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Chondrocranial and hyobranchial morphology in larvae of the genus *Rhinella* Fitzinger, 1826 (Amphibia, Anura, Bufonidae)

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The chondrocranium and hyobranchial skeleton of *Rhinella abei, R. crucifer, R. granulosa, R. henseli, R. hoogmoedi, R. icterica, R. jimi, R. ornata, R. pombali, R. pygmaea* and *R. schneideri* are described, and compared with each other and other bufonids based on the descriptions available in the literature. The chondrocranium and hyobranchial skeleton of *Rhinella* is conserved in many aspects. These similarities are associated with suprarostral components, the distal end of the cornua trabeculae, cartilago orbitalis, palatoquadrate and processes of the ceratohyal and ceratobranchial IV. *Rhinella granulosa* is the most distinct species of the genus described here, differing from other species in the shape of its pars corporis, its ethmoid plate width, the shape of the fenestra subocularis, the inclination of the processus muscularis quadrati, the commissura quadratoorbitalis, the length of the processus ascendens and the shape of the processus anterior hyalis.

Key words: Bufonids, cranial morphology, tadpoles, taxonomy

INTRODUCTION

The toad genus *Rhinella* Fitzinger, 1826 includes the most southern South American species of the former genus *Bufo* Garsault, 1764 (see Frost et al., 2006 for a comprehensive revision of the genus *Bufo*). Currently, the genus *Rhinella* is composed of 86 species distributed from the lower Rio Grande Valley region of southern Texas (U.S.A.) and southern Sonora (Mexico) south through tropical Mexico and Central America to southern South America. These species occur in open and forested areas (Maciel et al., 2010; Frost, 2014). Most species of this genus are allocated into six morphological groups (Duellman & Schulte, 1992): *R. crucifer*, *R. granulosa*, *R. margaritifera*, *R. marina*, *R. spinulosa* and *R. veraguensis* species groups. Pramuk (2006) recognised the monophyly of most species groups, except *R. spinulosa*, which comprises of several separate lineages.

Out of 86 species of the genus *Rhinella*, 26 have the tadpole described: *R. abei* (Fehlberg et al., 2012), *R. achavali* (Kolenc et al., 2013), *R. arequipensis* (Aguilar & Gamarra, 2004), *R. arenarum* (Cei, 1980), *R. castaneotica* (Caldwell, 1991), *R. chrysophora* (Lavilla & de Sá, 2001),

R. crucifer (Ruas et al., 2012), *R. cerradensis* (Maciel et al., 2007), *R. dorbignyi* (Borteiro et al., 2006), *R. fernandezae* (Cei, 1980), *R. granulosa* (Mercês et al., 2009), *R. hoogmoedi* (Mercês et al., 2009), *R. icterica* (Cei, 1980), *R. jimi* (Mercês et al., 2009; Tolledo & Toledo, 2010), *R. magnussoni* (Lima et al., 2007), *R. margaritifera* (Caldwell, 1991), *R. marina* (Cei, 1980), *R. ornata* (Heyer et al., 1990; Carvalho-e-Silva, 1988), *R. pombali* (Lourenço et al., 2010), *R. proboscidea* (Menin et al., 2006), *R. pygmaea* (Carvalho-e-Silva et al., 1994), *R. quecucha* (Aguayo et al., 2009), *R. rubescens* (Eterovick & Sazima, 1999), *R. schineideri* (Cei, 1980), *R. scitula* (Caramaschi & Niemeyer, 2003) and *R. spinulosa* (Aguilar & Gamarra, 2004). Features from the larval internal morphology have additionally been used in studies on the evolutionary relationships of anurans (e.g., Sokol, 1975; Wassersug, 1980; Haas, 1996, 1997; Maglia et al., 2001; Haas, 2003). Among traits of the internal morphology, the cranial morphology of tadpoles has received attention for having demonstrated taxonomic importance; many of them are phylogenetically informative (e.g., Haas, 1995; Larson & de Sá, 1998; Haas, 2003; Larson et al., 2003; Pugener et al., 2003; Vera Candioti, 2007).

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Among the recent studies on tadpoles, Haas (2003) proposed a phylogenetic hypothesis for the anurans using mainly larval characters of the internal and external morphology. This author provided diagnoses for 18 families of anurans, including the Family Bufonidae. In this family, nine species had their tadpoles analysed, from which two are from the genus *Rhinella*, *R. arenarum* (Hensel, 1867) and *R. marina* (Linnaeus, 1758).

The morphologies of the chondrocranium and of the hyobranchial skeleton have been poorly studied in the genus *Rhinella*. Currently, only seven species have the chondrocranium and the hyobranchial skeleton formerly described: *R. achavali* (Kolenc et al., 2013), *R. arenarum* (Fabrezi & Vera, 1997; Haas, 2003; Vera Candioti, 2007), *R. chrysophora* (Lavilla & de Sá, 2001), *R. quechua* (Aguayo et al., 2009), *R. marina* (Haas, 2003), *R. schneideri* (Fabrezi & Vera, 1997; Prado, 2006) and *R. spinulosa* (Vera Candioti, 2007).

Given the current need for additional knowledge concerning the internal anatomy of the *Rhinella* tadpoles and also because morphological characteristics of tadpoles are useful to taxonomic and phylogenetic studies, we have described and compared the chondrocranium and hyobranchial apparatus of tadpoles from 11 species of the genus *Rhinella*.

MATERIALS AND METHODS

We analysed tadpoles housed at the following Brazilian herpetological collections: CFBH (Célio F.B. Haddad Collection, Universidade Estadual Paulista, Campus de Rio Claro, São Paulo), DZSJRP (Departamento de Zoologia, Universidade Estadual Paulista, São José do Rio Preto, São Paulo), MNRJ (Museu Nacional, Rio de Janeiro), ZUFG (Zoological Collection, Universidade Federal de Goiás, Goiânia, Goiás), ZUFRJ (Zoological Collection, Universidade Federal do Rio de Janeiro, Rio de Janeiro), MZFS (Museu de Zoologia da Universidade Estadual de Feira de Santana, Feira de Santana, Bahia) and UFBA (Museu de Zoologia da Universidade Federal da Bahia, Salvador, Bahia).

Tadpoles of *Rhinella* described in this study belong to four species groups formerly described in the literature (Duellman & Schulte, 1992): the *Rhinella crucifer* species group—*R. abei* (Baldissera, Caramaschi & Haddad), *R. crucifer* (Wied-Neuwied), *R. henseli* (Lutz), *R. ornata* (Spix) and *R. pombali* (Baldissera, Caramaschi & Haddad); the *R. granulosa* species group—*R. granulosa* (Spix) and *R. pygmaea* (Myers & Carvalho); the *R. marina* species group—*R. icterica* (Spix), *R. jimi* (Stevaux) and *R. schneideri* (Werner); and the *R. margaritifera* species group—*R. hoogmoedi* (Caramaschi & Pombal).

To confirm the taxonomic identification of each tadpole specimen, we made comparisons with (i) specimens from the same lots of original tadpole descriptions, (ii) with the original tadpole descriptions, (iii) with specimens from the same locality of the original description. Additional information about the specimens analysed can be found in the Online Appendix.

Specimens were analysed using the *Leica* EZ4 stereomicroscope. The morphological description was based on individuals of 11 larval stages (28–39; Gosner,
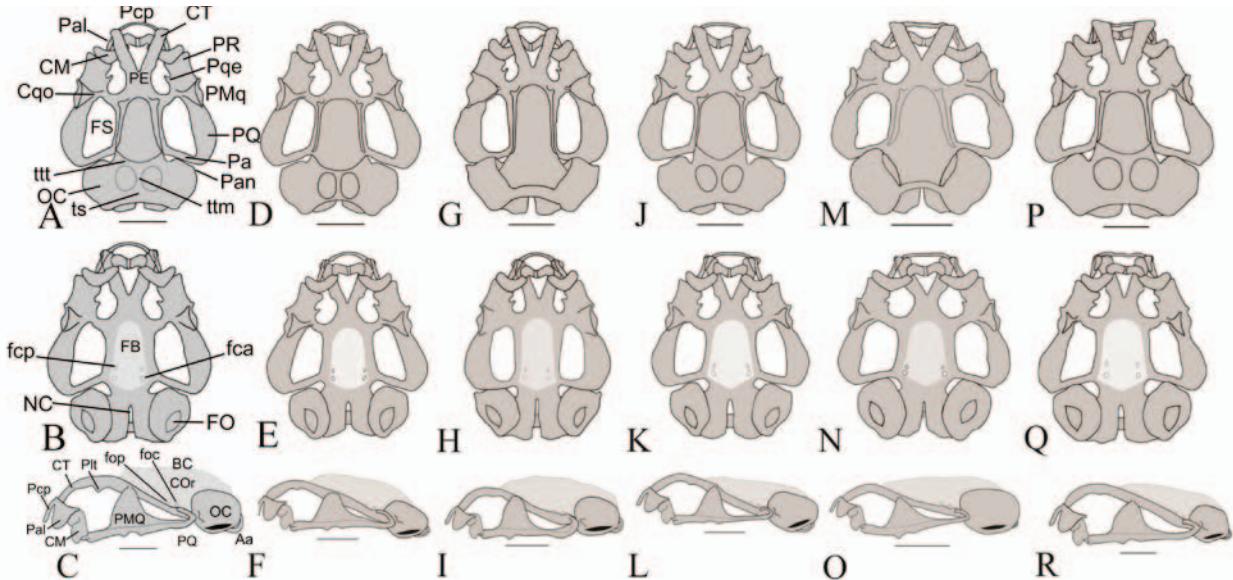
Fig. 1. Chondrocranium. Dorsal, ventral and lateral views (respectively) for *Rhinella abei* (A, B, C; stage 36, DZSJRP 908.1), *R. crucifer* (D, E, F, stage 31, UFBA 11819), *R. granulosa* (G, H, I; stage 36, MZEFS 110), *R. henseli* (J, K, L; stage 37, DZSJRP 1576.4), *R. hoogmoedi* (M, N, O; stage 34, UFBA 11105) and *R. icterica* (P, Q, R; stage 36, DZSJRP 15085). (Pcp) Pars corporis; (Pal) Pars alaris; (CT) Cornua trabeculae; (CM) Cartilago Meckeli; (Pqe) Processus quadratoethmoidalis; (PE) Ethmoid plate; (PR) Processus articularis quadrati; (PMq) Processus muscularis quadrati; (cqo) Commissura quadratoorbitalis; (cqc) Commissura quadratocranialis; (lon) Lamina orbitonasalis; (FS) Fenestra subocularis; (PQ) Palatoquadrate; (PA) Processus ascendens; (OC) Otic capsule; (ttt) Taenia tecti transversalis; (ttm) Taenia tecti medialis; (ts) Tectum synoticum; (Pan) Processus anterolateralis of the otic capsule; (BC) braincase; (Plt) Processus lateralis trabeculae; (COr) Cartilago orbitalis; (fop) Foramen opticum; (foc) Foramen oculomotorium; (Aa) Arcus occipitalis; (FB) Fenestra basicranialis; (fcp) Foramen craniopalatinum; (fca) Foramen caroticum primarum; (NC) Notocordal canal; (FO) Fenestra ovalis. (scale bar =1 mm).

1960). To document the structures of the chondrocranium and hyobranchial apparatus, specimens were cleaned and double-stained for bone and cartilage using a technique of Taylor & Van Dyke (1985); the terminology follows de Sá (1988) and Haas (1995, 1997). The illustrations of the chondrocranium and hyobranchial apparatuses were produced in Photoshop CS5 from photographs obtained under stereomicroscope with the image analyser.

RESULTS

Chondrocranial morphology, upper jaws and ethmoid region

The chondrocranium in *Rhinella* is longer than wide (Figs. 1, 2). The suprarostral cartilages consist of two elements, the pars corporis and the pars alaris. The pars corporis is constituted for the ventromedial fusion of two elements forming, in anterior view, a structure that is a "U" shape (Fig. 3A), as in most species, or a "V" shape (Fig. 3B), as in *R. granulosa*. The pars corporis are narrow cartilages (Fig. 3A), except in *R. granulosa* that has these cartilages that are broad and robust (Fig. 3B). The pars alaris is well developed for all species, has a processus anterior dorsalis round and one processus posterior dorsalis digitiform that is elongated (Figs. 3A, B). The pars alaris and pars corporis are ventrally separate, but fused dorsally through a narrow strip of cartilage just ventral to their point of articulation with the cornua trabeculae. The cornua trabeculae is articulate with the suprarostral cartilages at the connection point between the pars alaris and pars corporis. The cornua trabeculae are long (Figs. 1, 2); they are similar in width among most species, but they are thinner in *R. schneideri* (Fig. 2M) and broader in *R. icterica* (Fig. 1P). The cornua trabeculae width is not uniform throughout the whole length. All species have the distal end of the cornua trabeculae wider and triangular. The cornua trabeculae diverge, producing a

"V" design in dorsal view. There is a slight narrowing in the proximal region of the cornua trabeculae close to the ethmoid plate (Figs. 1, 2). The processus lateralis trabeculae is evident in all species (Figs. 1, 2), but it is not as prominent in *R. granulosa* (Fig. 1G).

Braincase

The braincase is open dorsally (Figs. 1, 2) and the braincase width corresponds to 30% of the chondrocranium width. Subdivisions in the frontoparietal fontanelle were not observed in *R. granulosa* (Fig. 1G), *R. hoogmoedi* (Fig. 1M), *R. jimi* (Fig. 2A) and *R. schneideri* (Fig. 2M). The braincase is bound on either side by taenia tecti marginalis. In *R. abei*, *R. crucifer*, *R. henseli*, *R. icterica* (Fig. 1A, D, J, P), *R. ornata*, *R. pombali* and *R. pygmaea* (Fig. 2D, G, J) the frontoparietal fontanelle showed subdivisions, with a larger frontoparietal fontanelle and two smaller parietal fenestrae delimited by taenia tecti medialis and taenia tecti transversalis. In one specimen of *R. pombali* (stage 28) we did not observe subdivisions in the frontoparietal fontanelle. The septum nasi is not observable and the lamina orbitonasalis is evident in all species. The cartilago orbitalis consists of a thin section that is poorly chondrified and only lightly stained with alcian blue.

In lateral view, and posteriorly to the cartilago orbitalis, there are the foramen opticum, most anterior and with the smaller diameter, and the foramen oculomotorium, with the largest diameter, most posterior and situated above the insertion point of the processus ascendens on the braincase (Fig. 1C, F, I, L, O, R; Fig. 2C, F, I, L, O). The foramen troclear and foramen prooticum are not visible.

The fenestra basicranialis is broad, delimited anteriorly by the planum trabecularum anticum, laterally by the trabeculae cranii and posteriorly by the planum basale. The fenestra basicranialis has a round anterior margin and a triangular posterior margin. The
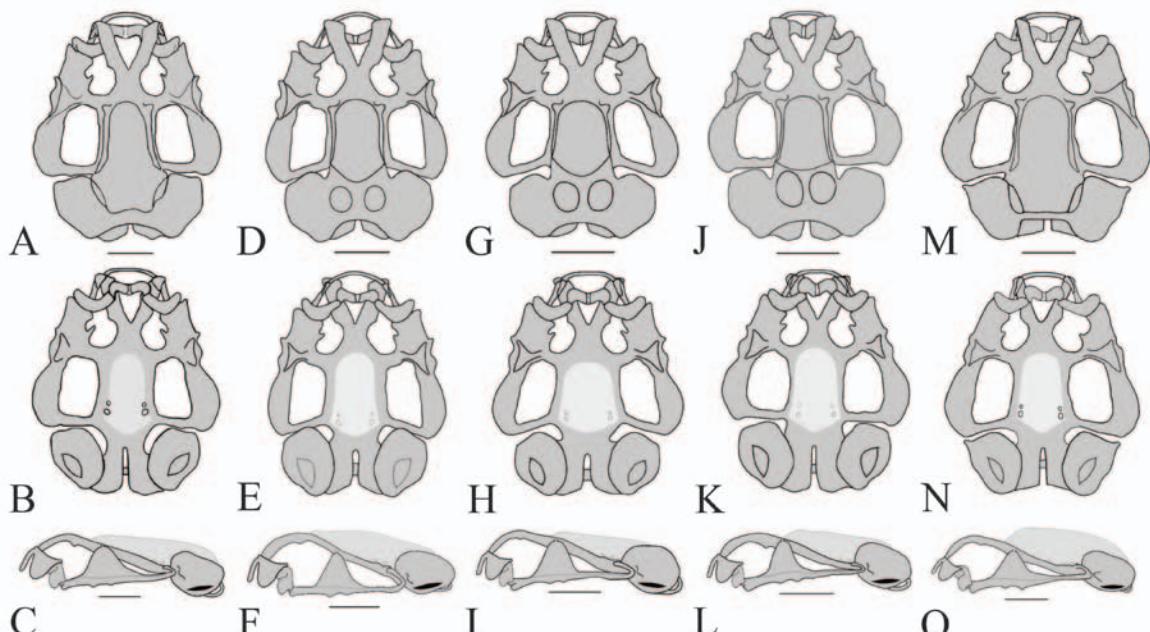
Fig. 2. Chondrocranium. Dorsal, ventral and lateral views (respectively) for *Rhinella jimi* (A, B, C; stage 32, UFBA11820), *R. ornata* (D, E, F; stage 36, MNRJ59456), *R. pombali* (G, H, I; stage 31, MNRJ 49738), *R. pygmaea* (J, K, L; stage 36, ZUFRJ 3318), *R. schneideri* (M, N, O; stage 31, DZSJRP711.1), (scale bar=1 mm).
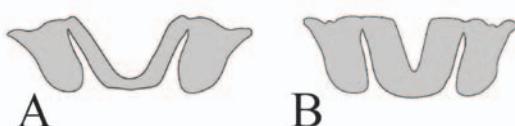
Fig. 3. Suprarostral cartilage. (A) Pars corporis and pars alaris of *R. henseli* in frontal view, stage 37, DZSJRP1576.4; (B) Pars corporis and pars alaris of *R. granulosa* in frontal view, stage 36, MZEFS110.

planum intertrabeculare is poorly chondrified between stages 28 (observed in one specimen of *R. pombali*) and 37, becoming thicker and more rigid between stages 38 (observed in one specimen of *R. icterica*) and 39 (observed in one specimen of *R. pombali*). On the braincase floor, there are the foramen caroticum primarum, more posterior and of a greater diameter, and the foramen craniopalatinum, more anterior and of a smaller diameter. In some specimens at an early larval stage, the foramen craniopalatinum are not clearly identifiable, possibly due to poor chondrification of the planum intertrabeculare (Fig. 1B, E, H, K, N, Q; Fig. 2B, E, H, K, N).

Otooccipital region
The otic capsule is large (Figs. 1, 2); its length is approximately 30% of the chondrocranium length and round, with a round processus anterolateralis in *R. abei*

(Fig. 1A, B, C), *R. crucifer* (Fig. 1D, F, G), *R. henseli* (Fig. 1J, K, L), *R. jimi* (Fig. 2A, B, C), *R. ornata* (Fig. 2D, E, F), *R. pombali* (Fig. 2G, H, I) and *R. pygmaea* (Fig. 2J, K, L); acute in *R. granulosa* (Fig. 1G, H, I) and *R. schneideri* (Fig. 2M, N, O); and absent in *R. hoogmoedi* (Fig. 1M, N, O). The larval processus oticus is absent. The fenestra ovalis is present ventrally on the otic capsule (Fig. 1B, E, H, K, N, Q; Fig. 2B, E, H, K, N). The otic capsules are dorsally connected by the tectum synoticum, which may vary in width between species. The otic capsules are connected to the braincase through the tectum synoticum, taenia tecti marginalis, planum basale and arcus occipitalis. At the posterior margin of the planum basale, the notocordal canal is long, corresponding to 30% of the braincase length in most species and 23% of the braincase length in *R. hoogmoedi*. The acoustic foramen is indistinct.

Palatoquadrate
The palatoquadrate is long and parallel to the braincase (Fig. 1C, F, I, L, O, R; Fig. 2C, F, I, L, O), with a smooth surface and margin. The palatoquadrate is attached to the braincase by three cartilaginous points, commissura quadratocranialis, commissura quadratoorbitalis and processus ascendens; the commissura quadratocranialis connects the palatoquadrate anteriorly to the braincase. In the anterior margin of the palatoquadrate there is a triangular and well developed processus quadratoethmoidalis; the commissura quadratoorbitalis
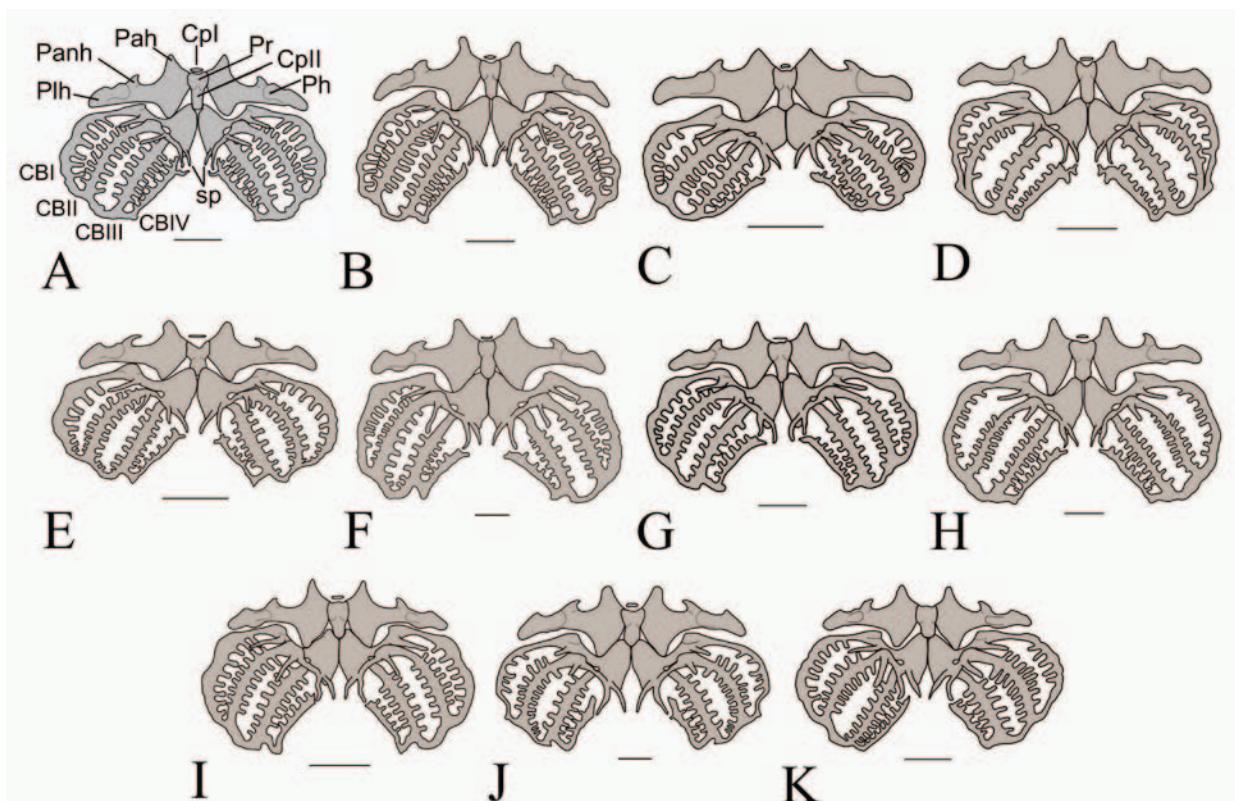
Fig. 4. Hyobranchial apparatus. Ventral view. (A) *Rhinella abei*, stage 36, DZSJRP917.1; (B) *R. crucifer* stage 31, UFBA 11819; (C) *R. granulosa*, stage 36, MZEFS 110; (D) *R. henseli*, stage 37, DZSJRP1576.4; (E) *R. hoogmoedi*, stage 34, UFBA 11105; (F) *R. icterica*, stage 36, DZSJRP 1508.5; (G) *R. jimi*, stage 32, UFBA 11820; (H) *R. ornata*, stage 36, MNRJ59456; (I) *R. pombali*, stage 31, MNRJ 49738; (J) *R. pygmaea*, stage 36, ZUFRJ 3318; (K) *R. schneideri*, stage 31, DZSJRP 711.1. (CpI) Copula anterior; (Pr) Par reuniens; (CpII) Copula posterior; (Ch) Ceratohyal; (Pah) Processus anterior hyalis; (Panh) Processus anterolateralis hyalis; (Plh) Processus lateralis hyalis; (Pph) Processus posterior hyalis; (Ph) Condylus articularis; (Phb) Hypobranchial plate; (sp) Spicules; (CB I, II, III, IV) Ceratobranchial cartilages. (scale bar=1 mm).

connects the anterior end of the processus muscularis quadrati to the dorsal surface of the commissura quadratocranialis (Fig. 1A, D, J, M, P; Fig. 2A, D, G, J, M); the commissura quadratoorbitalis is wide and distinct on most species, except in *R. granulosa* whose commissura is thin and poorly discernible (Fig. 1G); the processus ascendens is long on most species (Fig. 1A, D, J, M, P; Fig. 2A, D, G, J, M) and short in *R. granulosa* (Fig. 1G). In *R. granulosa* (Fig. 1G, H), *R. jimi* (Fig. 2A, B) and *R. pygmaea* (Fig. 2J, K), the processus ascendens is attached perpendicularly on the braincase; in *R. abei* (Fig. 1A, B), *R. crucifer* (Fig. 1D, E), *R. henseli* (Fig. 1J, K), *R. hoogmoedi* (Fig. 1M, N), *R. icterica* (Fig. 1P, Q), *R. ornata* (Fig. 2D, E), *R. pombali* (Fig. 2G, H) and *R. schneideri* (Fig. 2M, N) it is attached obliquely to the braincase, differing somewhat between species in the degree of inclination. In all species the processus ascendens is attached to the trabeculae cranii just below the foramen oculomotorium.

The processus ascendens is close to the otic capsule in *R. abei*, *R. crucifer*, *R. henseli*, *R. jimi*, *R. pygmaea* and *R. schneideri*; overlapping the otic capsule in *R. granulosa*, *R. hoogmoedi*, *R. icterica* and *R. ornata*; and away from the otic capsule in *R. pombali*. The processus pseudopterygoideus is absent.

The fenestra subocularis is broad, with the anterior margin round and the posterior margin straight in almost all species (Fig. 1A, B, D, E, J, K, M, N, P, Q; Fig. 2A, B, D, E, G, H, J, K, M, N), except in *R. granulosa*, whose fenestra is elliptical in shape (Fig. 1G, H). The posterior margin of the arcus subocularis is laterally round. The processus muscularis quadrati is high and its base wider than its apex; it is directed to the dorsum of the chondrocranium, moderately inclined towards the dorsum in most species (Fig. 1A, D, J, M, P; Fig. 2A, D, G, J, M), but has a more pronounced inclination in *R. granulosa* (Fig. 1G). The pars articularis quadrati is long and slightly expanded at the anterior region (Fig. 1).

Lower jaws
The cartilago Meckeli are sigmoid and robust. These cartilages articulate with the pars articularis quadrati. The infrarostral cartilages are short, rectangular-shaped, robust, independent and connected medially by the commissura intermandibularis.

Hyobranchial apparatus
The copula anterior is observed in almost all species (Fig. 4A–J), except in *R. schneideri* (Fig. 4K). The ceratohyal is composed of five processes: the processus anterior hyalis, the processus anterolateralis hyalis, the processus lateralis hyalis, the processus posterior hyalis and the condylus articularis. The ceratohyal is long and joined by a pars reuniens, which is wider than the copula posterior. The processus urobranchialis is ventrally located on the copula posterior; this structure is short and quadrangular (Fig. 4A–K). The processus anterior hyalis is high, with a round apex in all species (Fig. 4A, B, D–K) and a triangular apex in *R. granulosa* (Fig. 4C). The processus anterolateralis hyalis is short, with a narrow base, an acute apex and is inclined towards the midline of the body. In *R. hoogmoedi* the processus anterolateralis

hyalis is elongated and has a sharp slope to the midline of the body (Fig. 4E). The processus lateralis hyalis is robust and round in all species. The condylus articularis is a robust projection of the dorsal surface of the ceratohyal with a round apex (Fig. 4A–K).

The hypobranchial plates are high and have large projections to the sides forming a well-developed processus anterior branchialis. The hypobranchial plates are medially connected by the commissura inter-hyal. The ceratobranchial cartilages are long, have lateral projections and are united distally by commissura terminales. The ceratobranchial I is continuous to the hypobranchial plate and the ceratobranchial cartilages IV is free at the proximal end. There are four well developed cartilaginous spicules of equivalent shape and size; the spicule I, II and III are attached to the margin of the lateral extension of the hypobranchial plates and the spicule IV is attached to the posterior margin of the hypobranchial plates near the midline (Fig. 4A–K).

DISCUSSION

Cranial morphology has been poorly studied in bufonid tadpoles, being known only in *Amietophrynus regularis* (Reuss, 1833) (Sedra, 1950; Sedra & Michael, 1958), *Anaxyrus americanus* (Holbrook, 1836) (Larson, 2004), *Anaxyrus terrestris* (Bonnaterre, 1789) (Sokol, 1981), *Atelopus tricolor* (Boulenger, 1902) (Lavilla & de Sá, 2001; Haas, 2003), *Bufo brongersmai* Hoogmoed, 1972 (Haas, 2003), *B. bufo* (Linnaeus, 1758) (Haas, 1995, 2003), *Duttaphrynus melanostictus* (Schneider, 1799) (Haas, 2003); *Melanophryniscus montevidensis* (Philippi, 1902), *M. pachyrhynus* (Miranda-Ribeiro, 1920) (Haas, 2003; Larson et al., 2003), *M. sanmartini* Klappenbach, 1968 (Larson et al., 2003), *Peltophryne peltocephala* (Tschudi, 1838), *Pedostibes hosii* (Boulenger, 1892) (Haas, 2003), *Rhinella achavali* (Kolenc et al., 2013), *R. arenarum* (Fabrezi & Vera, 1997; Haas, 2003; Vera Candioti, 2007), *R. chrysophora* (Lavilla & de Sá, 2001), *R. quechua* (Aguayo et al., 2009), *R. marina* (Haas, 2003), *Rhinella schneideri* (Fabrezi & Vera, 1997; Prado, 2006) and *R. spinulosa* (Vera Candioti, 2007).

The chondrocranial and hyobranchial morphology of *Rhinella* is conserved; there are similarities in many aspects between the species analysed here and those previously described (Fabrezi & Vera, 1997; Lavilla & de Sá, 2001; Haas, 2003; Prado, 2006; Vera Candioti, 2007; Aguayo et al., 2009). In addition, many features are shared with other bufonids that have well-studied chondrocranial and hyobranchial morphologies (Sedra, 1950; Sedra & Michael, 1958; Lavilla & de Sá, 2001; Haas, 2003; Larson et al., 2003; Larson, 2004).

Based on the comparisons shown here, some variation observed between *Rhinella* tadpoles can be listed (i) pars corporis in anterior view forming a "U" or "V"; (ii) cartilage of the narrow or wide pars corporis; (iii) cornua trabeculae width; (iv) a wide or narrow ethmoid plate; (v) a more prominent or less prominent processus lateralis trabeculae; (vi) *fenestra subocularis* with a rounded anterior margin and a straight or elliptical-shaped posterior margin; (vii) processus muscularis

quadrati moderately inclined towards the dorsum or with a more pronounced inclination towards the dorsum; (viii) wide or thin commissura quadratoorbitalis; (ix) long or short processus ascendens; (x) processus ascendens perpendicularly or obliquely attached to the braincase; (xi) processus ascendens near, away or overlapping the otic capsule; (xii) processus anterolateralis of the otic capsule present with the round acute or absent apex; (xiii) present or absent copula anterior; (xiv) round or triangular processus anterior hyalis; and (xv) processus anterolateralis hyalis short and moderately inclined to the midline of the body or elongated and with pronounced inclination.

Four possible synapomorphies for Bufonidae were formerly suggested by Larson et al. (2003): (i) absent larval processus oticus; (ii) reduced or absent processus anterolateralis of the otic capsule (larval crista parotica); (iii) ceratobranchial IV not fused to the hypobranchial plate; and (iv) cartilago orbitalis poorly chondrified. The results presented here for *Rhinella* support such synapomorphies.

The tadpole of *Rhinella* (in this work; Fabrezi & Vera, 1997; Vera Candioti, 2007; Aguayo et al., 2009; Kolenc et al., 2013), *Anaxyrus americanus* (Larson, 2004), *Atelopus tricolor* (Lavilla & de Sá, 2001), *Bufo bufo* (Haas, 1995), *Amietophrynus regularis* (Sedra, 1950), *Melanophryniscus montevidensis*, *M. pachyrhynus* and *M. sanmartini* (Larson et al., 2003) share a pars corporis formed as a single element, dorsally fused to the pars alaris; these have the processus posterior dorsalis digitiform well-developed.

All species of *Rhinella* analysed here have the cartilago orbitalis poorly chondrified, even in later stages, similar to what was reported for *Anaxyrus americanus* (Larson, 2004). Such features are also present for *R. quechua* (Aguayo et al., 2009) and *R. chrysophora*, as well as for other bufonids including *Atelopus tricolor* (Lavilla & de Sá, 2001), *Anaxyrus terrestris* (Sokol, 1981), *Melanophryniscus montevidensis*, *M. pachyrhynus* and *M. sanmartini* (Larson et al., 2003). However, Sedra (1950) illustrated for *Amietophrynus regularis* an apparently rigid and well developed cartilago orbitalis. Vera Candioti (2007) did not describe details of the development of this cartilage in *R. arenarum* and *R. spinulosa*, but did not show the lateral walls of the braincase, only the trabeculae cranii and, dorsally, the taenia tecti marginalis for *R. spinulosa.*

The reason we did not observe the septum nasi in the *Rhinella* species (in this work), and subdivisions of the frontoparietal fontanelle for some species, may be due to the developmental stages of the larvae and to the poor or the late chondrification of these parts, so as that such regions are poorly stained by alcian blue as previously reported by Larson (2004) for *Anaxyrus americanus* (stage 36–37).

Among bufonids the processus anterolateralis of the otic capsule (larval crista parotica) may be absent as in *R. hoogmoedi* (in this work), *R. chrysophora* (Lavilla & de Sá, 2001), *Rhinella quechua* (Aguayo et al., 2009), *Anaxyrus americanus* (Larson, 2004), *Atelopus tricolor* (Lavilla & de Sá, 2001), *Bufo bufo* (Haas, 1995), *Melanophryniscus montevidensis*, *M. pachyrhynus* and *M. sanmartini* (Larson et al., 2003); poorly developed with a round aspect as in most

species of *Rhinella* described here and for *Amietophrynus regularis* (poorly developed in its early stages, although more developed in its stages near metamorphosis) (Sedra, 1950); well developed with an acute appearance as in *R. schneideri* and *R. granulosa* (in this work), which was described and illustrated for *R. arenarum* and *R. spinulosa* by Vera Candioti (2007). The larval processus oticus is absent in all bufonids with their chondrocranial morphology available (in this work; Sedra, 1950; Haas, 1995; Fabrezi & Vera, 1997; Lavilla & de Sá, 2001; Haas, 2003; Larson et al., 2003; Larson, 2004; Candioti, 2007; Kolenc et al., 2013). This character was included by Haas (2003) in the list of bufonid autapomorphies.

The palatoquadrate is similar among bufonids (in this work; Sedra, 1950; Fabrezi & Vera, 1997; Lavilla & de Sá, 2001; Larson, 2004; Vera Candioti, 2007; Kolenc et al., 2013), however, in *Rhinella quechua* (Aguayo et al., 2009), *R. chrysophora* and *Atelopus tricolor* there is a pronounced inclination that extends posteriorly toward the otic capsule and the anterior region of the palatoquadrate is distinctively broader (Lavilla & de Sá, 2001). In most species of bufonids that have had their chondrocranium described (in this work; Sedra, 1950; Fabrezi & Vera, 1997; Lavilla & de Sá, 2001; Haas, 2003 p. 69, character 78; Larson, 2004; Vera Candioti, 2007; Aguayo et al., 2009; Kolenc et al., 2013), the palatoquadrate is attached to the braincase by three cartilaginous points, the commissura quadratocranialis, processus ascendens and commissura quadratoorbitalis. The connection of the processus muscularis quadrati to the braincase through the commissura quadratoorbitalis is defined by Haas (2003 p. 69, character 78). However, the commissura quadratoorbitalis is absent in known tadpoles from *Melanophryniscus* according to Larson et al. (2003). The absence of the processus pseudopterygoideus from commissura quadratocranialis corresponds to one of the states of the character "77" from Haas (2003).

In *Rhinella* the connection of the processus ascendens to the braincase corresponds to a 'low' attachment condition, defined by Sokol (1975, 1981) and also applied by Larson & de Sá (1998 p. 299, Character n), Maglia et al. (2001 p. 548, Character 25), Haas (2003 p. 65 Character 71) and Vera Candioti (2007). In *Rhinella* tadpoles the processus ascendens is most commonly obliquely attached to the braincase, as in the *R. crucifer* species group, *R. icterica*, *R. schneideri* (in this work) and as illustrated for *R. chrysophora* (Lavilla & de Sá, 2001) and *R. quechua* (Aguayo et al., 2009). The same configuration exists for other bufonids including *Amietophrynus regularis* (Sedra, 1950 p. 449 Figure 2, 3), *Anaxyrus americanus* (Larson, 2004), *Atelopus tricolor* (Lavilla & de Sá, 2001), *Melanophryniscus montevidensis*, *M. pachyrhynus* and *M. sanmartini* (Larson et al., 2003). However, *R granulosa*, *R. jimi* and *R. pygmaea* (in this work) have the *processus ascendens* perpendicularly attached to the braincase, similar to what was found for *R. arenarum* and *R. spinulosa* by Vera Candioti (2007).

According to Larson et al. (2003), the copula anterior is highly variable between bufonids. Such a structure is present in *Anaxyrus americanus* (Larson, 2004), *Amietophrynus regularis* (Sedra & Michael, 1958), *Rhinella arenarum* (Fabrezi & Vera, 1997; Candioti, 2007), *R.*

quechua (Aguayo et al., 2009), *R. chrysophora* (Lavilla & de Sá, 2001), *R. spinulosa* (Candioti, 2007) and in most species of *Rhinella* described here, except for *R. schneideri* where such a structure was not observed. It was also not found in previous descriptions by Fabrezi & Vera (1997) and Prado (2006). Similar to *R. schneideri* the copula anterior is also absent in *Atelopus tricolor* (Lavilla & de Sá, 2001), *Bufo bufo* (Hass, 1995) and *R. achavali* (Kolenc et al., 2013).

The shape of the ceratohyal and the hypobranchial plate is similar among bufonids and it is common to have five processes associated with ceratohyal (in this work; Sedra & Michael, 1958; Fabrezi & Vera, 1997; Lavilla & de Sá, 2001; Larson et al., 2003; Larson, 2004; Vera Candioti, 2007; Aguayo et al., 2009). All tadpoles of *Rhinella* described here and most of those previously described have four ceratobranchial cartilages. However, *Rhinella chrysophora* (Lavilla & de Sá, 2001) is the only representative of the genus with only three ceratobranchial cartilages.

In most species of *Rhinella* (in this work; Fabrezi & Vera, 1997; Lavilla & de Sá, 2001; Haas, 2003; Prado, 2006; Vera Candioti, 2007; Aguayo et al., 2009) there exists four well-developed cartilaginous spicules, with equivalent shape and size; spicules I, II and III are attached to the margin of the lateral extension of the hypobranchial plate and spicule IV is attached to the posterior margin of the hypobranchial plate. However, Larson et al. (2003) described for *Melanophryniscus montevidensis*, *M. pachyrhynus* and *M. sanmartini* a rounded mass of cartilage attached to the posterior margin of the hypobranchial plate. A similar structure was reported in *Amietophrynus regularis* (Sedra & Michael, 1958). This mass is suggested by Sedra & Michael (1958) and Larson et al. (2003) as a possible vestige of the spicule IV.

Rhinella chrysophora as described by Lavilla & de Sá (2001), despite having common features with other species of *Rhinella*, is the member of the genus with a more distinct general appearance of the chondrocranium and hyobranchial apparatus. Among the features that distinguish this species include: a chondrocranium wider than long; suprarostral cartilages with two dorsolateral slits that serve as articulation points with the cornua trabeculae; a subocular bar of palatoquadrate that extends posteriorly and almost reaches the posterior margin of the otic capsule; a narrow fenestra subocularis; and an absent ceratobranchial IV. On many features, the chondrocranium and hyobranchial apparatus of *Rhinella chrysophora* resembles more that of *Atelopus tricolor*, the latter a representative of a basal genus of Bufonidae (*sensu* Pramuk et al., 2008).

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Appendix 1. Specimens analysed.

Rhinella abei. – DZSJRP 908.1, *n*=1, stage=37; DZSJRP 917.1, *n*=2, stage=36. São José dos Pinhais, Serro e Gemido, Paraná, Brasil. Specimens compared to the original description (Fehlberg et al., 2012).

R. crucifer. – UFBA 0107, *n*=2, stage=31. Guarajuba, Camaçari, Bahia, Brasil. Specimens compared to the original description (Ruas et al., 2012) and with specimens from the same locality of the original description. UFBA 11819, *n*=3, stage=31. Igrapiúna (13°49'25''S, 39°11'39''W), Bahia, Brasil (Specimens of the same locality of the specimens utilised in the original description).

R. granulosa. – MZEFS110, *n*=2, stage=34 and 36. Feira de Santana, Bahia, Brasil. Specimens from the same lots used in the original description.

R. henseli. – DZSJRP 1576.4, *n*=1, stage=36; DZSJRP 1716.1, *n*=1, stage=37. Parque Nacional das Araucárias, Santa Catarina, Brasil. Specimens from the same lots used in the original description.

R. hoogmoedi. – UFBA 11105, *n*=3, stage=34. Aritaguá, Ilhéus, Bahia, Brasil. Specimens compared to the original description (Mercês et al., 2009).

R. icterica. – DZSJRP 1508.5, *n*=2, stage=36. Parque Estadual Campos do Jordão, Campos do Jordão, São Paulo, Brasil. UFRJ 5130, *n*=1, stage=31; *n*=2, stage=38. Teresópolis, Rio de Janeiro, Brasil. Specimens compared to the original description (Cei, 1980).

R. jimi. – UFBA 11820, *n*=1, stage=31; *n*=2, stage=32; *n*=3, stage=33; *n*=1, stage=34; *n*=2, stage=35; *n*=1, stage=36. Brotas de Macaúbas, Bahia, Brasil. Specimens compared to the original description (Mercês et al., 2009, Tolledo & Toledo, 2010).

R. ornata. – CFBH 12098, *n*=3, stage=35. Córrego da Onça, Parque Estadual Morro do Diabo, Teodoro Sampaio, São Paulo. DZSJRP 1192.3, *n*=1, stage=35; DZSJRP 1175.2, *n*=1, stage=36. Bertioga, Parque das Neblinas, São Paulo, Brasil. MNRJ 59456, *n*=3, stage=35–37. Reserva Biológica União, Casimiro de Abreu, Rio De Janeiro, Brasil. UFRJ 5101, *n*=3, stage=31. Penha, Rio de Janeiro, Rio de Janeiro, Brasil. Specimens compared to the original description (Heyer, 1990).

R. pombali. – MNRJ 49738, *n*=3, stage=21, 31, 39. RPPN Santuário do Caraça, Catas Altas, Minas Gerais, Brasil. Specimens compared to the original description (Lourenço et al., 2010).

R. pygmaea. – UFRJ 3318, *n*=1, stage=36; *n*=1, stage=38. Arraial do Cabo, Rio de Janeiro, Brasil. Specimens compared to the original description (Carvalho-e-Silva & Carvalho-e-Silva, 1994).

R. schneideri. – DZSJRP711.1, *n*=3, stage=31. Sítio Boa Esperança, Icém, São Paulo, Brasil. UFG120, *n*=1, stage=36. Portelândia, Goiás, Brasil. Specimens compared to the original description (Cei, 1980; Fabrezi & Vera, 1997; Rossa-Feres & Nomura, 2006).

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