, *Thoropa miliaris*; TV, *Trachycephalus venulosus*.

Figure 5. Phylogeny of the studied species showing labial tooth shapes in several taxa. The meta-tree was based on Frost *et al*. (2006), Grant *et al*. (2006), Pramuk (2006), Ponssa (2008), Barrionuevo (2009), and Cei (1980). Abbreviations next to species names represent ecomorphological guilds (Altig & McDiarmid, 1999a). Clades that were significant in canonical phylogenetic ordination (Tables 1, 2) are marked with an asterisk. Outlines on the right are representatives of families, corresponding to observed labial teeth of single specimens (black outlines) and averages of families regarding the overall consensus shape (thin plate splines; Rohlf, 2005). Outlines on the left are optimized shapes for ancestral labial teeth (Catalano *et al*., 2010) in major clades and in significant clades after canonical phylogenetic ordination; vectors on the outlines depict the shape change from corresponding ancestors. AD, adherent; AR, arboreal; CA, carnivore; CL, clasping; FO, fossorial; GA, gastromyzophorous; LEB, lentic benthic; LOB, lotic benthic; NE, nektonic; SR, suspension-rasper; ST, semiterrestrial; SU, suctorial.

Table 1. Results of Monte Carlo permutation tests of individual ecomorphological groups and individual monophyletic clades for the labial tooth shape matrix ($\alpha = 0.05$; significance levels: * 0.05, ** 0.01, *** 0.001), *F*- and *P*-values after 999 Monte Carlo irrestrict permutations, and percentage of the variation explained (with respect to total unconstrained variation)

	F	*P*	% Variance	Cumulative % variance
Monophyletic clades				
Hyloides/Ranoides	14.45	0.001***	11	11
Anomocoela	14.07	0.001***	9	20
Lophiohylini + Hylini	7.93	0.001***	4	24
Sokolanura	4.83	0.010**	3	27
Bufonidae excluding *Melanophryniscus*	4.46	0.008**	3	30
Pelodryadinae + Phyllomedusinae	3.51	0.026*	2	32
Remaining partitions	1.41–2.50	0.082–0.225	3	35
Ecomorphological guilds				
Gastromyzophorous	6.16	0.002**	4	4
Suctorial	5.92	0.004**	3	7

and *L. chaquensis*. Although a marked overlap is evident, a tendency for species to separate on ES3 can be observed, especially among erupted labial teeth (Fig. 6). A multivariate analysis of variance on the whole eigenshape score matrix resulted in significant differences between species (Wilks' lambda = 0.241, $P \cong 0.00000$; differences were significant in univariate tests from ES3: $F = 29.242$, $P \leq 0.00001$).

DISCUSSION

Species ordination based on labial tooth shapes in lateral view shows obvious interspecific variations. Morphological variations relate mainly to the general curvature of the labial tooth axis; the angle between the labial tooth base and labial tooth tip; head shape; and sheath width. As shown by the canonical phylogenetic ordination, this variation is better explained by phylogenetic structure than by tadpole ecology.

PHYLOGENETIC PATTERNS

Labial tooth shape variations appear at several taxonomic levels. Most families overlap their distributions

Table 2. Summary of the results of the canonical phylogenetic ordination of labial tooth shape, ecomorphological guilds, and phylogeny of tadpoles of 108 species

	% Variance
Exclusively phylogeny	35%
Exclusively guilds	7%
Shared	7%
Not explained either by guilds or phylogeny	51%
	100%

in the ordination plot and share labial teeth similar to the average labial tooth; basal non-neobatrachians and *Heleophryne* tadpoles diverge with very curved labial teeth and varied sheath widths, whereas arthroleptid, hemisotid, and calyptocephalellid labial teeth are comparatively straight and narrow (Fig. 4). Curved, broad-based labial teeth optimize as ancestors in major clades, and labial teeth tend to be progressively straighter and narrower (Fig. 5). On the basis of labial tooth features of *Ascaphus* and its basal
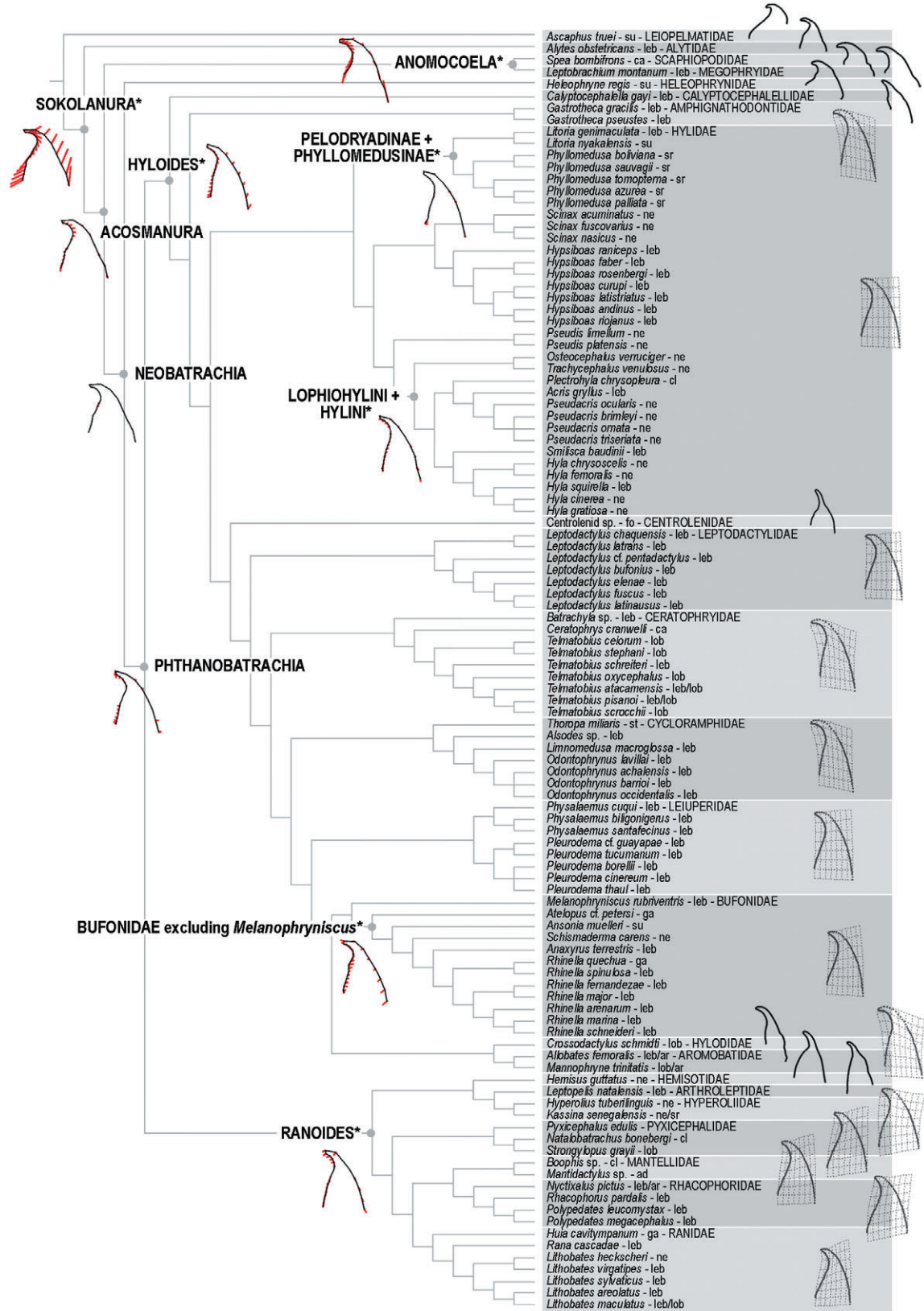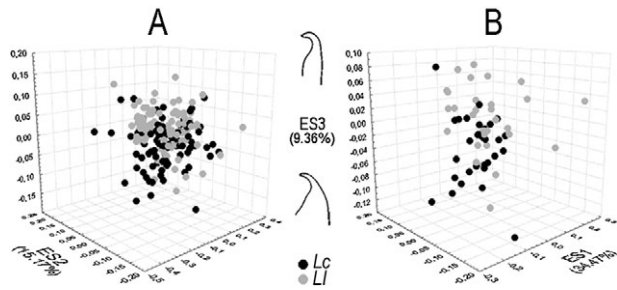
Figure 6. Three-dimensional scatterplots of *Leptodactylus chaquensis* and *Leptodactylus latrans* (LC and LL; $N = 180$) on the first three eigenshapes (ES1–ES2–ES3 ≅ 60% total variation) that illustrate intraspecific variation. A, labial teeth from several sectors of the labial tooth rows and replacement series. B, only erupted labial teeth. Note the tendency of both groups to separate along the ES3 related mainly to labial tooth sheath width.

position in most phylogenetic hypothesis, Noble (1926) and Altig (2006) suggested that the hypothetical ancestral tadpole likely had labial teeth with short, weakly spatulate heads, cusps small to lacking, and an initial function of stabilizing the oral disc. Conversely, Gosner (1959) noted that fully cusped labial teeth occur in basal Alytidae and Bombinatoridae, and that larvae of both *Ascaphus* and Anomocoela are so specialized ecologically and morphologically that it is reasonable that labial tooth form in these species may also be specialized. Consequently, Gosner (1959) interpreted labial tooth reduction in several advanced groups (i.e. hylids and ranids) as independently derived events. It is possible that the ancestral condition for anurans involved a multiserial, burr-like surface of firmly attached labial teeth as seen in the distal rows of *A. truei* (Altig, 2006). In that scenario, the evolution from short, curved, broad-based to slender, longer labial teeth is consistent with the acquisition of a more flexible oral disc, related to the progressive appearance of muscular control for the upper jaw (Lalagobatrachia), oral disc extrinsic musculature (Sokolanura), and uniserial labial tooth rows (Acosmanura) (Haas, 2001, 2003; Wassersug & Yamashita, 2001). The divergence of the major clades Hyloides and Ranoides included differences in labial teeth; the labial teeth of most Hyloides have a straight or obtuse oral angle and a long, curved head. The sample of Ranoides included in this analysis is much smaller than that of Hyloides and, although several families are represented, the labial tooth diversity is surely underestimated. When compared with hyloids, ranoid labial teeth are generally straighter, slightly broader, and have a straight or acute oral angle.

Several genera are distinct based on the labial tooth features. *Hyla* and *Hypsiboas* (until recently considered to be the same genus) differ in labial tooth curvature and sheath width. Ceratophryine genera range from labial teeth absent in *Lepidobatrachus*, straight, noncusped labial teeth in *Ceratophrys*, and curved, cusped labial teeth in *Chacophrys* (Quinzio, Fabrezi & Faivovich, 2006; S. Quinzio, unpubl. data). Basal species of the clade grouping all bufonids excepting *Melanophryniscus* have broad-based labial teeth with short, thick heads; conversely, labial teeth of *Anaxyrus* and *Rhinella* are narrow-based, curved, and have long, narrow heads. On the other hand, tadpoles of some genera have labial teeth that resemble those of tadpoles of closely-related taxa. For example, although different in the overall curvature and sheath width, labial teeth of *Pseudis* have triangular heads with two to four distal cusps, similar to other dendropsophini tadpoles with reduced mouthparts (Echeverría, 1997; Faivovich *et al*., 2005). Labial tooth shape variation is in general also consistent with intrageneric grouping within several genera (e.g. *Lithobates*, *Phyllomedusa*, *Physalaemus*, *Pleurodema*, and *Rhinella*; see Supporting Information, Table S1). For example, labial teeth of tadpoles of the *Pleurodema nebulosum* Group have shorter and less cuspate heads than those of the *P. cinereum* Group. In some cases, there is also variation within species groups; for example, *Leptodactylus elenae* (*L. fuscus* Group) differs from the remaining species within the group by having comparatively straighter labial teeth with very short cusps, and the sibling species *Physalaemus santafecinus* and *Physalaemus biligonigerus* differ in overall labial tooth curvature.

There are studies showing that the larval labial tooth row formula is achieved through sequential adding of tooth rows during early stages (Thibaudeau & Altig, 1988). Conversely, the ontogeny of individual labial tooth is not well studied, although some data suggest that the shape and size are changed during development; labial teeth are smaller at the beginning and the end of the larval period, with shorter heads and scarcer, short cusps (Fig. 7) (Hosoi *et al*., 1995; Grosjean, 2005). Echeverría (1997) commented that, in species with few labial teeth, individual labial teeth are often tiny or weakly developed. We also noted that several taxa in our sample have alternative configurations of labial teeth that vary from numerous, marginal cusps to few, distal cusps; in many of these groups, labial teeth with few cusps co-occur with fewer labial rows compared to related taxa. This occurs in genera relative to other genera (e.g. *Dendropsophus* and *Pseudis* compared to clade outgroups; *Eupsophus* and *Insuetophrynus* relative to other cycloramphids), within genera (e.g. *Leptodactylus pentadactylus* Group; *P. nebulosum* Group; *Scinax acuminatus* and *Scinax boulengeri* relative to other *Scinax*; *Osteopilus ocellatus* relative to other
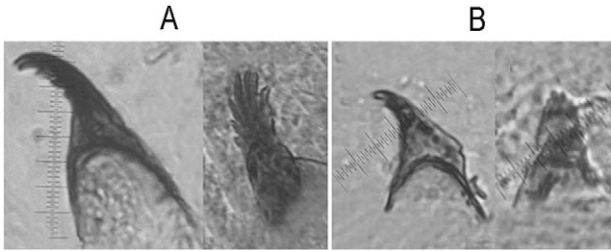
Figure 7. Ontogenetic variation in labial tooth shape in *Leptodactylus chaquensis*. Lateral (left) and frontal (right) views of a labial tooth of tadpoles at Gosner Stages (A) 31 and (B) 41. Images are to scale to show size reduction in the labial tooth of the older specimen.

Osteopilus; *Lithobates heckscheri* and *Lithobates areolatus* relative to other *Lithobates*; *Rana aurora* and *Rana pretiosa* relative to other *Rana*), and even within species groups (e.g. *Rhinella fernandezae* relative to *Rhinella major*) (Gosner, 1959; Cei, 1980; Lannoo, Townsend & Wassersug, 1987; Echeverría, 1997; Lavilla, Ponssa & Saleme, 2000; Borteiro *et al*., 2006; Rabanal & Formas, 2009; Vera Candioti, Nuñez & Ubeda, 2010). This may result from changes in developmental patterns, such that, from a generalized oral configuration (labial tooth row formula 2/3 and labial teeth with several marginal cusps), some species derive by modifying their oral ontogenies (e.g. by developmental truncation) and then show fewer labial rows and individual labial teeth with fewer cusps. This was already suggested for the arboreal tadpoles of *Osteopilus* and *Anotheca* by Wassersug (1980) and Lannoo *et al*. (1987), who proposed that the origin of reduced labial row number and individual labial teeth might be the result of a shift in the timing of development, related to macro- and oophagy. Comparative data on oral ontogenies of closely-related species with different labial tooth morphology together with phylogenetic hypotheses would be insightful for an understanding of the evolution of different oral apparatus configurations.

ECOMORPHOLOGICAL PATTERNS

Keratinized labial teeth are a synapomorphy of Anura (Frost *et al*., 2006), although they are secondarily lost in several groups, in some cases related to ecological aspects. Labial teeth are absent in pipids, rhinophrynids, microhylids, some neustonic forms with upturned oral discs (e.g. *Megophrys*, *Silverstoneia flotator*, and *Leptodactylodon*), megalophagous *Lepidobatrachus*, some macrophagous *Dendropsophus* and *Occidozyga*, burrowers such as *Cochranella* and *Leptobrachella*, and some lenthic/lotic benthic tadpoles of *Cardioglossa*, *Opisthothylax*, and *Taudactylus*. Also,

endotrophic tadpoles from various families lack labial teeth.

Among species with labial teeth, not all ecomorphological guilds could be included in the present study, and species per guild are not sufficient to define clear trends; however, in some cases, labial tooth morphology is related to tadpole ecology although it explained a small percentage of shape variation (7%) (Fig. 8; Tables 1, 2). As noted by Altig & Johnston (1989), labial tooth shape may affect the style and length of substrate contact and the pressure needed to keep the labial tooth implanted or in proper alignment; this would have consequences for substrate adhesion in different microhabitats. Some trends related to microhabitat are evident. Lotic tadpoles commonly have large, ventral oral discs with uninterrupted marginal papillae, and labial rows with smaller labial teeth arranged more densely than in lentic forms (Altig & Johnston, 1989). Furthermore, Van Buskirk (2009) found significant differences between stream and pond tadpoles, with stream species having arched anterior labial tooth rows, a narrow mouth, and a thin lower jaw sheath. Individual labial tooth shape may also affect substrate adhesion (Littlejohn & Martin, 1965; Odendaal & Bull, 1980; Odendaal, Bull & Nias, 1982). In the present study, a phylogenetically independent ecomorphological effect is significant for guilds of tadpoles from fast-flowing systems (gastromyzophorous and suctorial, $P \leq 0.004$) (Tables 1, 2). The gastromyzophorous tadpoles of *Atelopus* and *Huia* have labial teeth with broad sheaths, curved heads, and an acute oral angle. The four suctorial species we studied (i.e. *A. truei*, *H. regis*, *Litoria nyakalensis*, and *A. muelleri*) have similar labial teeth with some differences in the overall curvature and head shape (Fig. 9) (Inger, 1960, 1985). Functionally, broad-based labial teeth positioned on flattened, broad-based tooth ridges, and shallow interrow valleys (Altig & Johnston, 1989) likely constitute a stronger, resistant system for substrate adhesion. Also, the labial teeth of *A. truei* and other torrent tadpoles have extended front bases (which results in an acute oral angle) that extend into the interrow tissue; this is interpreted as a bracing mechanism to keep the labial teeth from either pulling out too easily or to keep the entire series from collapsing backwards when under pressure (Altig & Pace, 1974; Altig & Johnston, 1989). Cusp pattern could be also functionally correlated with substrate adhesion. In *Rhinella quechua*, the long, distal cusps, almost aligned at the labial tooth tip, could increase labial tooth contact and working surface. The curved labial teeth with numerous cusps of *Amolops*, *Huia*, and *Meristogenys* (Ranidae, gastromyzophorous) and *Rhacophorus gauni* (Rhacophoridae, torrent tadpole; Inger, 1985)
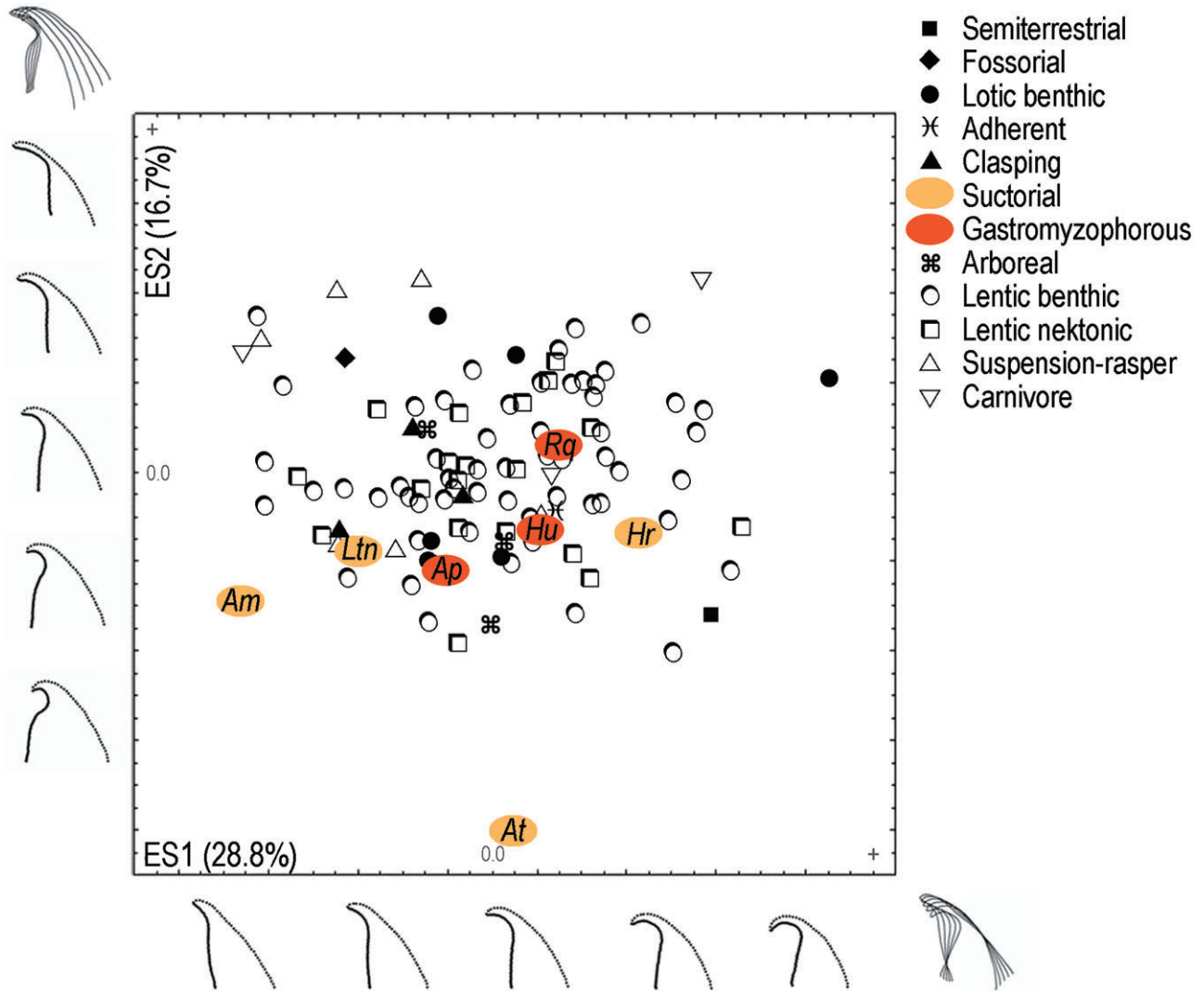
Figure 8. Scatterplot of species (N = 108) grouped by ecomorphological guilds (Altig & Johnston, 1989) on the first two eigenshapes (ES1–ES2 ≅ 46% total variation) plus models of morphological shape change along each axis; labial teeth modelled at minimum, 25%, 50%, 75%, and maximum scores. AM, *Ansonia muelleri*; AP, *Atelopus* cf. *petersi*; AT, *Ascaphus truei*; HR, *Heleophryne regis*; HU, *Huia cavitympanum*; LTN, *Litoria nyakalensis*; RQ, *Rhinella quechua*.

would have the same function. An interesting observation is that the labial teeth of *R. quechua* and *Leptodactylus* cf. *pentadactylus* are much alike in that they are relatively curved, with short, broad, thick heads, and long, distal cusps aligned at the labial tooth tip (Fig. 10). This feature would add to the similarities between macrophagous and rheophilous tadpoles that have been reported in studies of skeletal and muscular systems (Satel & Wassersug, 1981; Haas & Richards, 1998). By contrast, tadpoles in lotic clasping, benthic and fossorial guilds (e.g. species of *Boophis*, *Plectrohyla*, and *Natalobatrachus*, *Crossodactylus*, *Calyptocephalella*, and *Strongylopus*, and centrolenid sp., respectively) inhabit slower flowing water (Altig & Johnston, 1989; Altig & McDi-

armid, 1999b). These habitats apparently do not require special morphological traits, and labial teeth in these species are often straighter, with straight heads and an average sheath width (Figs 8, 9). At the opposite extreme, the labial teeth of the only semiterrestrial species that we included (i.e. *T. miliaris*) are among the most curved and have a strongly curved head and comparatively wide sheath. Likewise, semiterrestrial tadpoles of *Petropedetes martiensseni* (Petropedetidae) have labial teeth with a broad sheath and a strongly flexed head with numerous cusps (Drewes, Altig & Howell, 1989). The lack of data on labial teeth of other semiterrestrial tadpoles precludes a more profound discussion on the relationship with microhabitat and behavior of these
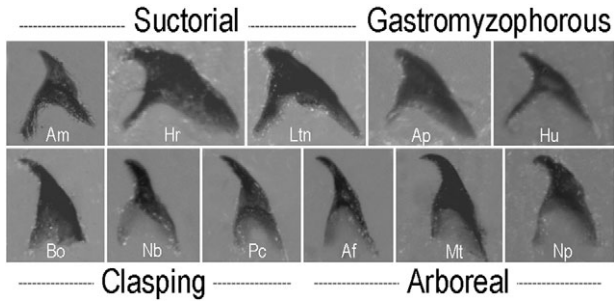
Figure 9. Labial teeth of tadpoles from some different ecomorphological guilds: Suctorial: AM, *Ansonia muelleri*; HR, *Heleophryne regis*; LTN, *Litoria nyakalensis*. Gastromyzophorous: AP, *Atelopus* cf. *petersi*; HU, *Huia cavitympanum*. Clasping: BO, *Boophis* sp.; NB, *Natalobatrachus bonebergi*; PC, *Plectrohyla chrysopleura*. Arboreal: AF, *Allobates femoralis*; MT, *Mannophryne trinitatis*; NP, *Nyctixalus pictus*. Images are not shown to scale.
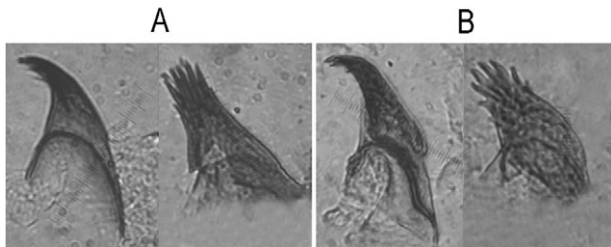

Figure 10. Comparison between labial teeth of (A) *Rhinella quechua* (Bufonidae, gastromyzophorous) and (B) *Leptodactylus* cf. *pentadactylus* (Leptodactylidae, carnivore). Lateral (left) and frontal (right) views showing short, wide, thick heads with long, distal cusps almost aligned at the labial tooth tip.

tadpoles. Finally, labial teeth of tadpoles inhabiting nonflowing and slow current water bodies are scattered on the morphospace, and no clear pattern is discernible among different microhabitats (i.e. benthic, nektonic, and arboreal) (Figs 8, 9).

No ecomorphological guild was significant for labial tooth shape ordination relative to feeding habits. Differences in feeding habits that surely result in feeding partitioning among sympatric tadpoles do occur (Schiesari, Werner & Ling, 2009; Whiles *et al*., 2009), although omnivory with a larger component of animal tissues than expected is common. The role of labial teeth on tadpole feeding mechanisms has been explored in a series of recent contributions that show how missing tooth rows alter feeding kinematics and change foraging efficiency, revealing some constraints that missing teeth have on feeding (Venesky, Parris & Storfer, 2010a; Venesky, Wassersug, Parris, 2010b,c). Regarding labial tooth shape, although specific data are lacking, the harvesting ability of a given labial

tooth shape likely has a wide latitude that could be particularly functional only under specific circumstances. Accordingly, data on gut contents obtained by Gosner (1959) showed no clear relationship between labial tooth shape and food preferences in most species. Macrophagous carnivorous tadpoles share several anatomical features (Wassersug & Hoff, 1979; Vera Candioti, 2007), although labial teeth are very different among the species we studied (Figs 2, 3, 4). Altig & Johnston (1989) hypothesized that, in labial teeth with numerous cusps, the large contact surface surely provides an efficient tool for food removal; the lack of cusps in some carnivorous tadpoles could be compensated by a high labial tooth density or numerous labial tooth rows (e.g. 95 per mm and LTRF 8/8 in *C. cranwelli*; Vera Candioti, 2005). On the other hand, *Leptobrachium*, *Leptolalax*, *Scaphiopus*, and even carnivorous and herbivorous morphs within *Spea* (all Anomocoela species) differ in several characters (Satel & Wassersug, 1981; Pfennig & Murphy, 2000, 2002; Storz, 2004), although labial tooth morphology remains the same regardless of the ecomorphological guild (Gosner, 1959; Altig & Pace, 1974; Inger, 1985; Hall, Larsen & Fitzner, 2002). The relationship between labial tooth configuration and feeding habits in other trophic guilds is also unclear. Finally, among nonfeeding tadpoles, Thibaudeau & Altig (1999) identify a continuum ranging from larvae morphologically almost identical to those of exotrophic species up to highly modified ones that lack several larval characters, including an oral apparatus. The configurations of keratinized mouthparts in species with reduced oral discs has been scarcely studied, and some results indicate that the reduction in number of rows may be accompanied by reduction in the morphology of individual labial teeth as well. In this regard, genera with both exotrophic and endotrophic species (e.g. *Cycloramphus* and *Gastrotheca*; Heyer, 1983; Wassersug & Duellman, 1984; Wiens, Kuczynski, Duellman & Reeder, 2007) likely represent a profitable group for studying the evolution of different oral configurations relative to developmental modes.

Tadpoles have long been considered to exhibit homodonty with labial teeth varying in size in various rows and parts (lateral versus medial) of rows. Hosoi *et al*. (1995) notes that the complexity of labial tooth morphology may increase ontogenetically, and the discussion by Viertel *et al*. (2007) likely represents a similar situation. A recent study by Haas *et al*. (2009) notes the profound differences in labial tooth morphology among rows of *Ansonia* tadpoles. Tooth functional differences related to both substrate adhesion and feeding mechanism in these suctorial tadpoles could be expected. Finally, atypical labial teeth that were not included in the present study warrant comment (e.g. *Phyllodytes gyrinaethes*, *Osteopilus*

brunneus, *Mantidactylus lugubris*, and species of *Hoplobatrachus*; Lannoo *et al.*, 1987; Peixoto, Caramaschi & Freire, 2003; Grosjean, Vences & Dubois, 2004; Altig, 2006). In all cases, the labial teeth are drastically different from either congeners or other close relatives, and all of them sit atop the local epidermis as a series of stacked cones that do not extend into the labial tooth ridge (Altig, Lathrop & Murphy, 2009). Comparative developmental and genetic control studies may help to elucidate whether these unusual labial teeth are in fact modifications of typical labial teeth or nonhomologous structures involving different development mechanisms.

SUMMARY AND PERSPECTIVES

Although this initial analysis did not consider all potential modifiers of labial tooth shape, the comparisons of shapes among taxa reveal similarities and differences that vary between and among various taxonomic levels and, in some cases, these are related to tadpole ecology. The pattern of a large consensus group with outliers of various kinds and distances repeats what is known about tadpole diversity in general. Such patterns imply that an average tadpole shares a number of features with many taxa from many families, even if lesser differences allow for ecological segregation once the details are known. Around this average cloud, there are various novelties that presumably represent morphological excursions into less competitive realms. At the same time, the story remains frustratingly incomplete because we lack important sets of data. There is no information that equates labial tooth morphology with any specifics of harvesting abilities or if, and in what cases (e.g. substrate thick/thin, stiff/flimsy, or discrete/fibrous), labial teeth versus the jaw sheaths are the primary harvesting structures. Knowing the effects of variations of labial tooth shape on withstanding the mechanical stresses (Freeman & Lemen, 2007) during feeding would be informative, and the internal structure of labial teeth (e.g. collagen fiber patterns and internal struts; analogue in Seki, Schneider & Meyers, 2005) as it affords strength to individual labial teeth needs to be studied. Much more information is needed on the mechanical structure and function of the labial tooth series. How the labial teeth in a series are interdigitated, the curvature of the series, how it is rooted in the labial tooth ridge, and how the series responds during a feeding bite comprise pertinent data that are needed to better understand labial tooth and labial tooth row functions. Many aspects of tadpole morphology, including some mouthparts (Bresler, 1954; Relyea & Auld, 2005), are quite plastic under various conditions, and the presence of such variations of labial teeth needs examination. Finally, additional information is needed on the ontogenetic changes of the sizes and shapes of labial teeth (Hosoi *et al.*, 1995) of different developmental generations and whether these changes reflect a phylogenetic progression and any changes in function and ecology. In this context, the present study represents a first approach to labial tooth shape variation that will be useful for future comparative and functional studies.

ACKNOWLEDGEMENTS

REFERENCES

Altig R. 2006. Discussions of the origin and evolution of the oral apparatus of anuran tadpoles. *Acta Herpetologica* **2:** 95–105.

Altig R. 2007. A primer for the morphology of anuran tadpoles. *Herpetological Conservation and Biology* **2:** 71–74.

Altig R, Johnston GF. 1989. Guilds of anuran larvae: relationships among developmental modes, morphologies and habits. *Herpetological Monographs* **3:** 81–109.

Altig R, Lathrop A, Murphy RW. 2009. Morphology of Southeast Asian tadpoles: *Hoplobatrachus chinensis* (Dicroglossidae), *Leptolalax pelodytoides* (Megophryidae), and other megophryids. *Russian Journal of Herpetology* **16:** 126–130.

Altig R, McDiarmid RW. 1999a. Body plan: development and morphology. In: McDiarmid RW, Altig R, eds. *Tadpoles: the biology of Anuran larvae*. Chicago, IL: University of Chicago Press, 24–51.

Altig R, McDiarmid RW. 1999b. Diversity: familial and generic characterization. In: McDiarmid RW, Altig R, eds. *Tadpoles: the biology of Anuran larvae*. Chicago, IL: University of Chicago Press, 295–337.

Altig R, Pace WL. 1974. Scanning electron photomicrographs of tadpole labial teeth. *Journal of Herpetology* **8:** 247–251.

Barrionuevo JS. 2009. Análisis filogenético de las especies del grupo meridional del género *Telmatobius* (Anura: Ceratophryidae). DPhil. Thesis, Universidad Nacional de Tucumán.

Borcard D, Legendre P, Drapeau P. 1992. Partialing out the spatial component of ecological variation. *Ecology* **73:** 1045–1055.

Borteiro C, Kolenc F, Tedros M, Prigioni C. 2006. The tadpole of *Chaunus dorbignyi* (Duméril & Bibron) (Anura, Bufonidae). *Zootaxa* **1308:** 49–62.

Bresler J. 1954. The development of labial teeth of salientian larvae in relation to temperature. *Copeia* **1954:** 207–211.

Briggs DEG, Crowther PR. 2003. *Paleobiology II*. Cornwall, United Kingdom: Blackwell Science Ltd.

Catalano SA, Goloboff PA, Giannini NP. 2010. Phylogenetic morphometrics (I): the use of landmark data in a phylogenetic framework. *Cladistics*. doi 10.1111/j.1096-0031.2010.00302.x.

Cei JM. 1980. Amphibians of Argentina. *Monitore zoologico italiano, Monografía* **2:** XII–609.

Drewes RC, Altig R, Howell KM. 1989. Tadpoles of three frog species endemic to the forests of the Eastern Arc Mountains, Tanzania. *Amphibia-Reptilia* **10:** 435–443.

Dubois A. 1995. Keratodont formulae in anuran tadpoles: proposals for a standardization. *Journal of Zoological Systematics and Evolutionary Research* **33:** 1–15.

Dujardin L, Duriez T. 1995. A mathematical model for the shape of the hooks of cestodae. *Acta Biotheoretica* **43:** 217–225.

Echeverría DD. 1997. Microanatomy of the oral apparatus and oral cavity of *Hyla minutus* Peters, 1872 larvae (Anura, Hylidae), with data on feeding habits. *Alytes* **15:** 26–36.

Fabrezi M. 2001. Variación morfológica de la dentición en anuros. *Cuadernos de Herpetología* **15:** 17–28.

Fabrezi M, Emerson SB. 2003. Parallelism and convergence in anuran fangs. *Journal of Zoology* **260:** 41–61.

Faivovich J, Haddad CFB, Garcia PCA, Frost DR, Campbell JA, Wheeler WC. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History* **294:** 1–240.

Fiorito de López LE, Echeverría DD. 1984. Morfogénesis de los dientes larvales y pico córneo de *Bufo arenarum* (Anura: Bufonidae). *Revista del Museo Argentino de Ciencias Naturales, Zoología* **13:** 573–578.

Fiorito de López LE, Echeverría DD. 1989. Microanatomía e histogénesis del aparato bucal en las larvas de *Bufo arenarum* (Anura: Bufonidae). *Cuadernos de Herpetología* **4:** 4–10.

Freeman PW, Lemen C. 2007. An experimental approach to modeling the strength of canine teeth. *Journal of Zoology* **271:** 162–169.

Frost DR. 2009. Amphibian Species of the World: an online reference version 5.3 (12 February, 2009). Electronic database available at: http://research.amnh.org/herpetology/amphibia/index.php (accessed March 2010) American Museum of Natural History, New York, NY.

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, De Sá R, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto BL, Moler P, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* **297:** 1–370.

Funk VA, Specht CD. 2007. Meta-trees: grafting for a global perspective. *Proceedings of the Biological Society of Washington* **120:** 232–240.

Geerinckx T, De Poorter J, Adriaens D. 2007. Morphology and development of teeth and epidermal brushes in loricariid catfishes. *Journal of Morphology* **268:** 805–814.

Giannini NP. 2003. Canonical phylogenetic ordination. *Systematic Biology* **52:** 684–695.

Goloboff PA. 2003. Parsimony, likelihood, and simplicity. *Cladistics* **19:** 91–103.

Gosner KL. 1959. Systematic variations in tadpole teeth with notes on food. *Herpetologica* **15:** 203–210.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16:** 183–190.

Grant T, Frost DR, Caldwell JP, Gagliardo R, Haddad CFB, Kok PJR, Means DB, Noonan BP, Schargel WE, Wheeler WC. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History* **299:** 1–268.

Grosjean S. 2005. The choice of external morphological characters and developmental stages for tadpole-based anuran taxonomy: a case study in *Rana* (*Sylvirana*) *nigrovittata* (Blyth, 1855) (Amphibia, Anura, Ranidae). *Contributions to Zoology* **74:** 61–76.

Grosjean S, Vences M, Dubois A. 2004. Evolutionary significance of oral morphology in the carnivorous tadpoles of tiger frogs, genus *Hoplobatrachus* (Ranidae). *Biological Journal of the Linnean Society* **81:** 171–181.

Haas A. 2001. Mandibular arches musculature of anuran tadpoles, with comments on homologies of amphibian jaw muscles. *Journal of Morphology* **247:** 1–33.

Haas A. 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* **19:** 23–89.

Haas A, Richards SJ. 1998. Correlations of cranial morphology, ecology and evolution in australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura: Hylidae: Pelodryadinae). *Journal of Morphology* **238:** 109–141.

Haas A, Wolter J, Hertwig ST, Das I. 2009. Larval morphologies of three species of stream toads, genus *Ansonia* (Amphibia: Bufonidae) from East Malaysia (Borneo), with a key to known Bornean *Ansonia* tadpoles. *Zootaxa* **2302:** 1–18.

Hall JA, Larsen JH Jr, Fitzner RE. 2002. Morphology of the prometamorphic larva of the spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae), with an emphasis on the lateral line system and mouthparts. *Journal of Morphology* **252:** 114–130.

Hanken J, Hall B. 1993. *The skull. Volume 3. Functional and evolutionary mechanisms*. Chicago, IL: University of Chicago Press.

Héron-Royer LF, Van Bambeke C. 1889. Le vestibule de la bouche chez les têtards des batraciens anoures d'Europe. *Archives de Biologie* **9:** 185–309.

Herrel A, Vanhooydonck B, Van Damme R. 2004. Omnivory in lacertid lizards: adaptive evolution or constraint? *Journal of Evolutionary Biology* **17:** 974–984.

Heyer R. 1983. Variation and systematics of frogs of the genus *Cycloramphus* (Amphibia, Leptodactylidae). *Arquivos de Zoologia* **30:** 235–339.

Hosoi M, Niida S, Yoshiko Y, Suemune S, Maeda N. 1995. Scanning electron microscopy of horny teeth in the anuran tadpole Rhacophoridae, *Rhacophorus arboreus* and *Rhacophorus schlegelii*. *Journal of Electron Microscopy* **44:** 351–357.

Inger RF. 1960. A review of the Oriental toads of the genus *Ansonia* Stoliczka. *Fieldiana Zoology* **39:** 473–503.

Inger RF. 1985. Tadpoles of the forested regions of Borneo. *Fieldiana Zoology n. s.* **26:** 1–89.

Krieger JD. 2008. *Extended eigenshape*, Version 3.0. Available at: http://www.morpho-tools.net

Krieger JD, Guralnick RP, Smith DM. 2007. Generating empirically determined, continuous measures of leaf shape for paleoclimate reconstruction. *Palaios* **22:** 212–219.

Lannoo MJ, Townsend DS, Wassersug RJ. 1987. Larval life in the leaves: arboreal tadpoles types, with special attention to the morphology, ecology, and behavior of the oophagous *Osteopilus brunneus* (Hylidae) larva. *Fieldiana Zoology n. s.* **38:** 1–31.

Lavilla EO, Ponssa ML, Saleme S. 2000. Caracterización de las larvas de *Bufo fenandezae* Gallardo, 1957 y *Bufo granulosus major* Müller & Hellmich, 1936 (Anura: Bufonidae) y clave para la identificación de las larvas de *Bufo* que habitan el Chaco Argentino. *Bollettino del Museo Regionale di Scienze Naturali di Torino* **17:** 333–344.

Littlejohn MJ, Martin AA. 1965. A new species of *Crinia* (Anura: Leptodactylidae) from South Australia. *Copeia* **1965:** 19–24.

Lohmann GP. 1983. Eigenshape analysis of microfossils: a general morphometric procedure for describing changes in shape. *Mathematical Geology* **15:** 659–672.

MacLeod N. 1999. Generalizing and extending the eigenshape method of shape space visualization and analysis. *Paleobiology* **25:** 107–138.

MacLeod N. 2002. Geometric morphometrics and geological shape-classification systems. *Earth-Science Reviews* **59:** 27–47.

Massare JA. 1987. Tooth morphology and prey preference of Mesozoic marine reptiles. *Journal of Vertebrate Paleontology* **7:** 121–137.

Noble GK. 1926. The importance of larval characters on the classification of South African Salientia. *American Museum Novitates* **237:** 1–10.

Odendaal FJ, Bull CM. 1980. Influence of water speed on tadpoles of *Ranidella signifera* and *R. riparia* (Anura: Leptodactylidae). *Australian Journal of Zoology* **28:** 79–82.

Odendaal FJ, Bull CM, Nias RC. 1982. Habitat selection in tadpoles of *Ranidella signifera* and *R. riparia* (Anura: Leptodactylidae). *Oecologia* **52:** 411–414.

Orrico VGD, Mongin MM, Carvalho-e-Silva AMPT. 2007. The tadpole of *Hypsiboas latistriatus* (Caramaschi & Cruz, 2004), a species of the *Hypsiboas polytaenius* (Cope, 1870) clade (Amphibia, Anura, Hylidae). *Zootaxa* **1531:** 25–37.

Padilla DK. 2003. Form and function of radular teeth of herbivorous mollusks: focus on the future. *American Malacological Bulletin* **18:** 1–6.

Peixoto OL, Caramaschi U, Freire EMX. 2003. Two new species of *Phyllodytes* (Anura: Hylidae) from the state of Alagoas, northeastern Brazil. *Herpetologica* **59:** 235–246.

Pfennig DW, Murphy PJ. 2000. Character displacement in polyphenic tadpoles. *Evolution* **54:** 1738–1749.

Pfennig DW, Murphy PJ. 2002. How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution* **56:** 1217–1228.

Ponssa ML. 2008. Cladistic analysis and osteological descriptions of the frog species in the *Leptodactylus fuscus* species group (Anura, Leptodactylidae). *Journal of Zoological Systematics and Evolutionary Research* **46:** 249–266.

Pramuk JB. 2006. Phylogeny of South American *Bufo* (Anura: Bufonidae) inferred from combined evidence. *Zoological Journal of the Linnean Society* **146:** 407–452.

Quinzio SI, Fabrezi M, Faivovich J. 2006. Redescription of the tadpole of *Chacophrys pierottii* (Vellard, 1948) (Anura, Ceratophryidae). *South American Journal of Herpetology* **1:** 202–209.

Rabanal FE, Formas JR. 2009. Complementary diagnosis of the genus *Insuetophrynus* (Anura, Cycloramphidae) based on larval characters. *Zootaxa* **2116:** 59–67.

Reilly SM, McBrayer LD, White TD. 2001. Prey processing in amniotes: biomechanical and behavioral patterns of food reduction. *Comparative Biochemistry and Physiology Part A* **128:** 397–415.

Relyea RA, Auld JR. 2005. Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. *Ecology* **86:** 1723–1729.

Rohlf FJ. 1986. Relationships among eigenshape analysis, Fourier analysis, and analysis of coordinates. *Mathematical Geology* **18:** 815–857.

Rohlf FJ. 2005. *tpsRelw*, Version 1.42. New York, NY: Ecology & Evolution, SUNY at Stony Brook. Available at: http://life.bio.sunysb.edu/morph

Rohlf FJ. 2008. *tpsDig*, Version 2.11. New York, NY: Ecology & Evolution, SUNY at Stony Brook. Available at: http://life.bio.sunysb.edu/morph

Rüber L, Adams DC. 2001. Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. *Journal of Evolutionary Biology* **14:** 325–332.

Satel S, Wassersug RJ. 1981. On the relative sizes of buccal floor depressor and elevator musculature in tadpoles. *Copeia* **1981:** 129–137.

Schiesari L, Werner EE, Ling GW. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. *Freshwater Biology* **54:** 572–586.

Seki Y, Schneider MS, Meyers MA. 2005. Structure and mechanical behavior of a toucan beak. *Acta Materialia* **53:** 5281–5296.

Storz BL. 2004. Reassessment of the environmental mechanisms controlling developmental polyphenism in spadefoot toad tadpoles. *Oecologia* **141:** 402–410.

Sumida SS, Murphy RW. 1987. Form and function of the tooth crown structure on gekkonid lizards (Reptilia, Squamata, Gekkonidae). *Canadian Journal of Zoology* **65:** 2886–2892.

Taylor CL, Altig R, Boyle CR. 1996. Oral disc kinematics of four lentic anuran tadpoles. *Herpetological Natural History* **4:** 49–56.

Ter Braak CJF, Smilauer P. 1997. *CANOCO for Windows,* Version 4.5. Wageningen, The Netherlands: Biometrics – Plant Research International.

Thibaudeau G, Altig R. 1988. Sequence of ontogenetic development and atrophy of the oral apparatus of six anuran tadpoles. *Journal of Morphology* **197:** 63–69.

Thibaudeau G, Altig R. 1999. Endotrophic anurans. In: McDiarmid RW, Altig R, eds. *Tadpoles: the biology of Anuran larvae*. Chicago, IL: University of Chicago Press, 170–188.

Ungar PS, M'Kirera F. 2003. A solution to the worn tooth conundrum in primate functional anatomy. *Proceedings of the National Academy of Sciences of the United States of America* **100:** 3874–3877.

Van Buskirk J. 2009. Getting in shape: adaptation and phylogenetic inertia in morphology of Australian anuran larvae. *Journal of Evolutionary Biology* **22:** 1326–1337.

Van Dijk DE. 1966. Systematic and filed keys to the families, genera and described species of the Southern African anuran tadpoles. *Annals of the Natal Museum* **18:** 231–286.

Venesky MD, Parris MJ, Storfer A. 2010a. Impacts of *Batrachochytrium dendrobatidis* infection on tadpole foraging performance. *EcoHealth* **6:** 565–575.

Venesky MD, Wassersug R, Parris MJ. 2010b. How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenocephalus*)? *Biological Bulletin* **218:** 160–168.

Venesky MD, Wassersug R, Parris MJ. 2010c. Fungal pathogen changes the feeding kinematics of larval anurans. *Journal of Parasitology* **96:** 552–557.

Vera Candioti MF. 2005. Morphology and feeding in tadpoles of *Ceratophrys cranwelli* (Anura: Leptodactylidae). *Acta Zoologica* **86:** 1–11.

Vera Candioti MF. 2007. Anatomy of anuran tadpoles from lentic water bodies: systematic relevance and correlation with feeding habits. *Zootaxa* **1600:** 1–175.

Vera Candioti MF, Nuñez JJ, Ubeda C. 2010. Development of the nidicolous tadpoles of *Eupsophus emiliopugini* (Anura: Cycloramphidae) until metamorphosis, with comments on systematic relationships of the species and its endotrophic developmental mode. *Acta Zoologica*. Early view. doi 10.1111/j.1463-6395.2010.00448.x.

Viertel B, Lötters S, Baumgart A, Oberst M, Eisenbeis G, Veith M. 2007. Larval morphology of reed frogs, *Hyperolius kivuensis* and *H. viridiflavus*, from western Kenya (Amphibia, Hyperoliidae). *Revue Suisse de Zoologie* **114:** 825–837.

Wassersug RJ. 1980. Internal oral features of larvae from eight anuran families. Functional, systematics, evolutionary and ecological considerations. *Miscellaneous Publications of the Museum of Natural History, University of Kansas* **65:** 1–146.

Wassersug RJ, Duellman WE. 1984. Oral structure and their development in egg-brooding hylid frog embryos and larvae: evolutionary and ecological implications. *Journal of Morphology* **182:** 1–37.

Wassersug RJ, Hoff K. 1979. A comparative study of the buccal pumping mechanism of tadpoles. *Biological Journal of the Linnean Society* **12:** 225–259.

Wassersug RJ, Yamashita M. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. *Comparative Biochemistry and Physiology Part A* **131:** 183–195.

Whiles MR, Galdyshev MI, Sushchik NN, Makhutova ON, Kalachova GS, Peterson SD, Regester KJ. 2009. Fatty acid analyses reveal high degrees of omnivory and dietary plasticity in pond-dwelling tadpoles. *Freshwater Biology* **55:** 1533–1547.

Wiens JJ, Kuczynski CA, Duellman WE, Reeder TW. 2007. Loss and re-evolution of complex life 658 cycles in marsupial frogs: does ancestral trait reconstruction mislead? *Evolution* **61:** 1886–1899.

Zahn CT, Roskies RZ. 1972. Fourier descriptors for plane closed curves. *IEEE Transactions, Computers* **C21:** 269–281.

SUPPORTING INFORMATION

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

Doc. S1. Tadpole species studied ($N = 108$, names followed by an asterisk), and species with previous published information. Following columns are the species group (*sensu* Frost, 2009), ecomorphological guild (*sensu* Altig & McDiarmid, 1999b), source of the image used (SEM, scanning electron microscopy; LM, light microscopy; PL, published in literature), distinctive labial tooth features, and literature references.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.