Comparative description and ossification patterns of *Dendropsophus labialis* (Peters, 1863) and *Scinax ruber* (Laurenti, 1758) (Anura: Hylidae)

Angélica Arenas-Rodríguez Corresp. ¹, Juan Francisco Rubiano Vargas ², Julio Mario Hoyos Corresp. ¹

¹ Facultad de Ciencias, UNESIS (Unidad de Ecología y Sistemática), Pontificia Universidad Javeriana, Bogotá, Colombia
² Facultad de Ciencias, Universidad del Bosque, Bogotá, Colombia

Corresponding Authors: Angélica Arenas-Rodríguez, Julio Mario Hoyos
Email address: angelica.arenas@javeriana.edu.co, jmhoyos@javeriana.edu.co

Although comparative studies of anuran ontogeny have provided new data on heterochrony in the life cycles of frogs, most of them have not included ossification sequences. Using differential staining techniques, we observe and describe differences and similarities of cranial and postcranial development in two hylid species, *Scinax ruber* (Scinaxinae) and *Dendropsophus labialis* (Hyline), providing new data of ontogenetic studies in these Colombian species. We examined tadpoles raining from Gosner Stages 25 to 45. We found differences between species in the infrarostral and suprarostral cartilages, optic foramen, planum ethmoidale, and gill apparatus. In both species, the first elements to ossify were the atlas and transverse processes of vertebral column and the parasphenoid. Both species exhibited suprascapular processes as described in other hylids. Although the hylids comprise a large group (over 700 species), postcranial ossification sequence is only known for 15 species. Therefore, the descriptions of the skeletal development and ossification sequences provided herein will be useful for future analyses of heterochrony in the group.
Comparative description and ossification patterns of *Dendropsophus labialis* (Peters, 1863) and *Scinax ruber* (Laurenti, 1758) (Anura: Hylidae)  
Angélica Arenas-Rodríguez¹, Juan Francisco Rubiano Vargas², Julio Mario Hoyos¹

¹Facultad de Ciencias, UNESIS (Unidad de Ecología y Sistemática), Pontifica Universidad Javeriana, Bogotá, Colombia  
²Facultad de Ciencias, Universidad del Bosque, Bogotá, Colombia

**Short Title:** Skeletal development of two hylids
Abstract Although comparative studies of anuran ontogeny have provided new data on heterochrony in the life cycles of frogs, most of them have not included ossification sequences. Using differential staining techniques, we observe and describe differences and similarities of cranial and postcranial development in two hylid species, *Scinax ruber* (Scinaxinae) and *Dendropsophus labialis* (Hylinae), providing new data of ontogenetic studies in these Colombian species. We examined tadpoles raining from Gosner Stages 25 to 45. We found differences between species in the infrarostral and suprarostral cartilages, optic foramen, planum ethmoidale, and gill apparatus. In both species, the first elements to ossify were the atlas and transverse processes of vertebral column and the parasphenoid. Both species exhibited suprascapular processes as described in other hylids. Although the hylids comprise a large group (over 700 species), postcranial ossification sequence is only known for 15 species. Therefore, the descriptions of the skeletal development and ossification sequences provided herein will be useful for future analyses of heterochrony in the group.
Introduction

Comparative morphological descriptions for a specific group of frogs have provided useful systematic characters since the 1960 (e.g. Canatella, 1999; Duellman, Marion and Hedges, 2016). However, most studies of frog morphological characters focus on adults (Faivovich, 2002; Faivovich et al., 2005; Maglia et al. 2007; Wiens et al, 2010; Pyron and Wiens, 2011; Yıldırım and Kaya, 2014; Duellman et al., 2016), and tadpoles have often been overlooked (Alcalde et al., 2011). Of those comparative studies that examine tadpoles, most consider external morphological characters and skeletal characters are often neglected (Fabrezi and Lavilla, 1992; Faivovich, 2002; Maglia et al., 2007; Hoyos et al., 2012; Yıldırım and Kaya, 2014). When skeletal features are considered, the chondrocranium is most often described, while the postcranium is frequently ignored (e.g. Orton, 1953; Starrett, 1973; Wassersug, 1980; Wassersug and Heyer, 1988; Haas, 2003). However, as with other groups, relatively few detailed comparative morphological studies of hylid tadpole skeletal development have been completed. Given the diversity and recent taxonomic re-arrangements of the hylids (Duellman et al., 2016; Jungfer, 2017) is important to amass as much comparative information about the group as possible. Thus, there continues to be a pressing need to conduct comprehensive comparative studies of hylids developmental morphology.

Interspecific variations in morphology help to clarify taxonomic groups within in the Hylidae. The family is predominantly distributed across the Neotropical region (Frost, 2018; Duellman et al., 2016) and comprises 706 species grouped into seven subfamilies: Acridinae, Cophomantinae, Dendropsophinae, Hylineae, Lophyophylinae, Pseudinae, and Scinaxinae (Faivovich et al., 2005; Wiens et al., 2010; Duellman et al.,
Ossification sequences are known for only 15 species, and only eight of those include the postcranial skeleton: *Acris blanchardi* (Havens, 2010; Maglia et al., 2007), *Boana lanciformis* (former *Hyla lanciformis*, De Sá, 1988), *Boana pulchella* (former *Hypsiboas pulchellus* Hoyos et al., 2012), *Dryophytes chrysoscelis* (former *Hyla chrysoscelis*, Sherman and Maglia, 2014), *Dryophytes versicolor* (former *Hyla chrysoscelis*, Sheil et al., 2014), *Hyla orientalis* (Yıldırım and Kaya, 2014), *Osteopilus septentrionalis* (Sheil et al., 2014), *Pseudacris crucifer* (Havens, 2010).

Because identifying variations in developmental morphology and ossification sequence can lead to informative phylogenetic characters (Weisbecker and Mitgutsch, 2010; Harrington, Harrison, Sheil, 2013), we provide a detailed anatomical comparison of the cranial and postcranial development (including the sequence of onset of ossification) between two species of Andean hylids, *Dendropsophus labialis* and *Scinax ruber*. 
**Materials and methods**

We cleared and double stained for bone and cartilage. We make to the standard protocol of Dingerkus and Uhler (1977): 1) proportion of ethanol (SIGMA Ref. 459836-2L) and acetic acid (SIGMA Ref. K36101663 620) was changed to 70:30; 2) the Alcian blue (SIGMA Ref. A5268-25G) was increased to 75 mg/add volume, which was dissolved in ethanol and acetic acid; and 3) staining duration of this last solution was increased to 72 hours. A number of specimens in our series of *Scinax ruber* did not show clear staining, thus we increased our sample size. The sample size for tadpoles and metamorphs of *Dendropsophus labialis* was N = 32, and *Scinax ruber* was N = 114. The number in each series corresponds to the availability of specimens stored at the Museo de Historia Natural “Lorenzo Uribe” at the Universidad Javeriana (MUJ) and the Instituto de Ciencias Naturales at the Universidad Nacional in Bogotá – Colombia (ICN). The larval stages of *D. labialis* were collected from the Municipio Tenjo, Cundinamarca Departament, 3200 m (MUJ 9250). The larval stages of *S. ruber* were collected from the Mun. Neiva, Huila Dep., 570 m; Mun. Granada, Meta Dep., 470 m (MUJ 3727, MUJ 6178, ICN 46015-46017). Tadpoles and metamorphs were staged according to Gosner’s (1960).

Observations and photographs were made with a stereomicroscope (Advanced optical) connected to a camera (Infinity 1 Lumenera Corporation) with white LED light and Image Pro Insight program (version 8.0.3). The drawings were made using a digitizing tablet (Wacom Bamboo Connect pen) and edited using Adobe Illustrator 5.

Anatomical nomenclature for tadpoles follows Parker (1876), Higgins (1921), Jolie (1962), Roček (1981), Duellman and Trueb (1986), Haas (1995), Haas (1997), Hall and
The ossification sequence was determined by the first appearance of ossified bone. We refer to metamorphic climax (MC) *sensu* Banbury and Maglia (2006) as the Gosner stages (GS) at which major modifications and fundamental structural changes occur, resulting in the loss of most of the larval characters. We also used the term "rank" to refer to the ordinal number within an ossification sequence at which an element begins to ossify. We note the first time any specimen at that stage showed stain. If two or more elements begin ossifying at the same time Gosner stage, they were assigned the same rank (i.e. a tie) as per Nunn and Smith (1998).
Results

Skeletal development and sequence of onset of ossification of the cranial and postcranial elements of *Dendropsophus labialis* and *Scinax ruber* are showed in Table 1 and 2. Many young specimens (between Stages 26 and 35) stained poorly, however we had sufficient sample size assess ossification sequence. Specimens older than Stage 35 stained more reliably.

Chondrocranium

We observed similar changes in the shape, size, and modification of structures in the development of chondrocranium in the two species. The elements of the skeleton were compared according to the initiation of ossification and not with a specific stage, because in the two study species the ossification occurred in different Gosner stages (Table 3). The overall width of the chondrocranium in *Dendropsophus labialis* and *Scinax ruber* is roughly 80-90% of this total length (Fig 1). The chondrocranium in *D. labialis* is wider (dorsal view) and lower (lateral view) than *S. ruber* (Fig 1A, 1B, 1C).

Basicranial fenestrae did not differentiate with Alcian Blue in either species. We perceived a stronger blue coloration in *D. labialis*, and the jugular, prootic, and oculomotor foramen were clearly defined, whereas in *S. ruber* we could not see the oculomotor foramen.

The cartilaginous regions of the taenia tecti medialis and tectum sinoticum both represent a quarter of the basis cranii, extending from the frontoparietal fontanelle in both species. The tectum nasi roofs the nasal region, and the ethmoid plate forms the floor. The tectum nasi is separated from the orbit by a wall, the lamina orbitonasalis (=planum antorbitale *sensu* Canatella, 1999). Because these regions are weakly
chondrified, the lamina orbitonasalis is not observable in the tadpole stages, and the
nasal capsules become visible after metamorphic climax (stage 42 and beyond). The
taenia tecti marginalis is evident and clearly differentiated by GS37 in *D. labialis* and by
GS35 in *S. ruber*. In neither species did we observe a frontoparietal fenestra, nor was a
taenia tecti transversalis visible on the edge of the frontoparietal fontanelle (Fig. 1A).

**Suprarostral cartilage.** In both species, the suprarostral cartilage is composed of a
discontinuous cartilaginous plate divided into a corpus suprarostralis and a pars alaris;
posterolaterally we observed a distal syndesmotic junction between the corpus and the
ala. The ala has three processes: two rounded anterolateral processes that join
syndesmotically with the cornu trabecula, and one process posterolaterally (Fig. 1C).
Fenestrations were not observed in the suprarostral cartilage nor, in the adrostral
cartilage near the processus posterodorsalis (=processus dorsalis posterior, *sensu*
Bowatte and Meegaskumbura, 2011). In *D. labialis* the corpus suprarostral is curved,
while in *S. ruber* it is straighter and wider distally, articulating proximally with the cornu
trabecula (trabecular horn, *sensu* Cannatella, 1999). The cornua trabeculae are
approximately 35% of the total length of chondrocranium (lateral view) in both species,
but they are shorter and narrower in *D. labialis* than in *S. ruber*. The cornua trabeculae
articulate anteriorly with the corpus rostrale and laterally with the pars alaris of the
suprarostral cartilage.

**Cartilago Meckeli.** The cartilago Meckeli (= Meckel's cartilage, *sensu* Cannatella,
1999) has three processes: the retroarticular (short and blunt), the dorsomedial, and the
ventromedial. These processes articulate with the infrarostral cartilage (commissura
intramandibularis, *sensu* Cannatella, 1999) which is composed of two syndesmotically,
joined flat plates, and the processus muscularis quadrati. The shape of the processus
dorsomedialis and the processus ventromedialis are the same in both species. The
palatoquadrate cartilage and the commissura quadratocranialis are joined anteriorly to
the base cranii. Laterally, the palatoquadrate cartilage forms the arcus subocularis. The
process muscularis quadrati is joined to the processus antorbitalis (= pars plana sensu
Parker, 1876; = lamina externa sensu Higgins, 1921; = processus antorbitalis sensu
Roček, 1981; = triangular plane sensu Hall and Larsen, 1998 = cartilaginous planum
triangulare sensu Pugener and Maglia, 2007) anterolaterally, projecting above the cornu
trabecula. The processus hyoquadrati of the palatoquadrate cartilage articulates
ventrally with the ceratohyalia of the hyobranchial apparatus (Fig. 1D).

Otic capsule. This structure is longer and higher than wide, occupying about a fifth of
the total length of the skull. The crista parotica exhibits a more pronounced lateral
projection in D. labialis than in S. ruber. The crista parotica is laterally developed,
forming a small processus posterolateralis (= processus lateralis posterior sensu
Bowatte and Meegaskumbura, 2011) and a small processus anterolateralis (more
developed in D. labialis). The processus anterolateralis projects vertically, descending
obliquely and overlapping the ventral posterolateral margin of the palatoquadrate
cartilage. The otic capsule is perforated by the fenestra ovalis, which occupies about
20% of the otic capsule.

Hyobranchial apparatus. The large ceratohyal has a processus anterioris hyalis, a
processus posterioris hyalis, and a processus anterolateralis hyalis. The first two
processes are longer than the third, which extends to meet the transverse crease of the
processus lateralis hyalis.
The basihyal plate is oval and extends proximally to the copula anterior (= Basibranchial I sensu Duellman and Trueb, 1986; = basihyale sensu Haas, 1995 and Haas, 1997; = copula I sensu Maglia and Pugener, 1998; Sheil and Alamillo, 2005) in D. labialis, but is absent in S. ruber. The basibranchial plate is semi-oval and located between the two hypobranchial plates (= planum hypobranchiale sensu Haas, 1999; = plate hyoid sensu Maglia and Pugener, 1998; = hyobranchial plate sensu Sheil and Alamillo, 2005), and a branchial bridge is present in both species, being wider in S. ruber than in D. labialis. The junction between each ceratobranchium and the planum hypobranchiale is syndesmotic. The ceratobranchia are united posteriorly by the commissura terminalis and bear three spicules anteriorly (Fig. 1D).

The chondrocranial morphology and hyobranchial apparatus is generally similar between the species examined here and those previously studied. However, we did identify several differences between S. ruber and D. labialis, including: 1) the shape of the suprarostral, 2) the size and width of infrarostral cartilages, 3) the length of processus articularis, 4) the thickness of palatoquadrate, 5) the size of optic foramen, 6) the presence of an operculum and processus posterolateralis of the otic capsule, 7) the thickness of the processus muscularis quadrati, 8) the attachment of the ascending process to the braincase, 9) the thickness of the planum ethmoidale, 10) the development of the branchial apparatus, 11) the presence of Copula I, and 12) the type of junction between the ceratobranchia and planum hypobranchiale (Fig. 1 and 2). These differences likely represent species-specific differences between the two taxa examined.

**Appendicular skeleton**
Shoulder girdle. The pectoral girdle is arciferal in both species. The earliest ossification of the clavicle, coracoid, and scapula appears at GS36 (Fig. 3A). The clavicle and the cleithrum are distinct, and an epicoracoid cartilage is prominent between the clavicle and the coracoid. The epicoracoids are not mineralized. In *D. labialis* the omosternum is elongated, and the sternum has two projections. The omosternum and the sternum are oval in *S. ruber*. The clavicle articulates with the coracoid, which is ossified in *D. labialis* at GS41 and in *S. ruber* at GS46. The sternum is formed by the epicoracoid and the mesosternum, which joins the medial junction of the epicoracoids (Fig. 2B).

Pelvic girdle. In both species, the primordium of the ilium appears at GS34 and is fully developed by GS41. The ilium begins to ossify by GS41 in *D. labialis* and by GS39/40 in *S. ruber* and articulates anteriorly with the ventral surface of the lateral margin of the sacral diapophyses by GS42. The iliac crest appears dorsally prominent. The primordia of the pubis and the ischium appear at GS36, and are synchondrotically fused by GS38 in both species. The sacral diapophyses is wider in *D. labialis* that in *S. ruber*. The pubis is completely fused by GS40. The pelvic girdle is completely ossified with the halves fused at the midline, extending antrodorsally forming an angle of 55º with the head of the femur by GS45 (Fig. 3).

Fore limb and hind limb. The first cartilaginous elements of the forelimbs (radius, ulna, and humerus) appear at GS32, and those of the hindlimbs at GS33 (femur, tibia, and fibula). The tibia and fibula are fused in *D. labialis* by GS41 and in *S. ruber* by GS38. We observed ossification of the radius and ulna in *D. labialis* (GS41) and *S. ruber* (post
metamorphic). The radius and ulna are fused in both species. Primordia of the four
carpal and five tarsal elements appear by GS33 and complete development by GS41.
The phalangeal carpal formula is 3-3-4-4 and the phalangeal tarsal formula is 3-3-4-5-4
in both species. Metacarpals are curved and phalanges are cylindrical, having a conical
shape at the tip of the terminal phalanges. Digits IV (manus and pes) and V (pes) begin
to ossify by GS42 in *D. labialis*, although all phalanges are ossified at GS45 in both
species (Fig. 3). The carpals were cartilaginous in all specimens and stages examined,
and the distal tarsals were cartilaginous in *S. ruber*. The relative size of carpal elements
is 3 < 4 < 2 < 1 < prehallux and the tarsal elements is 4 < 5 < 3 < 2 < 1 < prepollex.
Sesamoids are absent from GS25 to GS45. Figure 3A shows the limb elements
(central, fibulare, radiale, tibiale, ulnare, and intermedium) at Stage 45.

**Axial skeleton**

The vertebral column is composed of eight procoelous presacral vertebrae, the sacrum,
and the urostyle. The notochord diminishes as the tadpoles grow and is completely
resorbed by GS44 in both species (Fig. 4). We found that the axial skeleton was more
chondrified in *D. labialis* than in *S. ruber*. The first postcranial skeletal elements to
develop in both species were the nine pairs of semicircular cartilaginous primordia of
neural arches, including eight presacral vertebrae, the sacrum, the urostyle and the
hypochord. The sacral diapophyseal primordia are cylindrical. The last postsacral
vertebra (first coccygeal or Vertebra X *sensu* Haas, 1999) and the second coccygeal
vertebra ossify only in *D. labialis* by GS45. Simultaneously to the ossification of
presacral vertebrae, there is notochord absorption, fusion of the coccygeal elements,
and urostyle formation. The urostyle has a bicondylar articulation with the sacral
vertebra and the condyles are widely separated in both species (Fig. 4).

The atlas is concave at its point of articulation with the convex occipital condyles at the base of the skull. Semicircular procoelous (*sensu* Jolie, 1962) vertebral centra begin to develop as early as GS31 in *D. labialis* and GS32 in *S. ruber*, increasing the thickness of both the neural arches and the transverse process. The neural arches appear as cartilage at GS33 in both species, completing at GS34 in *D. labialis* and at GS38 in *S. ruber*. The arches are fused dorsally at the midline at GS38 in *S. ruber* and at GS38 in *D. labialis*. The transverse processes are the first elements to ossify in both species (Tables 1 and 2). Postzygapophyses and prezygapophyses are conspicuous in presacral vertebrae II, III, and IV in both species. Sesamoids are absent from GS25 to GS45.

**Ossification sequence**

The earliest stage examined in both species was GS25. Ossification in *D. labialis* appears by GS34 and in *S. ruber* by GS35 (Fig. 1A and Fig. 4). Ossification in *D. labialis* begins with the atlas and the transverse processes, whereas in *S. ruber* it begins with the parasphenoid, the transverse processes I-VII and neural arches I-III.

Metamorphic climax (MC) begins at GS41 in *D. labialis* and GS39-40 in *S. ruber*. We identified seven ranks (I–VII) in *D. labialis* and five ranks (I–V) in *S. ruber* (Tables 1 and 2). Ossified elements were perceptible in *D. labialis* from GS35 to GS45, with 46 ossified elements, and from GS36 to GS43 in *S. ruber*, with 26 ossified elements. Metamorphic climax in *D. labialis* was at GS45 with 14 ossified elements and in *S. ruber* at GS39-40 with seven ossified elements. Of these, the structures in common are the femur, tibia, fibula, humerus, ilium, and radioulna.
Discussion

Despite Colombia housing the second greatest number of hylid species on the planet, few previous studies have considered developmental ossification of Colombian hylids. The family Hylidae has gone through a number of taxonomic re-arrangements, as elucidated by various phylogenetic hypotheses based on molecular, chromosomal, and morphological data from both larvae and adults (Faivovich, 2002; Faivovich et al., 2005; Wiens et al., 2010; Pyron and Wiens, 2011; Duellman et al., 2016). Data from additional morphological studies of Colombia hylids may help to support or refute these hypotheses.

Previous studies of the cranial morphology in hylid tadpoles include *Acris crepitans* (Maglia et al., 2007), *Boana lanciformis* (de Sá, 1988; Alcalde and Rosset, 2003), *Boana pulchella* (Hoyos et al., 2012), *Boana raniceps* and *Dendropsophus nanus* (former *Hyla raniceps* and *Hyla nana* Fabrezi and Lavilla 1992; Vera Candioti and Haas. 2004), *Dryophytes versicolor* (former *Hyla versicolor*, Sheil et al. 2014), *Hyla orientalis* (Yıldırım and Kaya 2014), *Julianus acuminatus* (former *Scinax acuminatus*, Fabrezi and Lavilla 1992; Faivovich 2002; Alcalde and Rosset 2003; Alcalde et al. 2011), *J. uruguayus*, *J. aff. pinimus* (former *Scinax uruguayus* and *Scinax aff. pinima*, Alcalde et al., 2011; Rodrigues et al., 2017), *Oloolygon aromothyella* and *O. berthae* (former *Scinax berthae*, Rodrigues et al., 2017; Alcalde et al., 2011; Faivovich, 2002), *O. skuki* (Rodrigues et al., 2017); *Scinax granulatus* and *S. squalirostris* (Rodrigues et al., 2017; Alcalde and Rosset, 2003), *S. boulengeri* (Rodrigues et al., 2017; Vera Candioti, 2007), *S. fuscovariatus* (Fabrezi and Vera, 1997), *S. nasicus* (Rodrigues et al., 2017; Vera
Candioti, 2007; Vera Candioti et al., 2004; Fabrezi and Vera, 1997), \textit{S. ruber} (Haas, 1996). For a complete overview of the findings of these studies please see Appendix 1.

Several of the differences between the two species examined here present interesting avenues for future examination. For example, processus ethmoidalis of the quadrate in \textit{S. ruber} is wide, and it is not clearly distinct from the processus articularis. In contrast, the processes of \textit{D. labialis} are easily distinguishable and similar to that described by Alcalde and Rosset (2003), who found similar features in \textit{Boana raniceps} compared with to the \textit{Scinax} group (\textit{S. squalirostris} and \textit{S. granulatus}, \textit{Scinax ruber} group). The palatoquadrate is similar but the processus ascendsens of the palatoquadrate in \textit{D. labialis} is wider than in \textit{S. ruber}, and the distal side of the cornu trabecula extends posteriorly toward the otic capsule. The anterior region of the palatoquadrate is distinctively broader in \textit{S. ruber} than in \textit{D. labialis}, and in \textit{S. ruber} the dorsomedial process is wider than the ventromedial process in \textit{D. labialis}.

When comparing the development of \textit{D. labialis} with \textit{D. nanus} (Vera Candioti et al., 2004 and Alcalde and Rosset, 2003), we found that \textit{D. labialis} can be differentiated by the reduction of the buccopharyngeal and branchial basket structures and a missing sinus posterior hypobranchialis and processus quadrato-ethmoidale. On the other hand, the information that is available for \textit{Scinax} species (Fabrezi and Lavilla, 1992; Haas, 1996; Fabrezi and Vera, 1997; Faivovich, 2002; Alcalde and Rosset, 2003; Vera Candioti et al., 2004; Vera and Haas, 2007; Alcalde et al., 2011; Rodrigues et al., 2017) revel that there are a numerous variations that requires extending the morphological studies in tadpoles.

\textit{Scinax ruber} presents alae and corpus of suprarostral cartilage with deeper notches.
The chondrocranium, hyobranchial apparatus, and the suprarostral body are joined syndesmotically as found by Vera and Haas (2007) in microphagous tadpoles of S. nasicus and S. boulengeri. *Dendropsophus labialis* like *Dendropsophus nanus* shows a suprarostral cartilage with corpus and alae forming a continuous structure, which is evidently, associated with a deviation of the macrophagous mechanisms described by Alcalde and Rosset (2003).

The lateral development of the crista parotica is more prominent in *S. ruber* than in *D. labialis*. It is possible that some of the variation in the anatomical structures of the otic capsule are functionally related to perception of vocalizations (i.e. same species recognition) in adult stages, but experiments must be conducted to confirm the relationship of these anatomical structures with hearing physiological functions (Ruggero and Temchin, 2002; Boistel et al., 2013).

The chondrification of skull in *S. ruber* is faint when viewed laterally, and foramina are not clearly visualized. By contrast, in *D. labialis*, much more blue coloration was observed. This could be due to abundant chondrification of these parts or to the early developmental stages of this anatomical area in which allowed differentiation of craniopalatine carotid foramina.

Although the sample size for the *Dendropsophus labialis* is very small in comparison with *S. ruber*, *Dendropsophus labialis* exhibited more ossified elements with stronger chondrification and less intraspecific variation, while *S. ruber* showed more intraspecific variation and less overall chondrification in the samples (Fig. 1). *D. labialis* presented uniformly stained (ossified) elements in all individuals (Table 3).

This variation between *S. ruber* and *D. labialis* could be caused by intrinsic factors that
determine the timing of development or extrinsic factors affecting osteogenesis (Vera and Ponssa, 2014). It may not be a coincidence that *S. ruber* is a generalist species and *D. labialis* is an endemic one (Frost 2018).

Haas (1996) reported that the ceratohyalia II-IV are fused in *Scinax ruber* and *Megophrys montana nasuta*, characteristics that separate them from other species. We confirmed this observation in *S. ruber* but not in *D. labialis*. The ceratohyal in *D. labialis* has a process on the articular condyle that is not present in *S. ruber*. Alcalde and Rosset (2003) found this process in both *S. granulatus* and *S. squalirostris*. Spicules I-III on the posterior margin of the hypobranchial plate are present in *D. labialis* and *S. ruber*, but spicule IV is not.

Copula II is present in both species. Copula I is present in *D. labialis* as in *S. squalirostris*, but absent in *S. ruber* as in *S. granulatus*, *Boana raniceps* (Alcalde and Rosset, 2003), and *Tlalocohyla smithii* (Vera and Haas, 2004). Although the presence of copula I is extremely variable in hylids and is shared by all non-hylids (Vera and Haas, 2004), a relationship between this structure and the ecological function that it performs (e.g. prey utilization), has not been identified.

Additional characteristics of the developmental morphology of these species places align them with other hylids that have been studied previously. For example, the urostyle of *D. labialis* and *S. ruber* forms a bicondylar articulation with the sacral vertebra, and the condyles are widely separated. The shoulder girdle of both species present differences in the shape of the omosternum and sternum at GS 45. *Dendropsophus labialis* and *S. ruber* present suprascapular processes in tadpoles and adults similar to those in other hylids (*Hypsiboas lanciformis*, De Sá, 1988; *Boana*...
pulchella, Hoyos et al., 2012; Pseudacris crucifer and Acris blanchardi, Havens, 2010 and A. crepitans, Maglia et al., 2007) but is absent in Scinax catharinae clade (Faivovich et al., 2005).

Variations of larval characters between Scinax and Dendropsophus have been included in several phylogenetic studies (Fabrezi and Vera, 1997; Haas, 1996; 1999; 2003; Alcalde and Rosset, 2003; Vera, 2007). In our study, the skeleton shows significant differences between the species Scinax ruber and Dendropsophus labialis, beginning with fact that elements ossified in S. ruber exhibit more intraspecific variability than in D. labialis (see Table 3).

Regarding ossification sequence, the first bones ossified in the cranium were the exoccipital, the frontoparietal, and the parasphenoid by GS 36. Haas (1999) found that in S. ruber this occurred one stage later by GS37. Similar to those that Haas (1999) described for other hylids, the ossification of the vertebrae begins from the centra of the presacral vertebrae and continues ventrally along the notochord, forming osseous rings around the notochord in both species. We found that the ossification of the centra in both species we studied begins ventrally and proceeds dorsally. Haas (1999) recorded the transverse processes of presacral vertebrae II- III as the first to ossify, while we found that it was the ossification or neural arches proceeds from I to IX in D. labialis (GS37) and from I to III in S. ruber (GS36) (Fig 4).

The detection of more intraspecific variability in Scinax ruber than in Dendropsophus labialis could also be due to the presence of more intra-generic diversity in the Scinax ruber clade. Alcalde and Rosset (2003) associated the type of feeding with the development of the lateral anterohial process of the ceratohial, between species with
macrophage larvae (*Dendropsophus nanus*), and scraping microphages (*Boana pulchellus, Julianus acuminatus* and *S. nasicus*). This may indicate that morphological characteristics of the jaw may be involved in the particular feeding behaviors of these tadpoles, and therefore these traits would help to distinguish the species (Appendix 1).

Differences between the ossification sequences of these two species are also evident when examining the ossification ranks and number of ossified bones. In particular, *D. labialis* has more ranks in the sequence and more elements that begin ossification prior to metamorphosis. With respect to the postcranium, the number of elements ossified appears earlier in *D. labialis* than in *S. ruber*. Because Gosner stages are based on external characteristics that rely on underlying skeletal change, it is only a relative measure of timing and should not be used as a way to compare species. Instead, we compared the relative timing of events in the ossification sequence by examining the order of onset of ossification of each element. Nunn and Smith (1998:86) considered “ontogeny may be ordered by age, size, or stage; none of these measures are useful for comparing ontogeny across significantly divergent taxa”.

Table 4 outlines the ossification sequences of different species of the family Hylidae. The number of ranks that include elements of the skull and postcranium vary from one to five. The number of ranks increases when postcranial elements are included.

Weisbecker and Mitgutsch (2010), Harrington et al. (2013), and Sheil et al. (2014) used similar ranked ossification sequence data to reconstruct phylogenetic trees of amphibians in the families of Leptodactylidae, Ranidae and Bufonidae. These researchers suggested using cranium and post-cranium data, relating them to the type of development, and to include sequences of fossils, as far as possible.
Although the morphology and systematics of amphibians have been extensively studied (Canatella and Trueb, 1988; de Sá and Hillis, 1990; Baez and Pugener, 2003; Roelants and Bossuyt, 2005; Faivovich et al., 2005, Frost et al., 2006; Pyron and Wiens, 2011; Duellman et al., 2016), additional comprehensive descriptions of skeletal development and ossification sequences are needed to truly understand patterns of heterochrony in the group. Some of the biological implications of heterochrony, which are well known in amphibians (Alberch, 1985; Reilly, Wiley and Meinhardt, 1991), include changes in structure, and changes in the rate of growth of entire organisms (Raff, 1996; Smith, 2001; 2002; 2003). Some scholars have recognized that heterochrony may work as modules of developmental events with evolutionary implications that can promote or restrict the development of individual morphologies (Wagner 1996).

Studies that have used statistical methods (e.g. Parsimov) to analyze ossification sequences have revealed heterochrony in the timing of onset of ossification in some cranial elements such as parasphenoid and prootic in S. ruber vs. H. pulchellus (Hoyos et al., 2012), or the frontoparietal, dentary, and maxilla in D. labialis vs. Pseudis platensis (Fabrezi and Goldberg, 2009). In our study, we found that the parasphenoid was the first element to ossify in both D. labialis and S. ruber, and the exoccipital, frontoparietal, and prootic were the second elements to ossify in both species. It is possible that the difference in cartilage formation between the two species examined herein is due to paracrine factors induced in cells that express mesodermal transcription factors involved in the activation of genes specific to cartilage (Gilbert, 2000; Kozhemyakina et al., 2015); however, we did not account for these factors.
Additionally, the intraspecific variation in the ossified elements between these species could be linked to specific genes (Raff, 1996).

Conclusions

The contribution of ontogenetic data (development and ossification sequences of skeletal structures) provides further information to help understand the interactions between ontogeny and phylogeny in morphological and ecological diversity of frogs. Ossification sequence data combined with evolutionary hypotheses may shed light on patterns of development to be used in future phylogenetic hypotheses. As Larson de Sá and Arrieta (2003) suggested, “variation in chondrocranial morphology in larval anurans can be phylogenetically informative, even among closely related taxa”.

Acknowledgements

We want to thank Timothy Sosa for help with the English translation. We appreciate the contributions of the anonymous reviewers.

References

Alberch P (1985) Problems with the interpretation of developmental sequences. Systematic Zoology 34:46–58.

Alcalde L, Rosset SD (2003) Descripción y comparación del condrocráneo en larvas de Hyla raniceps (Cope, 1862), Scinax granulatus (Peters, 1863) y Scinax squalirostris (Lutz, 1925) (Anura: Hylidae). Cuadernos de Herpetología 17:33–49.

Alcalde L, Vera Candioti MF, Kolenc F, Bortiero C, Baldo D (2011) Cranial anatomy of tadpoles of five species of Scinax (Hylidae, Hylinae). Zootaxa 2787:19–36.
Avilán P, Hoyos JM (2006) Osteology of *Eleutherodactylus bogotensis* (Amphibia, Anura, Leptodactylidae) from the Parque Nacional Natural Chingaza (Cundinamarca, Colombia). *Caldasia* 28: 89-109.

Báez AM, Pugener LA (2003) Ontogeny of a new Palaeogene pipid frog from southern South American and xenopodinomorph evolution. *Zoological Journal of the Linnean Society* 139:439–476. DOI: 10.1046/j.1096-3642.2003.00085.x

Banbury B, Maglia AM (2006) Skeletal development of the Mexican spade foot, *Spea multiplicata* (Anura: Pelobatidae). *Journal of Morphology* 267:803–821.

Boistel R, Aubin T, Cloetens P, Peyrin F, Scotti T, Herzog P, Gerlach J, Pollet N, Aubry JF (2013) How minute sooglossid frogs hear without a middle ear. *Proceedings of the National Academy of Sciences of the United States of America* 2:15360–15364. DOI: 10.1073/pnas.1302218110

Bowatte G, Meegaskumbura M (2011) Morphology and ecology of tadpoles of *Ramanella obscura* (Anura: Microhylidae). *Ceylon Journal of Science* 40: 109-120. DOI: 10.4038/cjsbs.v40i2.3927

Cannatella DC (1999) Architecture: Cranial and axial musculoskeleton. In R. W. McDiarmid, and R. Altig (Eds.), *Tadpoles: The biology of anuran larvae* (pp. 52–91). United States of America: University of Chicago Press.

Cannatella DC, Trueb L (1988) Evolution of pipoid frogs: Intergeneric relationships of the aquatic frog family Pipidae (Anura). *Zoological Journal of the Linnean Society* 94:1–38. DOI: 10.1111/j.1096-3642.1988.tb00880.x

de Sá RO (1988) Chondrocranium and ossification sequence of *Hyla lanciformis*. *Journal of Morphology* 195:345–355. DOI: 10.1002/jmor.1051950308
de Sá RO, Hillis DM (1990) Phylogenetic relationships of the pipid frogs *Xenopus* and *Silurana*: an integration of ribosomal DNA and morphology. Molecular Biology and Evolution 7:365–376. DOI: 10.1093/oxfordjournals.molbev.a040612

Dingerkus G, Uhler LD (1977) Enzyme clearing of Alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technology 52:229–232.

Duellman WE, Trueb L (1986) *Biology of amphibians*. New York: McGraw-Hill Inc.

Duellman WE, Marion AB, Hedges SB (2016) Phylogenetics, classification, and biogeography of the tree frogs (Amphibia: Anura: Arboranae). Zootaxa 4104:1–109.

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. DOI: 10.1002/jmor.10680.

Fabrezi M, Lavilla EO (1992) Estructura del condrocráneo y esqueleto hiobranquial en larvas de algunos hílidos neotropicales (Anura: Hylidae). Acta Zoologica Lilloana 41: 155–164.

Fabrezi M, Vera R (1997) Caracterización morfológica de larvas de anuros del Noroeste argentino. Cuadernos de Herpetología 11:37–49.

Faivovich JC (2002) A cladistics analysis of *Scinax* (Anura: Hylidae). Cladistics 18:367–393. DOI: 10.1111/j.1096-0031.2002.tb00157.x

Faivovich JC, Haddad CFB, García P, Frost DR, Campbell JA, Wheeler WC (2005) Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bulletin of the American Museum of Natural History 294:1–240. DOI: 10.5531/sd.sp.12
Frost DR (2018) Amphibian Species of the World: an Online Reference. New York, USA: American Museum of Natural History New York, USA. Available at http://research.amnh.org/herpetology/amphibia/index.html (accessed 12 December 2017)

Frost DR, Grant T, Faivovich JC, Haddad CFB, Wheeler WC, et al. (2006) The amphibian tree of life. Bulletin of the American Museum of Natural History 297:1–370. DOI: 10.5531/sd.sp.13

Gilbert SF (2000) The anatomical tradition: Evolutionary Embryology: Embryonic homologies. Chapter 1 Developmental Biology. Sunderland (MA): Sinauer Associates, Inc.

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

Haas A (1995) Cranial features of dendrobatid larvae (amphibia: Anura: Dendrobatidae). Journal of Morphology 224: 241-264. DOI: 10.1002/jmor.1052240302

Haas A (1996) Das larvale cranium von Gastrotheca riobambae und seine metamorphose (Amphibia, Anura, Hylidae). Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg 36:33–162.

Haas A (1997) The larval hyobranchial apparatus of discoglossoid frogs: Its structure and bearing on the systematics of the Anura (Amphibia: Anura). Journal of Zoological Systematics and Evolutionary Research 35: 179-197. DOI: 10.1111/j.1439-0469.1997.tb00422.xh
Haas A (1999) Larval and metamorphic skeletal development in the fast-developing frog *Pyxicephalus adspersus* (Anura, Ranidae). *Zoomorphology* 119:23-35.

Haas A (2003) Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* 19:23–89. DOI: 10.1111/j.1096-0031.2003.tb00405.x

Hall JA, Larsen JH (1998) Postembryonic ontogeny of the spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae): Skeletal morphology. *Journal of Morphology* 238: 179–244.

Harrington SM, Harrison LB, Sheil CA (2013) Ossification sequence heterochrony among amphibians. *Evolution and Development* 15: 344–364. DOI: 10.1111/ede.12043.

Havens SB (2010) The role of skeletal development in body size evolution of two North American frogs. Master thesis, Missouri University of science and technology.

Higgins GM (1921) The nasal organ in amphibia. *Illinois biological monographs* 6: 3–90.

Hoyos JM, Sánchez-Villagra MR, Carlini AA, Mitgutsch C (2012) Skeletal development and adult osteology of *Hypsiboas pulchellus* (Anura: Hylidae). *Acta Herpetologica* 7: 119–138.

ICVAN, International committee on veterinary anatomical nomenclature (1973) *Nomina anatomica veterinaria*. 2nd edition. Vienna, Austria: International Committee on Veterinary Anatomical Nomenclature under the financial responsibility of the World Association of Veterinary Anatomists.

Jolie M (1962) *Chordata Morphology*. New York: Reinhold Publ. Co.

Jungfer KH (2017) On Warszewicz’s trail: the identity of *Hyla molitor* O. Schmidt, 1857. *Salamandra* 53:18–24.
Kozhemyakina E, Lassar AB, Zelzer E (2015) A pathway to bone: Signaling molecules and transcription factors involved in chondrocyte development and maturation. *Development* 142:817–831. DOI: 10.1242/dev.105536.

Larson, P. M.; de Sá, R. O.; Arrieta, D. (2003) Chondrocranial, hyobranchial and internal oral morphology in larvae of the basal bufonid genus *Melanophryniscus* (Amphibia: Anura). *Acta Zoologica Stockholm* 84(2): 145–154. DOI: 10.1046/j.1463-6395.2003.00140.x

Maglia AM, Pugener LA (1998) Skeletal development and adult osteology of *Bombina orientalis* (Anura: Bombinatoridae). *Herpetology* 54: 344-363.

Maglia AM, Pugener LA, Mueller JM (2007) Skeletal morphology and postmetamorphic ontogeny of *Acris crepitans* (Anura: Hylidae): a case of miniaturization in frogs. *Journal of Morphology* 268: 194–223. DOI: 10.1002/jmor.10508

Nunn CL, Smith KK (1998) Statistical analyses of developmental sequences: the craniofacial region in marsupial and placental mammals. *American Naturalist* 152:82–101.

Orton GL (1953) The systematics of vertebrate larvae. *Systematic Zoology* 2:63–75.

Parker WK (1876) On the structure and development of the skull in the Batrachia. Part II. *Philosophical Transactions of the Royal Society* 166:648–699.

Pugener LA, Maglia AM (2007) Skeletal morphology and development of the olfactory region of *Spea* (Anura: Scaphiopodidae). *Journal of Anatomy* 211:754–768. DOI: 10.1111/j.1469-7580.2007.00826.x

Pyron A, Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetic Evolution* 61:543–583. DOI: 10.1016/j.ympev.2011.06.012
Raff RA (1996) *The shape of life: genes, development and the evolution of animal form.* Chicago: University of Chicago Press.

Reilly SM, Wiley EO, Meinhardt DJ (1991) An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biological Journal of the Linnean Society* 60:119–43. DOI: 10.1111/j.1095-8312.1997.tb01487.x

Roček Z. (1981) Cranial anatomy of frogs of the family Pelobatidae Stannius, 1856, with outlines of their phylogeny and systematics. *Acta Universitatis Carolinae Biologica* 1980: 1–164.

Rodrigues GDV, do Nascimento FAC, de Almeida JPFA, Mott T (2017) The tadpole of *Scinax skuki* (Anura: Hylidae) from the type locality, with a description of its larval skeleton. *Studies on Neotropical Fauna and Environment* 52: 204-215. DOI: 10.1080/01650521.2017.1342485

Roelants K, Bossuyt F (2005) Archaeobatrachian paraphyly and pangaean diversification of crown-group frogs. *Systematic Biology* 54:111-26. DOI: 10.1080/10635150590905894

Ruggero MA, Temchin AN (2002) The roles of the external, middle, and inner ears in determining the bandwidth of hearing. *Proceedings of the National Academy of Sciences of the United States of America* 99:13206–13210. DOI: 10.1073/pnas.202492699

Shearman RM, Maglia AM (2014) Osteological development of Cope’s gray treefrog, *Hyla chrysoscelis*. *Acta Zoologica Stockholm* 96: 181–198. DOI: 10.1111/azo.120662
Sheil CA, Jorgensen M, Tulenko F, Harrington S (2014) Variation in timing of ossification affects inferred heterochrony of cranial bones in Lissamphibia. *Evolution and Development* 16:292–305. DOI: 10.1111/ede.120912

Sheil CA, Alamillo H (2005) Osteology and skeletal development of *Phyllomedusa vaillantii* (Anura: Hylidae: Phyllomedusinae) and a comparison of this arboreal species with a terrestrial member of the genus. *Journal of Morphology* 265: 343–368. DOI: 10.1002/jmor.103621

Smith KK (2001) Heterochrony revisited: the evolution of developmental sequences. *Biological Journal of the Linnean Society* 73:169–86. DOI: 10.1006/bijl.2001.0535

Smith KK (2002) Sequence heterochrony and the evolution of development. *Journal of Morphology* 252:82–97. DOI: 10.1002/jmor.10014

Smith KK (2003) Time’s arrow: heterochrony and the evolution of development. *International Journal of Biomedical Research* 47:613–21.

Starrett PH (1973) *Evolutionary patterns in larval morphology*. In J. L. Vial, editor, Evolutionary Biology of the Anurans: Contemporary Research on Major Problems. Columbia: University of Missouri Press. p 251–271.

Vera Candioti MF (2007) Anatomy of anuran tadpoles from lentic water bodies: Systematic relevance and correlation with feeding habits. *Zootaxa* 1600:1175–5326.

Vera Candioti MF, Haas A (2004) Hyobranchial Apparatus of *Hyla nana*. *Cuadernos de herpetología* 18:3–15.

Vera MC, Ponssa ML. (2014) Skeletogenesis in anurans: cranial and postcranial development in metamorphic and postmetamorphic stages of *Leptodactylus*
bufonius (Anura: Leptodactylidae). *Acta Zoologica* 95:44-62. DOI:

10.1111/azo.12007

Wagner GP (1996) Homologues, natural kinds and the evolution of modularity. *American Zoology* 36:36–43.

Wassersug RJ (1980) Internal oral features of larvae from eight families: Functional, systematic, evolutionary and ecological considerations. *Miscellaneous publication - University of Kansas, Museum of Natural History* 68:1–146. DOI:

10.5962/bhl.title.16230

Wassersug RJ and Heyer, W. R (1988) A survey of internal oral features of leptodactyloid larvae (Amphibia: Anura). *Smithsonian Contributions to Zoology* 457:1–99. Weisbecker V, Mitgutsch C (2010) A large-scale survey of heterochrony in anuran cranial ossification patterns. *Journal of Zoological Systematics and Evolutionary Research* 48:332–347. DOI: 10.1111/j.1439-0469.2010.00570.x

Wiens JJ, Kuczynski CA, Hua X, Moen DS (2010) An expanded phylogeny of tree frogs (Hylidae) based on nuclear and mitochondrial sequence data. *Molecular Phylogenetic Evolution* 55:871–82. DOI: 10.1016/j.ympev.2010.03.013

Yıldırım E, Kaya U (2014) Comparative skeletogenesis of the oriental tree frog *Hyla orientalis* (Anura: Hylidae). *Zoologischer Anzeiger* 253:361-71. DOI:

10.1016/j.jcz.2014.04.002
Table 1 (on next page)

Ossification sequence of cranial and postcranial elements in *Dendropsophus labialis* (Peters, 1863)
| Rank | Gosner stage (Number of specimens) | Elements ossified |  |
|------|-----------------------------------|-------------------|---|
|      | 26(3), 27(3), 28(2), 29(2), 31(3), 32(1), 33(1), 34(1) | **Cranium** | **Postcranium** |
| I    | 35 (1) |  | Transverse process I–V |
| II   | 36 (2) | Parasphenoid | Transverse process VI–VIII |
| III  | 37 (3) | Frontoparietal, exoccipital | Neural arches I–VIII |
| IV   | 38 (1) |  | Hypochord |
| V    | 41 (3) |  | Femur, tibiofibula, humerus, ilium, radioulna, clavicle, pubis, metatarsal III–V, coracoids |
| VI   | 42 (3) |  | Metacarpal IV, urostyle |
| VII  | 45 (1) | Mentomeckelian, premaxilla, maxilla, angulosplenial, dentary | Manus IV proximal phalange, Metacarpal III and V, scapula, pedal digit IV proximal phalange, Metacarpal I and II, metatarsal I, prepollex |
| VIII | 46 (1) | Neopalatine, nasal, pterygoid, vomer, septomaxilla, squamosal |  |
Ossification sequence of the cranial and postcranial elements in *Scinax ruber* (Laurenti, 1758).

**Table 2** (on next page)
| Rank | Gosner stage (Number of specimens) | Elements ossified | Cranium | Postcranium |
|------|-----------------------------------|------------------|---------|-------------|
| I    | 26(3), 29(8), 30(7), 31(9), 32(8), 33(9), 34(9), 35(14) |                  |         |             |
|      | 36 (10)                           | Parasphenoid     | Transverse process I–VII |
| II   | 37 (11)                           |                  | Neural arch I–III |
| III  | 38 (4)                            | Frontoparietal, exoccipital | Transverse process VIII, neural arch IV–VIII |
| IV   | 39–40 (3)                         |                  | Femur, tibiafibula, humerus, ilium, radioulna, scapula, hypochord |
|      | 41(4), 42(5)                      |                  |         |             |
| V    | 43 (3)                            |                  | Ischium |
|      | 44(4), 45(2)                      |                  |         |             |
| VI   | 46 (1)                            | Mentomeckelian, premaxilla, maxilla, angulosplenial, dentary, neopalatine, pterygoid, vomer, septomaxilla, squamosal |         |             |
Onset of ossification of cranial and poscranial elements of *Dendropsophus labialis* and *Scinax ruber*
| Species                              | **Dendropsophus labialis** | **Scinax ruber** |
|--------------------------------------|-----------------------------|------------------|
| **Gosner Stage**                     | 35 (1)                      | 36 (6)           |
| (No. Specimens with ossified elements)| 36 (2)                      | 37 (9)           |
| 37 (3)                               | 38 (1)                      | 39/40 (3)        |
| 41 (3)                               | 42 (3)                      | 41 (4)           |
| 43 (1)                               | 44 (1)                      | 42 (5)           |
| 45 (1)                               | 46 (1)                      | 43 (3)           |
| **Paraesfenoids**                    | 36 (7)                      | 37 (9)           |
| 38 (3)                               | 39 (4)                      | 40 (5)           |
| 41 (3)                               | 42 (3)                      | 43 (3)           |
| 44 (1)                               | 45 (1)                      | 46 (1)           |
| **Exoccipital**                      | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Frontopariental**                  | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Cranium**                          | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Transverse Process**               | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Ventral Process**                  | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Ventral Process**                  | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Pos-cranium**                      | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Neural arch**                      | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Manuscript**                       | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| **Metacarpal I**                     | 36 (6)                      | 37 (9)           |
| 38 (3)                               | 39/40 (3)                   | 41 (4)           |
| 42 (5)                               | 43 (3)                      | 44 (4)           |
| 45 (1)                               | 46 (1)                      | 47 (1)           |
| Bone Type                  | Metacarpal II | Metacarpal III | Metacarpal IV | Metacarpal V | Metatarsal distal tarsal IV | Metatarsal distal tarsal IV | Prepollex | Pes Metatarsal I | Metatarsal II | Metatarsal III | Metatarsal IV | Metatarsal V | Metatarsal V | Atlas       | Femur       | Fibula      | Tibia       | Tibiofibula | Scapula | Suprascapula |
|---------------------------|---------------|----------------|---------------|--------------|-----------------------------|-----------------------------|------------|-----------------|---------------|----------------|---------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|----------|-------------|
|                           | 1             | 1              | 1             | 2            | 1                           | 1                           |            | 1               | 1             | 1              | 1             | 2            | 1            | 1           | 1           | 1           | 1           | 1           | 1         | 1           |
|       | Clavicle | 1 | 2 | 2 | 1 | 1 | 3 | 1 | 3 | 1 |
|-------|----------|---|---|---|---|---|---|---|---|---|
| Coracoids | 1 | 2 | 2 | 1 | 1 |   |   |   |   |   |
| Humerus | 1 | 2 | 3 | 1 | 1 | 1 |   | 1 | 1 | 3 |
| Ulna   | 1 |   |   |   |   |   |   |   |   |   |
| Radioulna | 1 | 2 | 3 | 1 | 1 | 1 | 3 | 3 | 3 |   |
Table 4 (on next page)

Ossification sequences of different species of the family Hylidae including postcranial elements.
| Subfamily          | Specie                  | Element | No. ranks | Ossification sequence                                                                 | References          |
|-------------------|-------------------------|---------|-----------|--------------------------------------------------------------------------------------|---------------------|
| Acridinae         | *Acris blanchardi*      | C       | 9         | ps [ex, fp, po] [pm, sm]ma, ns, vo [an, de] [me, pt, qj, qu, sq] sp                     | Havens, 2010        |
|                   |                         | C, P    | 11        | ps, ve [ex, fe, fp, po] [fi, hu, mt, pf, ra, sc, ul, tf, ti] [cl, co, ct, il, mc, ph] [is, pm, sm] ma, ns, vo, [an, de] [me, pt, qj, qu, sq] sp | Havens, 2010        |
|                   | *Hyliola regilla*       | C       | 6         | ps, fp [ex, po] pv [ma, ns, pm, sm, sq] [ag, de, pt] [cm, me, pa, qj, sp]              | Gaudin, 1973        |
|                   | *Pseudacris crucifer*   | C       | 8         | ps [ex, fp] [pf, po] [ma, pm, sm] ns [an, vo] [me, pt, qj, qu, sq] sp                  | Havens, 2010        |
|                   |                         | C, P    | 13        | ps, ve, fe [hu, il, ra, su, ul] [ct, ex, fp, fr, sc, tf, ti] [cl, co, mt, pf, po] [mc, ph] is [ma, pm, sm] ns [an, vo] [me, pt, qj, qu, sq] sp | Havens, 2010        |
|                   | *Pseudacris triseriata* | C       | 4         | [ex, fp, pm] [de, ma, ns, pt, qj, sq, vo] [m, po] [cm, ha, pa, ps, sp]                 | Stokely & List, 1954 |
| Dendropsophinae   | *Dendropsophus labialis*| C       | 3         | ps [ex, fp, po] [an, de, ma, me, np, pm, sm, sq, vo]                                  | This study          |
|                   |                         | C, P    | 8         | [ve] [ps] [ex, fp, po] [cl, co, fe, hu, il, mt, ru, tf] [mc, sc] [an, de, ma, me, np, pm, sm, sq, vo] | This study          |
| Cophomantinae    | *Boana lanciformis*     | C       | 8         | fp, ps, ex, po [pm, sm] [ns, ma] [an, de, sq] [sp, me, qj, vo, pa, pt]                 | De Sá, 1988         |
|                   |                         | C, P    | 9         | fp [ps, ve] ex [fr, cl, co, ct, fe, hu, mc, mt, po, sc, tf, ti] pf [pm, sm] [ns, ma] [an, de, is, sq] [ap, me, pa, ph, pt, qj, sp, vo] | De Sá, 1988         |
|                   | *Boana pulchella*       | C       | 2         | [ex, fp, ps] [an, de, ma, pm, po, sq]                                                 | Hoyos et al., 2012  |
|                   |                         | C, P    | 4         | [ex, fp, ps, ve] [fe, hu, il, ru, sc, tf] [cl, co, ct, hy, mc, mt, pf, ph] [an, de, ma, pm, po, sq] | Hoyos et al., 2012  |
| Hylinae           | *Dryophytes chrysoscelis* | C     | 8         | ps [ex][fe][fp][sm, pm, po][ma][de, ns, an, sq][vo]                                     | Sherman and Maglia, 2014 |
| Family               | Genus                  | Species            | Characters | References               |
|----------------------|------------------------|--------------------|------------|--------------------------|
| Dryophytes versicolor|                         |                    | ps [ex, na, cn][fe][sc, cl, co, hu, ra, ul, il, ti, fi, tb, mc, mt][ph, pf][fp][sm, pm, po][ma, is][de, ns, an, sq][vo] | Sheil et al., 2014 |
| Hyla orientalis      |                         |                    | ps [ex, hu, na, po, ve][ct, fe, fr, il, mc, tf, ti, ru][fp, cl, co, mt, pf, ph][ns, sm][an, de, is, ma, pm, pt, sq, pu, vo] | Yıldırım and Kaya, 2014 |
| Smilisca baudinii    |                         |                    | fp [ex, ps, sm][ag, de, ma, pm, sq][ns, pt, qj][me, pa, pv][cm, sp] | Trueb, 1966 |
| Triprion petasatus   |                         |                    | fp, ns [an, de, ex, ma, me, pa, pm, ps, pt, qj, sm, sq][cm, pv] | Trueb, 1970 |
| Lophyohylinae        | Osteopilus septentrionalis|                  | fp, sm [ag, cm, de, et, ex, ma, me, ns, pa, pm, po, ps, pt, qj, sq, vo] | Trueb, 1966 |
|                       |                         |                    | [fp, sm][ag, de, ex, ns, ma, pm, ps, pt, pv, sq] | Trueb, 1970 |
|                       |                         |                    | ps, ve, fe [hu, il, ra, su, ul] [ct, ex, fp, fr, sc, tf, tl][cl, co, mt, pf, po][mc, ph] | Sheil et al., 2014 |
| Pseudinae            | Pseudis platensis      |                    | [fp, ps][ex, po][ns, pm, sq] | Fabrezi and Goldberg, 2009 |
| Scinaxinae           | Scinax ruber           |                    | ps, ve [ex, fp, po][fe, hu, il, ru, sc, tf, tl] | This study |

1. C, cranium; P, poscranium
2. ag, angular; an, angulosplenial; ap, plectral apparatus; cl, clavicle; cu, columella; co, coracoid; ct, cleithrum; de, dentary; et, ethmoid; ex, exoccipital; fe, femur; fi, fibula; fp, frontoparietalis; fr, fibulare; ha, hyoid apparatus; hu, humerus; hy,
hypochord; il, ilium; is, ischium; ma, maxilla; mc, metacarpals; me, mentomeckelian; mt, metatarsals; na, neural arches; nc, neural center; np, neopalatine; ns, nasal; pa, palatine; pc, coronoid process; pr, presacral vertebrae; pf, phalanges of feet; ph, phalanges of manus; pm, premaxilla; po, prootic; ps, parasperoid; pt, pterygoid; pv, pre vomer; qj, quadratojugal; qu, quadrate; ra, radius; ru, radioulna; sc, scapula; sm, septomaxilla; sp, sphenethmoid; sq, squamosal; su, suprascapula; ti, tibia; tf, tibiofibula; tl, tibiale; tp, transverse process; ul, ulna; ve, vertebra (including na, nc, pr, tp); vo, vomer.

C = Cranial elements; P = Postcranial elements.

[Rank = absolute time of ossification of various structures simultaneously]
Figure 1

Larval chondrocranium of Dendropsophus labialis (GS 34 - MUJ 9250) and Scinax ruber (GS 34 - MUJ 6178)

A. Dorsal view, B. Ventral view, C. Lateral view, D. Ventral views of hyobranchial apparatus in Dendropsophus labialis (GS36 - MUJ 9250) and Scinax ruber (GS36 - MUJ 3727).

Scale 1 mm. Chondrocranium: a, alae suprarostralis; ci, cartilago infrarostralis; cm, cartilago Meckeli; cqc, commissura quadratocranialis; cs, suprarostral cartilage; ct, cornu trabeculae; fo, fenestra ovalis; jf, jugular foramen; pal, processus anterolateralis; pmq, processus muscularis quadrati; pof, prootic foramen; pq, palatoquadrate; oc, otic capsule; of, oculomotor foramen; opf, optic foramen, ts, tectum sinoticum. Hyobranchial apparatus: ca (I), copula anterioris; cb I–IV; ceratobranchialis I–IV; cp (II), copula posterioris; ct; commissura terminalis; pah, processus anteriors hyalis; palh, processus anterolateralis hyalis; phb, planum hypobranchiale; plh, processus lateralis hyalis; pph, processus posterioris hyalis; pr, pars reuniens; sp, spicula. Blue: cartilage, light blue: fontanella.
Figure 2

Appendicular skeleton of *Dendropsopus labialis* (GS45 – MUJ497) and *Scinax ruber* (GS45 – MUJ6018).

A. Scapula, B. Pectoral girdle, C. Ventral view pelvic girdle. Scale 1 mm. c, clavicle; co, coracoid; e, epicoracoid; ps, processus suprascapularis; o, omosternum; st, sternum; il, ilium; is, ischium; pu, pubis Red, ossified; blue, condrificated.
Figure 3

Dorsal view of manus and pes of *Dendropsophus labialis* (GS45 – MUJ497) and *Scinax ruber* (GS45 – MUJ6018).

A. Manus, B. Pes. Scale 1 mm. ce, centrale; fi, fibulare; mc, metacarpal, mt, metatarsus; ph, prehallux; pr, prepollex; rd; radiale; rad, radioulna; ul, ulnare and intermedium; ti, tibiale. I–V, phalanges. Red, ossified; blue, condrisificated.
Manuscript to be reviewed

A

D. labialis

B

S. ruber

III

II

I

mc

pr

ce

rd

ul

rad

uln

-----

IV

V

mt

ph

ce

ti

fi

PeerJ reviewing PDF | (2017:09:20437:3:0:NEW 26 Feb 2018)
Figure 4

Ventral view of ossification development in vertebral column of *Dendropsophus labialis* and *Scinax ruber* at GS 26-45.

Scale 1 mm. h, hypochord; a, Atlas; np, neural process; d, diapophysis; sd, sacral diapophysis; u, urostyle
Red, ossified; blue, condrificated.