

RESEARCH ARTICLE

Growth and cranial development in the Andean frogs of the genus *Telmatobius* (Anura: Telmatobiidae): Exploring the relation of heterochrony and skeletal diversity

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Abstract

Andean frogs of the genus *Telmatobius* occur at high elevations, they have an aquatic mode of life and large tadpoles. There are more than 60 species that closely resemble one another and have low values of genetic divergence. However, the skeleton, particularly the cranium, is interspecifically variable with respect to the different levels of development of some elements. Heterochrony is considered to have played a prominent role in generating phenotypic variation, especially among closely related species. Herein, the developmental origins of the adult cranial configuration of two species of *Telmatobius* are explored. The interactions among larval and post-metamorphic growth, sexual maturation, and ossification sequence in *T. oxycephalus* and *T. rubigo* are studied. Although there are no substantial changes in the sequences of ossification of the cranium, it is likely that differential timing of larval periods is related to adult cranial characters. The prolonged larval development of *T. rubigo* may result in peramorphic configurations of bones that ossify during pre-metamorphosis. This long developmental time would also explain why the gonads of *T. rubigo* are highly differentiated by the end of metamorphosis. In this species, sexual maturation may be attained precociously in relation to metamorphosis, thereby reducing post-metamorphic developmental time of late-onset bones, which have paedomorphic configurations (e.g., vomer, neopalatine, and columella). An inverse pattern characterizes *T. oxycephalus*, suggesting that the duration of larval life is related to skeletal configuration in *Telmatobius*.

KEYWORDS

cranium, heterochrony, larvae, ontogeny

1 | INTRODUCTION

The genus *Telmatobius* comprises 63 species of frogs distributed in the Andes from Ecuador to Argentina (Frost, 2017) at some of the highest elevations known among anurans (Lavilla & De la Riva, 2005; Seimon et al., 2007). Unlike most other frogs, adult *Telmatobius* typically are highly or strictly aquatic. The larvae are remarkably large, with the tadpoles of some species nearly 200 mm long (e.g., *T. macrostomus*, Vellard, 1951). The large size of *Telmatobius* larvae is attributed to the effect of low temperature (Catenazzi, von May, & Vredenburg, 2013; Roček, Böttcher, & Wassersug, 2006; Vellard, 1951) that decreases the rate of development and prolong larval period, during which the larvae continue to grow (Berven, 1982; Bury & Adams, 1999).

Relative changes in timing of developmental events (heterochrony) are thought to be a potential mechanism of evolutionary change (Gould, 1977; Hall, 1999). Heterochronic shifts can explain the acquisition of novel morphological configurations in the absence of significant genetic divergence. Thus, closely related species may resemble one another morphologically, but differ in certain characters. Such features may reflect, for instance, morphological characters of an immature developmental stage—that is, a paedomorphic character (e.g., Davies, 1989; Emerson, 1986; Smirnov, 1991; Trueb, 1985). Evidence of heterochronic shifts occur in *Telmatobius*, which have a low genetic divergence (De la Riva, García-París, & Parra-Olea, 2010; Sáez et al., 2014), a highly conserved soft morphology (e.g., De la Riva, 2005; Fabrezi & Lavilla, 1993; Sinsch, Hein, & Glump, 2005; Vera

Candiotti, 2008) but variable skeletal characters (De la Riva, Trueb, & Duellman, 2012). In particular, the skulls of some species seem to exhibit paedomorphic features in the structure of the neopalatine, columella, vomer, and vomerine teeth (Barrionuevo, 2013; Brunetti, Muñoz Saravia, Barrionuevo, & Reichle, 2017; Lobo Gaviola, 1988; Wiens, 1993).

Our knowledge of anuran cranial development has increased substantially in the last few decades and general patterns of the sequence of anuran ossification have been identified (e.g., Hanken & Hall, 1984; Trueb, 1985; Weisbecker & Mitgutsch, 2010). Bones that ossify late in development may be absent or have a paedomorphic configuration relative to those of closely related species (Smirnov, 1991; Trueb, 1985). Heterochronic processes may be associated with environmental conditions that affect the life cycle; for example, larvae may remain in the water in response to a drop in temperature or a drought that renders the terrestrial habitat inhospitable (Roček, 1995; Wilbur & Collins, 1973). Variation in the duration of the anuran larval period has been associated with variation in adult morphology (e.g., lengths of the snout and hind limbs) intraspecifically (e.g., Blouin & Brown, 2000; Emerson, 1987; Newman, 1989), as well as interspecifically (e.g., Gómez-Mestre & Buchholz, 2006). The relationship of larval development with osteological features has been explored in only few anuran taxa (e.g., Fabrezi & Goldberg, 2009; Gómez-Mestre et al., 2010; Kerney, Wassersug, & Hall, 2010; Smirnov, 1992).

Given the low genetic divergence among most species of *Telmatobius*, the observed osteological diversity of adults may reflect heterochronic developmental events. However, the ontogeny of the skull is unknown. Herein, I describe the ossification sequences of two species representing different clades of *Telmatobius*. *Telmatobius oxycephalus* Vellard, 1946 is semiaquatic and belongs to the *T. bolivianus* species group (Barrionuevo, 2017); members of this group occur at intermediate elevations in Bolivia and Argentina. *Telmatobius rubigo* Barrionuevo & Baldo, 2009 is strictly aquatic and it is a member of the *T. marmoratus* species group (Barrionuevo, 2017); this clade comprises species mainly from the highland Altiplano-Puna Plateau in Argentina, Bolivia, Chile, and Peru. The disparate altitudinal distributions and ecological habits of these species (Barrionuevo, 2016) may be correlated with differences in larval growth patterns and cranial development—a proposition that is explored herein, along with the relation between heterochrony in larval period and adult cranial morphology.

2 | MATERIALS AND METHODS

2.1 | Size and growth

Total length (TL) and body length (BL) of 86 larvae (*Telmatobius rubigo* Barrionuevo & Baldo, 2009, $n = 51$; *T. oxycephalus*, Vellard 1946 $n = 35$) were measured to the nearest 0.1 mm with a digital caliper following the methods of Lavilla and Scrocchi (1986). One-way analyses of variance (ANOVA) were performed to test for significant interspecific differences in TL, BL, and the ratio of BL:TL. I measured snout-vent length (SVL) and head width (HW) of a total of 44 *T. rubigo* (juveniles, $n = 11$; males $n = 21$; females $n = 12$) and

48 *T. oxycephalus* (juveniles, $n = 16$; males, $n = 20$; females $n = 12$). All measurements are in millimeters. Sexual maturity of postmetamorphic male frogs was determined by the presence of nuptial pads.

2.2 | Cranial development and ovarian maturation

Cranial development is described based on examination of 53 larvae of *Telmatobius* (Table 1). The 26 tadpoles of *T. rubigo* are from the Puna Plateau at elevations between 3,750 and 3,900 m, whereas the 27 larvae of *T. oxycephalus* are from an inter-Andean valley at approximately 2,800 m. Comparison of the adult cranial structure is based on examination of four adult *T. oxycephalus* and three adult *T. rubigo*. The anurans were fixed in buffered 4% formalin, staged according to Gosner (1960), and cleared and double-stained for bone and cartilage following the protocol of Wassersug (1976). The onset time of

TABLE 1 Voucher specimens of *Telmatobius rubigo* and *T. oxycephalus* cleared and double-stained for the study of the ossification sequence. The specimens are ordered by Gosner stages. MACN = Museo Argentino de Ciencias Naturales; FML = Fundación Miguel Lillo

Stage	<i>T. rubigo</i>	<i>T. oxycephalus</i>
28	MACN 49350	MACN 49376
29	MACN 49351	MACN 49377
	MACN 49358	
30	MACN 49352	MACN 49378
	MACN 49361	MACN 49379
	MACN 49362	MACN 49388
31	MACN 49363	MACN 49380
		MACN 49381
		MACN 49389
32		MACN 49390
33	MACN 49364	MACN 49382
		MACN 49383
		MACN 49391
34	MACN 49365	MACN 49384
35	MACN 49366	MACN 49385
		MACN 49386
36	MACN 49367	MACN 49392
37	MACN 49359	
38	MACN 49360	MACN 49387
		MACN 49393
40	MACN 49353	MACN 49394
	MACN 49371	MACN 49395
	MACN 49368	
41	MACN 49354	MACN 49396
	MACN 49355	MACN 49397
	MACN 49369	
	MACN 49372	
42	MACN 49373	MACN 49398
	MACN 49374	MACN 49399
43	MACN 49356	MACN 49400
	MACN 49357	MACN 49401
44	MACN 49375	FML 1592
45	MACN 49370	

ossification of each bone was determined by the appearance of Alizarin Red S stain. Some variation in the recognized onset of ossification may result from the clearing-and-staining process (Haas, 1999); however, all specimens were processed with the same stock solutions and under similar conditions to reduce protocol artifacts.

To identify sequence heterochronic patterns, I conducted a graphic analysis as proposed by Smith (2001). The onset ossification events of 13 cranial bones were converted into a rank (from 1 to 13). Onset events that occur simultaneously are assigned the mean rank of all the events that occur at that time. In cases of intraspecific variability in onset time, the first-appearance criterion was followed (Sheil, Jorgensen, Tulenko, & Harrington, 2014). The sequences were compared on a simple graph, in which ossification events are the independent variable (x axis) and their rank number is the dependent variable (y axis). *Hylorina sylvatica* was selected as a reference taxon because it is the closest relative to *Telmatobius* (fide Pyron, 2014) for which there is an ontogenetic series available for comparison (Alcalde & Basso, 2013). The sequence of ossification of this taxon determines the arrangement of events on the x axis.

During development and differentiation, the ovaries undergo more superficial changes than do the testes, and these changes are evident on gross examination of the gonads. Thus, I estimated their relative stage of development by examining the differentiation of the ovaries that were extracted from the specimens before clearing and staining.

2.3 | Specimens examined

Specimens examined include larvae (L), juveniles (J), and adult males (M), and females (F), as well as cleared-and-double-stained preparations (CS). Some collection numbers refer to lots of specimens, in which case the number of individuals (*n*) is indicated. The institutional codes are: Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires (MACN); Fundación Miguel Lillo, Tucumán (FML); and Laboratorio de Genética Evolutiva, Posadas (LGE). In some cases, field numbers to be accessioned in these institutions are indicated: field numbers of Sebastián Barrionuevo (SB, to be accessioned in FML and MACN), Boris Blotto (BB, to be accessioned in MACN) and Diego Baldo (DB, to be accessioned in LGE). All specimens are from Jujuy Province in northwestern Argentina and a complete list of the specimens and its provenance is provided below.

2.3.1 | *Telmatobius oxycephalus*

Argentina: Jujuy Province: Valle Grande Department: El Duraznillo, Calilegua, FML 1592 (L, CS), 1589 (*n* = 1F, 8M); Quebrada Agua del Tigre, Calilegua, FML 2861 (*n* = 2 M); M. Belgrano Department: El Duraznillo, close to Tiraxí, FML 1758 (*n* = 2F, 3M), Río de Yala, 1,800 m, FML 2242 (M); Tilcara Department: Río Huasamayo, 2,852 m, MACN 39083 (M), 49376–49401 (L, CS), SB-MACN 557 (*n* = 10 L), 558 (*n* = 2L), 693 (*n* = 10L), 839 (*n* = 13L), 840 (*n* = 13L), SB-FML 016 (F), 018 (M), 019 (M, CS), 020–21 (M), 027(M), 022–26 (J), 028–31 (J), 101–103 (J), DB-LGE 3619–3620(J).

2.3.2 | *Telmatobius rubigo*

Argentina: Jujuy Province: El Queñoal, 3,966 m, MACN 39092 (M), 39093 (F), 49350–57(L, CS), SB-MACN 560 (*n* = 15L), FML 20829 (M), 21157 (*n* = 10L), SB-FML 090 (H), 091–92 (M), DB-LGE 3615 (F); Yoscaba, 3,750 m, MACN 41579 (J), 49371–75(L, CS), BB-MACN 2468 (*n* = 7L); Susques Department: Río Pastos Chicos close to Susques, 3,700 m, MACN 41658 (M), 41660–62 (F), 41663 (M), 41664 (F), 41665 (M), 41666 (F), 41668 (F), 41670 (J), 41671 (M, CS), 41673 (F, CS), 41675 (M), 41676 (F), 41677–79 (M), 41681–83 (M), 41684 (J), 41686 (M), 49361–70(L, CS); BB-MACN 2607 (*n* = 14L); Río Pastos Chicos, close to Sey, 3,800 m, MACN 41688 (F), 41689–90 (M), 41691 (J), 41692–94 (J), 41695 (M), 41696–98 (J), 49358–60 (L, CS), BB-MACN 2650 (*n* = 5L).

3 | RESULTS

3.1 | Size and growth

The growth of TL and of BL during larval development of *Telmatobius rubigo* and *T. oxycephalus* are markedly different. Examination of three blocks of grouped Gosner stages (Table 2), reveals that even in the youngest stages (28–33), the larvae of *T. rubigo* are significantly larger than those of *T. oxycephalus* (ANOVA, $F_{1,25} = 9.282$; $p = .005$). From Stage 34 to 39, the size differences between the species are more pronounced than in the younger group and the ranges barely overlap (ANOVA, $F_{1,31} = 39.988$; $p = <.001$). In the oldest group (Stages 40–43), the interspecific size disparity is even more pronounced than in the other groups (ANOVA, $F_{1,24} = 63.642$; $p = <.001$). The interspecific differences in BL reflect the same pattern as in TL (Table 2).

The scatter diagram of the ratio between BL and TL shows that this ratio is greater in *Telmatobius oxycephalus* than in *T. rubigo* in Stages 40–43 (Figure 1). An ANOVA of BL:TL between the species reveals that there are not significant differences in Stages 28–33

TABLE 2 Total length (TL) and body length (BL) of larvae of *T. oxycephalus* and *T. rubigo*. Tadpoles are grouped in three groups of Gosner stages; *n* = number of larvae; *SD* = standard deviation

	Stages (<i>n</i>)		Media (mm)	Range (mm)	SD
<i>T. oxycephalus</i>	28–33 (9)	TL	49.4	36.8–54.9	6.0
		BL	19.0	14.4–21.9	2.2
	34–39 (12)	TL	61.1	55.1–68.6	4.5
		BL	23.6	20.8–27.1	1.6
	40–43 (14)	TL	62.2	51.9–75.7	8.4
		BL	23.7	19.5–27.2	2.7
<i>T. rubigo</i>	28–33 (18)	TL	61.3	37.2–88.1	10.8
		BL	23.9	18.0–30.7	3.3
	34–39 (21)	TL	77.1	62.9–98.2	8.1
		BL	29.6	25.2–34.4	2.9
	40–43 (12)	TL	92.9	79.1–113.0	11.1
		BL	32.5	28.0–37.7	3.3

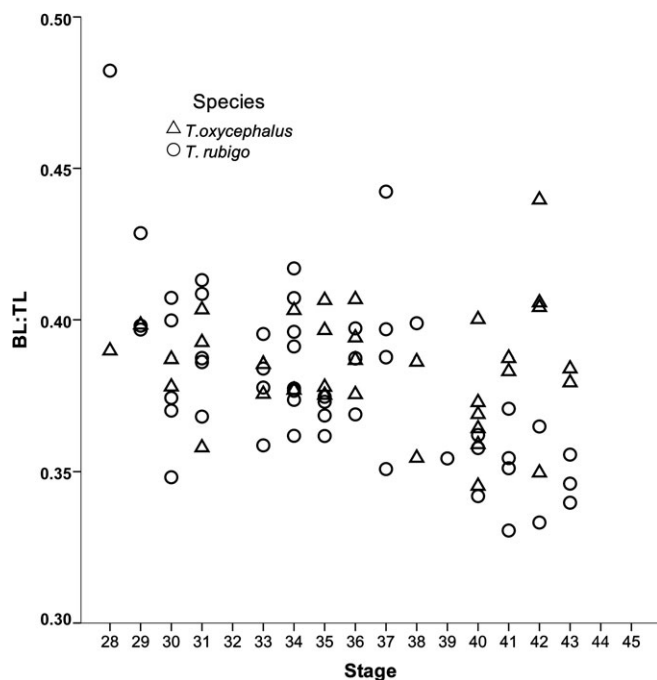


FIGURE 1 Ratio of body length (BL) and total length (TL) during larval development of *T. rubigo* (circles) and *T. oxycephalus* (triangles)

($F_{1,25} = 0.608$; $p = .443$) or Stages 34–39 ($F_{1,25} = 0.141$; $p = .710$); however, in Stages 40–43 the differences are significant ($F_{1,25} = 14.911$; $p = .001$). A higher ratio reflects that the tail is contributing less to TL than is the BL. Beginning with Stage 40, the tail of *T. oxycephalus* is proportionally shorter than the tail of *T. rubigo* (Figure 1).

Once metamorphosis is complete, *Telmatobius oxycephalus* and *T. rubigo*, experience considerable growth in SVL and HW (Figure 2). The size of the largest specimen of *T. oxycephalus* is about 164% of the size of the smallest postmetamorph. Likewise, the largest specimen of *T. rubigo* is about 168% of the smallest postmetamorph.

The nuptial pads of male *Telmatobius* are a conspicuous secondary sexual character. In *T. oxycephalus*, the smallest males in which they are present have SVL of 35.3 mm, whereas in *T. rubigo*, nuptial pads are present in males with a SVL of 37.1 mm (Figure 2). Comparing the sizes of the smallest metamorphosed individuals with the smallest sexually mature males (*T. oxycephalus*: 21.9 mm vs. 35.3 mm; and *T. rubigo*: 24.8 mm vs. 37.1 mm), it is observed that *T. oxycephalus* has a greater increase in size (62%) to reach sexual maturity than does *T. rubigo* (49%). Assuming that the total size span during postmetamorphic growth is a standardized unit (i.e., 100%), then *T. oxycephalus* is sexually mature when 37.5% of postmetamorphic growth is completed, whereas *T. rubigo* attains sexual maturity after reaching 29.5% of postmetamorphic growth.

3.2 | Changes in larval external morphology

The condition of the oral disc and vent tube are key characters used to define Gosner stages at the end of pro-metamorphosis (Stages 36–41). In *Telmatobius oxycephalus*, the oral disc is intact in all Stage-40 or younger larvae (Figure 3b). The oral disc begins to atrophy in

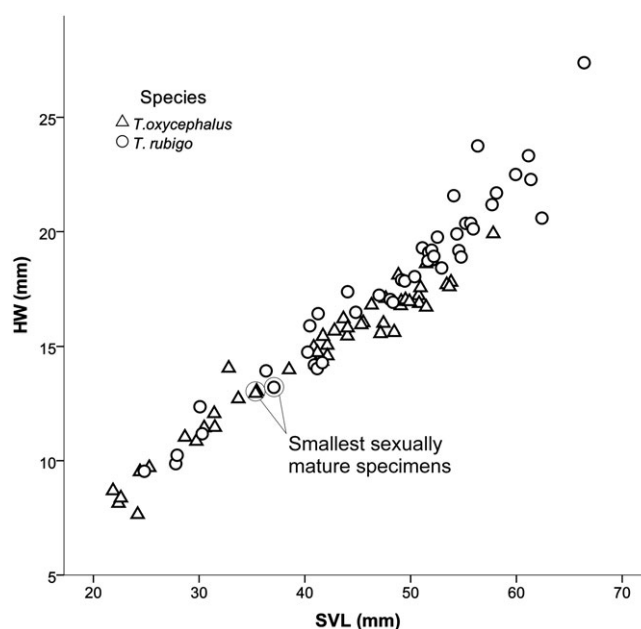


FIGURE 2 Relation of head width (HW) and snout-vent length (SVL) during postmetamorphic growth of *T. rubigo* (circles) and *T. oxycephalus* (triangles). The smallest males with sexual secondary characters are indicated

Stage 41 when jaw sheaths begin to erode in some individuals. When the forelimbs have emerged (Stage 42), the oral disc is absent in *T. oxycephalus* (Figure 3d). By contrast, the oral disc of some specimens of *T. rubigo* is intact in Stage 41 and even in Stage 42, after the forelimbs have emerged (Figure 3a,c). The vent tube is normally lost by Stage 41, before the forelimbs emerge in both taxa, but in one larval *T. rubigo*, the vent tube persists although the left forelimb has emerged (Figure 3a).

3.3 | Ossification sequence of the cranium

Skull bones are described in the order of their developmental appearance. Several cranial elements present in the adults (viz., neopalatine, quadratojugal, sphenethmoid, and columella) were not observed in the stages examined because they ossify after metamorphosis. The ossification sequences of the cranial elements in *Telmatobius oxycephalus* and *T. rubigo* are summarized in Table 3.

Parasphenoid: This dermal bone ossifies from three centers of ventral ossification; the anterior center corresponds to the cultriform process and the two posterolateral centers to the alae (Figure 4a, visible from dorsal view by transparency). The anterior center seems to ossify most rapidly, because the outline of the cultriform process is evident early. Subsequently, it fuses with each posterior center to form the alae (Figure 4b). A posteromedial notch persists between the alae until the two posterior centers fuse completely and the parasphenoid acquires its final shape (Figure 4c).

Frontoparietal: Each frontoparietal arises from two dermal centers of ossification dorsal to the taenia tecti marginalis. One is in the frontal region, anterior to the taenia tecti transversalis, and the other in the

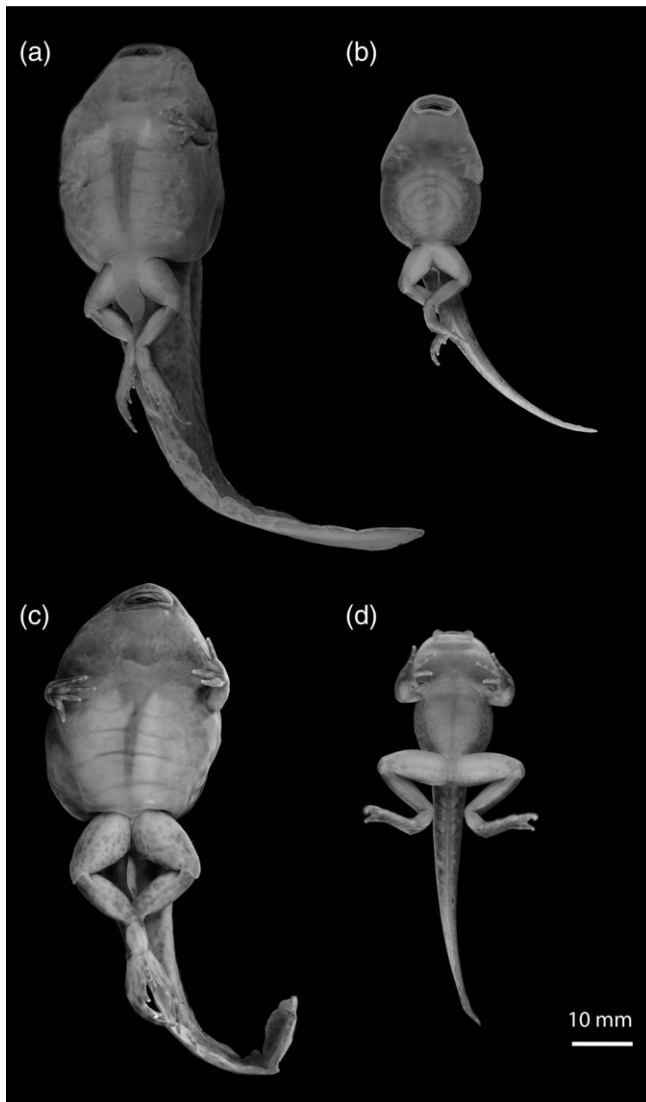


FIGURE 3 Ventral view of the larva of *T. rubigo* (a, c) and *T. oxycephalus* (b, d) showing the differences in size. In *T. rubigo* the vent tube may be present even after the forelimbs have begun to emerge (a), whereas in *T. oxycephalus* the vent tube is gone before the emergence of forelimbs (b). The larval mouthparts are still intact in *T. rubigo* after the emergence of forelimbs (c) whereas in *T. oxycephalus* most larval mouthparts have been lost (d)

parietal region (Figure 4a,b). The two-center state seems to last a very short time because in most specimens these centers are fused to form an elongated ossification. By the end of metamorphosis, each slender frontoparietals are still widely separated from one another in *Telmatobius oxycephalus*, whereas in *T. rubigo* they are robust and closely approximate one another in the parietal region (Figure 5e,j).

Exoccipital: Each endochondral bone ossifies from one center of ossification in the occipital arch (Figure 4a,b). During development, ossification spreads to the posterior margin of the otic capsule, tectum synoticum and the basal plate, and forms the occipital condyle. At the end of metamorphosis, the exoccipitals abut the posterior epiotic eminences in *Telmatobius rubigo*, whereas in *T. oxycephalus*, they do not (Figure 5). The paired exoccipitals are more narrowly separated dorsomedially and ventromedially at the foramen magnum in *T. rubigo* than in *T. oxycephalus*.

TABLE 3 Ossification sequences of the crania of *Telmatobius rubigo* and *T. oxycephalus*. Arrows indicate the number of developmental stages between the first stage in which each bone is recorded and the stage at which each element is present in all specimens examined

Stage	<i>T. Rubigo</i>	<i>T. Oxycephalus</i>
28		
29	Parasphenoid →→→→ 33 Frontoparietal →→→→→ 35 Exoccipital →→→→ 33	Parasphenoid
30		Frontoparietal → 31 Exoccipital →→ 32
31		
32		
33		
34		
35		
36	Prootic Nasal →→→→→→ 43 Premaxilla →→→→→→ 42	
37		
38		Prootic
39		
40		
41		Premaxilla
42	Septomaxilla → 43 Maxilla → 43	Nasal Septomaxilla Maxilla Angulosplenial Squamosal
43	Angulosplenial Squamosal Dentary	Dentary Pterygoid Vomer
44	Pterygoid Vomer Hyoid	Hyoid
45		

Prootic: Each bone arises from one center of ossification at the anteromedial margin of the cartilaginous otic capsule (Figure 4c). At the end of metamorphosis, the prootic ossification is more advanced in *Telmatobius rubigo* than in *T. oxycephalus* (Figure 5).

Nasal: This dermal element appears as a thin laminar ossification over the posterior region of nasal capsules. In *Telmatobius rubigo*, its onset time is variable (Table 3). In this species, the nasal has been observed as early as Stage 36, dorsal to the base of the chondrocranial cornu trabeculae (Figure 4c,d); it is present in all specimens from Stage 43 on. In *T. oxycephalus*, nasal ossification occurs at Stage 42.

Premaxilla: Each dermal bone ossifies from one center of ossification located at near the distal end of the pars alaris (Figure 4c,d), dorsal to the chondrocranial cornu trabeculae. Subsequently, the partes dentalis and palatina differentiate. In *Telmatobius rubigo*, the onset time of this bone is almost as variable as that of the nasal (Table 3), whereas in *T. oxycephalus*, the premaxilla ossifies in Stage 41. Premaxillary teeth are present at Stage 44 in both species.

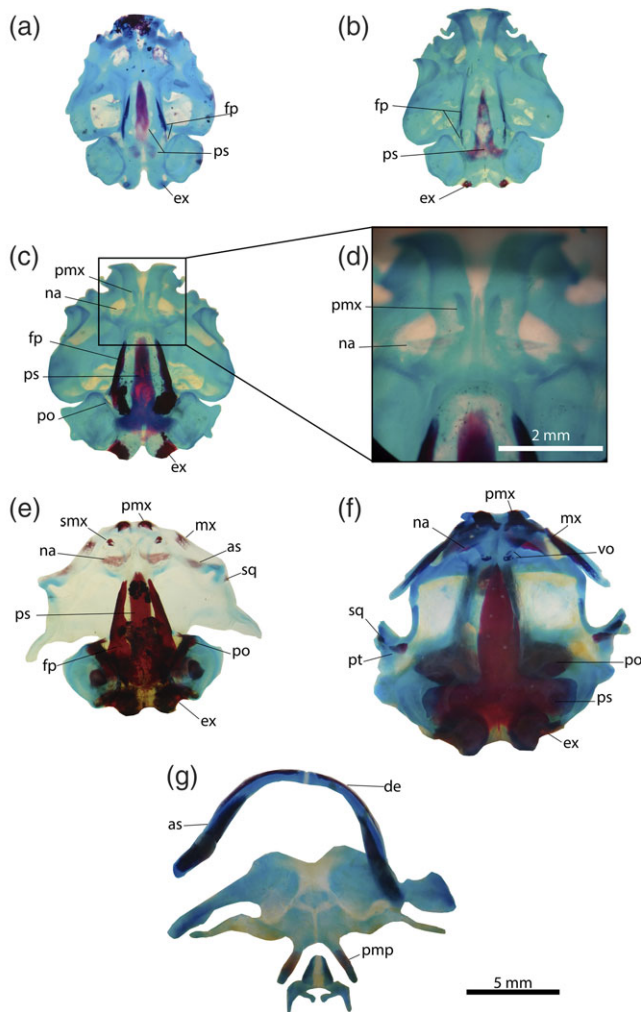


FIGURE 4 Ossification of the cranium of *T. rubigo*: MACN 49362, stage 30, dorsal view (a); MACN 49358, stage 29, dorsal view (b); MACN 49367, stage 36, dorsal view (c) and detail of the anterior part of the chondrocranium of the same specimen (d); MACN 49356, stage 43, dorsal view (e); MACN 49375, stage 44, ventral view (f) and ventral view of mandible and hyoid of the same specimen (g). Abbreviations are: as = angulosphenial; de = dentary; ex = exoccipital; fp = frontoparietal; mx = maxilla; na = nasals; ps = parasphenoid; pmp = postero medial process of hyoid; pmx = premaxilla; po = postotic; pt = pterygoid; qj = quadratojugal; smx = septomaxilla; sq = squamosal; vo = vomer

Septomaxilla: In both species of *Telmatobius*, the bone appears at Stage 42 but in *T. rubigo*, it is present in all individuals only from Stage 43 on.

Maxilla: The first part of this dermal bone to ossify is the pars facialis (Figure 4e). Subsequently, the partes dentalis and palatina differentiate. Maxillary teeth are present in Stage 44 in *Telmatobius rubigo*, but teeth were absent in the specimens of *T. oxycephalus* examined.

Squamosal: The ventral arm of the dermal squamosal ossifies first as a splint of bone (Figure 4e). The zygomatic and otic rami ossify postmetamorphically.

Angulosphenial: Each dermal bone ossifies as a thin laminar bone investing the posteromedial surface of Meckel's cartilage in the mandible (Figure 4e). At the end of metamorphosis, the articular region is still cartilaginous in both species.

Dentary: This dermal mandibular bone ossifies as a thin laminar bone investing the larval infrarostrals anteriorly (Figure 4g). By the end of metamorphosis, both the dentary and angulosphenial only partially invest Meckel's cartilage.

Pterygoid: This dermal bone arises at the base of the pterygoid process of the palatoquadrate in both taxa (Figure 4f). By the end of metamorphosis its three rami (anterior, medial, and posterior) are not defined.

Vomer: This dermal palatal bone arises from two centers of ossification in both species (Figure 4f). The anterior one corresponds to the plate and the posterior one to the dentigerous process. The onset of the vomerine teeth occurs postmetamorphically.

Hyoid apparatus: The initial ossification of this endochondral element is the posteromedial processes in both species (Figure 4g).

Intraspecific variability in the onset times of cranial elements is greater in *Telmatobius rubigo* than in *T. oxycephalus* (Table 3). From its first appearance, as many as seven stages elapse before nasals are present in all *T. rubigo*; other bones have a similar variation in onset time and several stages elapse before they are present in all specimens: frontoparietal (six stages), premaxilla (five stages), parasphenoid and exoccipital (four stages), and septomaxilla and maxillary (one stage). The only intraspecific variation in *T. oxycephalus* involves the exoccipital (two stages) and frontoparietal (one stage).

3.4 | Adult cranial osteology

Although the skull of adult *Telmatobius oxycephalus* and *T. rubigo* are superficially similar, some elements are markedly different, that is, the vomer, neopalatine, otic capsules, columella, and frontoparietal (Figure 6). In *T. oxycephalus*, the vomers are robust and have well-developed prechoanal and postchoanal processes. Each vomer bears from five to seven vomerine teeth. The neopalatines extend from the maxilla to the sphenethmoid and are notably arched, bearing a thin anterior flange (Figure 6d). The length of the otic capsules is <25% of total cranial length (Figure 6a). Each columella or stapes is relatively robust and well developed; and the stapedia footplate is evident (Figure 7a). The frontoparietals are delicate and can be separated or fused in the parietal region. In this region, the margins of the frontoparietals are clearly distinguishable from the subjacent neurocranium (Figure 6a).

In *Telmatobius rubigo* each vomer is slender and its prechoanal and postchoanal processes are less developed than in *T. oxycephalus*. There are three or fewer vomerine teeth, and these may be absent on one side (MACN 41673). The neopalatines are slightly slender, nearly straight and the anterior flange is absent (Figure 6f). The length of the otic capsules is more than 30% of total cranial length (Figure 6c). Each columella is thin, with a poorly developed stapedia footplate (Figure 7c). All these features resemble the configuration of juvenile *T. oxycephalus* (Figures 6b,e, 7b). On the contrary, the frontoparietals in *T. rubigo* are more robust and ossified than in *T. oxycephalus* and they are medially fused in the parietal region. In this region, the margins of the frontoparietal are difficult to distinguish from the subjacent neurocranium (Figure 6c).

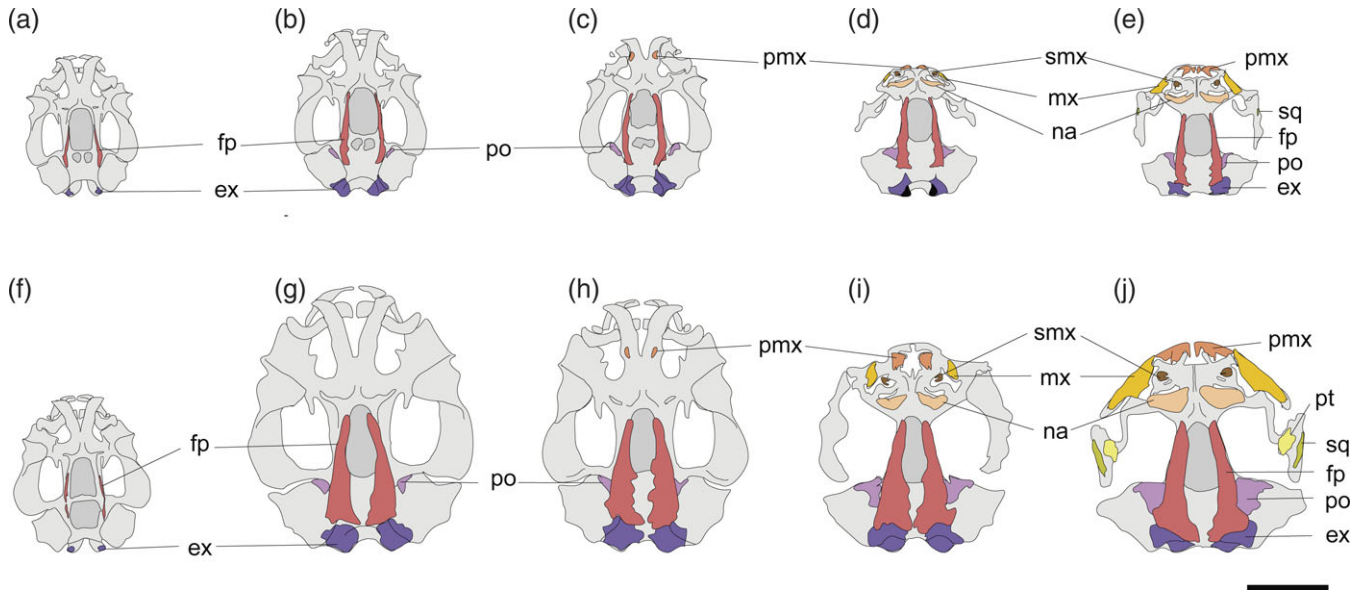


FIGURE 5 Comparative sequences of cranial ossification during larval development of *T. oxycephalus* (a–e) by Gosner stages 31 (a), 40 (b), 41 (c), 42 (d), and 43 (e); and *T. rubigo* (f–j) by stages 29 (f), 40 (g), 41 (h), 42 (i) and 44 (j); scale = 5 mm. Abbreviations are: ex = exoccipital; fp = frontoparietal; mx = maxilla; na = nasals; pmx = premaxilla; po = prootic; pt = pterygoid; smx = septomaxilla; sq = squamosal

3.5 | Aspect of ovaries

There are remarkable differences in the timing of ovarian differentiation between the two species of *Telmatobius* relative to larval limb development. The ovaries of *T. rubigo* are differentiated before hind-limb development is complete—that is, all toes are separate but metatarsal tubercles are absent (corresponding to Stage 37). At this stage, the ovaries have lobes and developing oocytes that are evident on gross examination (Figure 8a). The maximum width of the ovaries is about 86% the width of the kidneys, and the maximum

length is about 46% the length of the kidneys. By contrast, a comparable level of differentiation of the ovaries in *T. oxycephalus* is not achieved until the hind limbs are completely developed and the forelimbs have emerged (corresponding to Stage 42, Figure 8c). In this species, by Stage 38 the ovaries are barely differentiated and lobes and oocytes are not evident (Figure 8b); they are much smaller in relation to the kidneys than in *T. rubigo*, with the maximum width and length corresponding to 31% and 36%, respectively, of the size of the kidney.

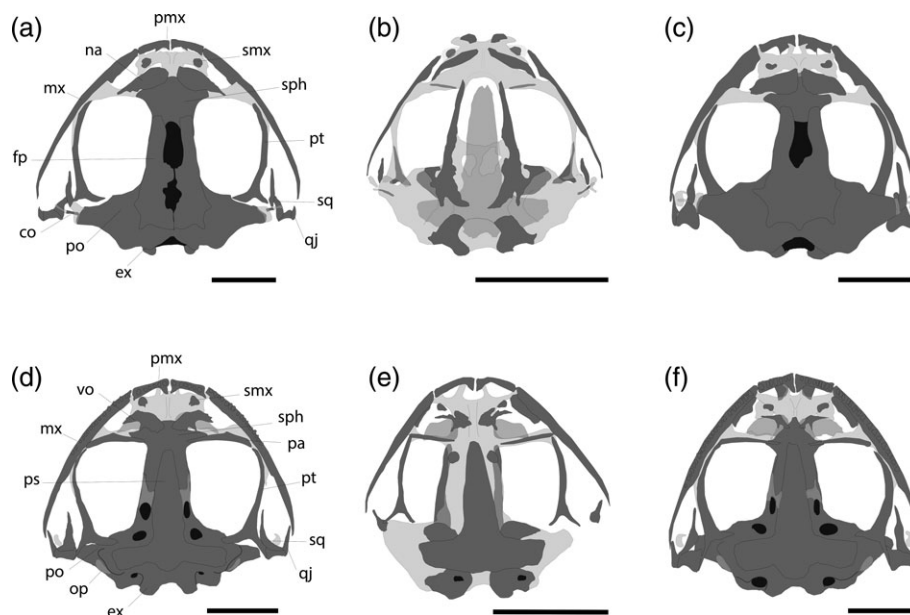


FIGURE 6 Dorsal view of the cranium of adult (a, FML 2867-II) and juvenile (b, SB-FML 102) *T. oxycephalus* and of an adult *T. rubigo* (c, FML 20829). Ventral view of the cranium of an adult (d, FML 2867-IV) and juvenile (e, SB-FML 031) *T. oxycephalus* and of an adult *T. rubigo* (f, FML 20829); scales = 5 mm. Abbreviations are: ex = exoccipital; fp = frontoparietal; mx = maxilla; na = nasals; op = operculum; ps = parasphenoid; pmx = premaxilla; po = prootic; pt = pterygoid; qj = quadratojugal; smx = septomaxilla; sq = squamosal; vo = vomer

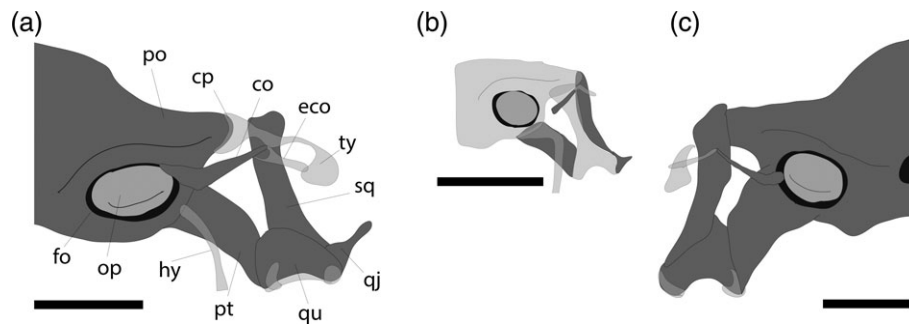


FIGURE 7 Posterior view of the otic region of an adult (a, FML 2867) and a juvenile (b, DB-LGE 3619) *T. oxycephalus* and of an adult *T. rubigo* (c, FML 20829) showing the middle ear and the configuration of the columella; scales = 2 mm. Abbreviations are: cp = crista parotica; co = columella; eco = extracolumella; fo = fenestra ovalis; hy = hyale; op = operculum; po = prootic; pt = pterygoid; qu = quadrate bone; qj = quadratojugal; sq = squamosal; ty = tympanic annulus

4 | DISCUSSION

4.1 | Growth and the loss of larval features

Although both species of *Telmatobius* described here have large tadpoles as is typical in most species of the genus, the larvae of *T. rubigo* are markedly larger than those of *T. oxycephalus*. The interspecific difference in larval size in the genus was first noted by Fernández (1927) and subsequently mentioned by others (e.g., Aguilar & Lehr, 2009; Barrionuevo & Baldo, 2009; Vellard, 1951). The geographic distribution of species of *Telmatobius* seems to be associated with larval size, because species with the largest tadpoles live in high elevation streams and lakes (e.g., *T. culeus*; *T. macrostomus*, *T. mayoloi*, *T. gigas*). Similarly, the tadpoles of *T. rubigo* are from a plateau at approximately 3,800 m, whereas the tadpoles of *T. oxycephalus*, significantly smaller, are from a valley about 1,000 m lower. As with other anuran species living in cold waters, large larval size may be correlated with lengthy developmental periods that include overwintering (Smith-Gill & Berven, 1979). Thus, it is inferred that the tadpole developmental rate decreases while overall growth continues.

Long-term studies of wild populations of *Telmatobius* are scarce, possibly because of the remote distribution of these

highland frogs; consequently, there are no studies based on marked individuals from which one could determine the duration of larval development. *Telmatobius jelskii* has been reported to have a larval period lasting from 3 to 7 months in the wild (Sinsch, 1990), whereas in laboratory-maintained *T. dankoi*, metamorphosis took place 13 months after the tadpoles were collected, although the age of the tadpoles at the time of their collection is not known (Formas, Veloso, & Ortiz, 2005).

The change in mouth configuration during the transition from larva to adult is a crucial step in anuran development. Loss of larval keratinized mouthparts usually occurs before, or coincident with, forelimb emergence (Stages 41 or 42; Gosner, 1960; Thibaudeau & Altig, 1988; Nodzenski & Inger, 1990). In *T. rubigo*, the larval oral disc is still intact after the forelimbs emerge in Stage 42, whereas in *T. oxycephalus* the larval mouthparts already have begun to atrophy at this stage. Apparently, the larvae of *T. rubigo* are able to feed after the forelimbs emerge. Other larval characters, as the vent tube, have probably delayed offsets in *T. rubigo*. In the youngest larval stages examined, there is no significant difference in the relative sizes of the tail between *T. rubigo* and *T. oxycephalus*; however, by the last larval stages, the tail of *T. rubigo* has become less reduced

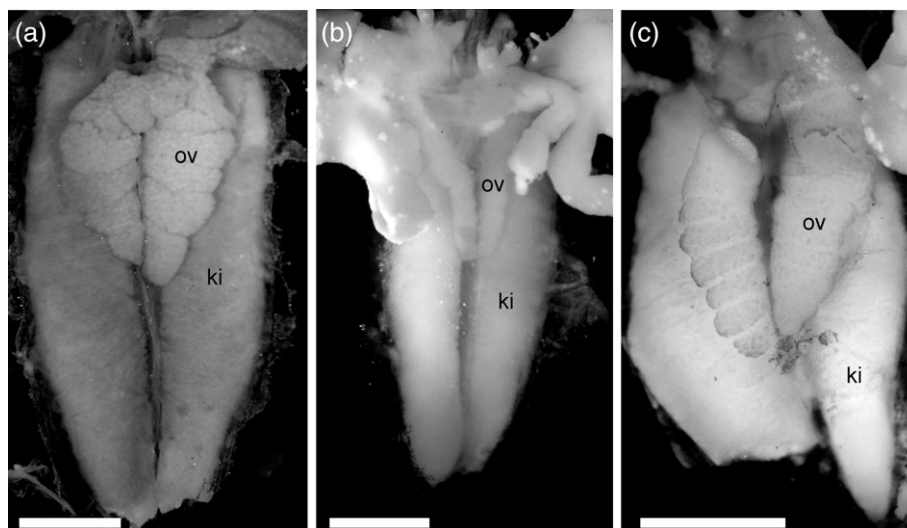


FIGURE 8 Ventral view of the kidneys (ki) and ovaries (ov) of *T. rubigo* by stage 37 (a, MACN 49359), and of *T. oxycephalus* by stages 38 (b, MACN 49393) and 42 (c, MACN 49399); scales = 2 mm

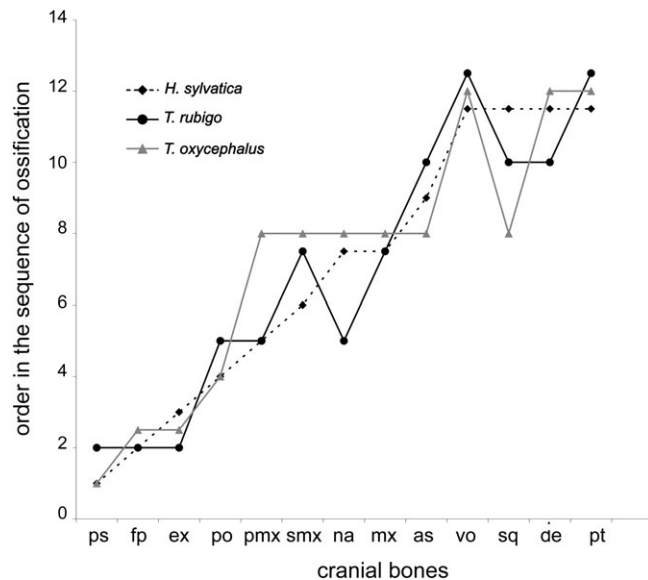


FIGURE 9 Comparison of cranial ossification sequences and events in *T. rubigo*, *T. oxycephalus*, and *Hylorina sylvatica*. The X-axis represents the developmental events (ossification of cranial bones) and is ordered following the reference taxon (*H. sylvatica*); ranks (sequences order) are plotted on the Y-axis. Abbreviations are: As = angulosplenic; de = dentary; ex = exoccipital; fp = frontoparietal; mx = maxilla; na = nasals; ps = parasphenoid; pmx = premaxilla; po = prootic; pt = pterygoid; smx = septomaxilla; sq = squamosal; vo = vomer

than that of *T. oxycephalus*. Thus, there may be a heterochronic shift in the offset of the tail of *T. rubigo*.

4.2 | Sequence of cranial ossification

The ossification sequence of cranial elements in *Telmatobius* generally corresponds to the typical sequence described for anurans (Trueb, 1985; Weisbecker & Mitgutsch, 2010). The comparison of the ontogenetic trajectories of the two species of *Telmatobius* and *Hylorina sylvatica* (Figure 9) reveals some shifts in the relative timing of ossification of cranial bones. Ossification of premaxilla, nasal, and squamosal are among the most divergent developmental events. The ossification of the premaxilla is delayed in *T. oxycephalus*. The ossification of the nasal occurs early in *T. rubigo*. In both species of *Telmatobius* (and especially in *T. oxycephalus*), the squamosal ossifies earlier than it does in *Hylorina*.

Within *Telmatobius*, the comparison of ossification sequences to the external features used to define Gosner stages reveals that in *T. rubigo*, the onset times of the prootic (Stage 34), and premaxilla and nasal (Stage 36) are earlier than in *T. oxycephalus* (Stages 38, 41, and 42, respectively); however, the ossification of the premaxilla and nasal is intraspecifically variable in *T. rubigo* (discussed below). Conversely, the onset times of squamosal and angulosplenic (Stage 43), and pterygoid and vomer (Stage 44) are post-displaced one stage in *T. rubigo* relative to *T. oxycephalus*. Additionally, in some specimens of *T. rubigo*, the septomaxilla and maxilla are post-displaced one stage. In this species, the later onset of the cranial elements of the jaw suspension and the mandible (i.e., squamosal, pterygoid, and angulosplenic) is associated with the delayed offset of larval mouth (see above).

4.3 | Extended larval life, “early” ossification, and intraspecific variation

In both species of *Telmatobius*, the cranium begins to ossify before the hind-limb buds are differentiated as foot paddles (Stages 29 and 30), whereas in most anurans for which there are ossification sequence data, the first cranial elements appear when the toes begin to differentiate—that is, from Stage 32 on (Gómez, Regueira, O’Donoghue, & Hermida, 2017). The characteristics of the few species that begin cranial ossification before Stage 30 apparently suggest that this may be associated with highly modified larvae or with larvae that have a prolonged development that may include overwintering (Table 4).

Extended larval development may imply that the “early” ossification might result from delayed differentiation of external features used to define Gosner stages (e.g., hind limbs). Given the relative independence of overall somatic development and ossification, one could anticipate that the more extended the larval period is, the more variable the ossification timing might be in relation to Gosner stages. Among anurans, *Ascaphus truei* is a model case of long larval development because in populations distributed at higher elevations, larval period may last up to 4 years (Bury & Adams, 1999). This is one of the most striking cases of intraspecific variation in the onset time of ossification (Moore & Townsend, 2003). From its first appearance, as many as 14 Gosner stages may elapse before the parasphenoid is present in all specimens. The frontoparietals and exoccipitals are similar, 11 and

TABLE 4 Anuran species with the earliest cranial ossification onset time relative to Gosner stages (GS). Some species have suctorial (1) or macrophagous larvae (2), whereas others (3) have a prolonged larval period that may include overwintering. In the remaining species (4) the duration of the larval periods are unknown, but each has at least one related species for which overwintering has been recorded

GS	Species	References
25	<i>Ranoidea nannotis</i> ¹	Haas & Richards (1998)
26	<i>Huia cavitympanum</i> ¹	Gan, Hertwig, Das, & Haas (2016)
	<i>Meristogenys jerboa</i> ¹	Gan et al. (2016)
	<i>Nasikabatrachus sahyadrensis</i> ¹	Senevirathne, Thomas, Kerney, Hanken, Biju et al. (2016); Raj, Vasudevan, Deepak, Sharma, Singh et al. (2012)
	<i>Taruga eques</i> ⁴	Senevirathne, Kerney, & Meegaskumbura (2017)
27	<i>Hymenochirus boettgeri</i> ²	de Sa & Swart (1999); Sokol (1962)
	<i>Polypedates crucifer</i> ⁴ ; <i>P. maculatus</i> ⁴	Senevirathne et al. (2017); Hsu, Kam, & Fellers (2012)
	<i>Taruga longinasus</i> ⁴	Senevirathne et al. (2017)
28	<i>Hadromophryne natalensis</i> ¹	Haas (2003)
	<i>Odontophrynus cultripes</i> ⁴	Do Nascimento, Mott, Langone, Davis, & de Sá (2013); Grenat, Gallo, Salas, & Martino (2011)
29	<i>Alsodes verrucosus</i> ⁴	Formas & Brieva (2004); Corbalán, Debandi, Martínez, & Úbeda (2014)
	<i>Ascaphus truei</i> ^{1,3}	Altig (1969), Yeh (2002)
	<i>Lithobates pipiens</i> ³	Kemp & Hoyt (1969)
	<i>Telmatobius oxycephalus</i> ³ ; <i>T. rubigo</i> ³	This study

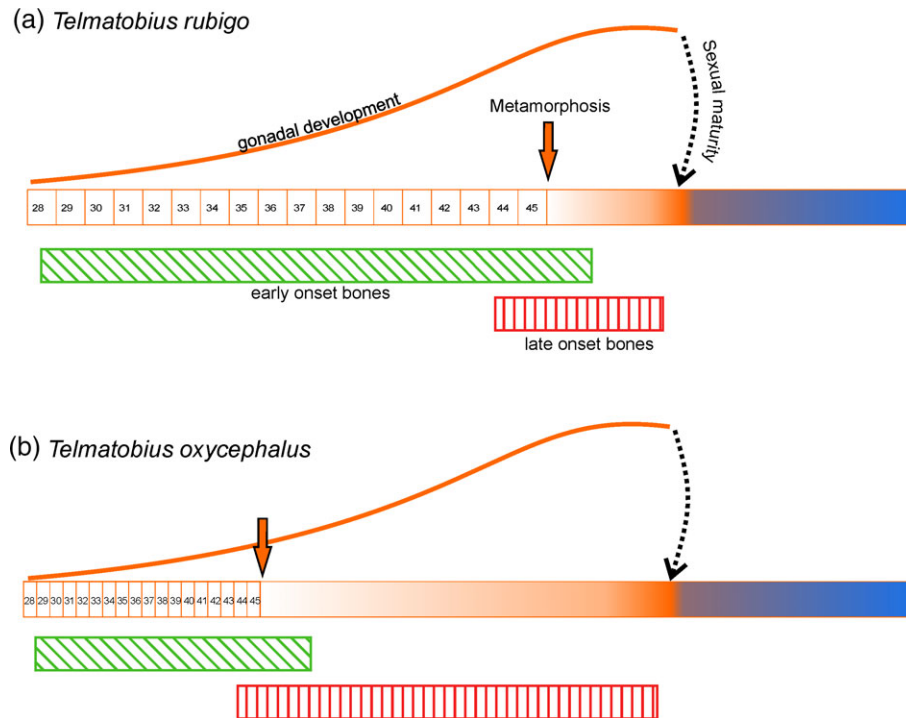


FIGURE 10 Simplified diagram depicting the duration of larval development in *Telmatobius rubigo* (a) and *T. oxycephalus* (b) and the temporal relationship between gonadal development and ossification of bones with both early-onset and late-onset times

6 stages elapse, respectively, before these bones are present in all specimens. Intraspecific variability in the onset times of cranial elements is greater in *Telmatobius rubigo* than in *T. oxycephalus*. Therefore, this may be related to the longer larval period in *T. rubigo*

4.4 | Ovarian maturation

The remarkable differences in timing of ovarian maturation between the two species of *Telmatobius* might be interpreted as either accelerated or deaccelerated ovarian developmental rates; nevertheless, gonadal development is thought to be a more accurate gauge of age than development of external larval features (Ogielska & Kotusz, 2004). Thus, at a comparable level of somatic development, the larval specimens examined of *T. rubigo*, exhibiting an advance level of ovarian maturation, probably are older than those of *T. oxycephalus*. Assuming that gonadal maturation timing is similar in both species and using it as a baseline, it can be inferred that somatic development in *T. rubigo* is post-displaced relative to somatic development in *T. oxycephalus*. The same pattern seems to characterize spadefoot toads (Scaphiropodidae) in which the gonads differentiate at earlier somatic developmental stages in those species having a longer larval period (*Spea multiplicata*) than in those with a shorter one (*Scaphiopus couchii*, Buchholz & Hayes, 2005).

4.5 | Extended larval development and its implications on postmetamorphic morphology

In summary, the data at hand shows that in *Telmatobius rubigo* the larvae are significantly larger, there is a higher intraspecific variation in the timing of ossification of cranial bones, and the ovaries are already differentiated at early stages, in comparison to *T. oxycephalus*.

Furthermore, almost all larval stages of *T. rubigo* can be found at the same time in the same place. In combination, these features indicate that the larval period of *T. rubigo* is longer than that of *T. oxycephalus*, probably related to the low temperatures of its habitat in the high elevation Puna Plateau. In the cold permanent streams of this region it is likely that the larval period in *T. rubigo* may last more than a year.

The data presented herein also suggest that the duration of larval life may influence the osteological diversity of adult *Telmatobius*, as was proposed for *Pelobates fuscus* by Smirnov (1992). Comparison of the adult skull structure reveals that some bones of *T. rubigo* resemble those of immature *T. oxycephalus* (Barrionuevo, 2013). For example, the vomer and vomerine teeth, neopalatine, otic capsules, and columella have a clearly paedomorphic morphology in *T. rubigo* in contrast to those elements in *T. oxycephalus*. However, other bones (e.g., well-developed frontoparietals) of *T. rubigo* seem to have a peramorphic configuration relative to their condition in *T. oxycephalus*.

Early onset cranial bones seem to have more time to ossify during the prolonged larval period of *T. rubigo* (Figure 10a). This may account for the better-developed frontoparietals of *T. rubigo* during the course of larval development. At the end of metamorphosis, each frontoparietal in *T. rubigo* is far more developed than its counterpart in *T. oxycephalus*.

Prolongation of the larval period also may affect late-onset bones. The more highly differentiated gonads of *T. rubigo* at the end of metamorphosis may signal a shorter time to attain sexual maturity during postmetamorphic growth (Figure 10a). The data on postmetamorphic growth support this hypothesis, because at sexual maturity, individual *T. rubigo* have reached only 30% of their maximum postmetamorphic size (Figure 2). Thus, bones that begin to ossify at the end or after metamorphosis have less time to complete their development before

the rate of differentiation diminishes. This may explain the paedomorphic configuration of the vomers, columella, and neopalatines in *T. rubigo*.

During the relatively shorter larval period of *Telmatobius oxycephalus*, early onset bones would have less time to ossify than in *T. rubigo* and this may explain the less developed frontoparietals of *T. oxycephalus*. Additionally, the shorter the larval period, the less time have the gonads to develop; consequently, the gonads are less differentiated at the end of metamorphosis (Figure 10b). This is also supported by the fact that close to 40% of the total postmetamorphic growth has occurred at sexual maturity of male *T. oxycephalus* (Figure 2). The longer postmetamorphic growth period before sexual maturity would allow to late-onset bones have more time to develop a fully adult configuration.

In general, interspecific genetic divergence in *Telmatobius* is minimal, especially among certain clades (De la Riva et al., 2010; Sáez et al., 2014). Low genetic divergence may explain the conserved phenotype of *Telmatobius* and suggest a recent origin of most lineages. However, some osteological characters are remarkably variable and the extension of larval life may account for this variation. It is plausible that *Telmatobius* evolved during the Andean uplift when populations may have become isolated at different elevations. As discussed herein, differences in elevation and consequently in habitat temperature can be correlated with substantial differences in developmental timing, and these may result in a high level of phenotypic plasticity that might underlie speciation events in *Telmatobius* during the Andean orogeny.

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REFERENCES

- Aguilar, C., & Lehr, E. (2009). Tadpole of *Telmatobius mayoloi* (Anura: Ceratophryidae). *Journal of Herpetology*, 43(1), 159–164.
- Alcalde, L., & Basso, N. G. (2013). Old and new hypotheses about the homology of the compound bones from the cheek and otico-occipital regions of the anuran skull. *Zoology*, 116, 232–245.
- Altig, R. (1969). Notes on the ontogeny of the osseous cranium of *Ascaphus truei*. *Herpetologica*, 25, 59–62.
- Barrionuevo, J. S., & Baldo, D. (2009). Description of the tadpoles of *Telmatobius platycephalus* and *Telmatobius pinguiculus* (Anura: Ceratophryidae) from Argentina. *The Herpetological Journal*, 19(1), 21–27.
- Barrionuevo, J. S. (2013). Osteology and postmetamorphic development of *Telmatobius oxycephalus* (Anura: Telmatobiidae) with an analysis of skeletal variation in the genus. *Journal of Morphology*, 274, 73–96.
- Barrionuevo, J. S. (2016). Independent evolution of suction feeding in Neobatrachia: Feeding mechanisms in two species of *Telmatobius* (Anura: Telmatobiidae). *The Anatomical Record*, 299, 181–196.
- Barrionuevo, J. S. (2017). Frogs at the summits: Phylogeny of the Andean frogs of the genus *Telmatobius* (Anura, Telmatobiidae) based on phenotypic characters. *Cladistics*, 33, 41–68.
- Berven, K. A. (1982). The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. II. An experimental analysis of larval development. *Oecologia*, 52, 360–369.
- Blouin, M. S., & Brown, S. T. (2000). Effects of temperature-induced variation in anuran larval growth rate on head width and leg length at metamorphosis. *Oecologia*, 125, 358–361.
- Brunetti, A. E., Muñoz Saravia, A., Barrionuevo, J. S., & Reichle, S. (2017). Silent sounds in the Andes: Underwater vocalizations of three frog species with reduced tympanic middle ears (Anura: Telmatobiidae: *Telmatobius*). *Canadian Journal of Zoology*, 95, 335–343.
- Buchholz, D. R., & Hayes, T. B. (2005). Variation in thyroid hormone action and tissue content underlies species differences in the timing of metamorphosis in desert frogs. *Evolution & Development*, 7(5), 458–467.
- Bury, R. B., & Adams, M. J. (1999). Variation in age at metamorphosis across a latitudinal gradient for the tailed frog, *Ascaphus truei*. *Herpetologica*, 55, 283–291.
- Catenazzi, A., von May, R., & Vredenburg, V. T. (2013). Conservation of the high Andean frog *Telmatobius jelskii* along the PERU LNG pipeline in the regions of Ayacucho and Huancavelica, Peru. In A. Alonso, F. Dallmeier, & G. Servat (Eds.), *Monitoring biodiversity: Lessons from a trans-Andean megaproject*. Washington DC: Smithsonian Scholarly Press.
- Corbalán, V., Debandi, G., Martínez, F., & Úbeda, C. A. (2014). Prolonged larval development in the critically endangered Pehuenche's frog *Alsodes pehuenche*: Implications for conservation. *Amphibia-Reptilia*, 35, 283–292.
- Davies, M. (1989). Ontogeny of bone and the role of heterochrony in the myobatrachine genera *Uperoleia*, *Crinia*, and *Pseudophryne* (Anura: Leptodactylidae: Myobatrachinae). *Journal of Morphology*, 200, 269–300.
- De la Riva, I. (2005). Bolivian frogs of the genus *Telmatobius*: Synopsis, taxonomic comments, and description of a new species. *Monografías de Herpetología*, 7, 65–101.
- De la Riva, I., García-París, M., & Parra-Olea, G. (2010). Systematics of Bolivian frogs of the genus *Telmatobius* (Anura, Ceratophryidae) based on mtDNA sequences. *Systematics and Biodiversity*, 8(1), 49–61.
- De la Riva, I., Trueb, L., & Duellman, W. E. (2012). A new species of *Telmatobius* (Anura: Telmatobiidae) from montane forests of southern Peru, with a review of osteological features of the genus. *South American Journal of Herpetology*, 7(2), 91–109.
- de Sa, R. O., & Swart, C. C. (1999). Development of the suprarostal plate of pipoid frogs. *Journal of Morphology*, 240(2), 143–153.
- Do Nascimento, F. A., Mott, T., Langone, J. A., Davis, C. A., & de Sá, R. (2013). The genus *Odontophrynus* (Anura: Odontophrynidae): A larval perspective. *Zootaxa*, 3700, 140–158.
- Emerson, S. B. (1986). Heterochrony and frogs: The relationship of a life history trait to morphological form. *The American Naturalist*, 127, 167–183.
- Emerson, S. B. (1987). The effect of chemically produced shifts in developmental timing on postmetamorphic morphology in *Bombina orientalis*. *Experimental Biology (Berlin)*, 47(2), 105–109.
- Fabrezi, M., & Goldberg, J. (2009). Heterochrony during skeletal development of *Pseudis platensis* (Anura, Hylidae) and the early offset of skeleton development and growth. *Journal of Morphology*, 270, 205–220.
- Fabrezi, M., & Lavilla, E. O. (1993). Anatomía del condrocraqueo en larvas de tres especies de *Telmatobius* del grupo meridional (anura: Leptodactylidae). *Physis*, 48, 39–46.
- Fernández, K. (1927). Sobre la biología y reproducción de batracios argentinos (segunda parte). *Boletín Academia Nacional de Ciencias de Córdoba*, 29, 271–320.
- Formas, J. R., & Brieva, L. (2004). The tadpoles of *Alsodes vanzolinii* and *A. verrucosus* (Anura: Leptodactylidae) with descriptions of their internal oral and chondrocranial morphology. *Amphibia-Reptilia*, 25, 151–164.

- Formas, J. R., Veloso, A., & Ortiz, J. C. (2005). Sinopsis de los *Telmatobius* de Chile. *Monografías de Herpetología*, 7, 103–114.
- Frost, D. R. (2017). Amphibian species of the world: An online reference. Version 6.0 (Date of access). New York, USA: American Museum of Natural History. Retrieved from <http://research.amnh.org/herpetology/amphibia/index.html>.
- Gan, L. L., Hertwig, S. T., Das, I., & Haas, A. (2016). The anatomy and structural connectivity of the abdominal sucker in the tadpoles of *Huia cavitympanum*, with comparisons to *Meristogenys jerboa* (Lissamphibia: Anura: Ranidae). *Journal of Zoological Systematics and Evolutionary Research*, 54, 46–59.
- Gómez, R. O., Regueira, E., O'Donohoe, M. E. A., & Hermida, G. N. (2017). Delayed osteogenesis and calcification in a large true toad with a comparative survey of the timing of skeletal ossification in anurans. *Zoologischer Anzeiger*, 267, 101–110.
- Gómez-Mestre, I., & Buchholz, D. R. (2006). Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proceedings of the National Academy of Sciences*, 103(50), 19021–19026.
- Gómez-Mestre, I., Saccoccio, V. L., Iijima, T., Collins, E. M., Rosenthal, G. G., & Warkentin, K. M. (2010). The shape of things to come: Linking developmental plasticity to post-metamorphic morphology in anurans. *Journal of Evolutionary Biology*, 23, 1364–1373.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Gould, S. J. (1977). *Ontogeny and phylogeny*. Cambridge, Massachusetts: Harvard University Press.
- Grenat, P. R., Gallo, L. M. Z., Salas, N. E., & Martino, A. L. (2011). External changes in embryonic and larval development of *Odontophrynus cordobae* Martino et Sinsch, 2002 (Anura: Cycloramphidae). *Biología*, 66, 1148–1158.
- Haas, A., & Richards, S. J. (1998). Correlations of cranial morphology, ecology, and evolution in Australian suctorial tadpoles of the genera *Litoria* and *Nyctimystes* (Amphibia: Anura: Hylidae: Pelodryadinae). *Journal of Morphology*, 238, 109–141.
- Haas, A. (1999). Larval and metamorphic skeletal development in the fast developing frog *Pixicephalus adpersus* (Anura, Ranidae). *Zoomorphology*, 119, 23–35.
- Haas, A. (2003). Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics*, 19, 23–89.
- Hall, B. K. (1999). *Evolutionary developmental biology* (2nd ed.). Dordrecht, Netherlands: Kluwer Academic Publishers.
- Hanken, J., & Hall, B. K. (1984). Variation and timing of the cranial ossification sequence of the oriental fire-bellied toad, *Bombina orientalis* (Amphibia, Discoglossidae). *Journal of Morphology*, 182, 245–255.
- Hsu, J. L., Kam, Y. C., & Fellers, G. M. (2012). Overwintering tadpoles and loss of fitness correlates in *Polypedates braueri* tadpoles that use artificial pools in a lowland agroecosystem. *Herpetologica*, 68(2), 184–194.
- Kemp, N. E., & Hoyt, J. A. (1969). Sequence of ossification in the skeleton of growing and metamorphosing tadpoles of *Rana pipiens*. *Journal of Morphology*, 129, 415–444.
- Kerney, R., Wassersug, R., & Hall, B. K. (2010). Skeletal advance and arrest in giant non-metamorphosing African clawed frog tadpoles (*Xenopus laevis*: Daudin). *Journal of Anatomy*, 216(1), 132–143.
- Lavilla, E. O., & De la Riva, I. (2005). Estudios sobre las ranas andinas de los géneros *Telmatobius* y *Batrachophrynus* (Anura: Leptodactylidae). *Monografías de Herpetología*, 7, 7–349.
- Lavilla, E. O., & Scrocchi, G. J. (1986). Morfometría larval de los géneros de *Telmatobiidae* (Anura: Leptodactylidae) de Argentina y Chile. *Physis*, 44(106), 39–43.
- Lobo Gaviola, F. J. (1988). *Osteología comparada de tres especies de Telmatobius (Anura: Leptodactylidae) de la Provincia de Tucumán (Argentina)* (p. 65). Tucumán: Universidad Nacional de Tucumán.
- Moore, M. K., & Townsend, V. R. (2003). Intraspecific variation in cranial ossification in the tailed frog, *Ascaphus truei*. *Journal of Herpetology*, 37(4), 714–717.
- Newman, R. A. (1989). Developmental plasticity of *Scaphiopus Couchii* tadpoles in an unpredictable environment. *Ecology*, 70, 1775–1787.
- Nodzinski, E., & Inger, R. F. (1990). Uncoupling of related structural changes in metamorphosing torrent-dwelling tadpoles. *Copeia*, 1990(4), 1047–1054.
- Ogielska, M., & Kotusz, A. (2004). Pattern and rate of ovary differentiation with reference to somatic development in anuran amphibian. *Journal of Morphology*, 259, 41–54.
- Pyron, R. A. (2014). Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. *Systematic Biology*, 63, 779–797.
- Raj, P., Vasudevan, K., Deepak, V., Sharma, R., Singh, S., Aggarwal, R. K., & Dutta, S. K. (2012). Larval morphology and ontogeny of *Nasikabatrachus sahyadrensis* Biju & Bossuyt, 2003 (Anura, Nasikabatrachidae) from western Ghats, India. *Zootaxa*, 3510, 65–76.
- Roček, Z. (1995). Heterochrony: Response of Amphibia to cooling events. *Geolines*, 3, 55–58.
- Roček, Z., Böttcher, R., & Wassersug, R. (2006). Gigantism in tadpoles of the Neogene frog *Palaebatrachus*. *Paleobiology*, 32(4), 666–675.
- Sáez, P. A., Fibla, P., Correa, C., Sallaberry, M., Salinas, H., Veloso, A., ... Méndez, M. (2014). A new endemic lineage of the Andean frog genus *Telmatobius* (Anura, Telmatobiidae) from the western slopes of the Central Andes. *Zoological Journal of the Linnean Society*, 171, 769–782.
- Seimon, T. A., Seimon, A., Daszak, P., Halloy, S. R. P., Schloegel, L. M., Aguilar, C. A., ... Simmons, J. E. (2007). Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biology*, 13(1), 288–299.
- Senevirathne, G., Kerney, R., & Meegaskumbura, M. (2017). Comparative postembryonic skeletal ontogeny in two sister lineages of Old World tree frogs (Rhacophoridae: *Taruga*, *Polypedates*). *PLoS One*, 12, e0167939.
- Senevirathne, G., Thomas, A., Kerney, R., Hanken, J., Biju, S. D., & Meegaskumbura, M. (2016). From clinging to digging: The postembryonic skeletal ontogeny of the Indian purple frog, *Nasikabatrachus sahyadrensis* (Anura: Nasikabatrachidae). *PLoS One*, 11, e0151114.
- Sheil, C. A., Jorgensen, M., Tulenko, F., & Harrington, S. (2014). Variation in timing of ossification affects inferred heterochrony of cranial bones in Lissamphibia. *Evolution & Development*, 16(5), 292–305.
- Sinsch, U. (1990). Froschlurche (Anura) der zentral-peruanischen Anden: Artdiagnose, Taxonomie, Habitate, Verhaltensökologie. *Salamandra*, 26(2/3), 177–214.
- Sinsch, U., Hein, K., & Glump, B. (2005). Reassessment of central peruvian *Telmatobiinae* (genera *Batrachophrynus* and *Telmatobius*): Osteology, palmar morphology and skin histology. *Monografías de Herpetología*, 7, 239–260.
- Smirnov, S. V. (1991). The anuran middle ear: Developmental heterochronies and adult morphology diversification. *Belgian Journal of Zoology*, 121, 99–110.
- Smirnov, S. V. (1992). The influence of variation in larval period on adult cranial diversity in *Pelobates fuscus* (Anura: Pelobatidae). *Journal of Zoology London*, 226, 601–612.
- Smith, K. K. (2001). Heterochrony revisited: the evolution of developmental sequences. *Biological Journal of the Linnean Society*, 73(2), 169–186.
- Smith-Gill, S. J., & Berven, K. A. (1979). Predicting amphibian metamorphosis. *The American Naturalist*, 113(4), 563–585.
- Sokol, O. M. (1962). The tadpole of *Hymenochirus boettgeri*. *Copeia*, 1962, 272–284.
- Thibaudeau, G. D., & Altig, R. (1988). Sequence of ontogenetic development and atrophy of the oral apparatus of six anuran tadpoles. *Journal of Morphology*, 197, 333–369.
- Trueb, L. (1985). A summary of the osteocranial development in anurans with notes on the sequence of cranial ossification in *Rhinophrynus dorsalis* (Anura: Pipioidea: Rhinophrynidae). *South African Journal of Science*, 81, 181–185.
- Vellard, J. (1951). Estudios sobre batracios andinos. I. El grupo *Telmatobius* y formas afines. *Memorias del Museo de Historia Natural Javier Prado*, 1, 1–89.
- Vera Candiotti, M. F. (2008). Larval anatomy of Andean tadpoles of *Telmatobius* (Anura: Ceratophryidae) from northwestern Argentina. *Zootaxa*, 1938, 40–60.

- Wassersug, R. J. (1976). A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technology*, 51(2), 131–134.
- Weisbecker, V., & Mitgutsch, C. (2010). A large-scale survey of heterochrony in anuran cranial ossification patterns. *Journal of Zoological Systematics and Evolutionary Research*, 48(4), 332–347.
- Wiens, J. J. (1993). *Systematics of the leptodactylid frog genus Telmatobius in the Andes of northern Peru*. Occasional Papers of the Museum of Natural History, University of Kansas, 162, 1–76.
- Yeh, J. (2002). The evolution of development: Two portraits of skull ossification in pipoid frogs. *Evolution*, 56(12), 2484–2498.
- Wilbur, H. M., & Collins, J. P. (1973). Ecological aspects of amphibian metamorphosis: nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. *Science*, 182(4119), 1305–1314.

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