

ECOMORPHOLOGICAL GUILDS IN ANURAN LARVAE: AN APPLICATION OF GEOMETRIC MORPHOMETRIC METHODS

M. FLORENCIA VERA CANDIOTI

Instituto de Herpetología, Fundación Miguel Lillo, Miguel Lillo 251, 4000 San Miguel de Tucumán, Argentina

Ecomorphological guilds for anuran larvae are based on developmental modes, external morphology and habitat. Furthermore, several authors have investigated relationships between internal morphology and ecological habits. However, the relationships between internal morphology and tadpole ecological habits are not well established. In the present paper the quantitative methodology of geometric morphometrics is applied to look for correlation between the anatomy hyobranchial skeleton and the ecology of anuran larvae. Tadpoles of 14 species belonging to six different ecomorphological guilds were studied. The specimens were cleared and stained, and the hyobranchial apparatuses removed and drawn in ventral view. To record the shape variation, landmark-based geometric morphometric methodology was applied, involving a Relative Warp Analysis followed by multivariate statistics. Results show that species classify into four significantly different groups, according to their hyobranchial apparatus shape. Macrophagous tadpoles have well-developed ceratohyals and hypobranchial plate developed, and branchial baskets highly reduced. Generalized tadpoles have a large ceratobranchial area, with the hypobranchial plate covering a smaller area. Microphagous tadpoles have a very developed and complex branchial basket, and their hypobranchial plates are strongly reduced. Megalophagous tadpoles have the ceratohyals laterally expanded. These four groups are in general maintained after the inclusion of more species from the literature. Morphological groups can be related to size of food particles consumed, from very large in megalophages and macrophages, to very small, in highly efficient microphages.

Key words: feeding habit, frog, hyobranchial skeleton, morphology, tadpole

INTRODUCTION

Altig and Johnston (1989) define ecomorphological guilds for tadpoles on the basis of developmental modes, habitat, and external morphological characters, such as body shape, eye position, and oral disc configuration and orientation. They argue that it is possible to find general morphological patterns linked to specific habitats and feeding habits. Several researchers have found that not only external, but also internal morphology can be related to tadpole ecology. Thus, there exist a number of published studies on the relations between buccal, skeletal, and muscular characteristics and feeding habits (Wassersug, 1980; Satel & Wassersug, 1981; Hall *et al.*, 2002; Alcalde & Rosset, 2003; Vera Candiotti & Haas, 2004; Vera Candiotti *et al.*, 2004; Vera Candiotti, 2005), and between internal morphology and microhabitat of these organisms (Noble, 1929; Haas & Richards; 1998).

Although most of the studies of tadpole chondrocrania are qualitative in nature, Larson (2002; 2005) analysed the ontogenetic changes in the chondrocranium of *Rana* spp. tadpoles, using a quantitative method for shape analysis. Geometric morphometric methods, followed by multivariate statistics, have been employed to show ontogenetic and allometric shape changes in other taxa (i.e., Monteiro & Abe, 1997; Monteiro *et al.*, 1999). In other studies, this methodology has been used to establish comparisons of shape among organisms of different spe-

cies (i.e., Rohlf, 1993; Fink & Zelditch, 1995; Monteiro & Abe, 1999; Giri & Collins, 2004; Stayton, 2005). These methods quantify shape change and allow the visualisation of patterns of morphological change through the use of thin plate splines and vectors. This kind of analysis provides a valuable option by transposing the application of geometric morphometrics to shape variations among species with different morphologies linked to varied ecology. In this study, geometric morphometric methods are applied to the analysis of the shape variation in the hyobranchial skeleton of tadpoles. The hyobranchial apparatus constitutes the floor of the buccal cavity and supports the gill filters and gills. It relates directly to feeding, since it intervenes in the buccal pump mechanism and in sorting and entrapment of food particles.

MATERIAL AND METHODS

Fourteen species of tadpoles from lentic environments were studied. They were selected according to the guilds mentioned by Altig & Johnston (1989). One to five individuals per species (46 specimens in total), at Gosner (1960) developmental stages 25-36 were analysed (see Appendix 1). The specimens were cleared and stained following the Wassersug (1976) protocol, then dissected using a stereomicroscope. The hyobranchial skeletons were removed and drawn in a ventral view, employing a camera lucida.

Variation in the shape of the hyobranchial skeletons across species was quantified following the geometric morphometric method described in Rohlf & Bookstein

TABLE 1. Tadpole guilds according to Altig & Johnston (1989). *Telmatobius* cf. *atacamensis* is placed in this guild because, even though it breeds in mountain streams, its larvae live in small pools without currents.

CARNIVORES	
<i>Ceratophrys cranwelli</i>	Type I
<i>Lepidobatrachus llanensis</i>	Type II
MACROPHAGOUS	
<i>Hyla nana</i>	Type II
<i>Hyla microcephala</i>	
SUSPENSION FEEDERS	
<i>Elachistocleis bicolor</i>	Type II
<i>Chiasmocleis panamensis</i>	
SUSPENSION RASPERS	
<i>Phyllomedusa hypochondrialis</i>	
BENTHIC	
<i>Bufo arenarum</i>	
<i>Bufo spinulosus</i>	
<i>Hyla rosenbergi</i>	
<i>Physalaemus santafecinus</i>	
<i>Telmatobius</i> cf. <i>atacamensis</i>	
NEKTONIC	
<i>Lysapsus limellus</i>	
<i>Scinax nasicus</i>	

(1990) and Larson (2002). The removed hyobranchial skeletons were placed in a slide, trying to maintain the same orientation in each case. A set of landmarks was then marked on the right side of the skeleton, with the camera lucida. Landmark selection was based on Haas & Richards (1998), with some of the points redefined. The sixteen landmarks are (see also Fig. 1): (1) nostral margin of pars reuniens; (2) tip of processus anterior

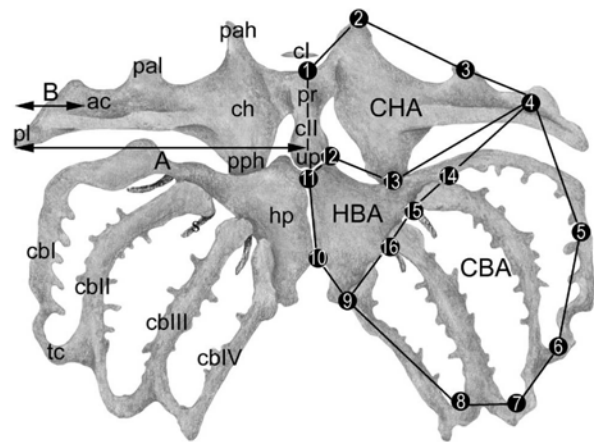


FIG. 1. Landmarks and measurements recorded on the hyobranchial skeleton, ventral view. 1-16, landmarks. Descriptions in text. A, width of ceratohyal; B, distance between the tip of the processus lateralis and the articular condyle; CBA, area of the ceratobranchials; CHA, area of the ceratohyal; HBA, area of the hypobranchial plate; ac, articular condyle; cb, ceratobranchial (I-IV); ch, ceratohyal; cI, copula I; cII, copula II; hp, hypobranchial plate; pah, processus anterior hyalis; pal, processus anterolateralis; pl, processus lateralis; pph, processus posterior hyalis; pr, pars reuniens; s, spiculum; tc, terminal commissure; up, urobranchial process.

hyalis; (3) tip of processus anterolateralis; (4) articular condyle; (5) most lateral internal point of branchial basket; (6) most caudal point of gill slit I; (7) most caudal point of gill slit II; (8) most caudal and medial point of ceratobranchial III; (9) most nostral and medial point of ceratobranchial IV; (10) most caudal point of hypobranchial plates junction; (11) most caudal point of copula II; (12) lateral point of hypobranchial plate - copula II junction; (13) tip of processus posterior hyalis; (14) most caudal point of ceratobranchial I - hypobranchial plate junction; (15) most caudal point of ceratobranchial II - hypobranchial plate junction; (16) most caudal point of ceratobranchial III - hypobranchial plate junction.

The configurations of landmarks were next digitized, using the program tpsDig (Rohlf, 2004), and translated, standardised to centroid size=1, and aligned through the Generalized Procrustes Analysis (GPA) to produce a consensus configuration, using the program tpsRelw (Rohlf, 2003). This method removes differences in size, position or rotation of the objects. A Relative Warp Analysis (i.e., Principal Component Analysis on the residuals from superimposition) was performed to obtain a plot of specimens scattered in a space defined by variability axes (the relative warps). Variation in shapes was depicted with thin-plate spline deformation grids, which reveals the modified shape compared to the consensus configuration (for further explanations see Rohlf & Bookstein, 1990; Bookstein, 1991; Fink & Zelditch, 1995; Monteiro & Reis, 1999; Adams *et al.*, 2004, among others). Finally, multivariate analyses were run (SPSS, 1998) on the scores of each specimen on the first relative warps, to test for significant differences among groups formed.

In addition to landmarks, some measurements already reported to be variable across species (Wassersug and Hoff, 1979; Haas & Richards, 1998) were recorded: in-lever arm proportion (distance between the lateral tip of processus lateralis and the articular condyle / total width of ceratohyal); ceratohyal area; hypobranchial area, ceratobranchials area (relative to the total hyobranchial apparatus area). Landmarks and measurements are shown in Fig. 1.

RESULTS

MORPHOLOGY OF THE HYOBRANCHIAL APPARATUS

Fig. 2 shows drawings of the hyobranchial apparatus of each of the 14 species. In *Bufo arenarum* tadpoles, the ceratohyals are elongated and have very prominent processes: processus anterior hyalis, anterolateralis, lateralis, posterior hyalis and the articular condyle, which is the point of articulation with the palatoquadrate. Copula I is small, and copula II is almost twice as long as the pars reuniens, and with a short urobranchial process. The ceratobranchials have numerous lateral projections and are distally joined by terminal commissures. Ceratobranchials I and II are synchondrotically attached to the hypobranchial plate,

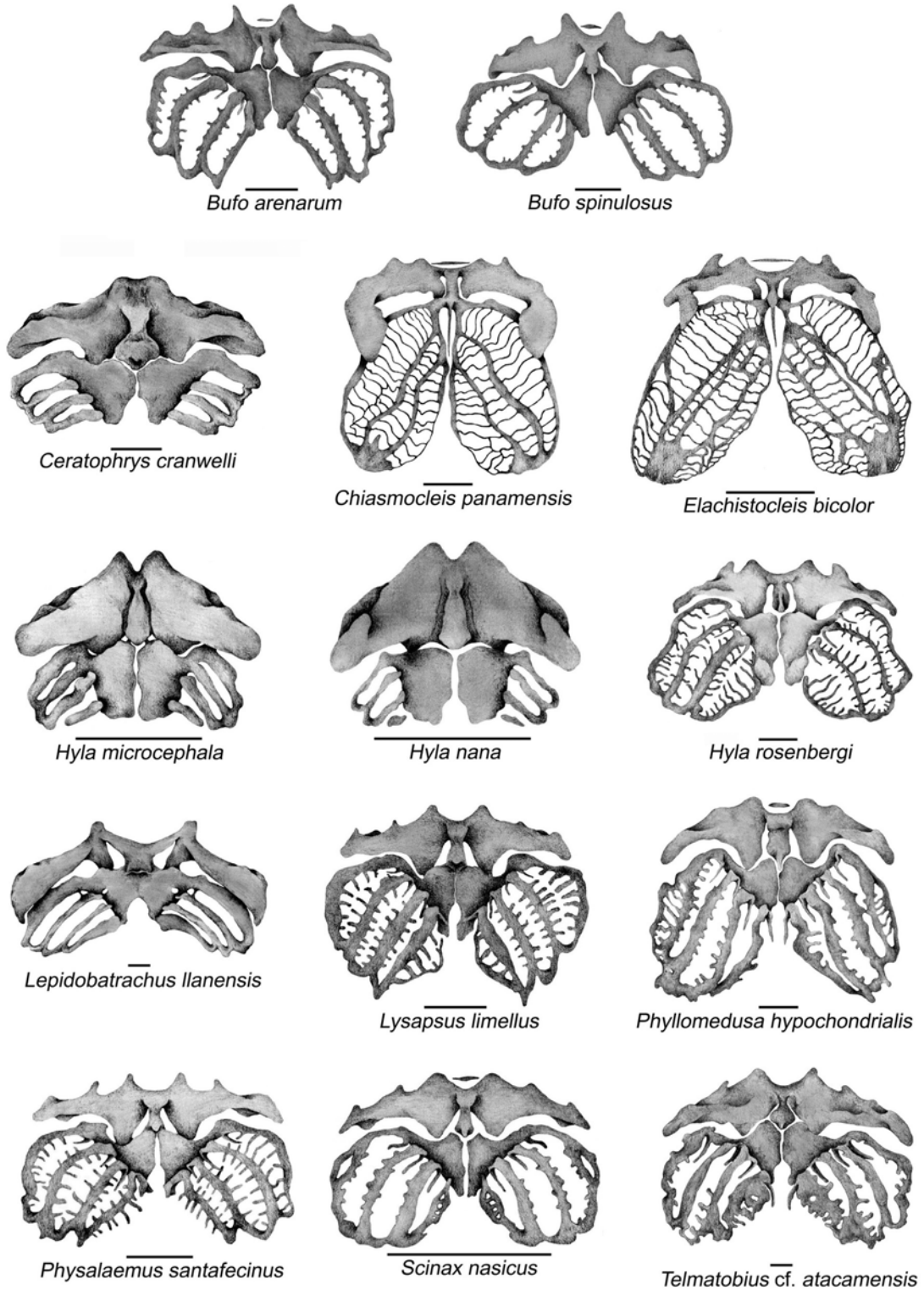


FIG. 2. Hyobranchial skeletons of the 14 species examined, ventral view. Scale bars = 1mm.

and the III and IV are connected by conjunctive tissue. Dorsally, there are four spicula extending from the base of each ceratobranchial; the fourth one is reduced. Tadpoles of *B. spinulosus*, *Hyla rosenbergi*, *Physalaemus santafecinus*, *Scinax nasicus*, *Telmatobius* cf. *atacamensis*, *Lysapsus limellus* and *Phyllomedusa hypochondrialis* have very similar hyobranchial skel-

etons. In *L. limellus* the first three spicula are long and thin, and the fourth one forms a quadrangular, poorly chondrified plate, continuous with hypobranchial plate. *Phyllomedusa hypochondrialis* ceratohyals have small processus anterior hyalis and anterolateralis, and the urobranchial process is longer than in the other species (28% of the length of the copula II). Copula I is absent

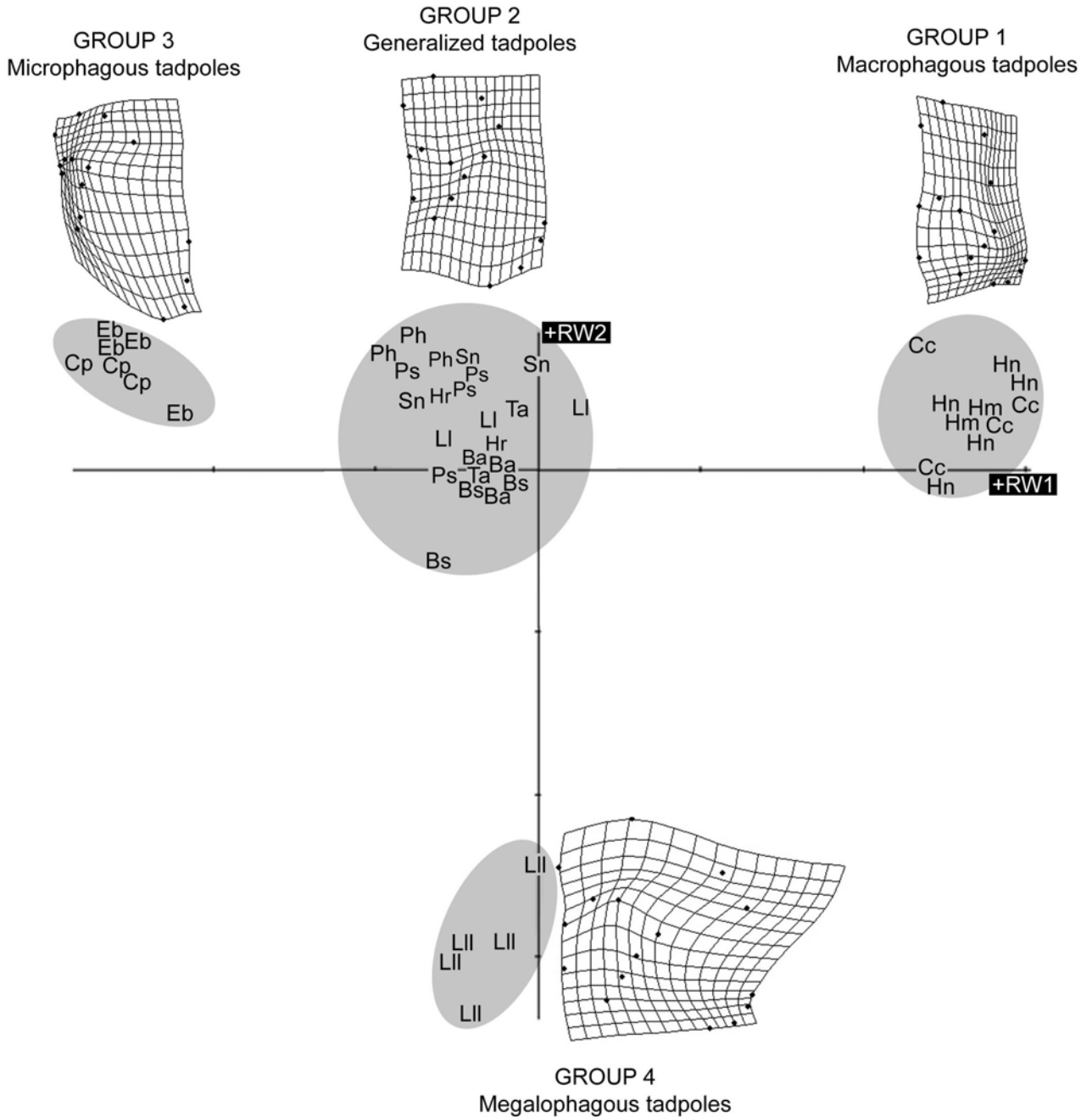


FIG. 3. Relative Warp Analysis of 14 species examined. Scores of 46 specimens on the first two relative warp axes. Shape variation among hyobranchial landmark configuration indicates four distinct clusters of specimens. Thin plate spline deformation grids describe shape variation at positive and negative extremes along each relative warp axis. The grids shown correspond to the highest and lowest scored specimen on each axis. Ba, *Bufo arenarum*; Bs, *Bufo spinulosus*; Cc, *Ceratophrys cranwelli*; Cp, *Chiasmocleis panamensis*; Eb, *Elachistocleis bicolor*; Hm, *Hyla microcephala*; Hn, *Hyla nana*; Hr, *Hyla rosenbergi*; LII, *Lepidobatrachus llanensis*; LI, *Lysapsus limellus*; Ph, *Phyllomedusa hypochondrialis*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ta, *Telmatobius cf. atacamensis*.

TABLE 2. Measurements of the hyobranchial skeleton of 14 species grouped in four clusters after the RW Analysis. Values are averages from the species within each group.

	In-lever arm proportion	Ceratohyal area	Hypobranchial area	Ceratobranchial area
Macrophagous tadpoles	0.51	44%	32%	24%
Generalized tadpoles	0.34	30%	20%	54%
Microphagous tadpoles	0.22	16%	7%	76%
Megalophagous tadpoles	0.21	35%	24%	41%

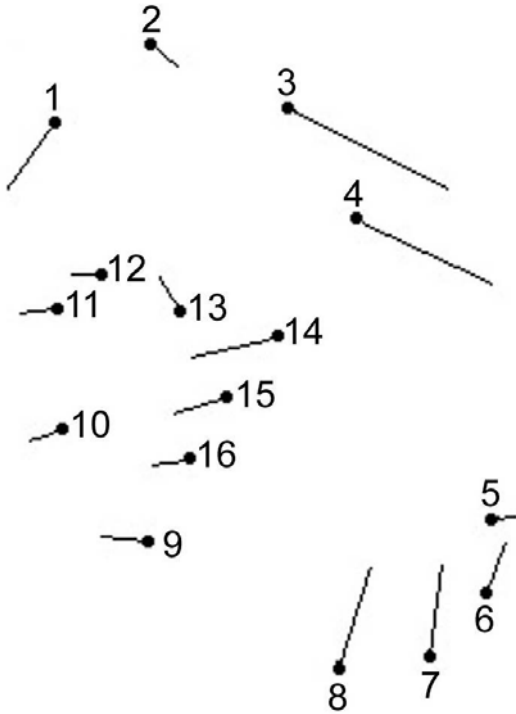


FIG. 4. Vector depiction of shape variation in *Lepidobatrachus llanensis* (megalophagous tadpoles) relative to the consensus. This species exhibit the lowest scores on the second relative warp. Black dots are landmarks of the consensus configuration and landmark numbers have been added to simplify interpretation. Note the longest vectors, showing the lateral translation of the ceratohyal processes, and the anterolateral translation of landmarks of the caudal region of the branchial basket.

in *H. rosenbergi*, *P. santafecinus*, *L. limellus* and *T. cf. atacamensis*.

In *Ceratophrys cranwelli*, the processes of the ceratohyals are short and massive, and the processus anterolateralis is not conspicuous. Copula I is absent and the ceratobranchials are devoid of lateral projections and spicula.

Hyla microcephala and *H. nana* have triangular and robust ceratohyals, with well-developed processus anterior hyalis, posterior hyalis, lateralis, and articular condyle. The processus anterolateralis is not conspicuous. Copula I and urobranchial process are absent. The pars reuniens is not clearly defined and appears vestigial, which makes the ceratohyals appear contiguous. The hypobranchial plates are thick, rectangular and continuous with copula II. The branchial basket is highly reduced; the ceratobranchials are bar-like, short, devoid of lateral projections and spicula. Terminal commissure III is absent in *H. microcephala* and some specimens of *H. nana*.

The microhylids *Chiasmocleis panamensis* and *Elachistocleis bicolor* have a very different hyobranchial apparatus. The processus lateralis is very developed and possesses a wide ventral laminar projection, caudally oriented. The processus posterior hyalis partially overlaps the proximal dorsal region of ceratobranchial I, and the articular condyle is a small

protuberance on the lateral posterior margin of the ceratohyal. Copula I is a slender cartilaginous bar. The pars reuniens is continuous with the ceratohyals and copula II. Copula II is small and bears a very long, thin urobranchial process, approximately 1.5 times as long as the copula II. Caudally, copula II is fused to the hypobranchial plates, which are also fused together. The ceratobranchials are fused to the hypobranchial plates and constitute a large, complexly reticulated branchial basket. There are three long spicula, the first one wider, possibly resulting from fusion of spicula I and II.

Lepidobatrachus llanensis have highly elongated ceratohyals, laterally wider, and with prominent processus anterior and posterior hyales. The processus anterolateralis is located near the lateral edge. The pars reuniens is V-shaped, with thin branches fused to processus anteriores hyales. Copula I is absent, and copula II is wide, 1.6 times as long as the pars reuniens, and bears a small, quadrangular urobranchial process. The hypobranchial plates are medially fused, forming a structure with a concave caudal margin. The ceratobranchials are bar-like and joined by terminal commissures. There are neither lateral projections nor spicula.

GEOMETRIC MORPHOMETRIC ANALYSIS

Four clusters are detected by the Relative Warp Analysis, and are shown in the scatterplot of the first two relative warps (Fig. 3). The first relative warp (RW1) captures a high percentage of the total shape variation, 55.49%, accumulating 77.84% together with RW2. The species reported as macrophages and Type I carnivores score high on RW1, whereas the suspension-feeder microhylids are located at the opposite extreme, with the lowest values. Suspension-rasper, bentic and nektonic tadpoles score with intermediate values on RW1. All these three groups share high values on RW2. Finally, *Lepidobatrachus llanensis* constitutes a separate cluster, with intermediate and low values on RW1 and RW2 respectively. On the basis of hyobranchial apparatus shape, the species are clustered in four groups defined here as:

1. Macrophagous tadpoles: *Ceratophrys cranwelli*, *Hyla microcephala*, *Hyla nana*;
2. Generalized tadpoles: *Bufo arenarum*, *Bufo spinulosus*, *Hyla rosenbergi*, *Lysapsus limellus*, *Phyllomedusa hypochondrialis*, *Physalaemus santafecinus*, *Scinax nasicus*, *Telmatobius cf. atacamensis*;
3. Microphagous tadpoles: *Chiasmocleis panamensis*, *Elachistocleis bicolor*;
4. Megalophagous tadpoles (as mentioned by Ruibal & Thomas, 1988): *Lepidobatrachus llanensis*.

The deformation grids describe positive and negative deviations from the mean form along the relative warp axis. On the RW1 axis, they show that the main changes are associated with the position of landmarks 5-8 with respect to 9 and 14-16, i.e., the size of the branchial basket. From the consensus shape, which is very similar to

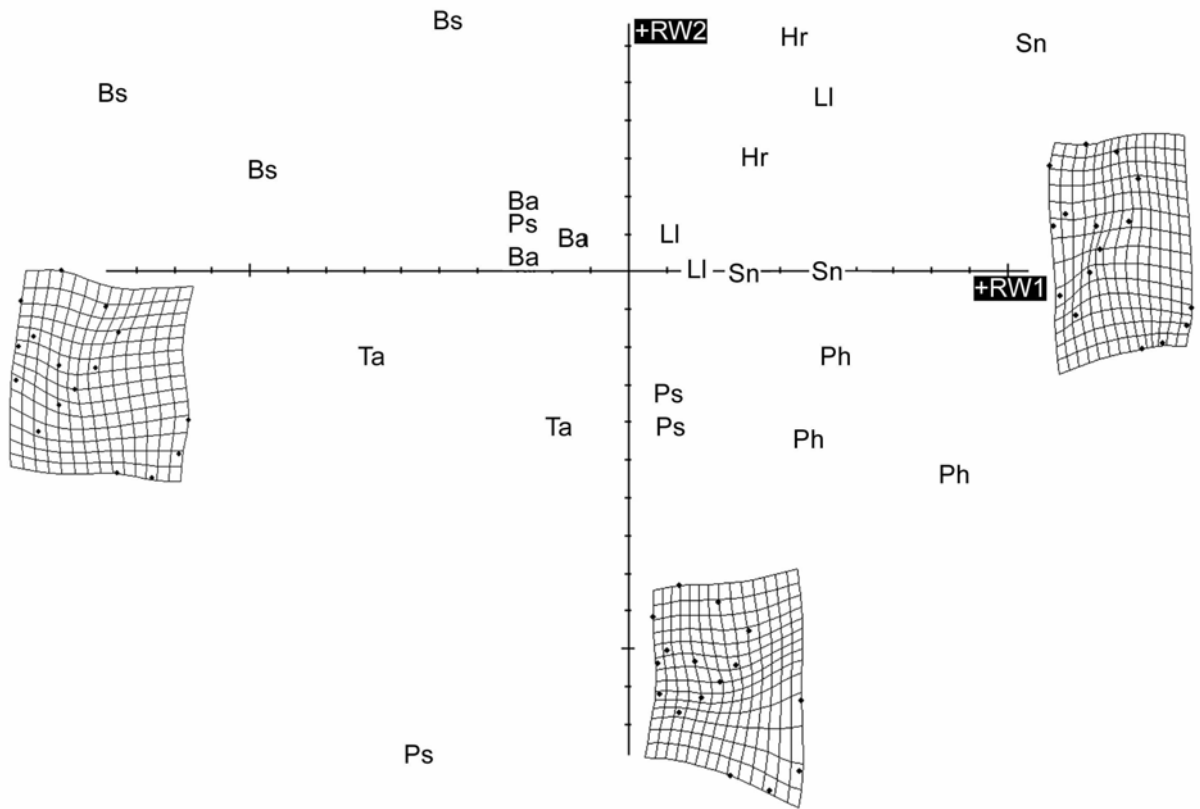


FIG. 5. Relative Warp Analysis of the generalist tadpoles group. Scores of 23 specimens on the first two relative warp axes. Ba, *Bufo arenarum*; Bs, *Bufo spinulosus*; Hr, *Hyla rosenbergi*; LI, *Lysapsus limellus*; Ph, *Phyllomedusa hypochondrialis*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ta, *Telmatobius cf. atacamensis*. Variation of hyobranchial landmark configurations indicates no clusters. Thin plate spline deformation grids describe shape variation at positive and negative extremes along the relative warp axes. The grids correspond to the highest and lowest scored specimens.

the shape of generalized tadpoles, species with high scores on RW1 (macrophagous tadpoles: *Ceratophrys cranwelli* and *Hyla microcephala* group tadpoles) have reduced branchial baskets, whereas species with low scores (microphagous tadpoles: microhylids) have enlarged branchial baskets. Landmarks 9 and 14-16 also vary in their locations compared to landmarks 10-13, indicating a change in size of the hypobranchial plate from high to low scored species (macrophagous to microphagous tadpoles). On RW2, the main shape variation is due to landmarks 1-4, which indicate a lateral expansion of the ceratohyals in low scoring specimens. Landmark 12, which defines the width of the copula II, also varies, with its greatest width in *Lepidobatrachus llanensis*. Variation can also easily be observed using vector mode. For example, in the vector depiction of shape deviation from the consensus in *Lepidobatrachus llanensis*, the translation of landmarks 1-4, and 7-8 can be easily observed (Fig. 4).

A MANOVA was performed on the scores of each specimen on the first four relative warps, which accumulate almost 90% of the variation. The four groups differ significantly (Wilks' lambda = 0.0001; $P < 0.001$). However, with *post hoc* tests, *Lepidobatrachus llanensis* (megalophagous tadpoles) is not different from generalized tadpoles on RW1 ($P = 0.821$). On RW2, only *Lepidobatrachus llanensis* differs

($P < 0.001$), whereas among the three remaining groups, there is significant difference between generalized and microphagous tadpoles ($P = 0.021$), yet no difference between macrophagous and generalized tadpoles ($P = 0.925$) nor between microphagous and macrophagous tadpoles ($P = 0.131$).

A MANOVA was also performed on the measurements recorded from each specimen, using the four recently formed groups as a grouping variable. This analysis reveals significant differences among groups (Wilks' = 0.006; $P < 0.001$). *Post hoc* tests show no significant differences between the in-lever arm proportion of *Lepidobatrachus llanensis* and microphagous tadpoles ($P = 0.908$). A summary of the measurements in each group is shown in Table 2.

The generalized tadpoles group was considered in a separate analysis, and a RWA run (Fig. 5). This time, no definite groups are formed, and the first relative warps explain low and similar percentages of the total variation (RW1=30.44%; RW2=18.63%; RW3=12.48%; RW4=8.00%, totalling 69.55%). *Bufo* species and *Telmatobius cf. atacamensis* tend to score low on the RW1, whereas *Phyllomedusa hypochondrialis*, *Hyla rosenbergi* and *Scinax nasicus* show high values. *Physalaemus santafecinus* and *Lysapsus limellus* show intermediate values, overlapping both extreme groups. The first axis can be interpreted as a lateral and poste-

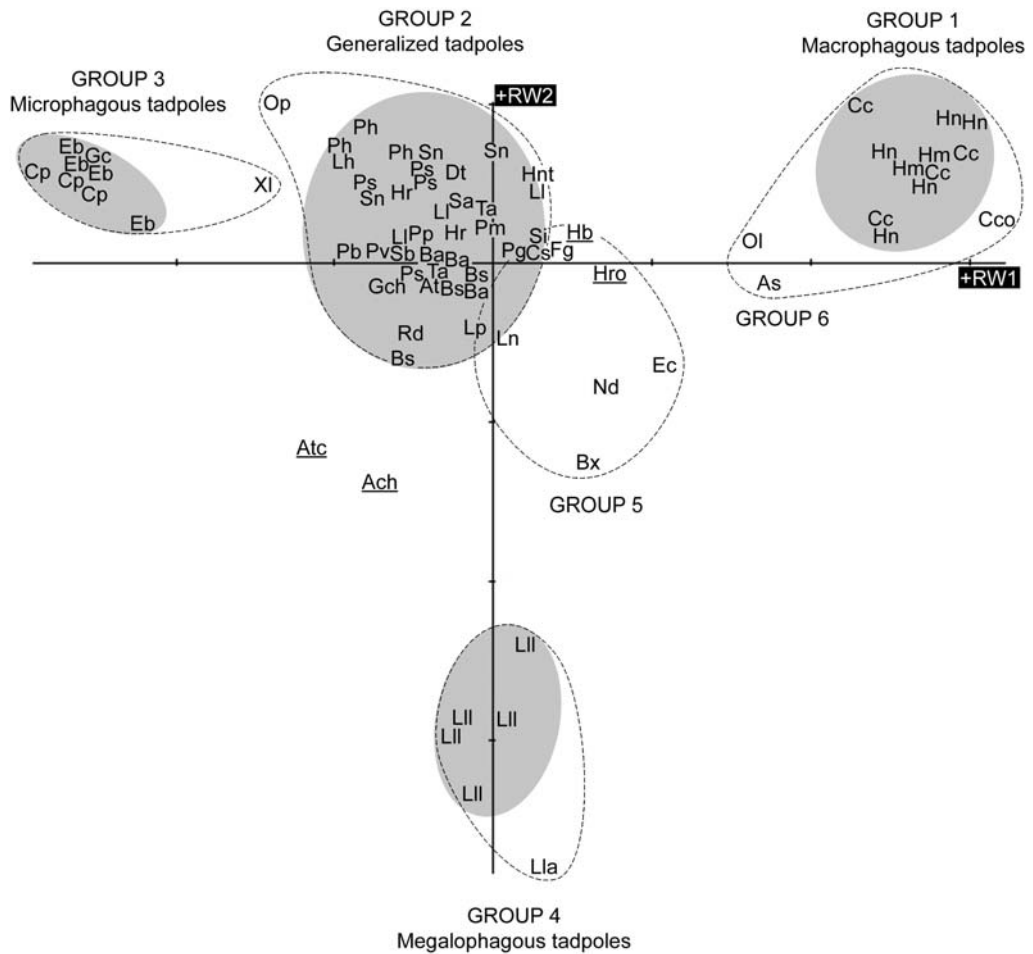


FIG. 6. Relative Warp Analysis of 14 species examined, plus 32 species from the literature (see Appendices; SET 1 and SET 2 respectively). Scores of the total of 78 specimens on the first two relative warp axes. SET 1: Ba, *Bufo arenarum*; Bs, *Bufo spinulosus*; Cc, *Ceratophrys cranwelli*; Cp, *Chiasmocleis panamensis*; Eb, *Elachistocleis bicolor*; Hm, *Hyla microcephala*; Hn, *Hyla nana*; Hr, *Hyla rosenbergi*; LII, *Lepidobatrachus llanensis*; LI, *Lysapsus limellus*; Ph, *Phyllomedusa hypochondrialis*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ta, *Telmatobius cf. atacamensis*. SET 2: As, *Anotheca spinosa*; Ach, *Atelophryniscus chrysophorus*; At, *Ascaphus truei*; Atc, *Atelopus tricolor*; Bx, *Boophis* sp.; Cco, *Ceratophrys cornuta*; Cs, *Cycloramphus stejnegeri*; Dt, *Dendrobates tinctorius*; Ec, *Eupsophus calcaratus*; Fg, *Flectonotus goeldii*; Gc, *Gastrophryne carolinensis*; Gch, *Gastrotheca christiani*; Hb, *Hymenochirus boettgeri*; Hnt, *Heleophryne natalensis*; Hro, *Hoplophryne rogersi*; Lh, *Leptobranchium hasselti*; Lla, *Lepidobatrachus laevis*; Ln, *Litoria nanotis*; Lp, *Leptodactylus pentadactylus*; Nd, *Nyctimystes dayi*; Ol, *Occidozyga laevis*; Op, *Otophryne pyburni*; Pb, *Phyllomedusa boliviana*; Pg, *Phasmahyla guttata*; Pm, *Pseudis minuta*; Pp, *Pseudis paradoxa*; Pv, *Phrynohyas venulosa*; Rd, *Rhinoderma darwini*; Sa, *Scinax acuminatus*; Sb, *Scaphiopus intermontanus*; Sp, *Spea bombifrons*; XI, *Xenopus laevis*. Grey ellipses are the original four groups from Fig. 3. Dash-lines are groups formed on the basis of probabilities of group membership (see Discussion for further explanations). Underlined names are the four species with a 10-landmark configuration. Note the location of most of the species within the original groups.

rior translation of processes anteriores hyales and anterolaterales, and the articular condyles, plus a slight widening of the branchial basket, due to the lateral translation of landmarks 5-8, from high to low scored specimens. The RW2 axis indicates an enlargement of the branchial basket, as a consequence of the posterior translation of the landmarks 6-8, in low scored specimens.

In order to test if the groups formed were also maintained after the inclusion of more specimens, a second set of species was added, consisting of published pictures of the hyobranchial apparatus of several taxa (a list of the species and their references is given in Appendix 2). A variety of species were included, in an attempt to complete the remaining guilds. Nonfeeding

tadpoles were also considered. All these illustrations were treated as mentioned above. The results are shown in Fig. 6, which shows a scatterplot of the specimens on the first two relative warps. Most of the added species fall into the four groups defined in the former analysis. *Lepidobatrachus laevis* groups with *L. llanensis* within megalophagous tadpoles; *Gastrophryne carolinensis* groups within the previously examined microphagous tadpoles; *Ceratophrys cornuta* fits with the macrophagous tadpoles, and most of the remaining species distribute among generalized tadpoles. To determine if they constitute separate clusters in their own right, a Discriminant Analysis was performed on the scores of the species on the first four relative warps.

Group membership was determined a priori on the basis of the four groups formed by the RWA. Doubtful species were coded as separate groups, and probabilities of group membership and predicted group were saved. The species to confirm were: *Boophis* sp., *Eupsophus calcaratus* and *Nyctimystes dayi*, considered as Group 5, and *Anotheca spinosa* and *Occidozyga laevis*, as Group 6. The analysis confirms the new groups, with high probability values ($P > 0.97$ in all five cases), and lower probabilities that the species belonged to Groups 2 and 1 respectively. Additionally, three species were misclassified. They are *Xenopus laevis*, coded as Group 2 (generalized tadpoles), but assigned to Group 3 by the analysis (microphagous tadpoles; $P = 0.99$), and *Flectonotus goeldii* and *Litoria nannotis*, coded as Group 2, but assigned to the new Group 5, although this time with lower P -values ($P = 0.53$ and $P = 0.54$ respectively).

A constraint on landmark-based geometric morphometric methodology is that it requires a complete and comparable set of landmarks, thus precluding the study of the origination or elimination of structures. Focusing only on landmarks common to all specimens can clearly affect the results of such a study (Adams *et al.*, 2004). Bookstein & Smith (2000) offer a possible solution to the appearance of new structures, but this method is not yet generalised. Consequently, in the present paper, tadpoles of *Atelopus tricolor*, *Atelophryniscus chrysophorus* (gastromizophorous), *Hymenochirus boettgeri* (Type III carnivore), and *Hoplophryne rogersi* (Arboreal Type I), even when there are available pictures in the literature, could not be included at the start, because all of them lack 1-3 ceratobranchials and thus landmarks 6-9 and 15-16. However, because it is of interest to analyse where these species would locate with relation to the remaining taxa, a second analysis was performed to include them, eliminating the said landmarks. Although such analysis entails loss of information due the elimination of landmarks, it results in a distribution of the species almost identical to the original one, implying that morphological shape variation can be captured by examining only the ceratohyals, hypobranchial plates and the first ceratobranchial. The composition of the microphagous, generalized, and megalophagous tadpoles groups remains unmodified, the only difference in macrophagous tadpoles group being the location of *Occidozyga laevis* (closer to macrophagous tadpoles), and of *Anotheca spinosa*, which moves away from this group to align itself with the most marginal sector of the generalized tadpoles group. In order to avoid repetition of figures, the four mentioned species have been added to Fig. 6 (names underlined), to show the approximate position where their 10-landmark configurations place them. *Hymenochirus boettgeri* and *Hoplophryne rogersi* locate in the 'macrophagous' extreme of the generalized tadpoles group, whereas gastromizophorous larvae fall between generalized and megalophagous tadpoles.

DISCUSSION

Some of the groups formed by RWA in the first analysis are in fact quite expected. A glance at the hyobranchial apparatus of macrophagous tadpoles (*Ceratophrys cranwelli* and *Hyla microcephala* group tadpoles) versus microhylids for example, suggests that these species would be placed in different groups. Generalized tadpoles include all the other species that are neither macrophagous nor microhylids tadpoles. The exception is *Lepidobatrachus llanensis*, which constitutes its own group, rather than clustering with the macrophagous tadpoles. The thin plate splines reveal that the ceratohyals of this species is different enough for it to justify a separate group. *Lepidobatrachus llanensis* tadpoles in fact, possess a mixture of both microphagous microhylids (low value of in-lever arm proportion) and generalized tadpoles (relative areas of the hyobranchial apparatus) characteristics.

After the inclusion of the second set of species taken from the literature, the original four groups are generally maintained. Group 6 (*Occidozyga laevis* and *Anotheca spinosa*) could probably be linked to the macrophagous tadpoles group, with the addition of more species and the subsequent translation of the centroids of both groups. In Group 5 this tendency is more pronounced, as visualised in the analysis of species located near the margins of the cluster: *Flectonotus goeldii* and *Litoria nannotis* show similar probabilities values 50% of being assigned to Groups 5 and 2 (i.e., to be considered as a new group or clustered with the generalized tadpoles). This results in Group 5 appearing to include itself within Group 2. It is thus possible to visualise a sort of continuum within this large Group 2, with suctorial and nidicolous tadpoles (viz., *Boophis* sp., *Eupsophus calcaratus*, *Nyctimystes dayi*) located on one of the extremes, toward microphagous and megalophagous tadpoles groups, and psammonic, fossorial and suspension-raspers (viz., *Otophryne pyburni*, *Leptobranchium hasselti*, *Phyllomedusa hypochondrialis*) tending to the opposite extreme, near microphagous tadpoles. Conversely, Groups 2 and 3 (generalized and microphagous tadpoles groups) remain well defined, because the most marginal species (*Xenopus laevis* and *Otophryne pyburni*) show high probability of membership in their own groups ($P = 0.99$ and $P = 0.97$ respectively).

Considering all of the above, how are these results to be interpreted in the context of the Altig and Johnston's ecomorphological guilds for tadpoles? To begin with, the 18 groups defined on the basis of external morphology and habitat could be consolidated into four to six (depending on the strict consideration of Groups 5 and 6) groups based on hyobranchial skeleton morphology. This conclusion is preliminary though, because I did not include enough samples from all 18 Altig and Johnston's groups. The tentative four groups are as follows:

1. *Macrophagous tadpoles*. It includes Altig and Johnston's Type II macrophages (*Hyla nana* and *H. microcephala*) some Type I carnivores (*Ceratophrys cranwelli* and *C. cornuta*) and probably, Type I macrophages and Type II arboreal (Group 6; *Occidozyga laevis* and *Anotheca spinosa*).
2. *Generalized tadpoles*. Considering Group 5, it includes all the endotrophs (exoviviparous, paraviviparous, and nidicolous: *Rhinoderma darwinii*, *Gastrotheca christiani*, *Eupsophus calcaratus*, *Cycloramphus stejnegeri* and *Flectonotus goeldii*), and among exotrophs, clasping (*Boophis* sp.), suctorial (*Ascaphus truei*, *Heleophryne natalensis*, *Nyctimystes dayi* and *Litoria nannotis*), neustonic (*Phasmahyla guttata*), psammonic (*Otophryne pyburni*), Type II fossorial (*Leptobranchium hasselti*), Type I and unidentified Type arboreal (*Hoplophryne rogersi* and *Dendrobates tinctorium*), some Type I and Type III carnivores (*Leptodactylus pentadactylus* and *Spea bombifrons*, and *Hymenochirus boettgeri*), nektonic (*Lysapsus limellus*, *Phrynohyas venulosa*, *Pseudis minuta*, *P. paradoxa*, *Scinax acuminatus* and *S. nasicus*), benthic (*Bufo arenarum*, *B. spinulosus*, *Hyla rosenbergi*, *Physalaemus santafecinus*, *Scaphiopus intermontanus* and *Telmatobius* cf. *atacamensis*), and suspension-rasper (*Phyllomedusa boliviana* and *P. hypochondrialis*). Gastromizoporous tadpoles (*Atelopus tricolor* and *Atelophryniscus chrysophorus*) seem to cluster with this group also.
3. *Microphagous tadpoles*. It includes suspension-feeders (*Chiasmocleis panamensis*, *Elachistocleis bicolor*, *Gastrophryne caroliniensis* and *Xenopus laevis*).
4. *Megalophagous tadpoles*. It includes Type II macrophages (*Lepidobatrachus laevis* and *L. llanensis*).

Wassersug & Hoff (1979) also detected four groups by analysing several measurements of the hyobranchial apparatus, in-lever arm proportion, buccal cavity volume, and angle of rotation of the ceratohyal, but *Lepidobatrachus* spp. were not included, and the fourth group is composed of benthic and torrent tadpoles. With the methodology applied in the present paper, and considering shape variables, such larvae do not differ significantly from generalized tadpoles.

The species in the macrophagous tadpoles group are characterised mainly by the reduced size of the branchial basket and large ceratohyals and hypobranchial plates. Additionally, in-lever arm proportion values are the highest of all groups. For tadpoles to ingest large food items requires either a large buccal aperture and buccal cavity, or a jaw apparatus capable of tearing the prey apart before engulfing it. The species in the macrophagous tadpoles group share the fact that they have achieved a large buccal cavity volume by the enlargement of the buccal floor. Tadpoles of the *Hyla microcephala* group, *Ceratophrys* spp., *Anotheca*

spinosa and *Occidozyga laevis* possess very robust ceratohyals, with greater rostrocaudal length than that of the hypobranchial plate. These features are in most of the species accompanied by a conspicuous development of the musculature responsible for depressing the buccal floor (Satel & Wassersug, 1981; Vera Candioti *et al.*, 2004; Vera Candioti, 2005).

With regard to feeding habits, all of these species consume large items, compared to the microscopic particles typically found in the guts of generalized tadpoles. *Ceratophrys cranwelli*, for instance, feeds on varied items whose maximum diameters represent 50% of the snout-vent length of the tadpole (Vera Candioti, 2005). *Hyla nana* tadpoles have a diet mainly composed of oligochaetes with average length that may represent 120% of the tadpole length (Vera Candioti *et al.*, 2004; pers. obs.). *Hyla microcephala* tadpoles examined in this study also had oligochaetes in their gut contents, and Wassersug and Hoff (1979) reported large filamentous plants in their diet. Finally, *Anotheca spinosa* and *Occidozyga laevis* are also macrophagous, sometimes oophagous (Jungfer, 1996; Altig & McDiarmid, 1999).

In the megalophagous *Lepidobatrachus*, the hyobranchial skeleton has large lateral extension. The branchial basket, even if it has bar-like ceratobranchials with neither spicules nor lateral projections, is not reduced in area. In this genus, unlike macrophagous tadpoles, the large buccal cavity volume is achieved by widening the whole body. The large lateral extension of the ceratohyals is, in fact, part of a lateral extension of the anterior and middle regions of the chondrocranium. The wide suprarostal and the lower jaw, both laterally expanded, create a large mouth opening. The lateral extension of palatoquadrates and ceratohyals, in turn, yield a large buccal volume. This implies that prey can be not only exceptionally large (nearly equal to the predator size), but can also be engulfed intact. Despite the low in-lever arm proportion value, the m. orbitohyoideus (responsible for the descent of the buccal floor) is very developed, inserting on the anterior margin of the quadratocranial commissure (Palavecino, 1999; pers. obs.). This indicates the generation of a strong negative pressure inside the buccal cavity. Ruibal & Thomas (1988) report for *Lepidobatrachus* spp. a diet mainly composed of live prey such as *Artemia*, worms, tadpoles and small fishes.

Most of the species fall into the generalized tadpoles group. Around 50% of the total area of the hyobranchial apparatuses is occupied by the branchial basket, and the hypobranchial area is smaller than the ceratohyal area. Mean in-lever arm proportion is 0.34, consistent with what is reported in Wassersug & Hoff (1979). As already mentioned, it is possible to detect a general tendency within this group, explained mainly by habitat. The species inhabiting fast water locate at one extreme, near macrophagous tadpoles. A similarity between hyobranchial skeletons of suctorial and macrophagous

larvae has been reported, and is explained by the fact that both kinds of tadpoles generate high negative pressures inside their buccal cavities, either to capture and retain large prey, or to adhere to the substrate (see Wassersug & Hoff, 1979; Haas & Richards, 1998). At the opposite extreme, near the microphagous tadpoles, are species with low mobility, inhabitants of lentic environments, or slow water microhabitats in lotic environments. Morphologically, the spectrum just described is characterised by a progressive increase of the size of the branchial basket, which results in an increase of the filtering capability for feeding. The 'macrophagous' extreme joins some of the species without active feeding, and suctorial tadpoles. Haas & Richards (1998) mentioned whole ephemeropteran larvae inside the digestive tract of *Boophis* sp. *Ascaphus truei* tadpoles, which are also suctorial but with a less modified hyobranchial apparatus, scrape algae from the rocks they cling to (Wassersug, 1972). Some endotrophic larvae have reduced branchial baskets (Lavilla, 1991; Haas, 1996a; Vera Candiotti *et al.*, 2005); like macrophagous tadpoles, they do not depend on an efficient filtering mechanism to obtain their food. At the 'microphagous' extreme are suspension-rasper tadpoles, which generate a suspension of small particles (*Phyllomedusa hypochondrialis*, mean=1.7% of the snout-vent length; pers. obs.), and *Otophryne pyburni*, known to filter microscopic particles from the sandy bottom where it lives (Wassersug & Pyburn, 1987). The remaining species of the generalized tadpoles group are mostly benthic, nektonic, and neustonic, feeding on small particles obtained in various ways and from different microhabitats (i.e., *Bufo spinulosus* mean = 1.8% of the snout-vent length; *Physalaemus santafecinus* mean = 5.5% of the SVL; *Telmatobius* cf. *atacamensis* mean = 13% of the SVL; pers. obs.). Also included here is the enormous tadpole of *Pseudis paradoxa* which, despite its size, has a generalized diet, mainly composed of macrophyte fragments, arthropod remnants and microalgae, with sizes that represent 11% of the snout-vent length (Rada & Bello, 1988; Arias *et al.*, 2002; pers. obs.).

Some species show that the concordance among feeding habit and morphology does not always hold. *Rhinoderma darwinii* and *Gastrotheca christiani* are endotrophic species, which retain a hyobranchial apparatus similar to that of the generalized tadpoles (Lavilla, 1987; Lavilla & Vaira, 1997), instead of the reduced one of other endotrophic tadpoles (i.e., *Cycloramphus stejnegeri*, *Flectonotus goeldii*, *Eupsophus calcaratus*; Lavilla, 1991; Haas, 1996a; Vera Candiotti *et al.*, 2005). *Leptodactylus pentadactylus* are facultatively carnivorous tadpoles (Heyer *et al.*, 1975), yet maintain a generalized hyobranchial apparatus, very similar to those of most of the species of the genus (Larson & De Sá, 1998). Finally, *Spea bombifrons* can be a macrophagous carnivore too, and although its buccal volume, ceratohyal development and in-lever arm proportion are all similar to those of macrophagous

tadpoles (Wassersug & Hoff, 1979), its branchial basket is not reduced enough to cluster it with them. The genera *Spea* and *Scaphiopus* are an interesting case, because some species have both generalized and carnivorous morphs, the latter characterised by modifications in the oral apparatus, intestinal length, fat bodies, and buccal floor levator and depressor muscles (Bragg, 1956; Bragg & Bragg, 1959; Acker & Larsen, 1979; Wassersug & Hoff, 1979; Hall & Larsen, 1998; Hall *et al.*, 2002). The location within generalized tadpoles group of both *Spea bombifrons* (carnivorous morph) and *Scaphiopus intermontanus* (herbivorous morph) suggests that apparently macrophagy does not necessitate major changes in the hyobranchial skeleton of these taxa.

The four species not included in the complete 16-landmark analysis apparently fit into the generalized tadpoles group. The location of gastromizoporous tadpoles, near *Lepidobatrachus*, may be explained by the lateral expansion of the hyobranchial skeleton, also accompanied in this case by a lateral expansion of the whole chondrocranium (Lavilla & De Sá, 2001). However, since the mouth opening is much smaller in gastromizoporous larvae than in *Lepidobatrachus* sp. (as inferred from mandible width), the lateral expansion of the head seems to be related more to the presence of a belly sucker presence than to the size of the food items ingested by these tadpoles. Data on the diet of these species would be needed in order to confirm this hypothesis. *Hoplophryne rogersi* tadpoles are macrophagous; their gut contents are mainly debris from arthropods and vegetal tissues, plus frog eggs (Noble, 1929). *Hymenochirus boettgeri* tadpoles are macrophagous too; they pursue and ingest live prey such as microcrustaceans, *Culex* larvae, and also small tadpoles (Sokol, 1962; Deban & Olson, 2002). These two latter species are located at the 'macrophagous' extreme of the generalized tadpoles group.

Finally, microphagous group tadpoles possess a highly developed branchial basket, with a relative area greater than 70% of the total hyobranchial area. The hypobranchial area is greatly reduced. The low in-lever arm proportion for tadpoles of this group is consistent with what Wassersug & Hoff (1979) reported. These authors calculated a large angle of rotation of the ceratohyal during the descent of the buccal floor. This allows microphagous tadpoles to draw large volumes of water into the buccal cavity without having to generate high negative pressure. Additionally, the region of mucous secretory ridges is extensive in these tadpoles, implying a more efficient mucous entrapment process (Seale & Wassersug, 1979).

Elachistocleis bicolor consume especially small particles (0.03 mm - 0.06 mm; <1% - 1% of the SVL; pers. obs.). Wassersug (1972) explored the efficiency of some species of tadpoles removing solid particles of different sizes from a suspension. *Xenopus laevis* tadpoles are capable of filtering the smallest particles (0.126 μ), in comparison with generalized tadpoles

(*Ascaphus truei* and *Rana pipiens*, 0.557 μ). Due to the similarity between most microhylids and *Xenopus laevis* branchial baskets, it seems probable that *Elachistocleis*, *Chiasmocleis* and *Gastrophryne* can also feed on particles smaller than 0.03 mm. This kind of highly efficient microphagy seems to be exclusive to microhylids, pipids and rhinophrynids. Although microhylid species included in this work are few, the morphological constancy in the hyobranchial skeleton across the family would seem to indicate that new taxa included would fit within this group. Exceptions with a less specialised hyobranchial skeleton (besides *Hoplophryne rogersi*) could be *Microhyla heymonsi*, a neustonic form capable of macrophagy, and *Scaphiophryne* spp., reported to be bottom grazers and carnivorous (Blommer-Schlösser, 1975; Wassersug, 1984; 1989; Altig & McDiarmid, 1999). *Rhinophrynus dorsalis*, with a hyobranchial skeleton similar to that of microhylids (Swart & De Sá, 1999), is both a proficient suspension-feeder and a predator of large zooplankton (Starrett, 1960).

In sum, macrophagy seems possible across a very broad array of hyobranchial skeletal morphologies. First, it occurs in macrophagous and 'near' generalized tadpoles, associated with very developed, robust, and long ceratohyals, and a marked reduction of the branchial basket. Secondly, it occurs in megalophagous species (*Lepidobatrachus* spp.), where the large buccal cavity volume is a result of the widening of the hyobranchial skeleton and neurocranium with the hyobranchial basket only being slightly reduced. Thirdly, it occurs among generalized tadpoles, with a scarcely or unmodified hyobranchial skeleton. In this latter case, the large buccal cavity volume results from a simple enlargement of the body size, with an absolute value that increases exponentially with linear measurements such as snout-vent length and width at eyes (Wassersug & Hoff, 1979). This also coincides with the observations of Petranka & Kennedy (1999), who discuss the capability of a number of generalized-morphology tadpoles to ingest large prey. Finally, some forms with a hyobranchial apparatus typically characterised as microphagous, such as *Rhinophrynus dorsalis* and *Microhyla heymonsi*, are also capable of macrophagy. There is a strong relation between macrophagy and muscular features. Most of the species feeding on large prey have an extensive development of the buccal floor depressor muscles, regardless of the configuration of their hyobranchial skeleton (Satel & Wassersug, 1981). An example is *Spea bombifrons*, with a very low IH/OH ratio (mm. interhyoideus / mm. orbitohyoideus; buccal floor levator and depressor muscles respectively) in the carnivorous morph, but similar to the generalized tadpoles in the omnivorous morph. On the contrary, highly efficient microphagy would only occur in tadpoles with large branchial baskets, which support complex filtering and particle entrapment structures.

The groups formed on the basis of hyobranchial morphology can be generally related to size of food particles. Species for which data on diet are available locate in different groups, showing a tendency for a decrease in average food-particle size, from very large within macrophagous tadpoles, to very small in microphagous forms. Additional work must be done to further address the question of whether these morphological groups effectively correlate with a gradient of food-particle sizes.

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REFERENCES

- Acker, R. L. & Larsen, J. H. Jr. (1979). A functional analysis of morphological variation in larval *Scaphiopus intermontanus*. *American Zoologist* **19**, 1012.
- Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology* **71**, 5-16.
- Alcalde, L. & Rosset, S. D. (2003). Descripción y comparación del condrocáneo en larvas de *Hyla raniceps* (Cope, 1862), *Scinax granulatus* (Peters, 1871) y *Scinax squalirostris* (A. Lutz, 1925) (Anura: Hylidae). *Cuadernos de Herpetología* **17**, 33-49.
- Altig, R. & Johnston, G. F. (1989). Guilds of anuran larvae: relationships among developmental modes, morphologies and habitats. *Herpetological Monographs* **3**, 81-109.
- Altig, R. & McDiarmid, R. W. (1999). Diversity. Familial and generic characterisation in tadpoles. In *The biology of anuran larvae*, 295-337. McDiarmid, R. W. & Altig, R. (Eds). Chicago: The University of Chicago Press.
- Arias, M. M., Peltzer, P. M., & Lajmanovich, R. C. (2002). Diet of the giant tadpole *Pseudis paradoxa platensis* (Anura: Pseudidae) from Argentina. *Phyllomedusa* **1**, 97-100.
- Blommers-Schlösser, R. M. A. (1975). Observations on the larval development of some Malagasy frogs, with notes on their ecology and biology (Anura; Dyscophynae, Scaphiophryninae and Cophylinae). *Beaufortia* **24**, 7-26.

- Bookstein, F. L. (1991). Morphometric tools for landmarks data. Cambridge: Cambridge University Press.
- Bookstein, F. L. & Smith, B. R. (2000). Inverting development: geometric singularity theory in embryology. In *Mathematical modelling, estimation and imaging*, 139-174. Wilson, D., Tagare, H., Bookstein, F. L., Préteaux, F. & Dougherty, E. (Eds). Washington: Proc. SPIE. vol. 4121.
- Bragg, A. N. (1956). Dimorphism and cannibalism in tadpoles of *Scaphiopus bombifrons* (Amphibia: Salientia). *Southwestern Naturalist* **1**, 105-108.
- Bragg, A. N. & Bragg, W. N. (1959). Variation in the mouth parts in tadpoles of *Scaphiopus* (Spea) *bombifrons* Cope (Amphibia: Salientia). *Southwestern Naturalist* **3**, 55-59.
- Deban, S. M. & Olson, W. M. (2002). Suction feeding by a tiny predatory tadpole. *Nature* **420**, 41-42.
- Fabrezi, M. & Lavilla, E. O. (1992). Estructura del condrocáneo y esqueleto hiobranquial en larvas de algunos hílidos neotropicales (Anura: Hylidae). *Acta Zoológica Lilloana* **41**, 155-164.
- Fabrezi, M. & Vera, R. (1997). Caracterización morfológica de larvas de anuros del Noroeste argentino. *Cuadernos de Herpetología* **11**, 37-49.
- Fink, W. L. & Zelditch, M. L. (1995). Phylogenetic analysis of ontogenetic shape transformations: a reassessment of the piranha genus *Pygocentrus* (Teleostei). *Systematic Biology* **44**, 343-360.
- Giri, F. & Collins, P. A. (2004). A geometric morphometric analysis of two sympatric species of the family Aeglididae (Crustacea, Decapoda, Anomura) from La Plata basin. *Italian Journal of Zoology* **71**, 85-88.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.
- Haas, A. (1995). Cranial features of dendrobatid larvae (Amphibia: Anura: Dendrobatidae). *Journal of Morphology* **224**, 241-264.
- Haas, A. (1996a). Non-feeding and feeding tadpoles in hemiphractine frogs: larval head morphology, heterochrony, and systematics of *Flectonotus goeldii* (Amphibia: Anura: Hylidae). *Journal of Zoological Systematics and Evolutionary Research* **34**, 163-171.
- Haas, A. (1996b). The larval hyobranchial apparatus of discoglossoid frogs: its structure and bearing on the systematics of the Anura (Amphibia: Anura) *Journal of Zoological Systematics and Evolutionary Research* **35**, 179-197.
- Haas, A. (2003). Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* **19**, 23-89.
- Haas, A. & Richards, S. J. (1998). Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Hylidae: Pelodyadinae). *Journal of Morphology* **238**, 109-141.
- Hall, J. A. & Larsen, J. H. Jr. (1998). Postembryonic ontogeny of the Spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae): skeletal morphology. *Journal of Morphology* **238**, 179-244.
- Hall, J. A., Larsen, J. H. Jr. & Fitzner, R. E. (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.
- Heyer, W. R., McDiarmid, W. R. & Weigmann, D. L. (1975). Tadpoles, predation and pond habitats in the tropics. *Biotropica* **7**, 100-111.
- Jungfer, K. H. (1996). Reproduction and parental care of the Coronated treefrog *Anotheca coronata* (Steindachner 1864) (Anura: Hylidae). *Herpetologica* **52**, 25-32.
- Larson, P. M. (2002). Chondrocranial development in larval *Rana sylvatica* (Anuran: Ranidae): morphometric analysis of cranial allometry and ontogenetic shape change. *Journal of Morphology* **252**, 131-144.
- Larson, P. M. (2005). Ontogeny, phylogeny, and morphology in anuran larvae: morphometric analysis of cranial development and evolution in *Rana* tadpoles (Anura: Ranidae). *Journal of Morphology* **264**, 34-52.
- Larson, P. M. & De Sá, R. O. (1998). Chondrocranial morphology of *Leptodactylus* larvae (Leptodactylidae: Leptodactylinae): its utility in phylogenetic reconstruction. *Journal of Morphology* **238**, 287-306.
- Lavilla, E. O. (1987). La larva de *Rhinoderma darwini* D. & B. (Anura: Rhinodermatidae). *Acta Zoológica Lilloana* **39**, 81-88.
- Lavilla, E. O. (1991). Condrocáneo y esqueleto visceral en larvas de *Cycloramphus stejnegeri* (Leptodactylidae). *Amphibia-Reptilia* **12**, 33-38.
- Lavilla, E. O. & De Sá, R. O. (2001). Chondrocranium and visceral skeleton of *Atelopus tricolor* and *Atelophryniscus chrysophorus* tadpoles (Anura, Bufonidae). *Amphibia-Reptilia* **22**, 167-177.
- Lavilla, E. O. & De Sá, R. O. (1999). Estructura del condrocáneo y esqueleto visceral de larvas de *Pseudis minuta* (Anura: Pseudidae). *Alytes* **16**, 139-147.
- Lavilla, E. O. & Vaira, M. (1997). Larval chondrocranium of *Gastrotheca christiani* (Hylidae), with comments on the chondrocrania of non-feeding larvae in Anura. In *Abstracts of the Third World Congress of Herpetology*, 124. Zbynek, R. & Hart, S. (Eds). Prague.
- Monteiro, L. R. & Abe, A. S. (1997). Allometry and morphological integration in the skull of *Tupinambis merianae* (Lacertilia: Teiidae). *Amphibia-Reptilia* **18**, 397-405.
- Monteiro, L. R. & Abe, A. S. (1999). Functional and historical determinants of shape in the scapula of xenarthran mammals: evolution of a complex morphological structure. *Journal of Morphology* **241**, 251-263.
- Monteiro, L. R. & Furtado dos Reis, S. (1999). *Princípios de morfometria geométrica*. Brasil: Holos Editora Ltda-Me.
- Monteiro, L. R., Lessa, L. G. & Abe, A. S. (1999). Ontogenetic variation in skull shape of *Trichomys apereoides* (Rodentia: Echimyidae). *Journal of Mammalogy* **80**, 102-111.

- Noble, G. K. (1929). The adaptive modifications of the arboreal tadpoles of *Hoplophryne* and the torrent tadpoles of *Staurois*. *Bulletin of the American Museum of Natural History* **58**, 291-337.
- Palavecino, P. (1999). Musculatura asociada al primer y segundo arco visceral de algunos anuros leptodactílidos. *Cuadernos de Herpetología* **13**, 37-46.
- Petranka, J. W. & Kennedy, C. A. (1999). Pond tadpoles with generalised morphology: is it time to reconsider their functional roles in aquatic communities? *Oecologia* **120**, 621-631.
- Rada, D. & Bello, R. (1988). Morfología e histología del sistema digestivo de la larva de *Pseudis paradoxa caribensis* Gallardo, 1961 (Amphibia, Pseudidae). *Iheringia Série Miscellanea* **2**, 69-91.
- Ridewood, W. G. (1898). On the larval hyobranchial skeleton of the anurous batrachians, with special reference to the axial parts. *Journal of the Linnaean Society of London* **26**, 474-487.
- Rohlf, F. J. (1993). Relative-warp analysis and an example of its application to mosquito wings. In *Contributions to morphometrics*, 131-159. Marcus, L. F., Bello, E., García Valdecasas, A. (Eds). Madrid: Museo Nacional de Ciencias Naturales.
- Rohlf, F. J. (2003). tpsRelw, Version 1.37 Copyright © 2003. New York: Ecology & Evolution, SUNY at Stony Brook.
- Rohlf, F. J. (2004). tpsDig, Version 1.40 Copyright © 2004. New York: Ecology & Evolution, SUNY at Stony Brook.
- Rohlf, F. J. & Bookstein, F. L. (1990). *Proceedings of the Michigan morphometrics workshop*. Michigan: University of Michigan, Museum of Zoology, Special Publications.
- Ruibal, R. & Thomas, E. (1988). The obligate carnivorous larvae of the frog *Lepidobatrachus laevis* (Leptodactylidae). *Copeia* **1988**, 591-604.
- Satel, S. & Wassersug, R. J. (1981). On the relative sizes of buccal floor depressor and elevator musculature in tadpoles. *Copeia* **1981**, 129-137.
- Seale, D. B. & Wassersug, R. J. (1979). Suspension feeding dynamics of anuran larvae related to their functional morphology. *Oecologia* **39**, 259-272.
- Sokol, O. M. (1962). The tadpole of *Hymenochirus boettgeri*. *Copeia* **1962**, 272-284.
- SPSS for Windows. (1998). Release 9.0.0. Copyright © SPSS Inc. 1989-1999.
- Stayton, C. T. (2005). Morphological evolution of the lizard skull: a geometric morphometrics survey. *Journal of Morphology* **263**, 47-59.
- Starrett, P. H. (1960). Description of tadpoles of Middle America frogs. Miscellaneous Publication of the Museum of Zoology, *University of Michigan* **110**, 1-37.
- Swart, C. C. & De Sá, R. O. (1999). The chondrocranium of the Mexican burrowing toad, *Rhinophrynus dorsalis* (Anura: Rhinophrynidae). *Journal of Herpetology* **33**, 23-28.
- Vera Candiotti, M. F. (2005). Morphology and feeding in tadpoles of *Ceratophrys cranwelli* (Anura: Leptodactylidae). *Acta Zoologica* **86**, 1-11.
- Vera Candiotti, M. F. & Haas, A. (2004). Three dimensional reconstruction of the hyobranchial apparatus of *Hyla nana* tadpoles (Anura: Hylidae). *Cuadernos de Herpetología* **18**, 3-15.
- Vera Candiotti, M. F., Lavilla, E. O. & Echeverría, D. D. (2004). Feeding mechanisms in two treefrogs, *Hyla nana* and *Scinax nasicus* (Anura: Hylidae). *Journal of Morphology* **261**, 206-224.
- Vera Candiotti, M. F., Úbeda, C. & Lavilla, E. O. (2005). Morphology and metamorphosis of *Eupsophus calcaratus* tadpoles (Anura: Leptodactylidae). *Journal of Morphology* **264**, 161-177.
- Wassersug, R. J. (1972). The mechanism of ultraplanktonic entrapment in anuran larvae. *Journal of Morphology* **137**, 279-288.
- Wassersug, R. J. (1976). A procedure for differential staining of cartilage and bone in whole formalin fixed vertebrates. *Stain Technology* **51**, 131-134.
- Wassersug, R. J. (1980). Internal oral features of larvae from eight anuran families. Functional, systematic, evolutionary and ecological considerations. University of Kansas *Museum of Natural History Miscellaneous Publications* **65**, 1-146.
- Wassersug, R. J. (1984). The *Pseudohemisus* tadpole: a morphological link between microhylid (Orton Type 2) and Ranoid (Orton Type 4) larvae. *Herpetologica* **40**, 138-149.
- Wassersug, R. J. (1989). What, if anything, is a microhylid (Orton Type II) tadpole? *Fortschritte der Zoologie* **35**, 534-538.
- Wassersug, R. J. & Hoff, K. (1979). A comparative study of the buccal pumping mechanism of tadpoles. *Biological Journal of the Linnaean Society of London* **12**, 225-259.
- Wassersug, R. J. & Pyburn, W. F. (1987). The biology of the Pe-ret' toad, *Otophryne robusta* (Microhylidae), with special consideration of its fossorial larva and systematic relationships. *Zoological Journal of the Linnaean Society of London* **91**, 137-169.
- Wild, E. R. (1997). Description of the adult skeleton and developmental osteology of the hyperossified Horned frog, *Ceratophrys cornuta* (Anura: Leptodactylidae). *Journal of Morphology* **232**, 169-206.

APPENDIX 1. Species examined.

Species	Family	Procedence	N	Stage
<i>Bufo arenarum</i>	Bufoidae	Salta, Argentina. December 2003	3	36
<i>Bufo spinulosus</i>	Bufoidae	Jujuy, Argentina. November 2001	3	34
<i>Ceratophrys cranwelli</i>	Leptodactylidae	Salta, Argentina. January 1996	4	33
<i>Chiasmocleis panamensis</i>	Microhylidae	Gamboa, Panamá. July-August 2001	3	35
<i>Elachistocleis bicolor</i>	Microhylidae	Santa Fe, Argentina. January-February 2001	4	34, 35
<i>Hyla microcephala</i>	Hylidae	Gamboa, Panamá. July-August 2001	2	25, 28
<i>Hyla nana</i>	Hylidae	Santa Fe, Argentina. January-February 2001	5	33, 35
<i>Hyla rosenbergi</i>	Hylidae	Gamboa, Panamá. July-August 2001	2	33, 34
<i>Lepidobatrachus llanensis</i>	Leptodactylidae	Salta, Argentina. November 1996	5	33
<i>Lysapsus limellus</i>	Hylidae	Santa Fe, Argentina. January-February 2001	3	31, 35
<i>Phyllomedusa hypochondrialis</i>	Hylidae	Formosa, Argentina. January 2004	3	31, 33, 34
<i>Physalaemus santafecinus</i>	Leptodactylidae	Santa Fe, Argentina. January-February 2001	4	32, 33
<i>Scinax nasicus</i>	Hylidae	Santa Fe, Argentina. January-February 2001	3	32, 35
<i>Telmatobius cf. atacamensis</i>	Leptodactylidae	Salta, Argentina. November 2003	2	26, 34

APPENDIX 2. Species from bibliography (categorised according to Altig and Johnston, 1989).

Guild	Species	Family	Reference
ENDOTROPHIC TADPOLES			
Paraviviparous	<i>Gastrotheca christiani</i>	Hylidae	Lavilla & Vaira, 1997
Exoviviparous	<i>Rhinoderma darwinii</i>	Rhinodermatidae	Lavilla, 1987
Nidicolous	<i>Eupsophus calcaratus</i>	Leptodactylidae	Vera Candiotti <i>et al.</i> , 2005
	<i>Cycloramphus stejnegeri</i>	Leptodactylidae	Lavilla, 1991
	<i>Flectonotus goeldii</i>	Hylidae	Haas, 1996a
EXOTROPHIC TADPOLES			
<i>Lotic</i>			
Neustonic	<i>Phasmahyla guttata</i>	Hylidae	Fabrezi & Lavilla, 1992
Gastromizoporous	<i>Atelopus tricolor</i>	Bufoidae	Lavilla & De Sá, 2001
Clasping	<i>Atelophryniscus chrysophorus</i>	Bufoidae	Lavilla & De Sá, 2001
Suctorial. Type I	<i>Boophis</i> sp.	Mantellidae	Haas & Richards, 1998
Suctorial. Type II	<i>Ascaphus truei</i>	Ascaphidae	Haas, 1996b
	<i>Heleophryne natalensis</i>	Heleophrynidae	Wassersug & Hoff, 1979
	<i>Nyctimystes dayi</i>	Hylidae	Haas & Richards, 1998
Psammonic	<i>Litoria nannotis</i>	Hylidae	Haas & Richards, 1998
	<i>Otophryne pyburni</i>	Microhylidae	Wassersug & Pyburn, 1987
Fossorial. Type II	<i>Leptobrachium hasselti</i>	Megophryidae	Ridewood, 1898
<i>Lentic</i>			
Arboreal. Type I	<i>Hoplophryne rogersi</i>	Microhylidae	Noble, 1929
Arboreal. Type II	<i>Anotheca spinosa</i>	Hylidae	Wassersug & Hoff, 1979
Arboreal. Type ?	<i>Dendrobates tinctorius</i>	Dendrobatidae	Haas, 1995
Benthic?	<i>Scaphiopus intermontanus</i>	Scaphiopodidae	Hall & Larsen, 1998
Suspension-Rasper	<i>Phyllomedusa boliviana</i>	Hylidae	Fabrezi & Lavilla, 1992
Suspension-Feeder. Type I	<i>Xenopus laevis</i>	Pipidae	Wassersug & Hoff, 1979
Suspension-Feeder. Type II	<i>Gastrophryne carolinensis</i>	Microhylidae	Wassersug & Hoff, 1979
Carnivore. Type I	<i>Ceratophrys cornuta</i>	Leptodactylidae	Wild, 1997
	<i>Spea bombifrons</i>	Scaphiopodidae	Wassersug & Hoff, 1979
	<i>Leptodactylus pentadactylus</i>	Leptodactylidae	Larson & De Sá, 1998
Carnivore. Type II	<i>Lepidobatrachus laevis</i>	Leptodactylidae	Ruibal & Thomas, 1988
Carnivore. Type III	<i>Hymenochirus boettgeri</i>	Pipidae	Wassersug & Hoff, 1979
Macrophagous. Type I	<i>Occidozyga laevis</i>	Ranidae	Ridewood, 1898
Nectonic. Type I	<i>Scinax acuminatus</i>	Hylidae	Fabrezi & Lavilla, 1992
	<i>Pseudis paradoxa</i>	Hylidae	Haas, 2003
	<i>Pseudis minuta</i>	Hylidae	Lavilla & De Sá, 1999
Nectonic. Type II	<i>Phrynohyas venulosa</i>	Hylidae	Fabrezi & Vera, 1997