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

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**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 of Neotropical frogs, Colombian species. Using differential staining techniques, we observe and describe the cranial and postcranial development in two hylid species, *Scinax ruber* and *Dendropsophus labialis*, providing new data for more comprehensive ontogenetic studies of Colombian species, Neotropical frogs. We examined specimens tadpoles ranging from Gosner stages 25 to 45. We found differences between the species in the infrarostral and suprarostral cartilages, optic foramen, planum ethmoidale, and the gill apparatus. In both species, one of the first elements to ossify were the atlas and transverse processes of spinal vertebral column and atlas in both species, and the parasphenoid in the skull. These two species showed exhibited the suprascapular processes that has characterized the Hylids that have been described in other Hylids species cleared and stained until now. Although the hylids comprise a large group (over 700 species), postcranial ossification sequence is only known for 15 species. New, therefore, the descriptions of the skeletal development and ossification sequences of larval stages of these two species provided herein, mainly especially data concerning the postcranium, should be contribute with useful information for future analysis of sequential heterochrony in the group, because although the hylids are widely known, there are few works (15 of 700 species) about ossification sequence that include the whole skeleton.
**Introduction**

Comparative morphological descriptions have that compare species and offer information that useful systematists systematic characters have been assessing about anuran relationships from the since the 1960s to the present day, using both molecular and morphological methods. However, most studies of frog morphological characters have widely privileged focus on adults, and tadpoles are often overlooked (Alcalde et al. 2011). Traditionally, of those comparative studies of examining frog larval tadpoles, have most considered external morphological characters and, while most skeletal characters (bones and cartilages) have been often neglected. When, and when skeletal features are considered, studies have been concentrated on describing the chondrocranium is most often more-described, and the than the postcranium is frequently ignored (e.g., Orton 1953; Starrett 1973; Wassersug 1980; Wassersug and Heyer 1988; Haas 2003). The frog family Hylidae is one of the families of frogs that contribute with large and diverse, and frequently the subject of morphological data is Hylidae studies. However, as with other groups, relatively few detailed comparative morphological studies of hylid tadpole skeletal development have been completed. Given the size and most diverse and that has recent taxonomic re-arrangements of the hylids (Duellman et al. 2016; Jungfer 2017), due widely to the diversity and great number of species it represents, it is important to amass as much comparative information. Each character, why not just describe each species individually? What new information does comparing the two species provide? Do the developmental differences relate to adult morphological differences? Providing more justification for the comparative approach in the introduction would make the paper more interesting, and the significance and new knowledge gained by comparing them should be discussed in the conclusions.
description of amphibian is important for development studies for such a large group as hylids. Additionally, the order to give comparative characters for anuran morphology and systematics of species belonging to the family Hylidae, about the group as possible. Thus, there continues to be a pressing need to conduct comprehensive comparative studies of hylid developmental morphology one of the most diverse and that has recently had taxonomic arrangements (Duellman et al., 2016; Jungfer 2017; Heyer 1988; Haas 2003).

Interspecific variations in morphology the family Hylidae presents one of the largest families of frogs with a great number of interspecific variations that have helped to clarify specific taxonomic groups within the Hylidae. It is The family is predominantly distributed across the Neotropical region (Frost 2017; Duellman et al., 2016; Frost, 2017, Duellman et al., 2016) and comprises 706 species commonly subdivided into seven subfamilies: Acridinae, Cophomantinae, Dendropsophinae, Hylinae, Lophophyllinae, Pseudinae, and Scinaxinae (Faivovich et al., 2005; Wiens et al., 2010; Duellman et al., 2016; Frost, 2017). Within the Hylidae, the ossification sequences have been studied for are known for only 15 species, and only of which only eight of those include the postcranial skeleton: eight species: Acris blanchardi (Havens, 2010; Maglia et al., 2007), (Havens, 2010), Boana lanciformis (De Sá, 1988), (De Sá, 1988), Boana pulchella (Hoyos et al., 2012), (Hoyos et al., 2012), Dryophytes chrysoscelis (Sherman and Maglia, 2014), (Sherman and Maglia, 2014), Dryophytes versicolor (Sheil et al., 2014), (Sheil et al., 2014), Hyla orientalis (Yıldırım and Kaya, 2014), (Yıldırım and Kaya, 2014), Osteopilus...
Because identifying variations in developmental morphology and the ossification sequence, placed in a phylogenetic context, will help to recognize and lead to potential informative phylogenetic characters and evolutionary relationships among species (Weisbecker and Mitgutsch, 2010; Harrington et al., 2013). The goal of the present study is to provide a detailed anatomical comparison of the larval morphology (cranial and postcranial development) and (including the sequence of onset of the ossification sequence) information between two species of frage Andean hylids, Dendropsophus labialis and Scinax ruber. These species were chosen to give with the aim of giving a contribution to the knowledge on the timing and sequences of ossification, this baseline information to be used later in comparisons of heterochrony within a phylogenetic context. Additionally, in order to provide comparative characters for anuran morphology and systematics of species belonging to the family Hylidae, one of the most diverse and that has recently had taxonomic arrangements (Duellman et al., 2016; Jungfer, 2017).
Materials and methods

According to the availability of biological material, we cleared and double-stained for cartilage and bone (Dingerkus and Uhler 1977) tadpoles and metamorphs of *Dendropsophus labialis* (N = 32), and *Scinax ruber* (N = 114); the number in each series corresponds to the availability of specimens in 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 Department, 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 metaphorphs were staged according to Gosner (1960). The larval stages of *D. labialis* were collected from the Municipio Tenjo, Cundinamarca Department, 3200 m (MUJ 9250), and adult stages from the Mun. Fomeque, Cundinamarca Dep., 3150 m (MUJ 497). 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 metaphorphs) and adult stages from the Mun. La Dorada, Caldas Dep., 480 m (MUJ 9037). All of these were staged according to Gosner (1960) (1960).

Observations and photographs were made with a stereomicroscope (Advanced optical) equipped with a digital camera (Infinity 1 Lumenera Corporation) with white LED light and Image Pro Insight software (version 8.0.3). 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 Larsen 1998; Maglia and Púgener 1998; Cannatella 1999; Haas 1999; Sheil and Alamillo 2005; Púgener and Maglia 2007; Bowatte and Megaskumbura 2011; Hoyos et al. 2012; adult nomenclature is based on Avilán and Hoyos (2006), using Latin names given by the ICVAN (1973), and taking into account that a Nomina Anatomica Batrachologica does not exist.

We refer to metamorphic climax (MC) sensu Banbury and Maglia (2006) as the Gosner stages at which major modifications and fundamental structural changes occur, resulting in the loss of most larval characters. We also use the term “rank” to refer to the ordinal number within an ossification sequence at which an element begins to ossify. If two or more elements begin ossifying at the same Gosner stage, they were assigned the same rank (i.e., a tie) as per Nunn and Smith 1998. We refer to metamorphic climax (MC) sensu Banbury and Maglia (2006) as the Gosner stages at which major modifications and fundamental structural changes occur, resulting in the loss of most larval characters. We also use the term “rank” to refer to the ordinal number within an ossification sequence at which an element begins to ossify. If two or more elements begin ossifying at the same Gosner stage, they were assigned the same rank (i.e., a tie) as per Nunn and Smith 1998.
Results

Individual skeletal development and sequence of onset of ossification of the cranial and poscranial elements in *Dendropsophus labialis* and *Scinax ruber* are showed in Table 1 and 2, each one showing ossification sequences. Because a marked clear staining ossified elements, thus we increased our sample size. Most of the poorly stained specimens we must element so touched use more specimens than in *D. ruber*, *D. labialis*. Besides, the were between largest stages 26 to 35, the total number of individuals that did not show ossification (GS stages 26 and 35); after Stage 35, specimens stained more clearly specimens with red coloration which allowed to compare these two species stages from 36 onwards.

Chondrocranium

We observed similar the changes in the shape, size, and increase in absorption modification of structures in the development of chondrocranium in *Dendropsophus labialis* and *Scinax ruber* is approximately 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). The basicranial fenestrae did not differentiate with Alcian Blue in both species. We observed a stronger blue coloration in *D. labialis*, and the jugular, (pt), prootic (po) and oculomotor (of) foramen were clearly differentiated, whereas in *S. ruber*, changes in skull were not reflected. In the section "ossification sequence" there is a summary on this subject, but it is not useful to transmit to the reader what are the elements (each one) that appear before or later, and what would be the difference between both species studied in this aspect.

Comment [FS]: 1) I suggest to present the results in a comparative way, and that the idea of the development of the described structures be clearly reflected:

Results:
The data of the tables 1 and 2 would be useful to see in a comparative way. The authors study the development of the skeletal system of two species of anurans, but in the description is not recorded in which stages / moments of development the structures, ossifications, etc., appear. This is a serious problem since the main objective of the work is to describe the development, and such as the results are presented, this is not reflected. In the section "ossification sequence" there is a summary on this subject, but it is not useful to transmit to the reader what are the elements (each one) that appear before or later, and what would be the difference between both species studied in this aspect.

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rubera we could not see the pseudomotor foramen.

tectum sinoticum (i.e.) both represents a quarter of that basis cranii, extending from the frontoparietal fontanelle in both species. The tectum nasi is a roof structure located anteriorly in the nasal region and the ethmoidal plate is forms the floor structure. The tectum nasi is separated from the orbit by a wall, the lamina orbitonasalis (= planum antorbitale sensu XXX). Because of these regions remain are only weakly chondrified, the lamina orbitonasalis is not observable in the tadpole stages, and the nasal capsules are becomes visible in on after metamorphic climax later stages (posterior Stage to the GS42 and beyond). The taenia tecti marginalis is evident and clearly differentiated at by GS37 in D. labialis and at GS35 in S. ruber. In neither species did we observed a frontoparietal fenestra, nor was a taenia tecti transversalis perceived visible directly on the edge of the frontoparietal fontanelle (Fig. 1A). Supraerostral cartilage (cs). In both species, the supraerostral cartilage is composed of a discontinuous cartilaginous plate divided into a corpus supraerostral and a pars alaris, while 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). The fenestrations were not observed in the supraerostral cartilage nor and in the adrostral cartilage near the processus posterodorsalis = processus dorsalis.
posterior, sensu Bowatte and Meegaskumbura 2011, and Bowatte and Meegaskumbura, 2011) and 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) trabecula. The cornua trabeculae are approximately 35% of the total length of chondrocranium (lateral view) in both species; they are and this is shorter and narrower in *D. labialis* than in *S. ruber*. The cornua trabeculæ articulatæ (trabecular horn, sensu Cannatella, 1999) trabecula anteriorly with the corpus rostrale and laterally with the pars alaris of the suprarostral cartilage.

*Cartilago Meckeli* (cm). 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 both the infrarostral cartilage (= commissura intramandibularis sensu Cannatella, 1999) which is composed of two syndesmotically joined flat plates (commissura intramandibularis, sensu Cannatella, 1999) and the processus muscularis quadrati (pmq); the shape of the processus dorsomedialis and the processus ventromedialis are the same in both species. The palatoquadrate cartilage and the commissura quadratocranialis (eqq) are joined anteriorly to the base crani. Laterally, the palatoquadrate cartilage (pq) forms the arcus subocularis. The processus muscularis quadrati is joined to the processus antorbitalis (= pars plana sensu Parker 1876; Parker, 1876; = lamina externa sensu Higgins 1921; Higgins, 1921; = processus antorbitalis sensu Roček 1981, Roček, 1981; = triangular plane sensu Hall and Larsen 1998; Hall & Larsen, 2010).
which 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 & 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 occupying occupies about 20% of the otic capsule.

*Hyobranchial apparatus*. The large ceratohyal has a processus anterioris hyalis, a processus posterioris hyalis (*= more pronounced lateral projection in *D. labialis* than in *S. ruber*). The ceratohyal has a processus anterioris hyalis, and a processus anterolateralis. The first two processes are longer than the processus anterolateralis.

The basihyal plate is oval and extends proximally to the copula anterior (*= basibranchial I* *sensu* Duellman and Trueb 1986; Duellman & Trueb, 1986; *= basihyale* *sensu* Haas 1995; Haas, 1996; and *= sensu* Haas 1997; Haas, 1997; *= copula* *sensu* Maglia and Pügener 1998; Sheil and Alamillo 2005).
and Púgener, 1998; Sheil and Alamillo, 2005) in *D. labialis*, but is absent in *S. ruber*. The basibranchial plate is semi-oval and placed between the two hypobranchial plates (planum hypobranchiale, phb) (= planum hypobranchiale sensu Haas 1999; Haas, 1999; Púgener 1998; Maglia & Púgener, 1998; = plate hyoid sensu Sheil and Alamillo 2005); the 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 (ct) bearing three spicules anteriorly (sp) (Fig. 1D).

**Appendicular skeleton**

*Shoulder girdle*. The pectoral girdle is arciferal in both species. The earliest ossification of clavicle (c), coracoid (co) and scapula appears at GS36 (Fig. 3A). The clavicle and the cleithrum are distinct, and the an epicoracoids cartilage (pe) is prominent between the clavicle and the coracoid. The epicoracoids are not mineralized in tadpoles. In *D. labialis*, the omosternum (o) in *D. labialis* 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 are formed by the epicoracoidsss and the sternum element (mesosternum) joining 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 in both species. The ilium begins to ossify during development in D. labialis at GS41 and in S. ruber at GS39/40, and articulates anteriorly with the ventral surface of the lateral margin of the sacral diapophyses at GS42. The iliac crest appears dorsally prominent; the primordia of the pubis and the ischium appear at GS36, and are synchondrotically fused at GS38 in both species. The sacral diapophysis is wider in D. labialis than in S. ruber. The pubis is totally fused at GS40. The pelvic girdle is completely ossified with the halves fused at the midline, extending anterodorsally forming an angle of 55° with the head of the femur by GS45 (Fig. 3B).

Forelimb and hindlimb. 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 by GS41 and S. ruber (postmetamorphic); these elements are fused in both species. Primordia of the four carpal and five tarsal elements appear at 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 (mc) 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 only in D. labialis, although all phalanges are ossified at GS45 in both species (Fig. 3). The carpals
stay remain were cartilaginous in the all specimens and stages examined, and the
distal tarsals remain were cartilaginous in *S. ruber*. The relative size of carpal
elements *(mc)* is 3 < 4 < 1 < prehallux *(ph)* and the tarsal elements *(mt)* is 4 < 5
< 3 < 2 < 1 < propollex *(pr)*. Sesamoids are absent from GS25 to GS45. In the
Figure 3A shows the limb elements *(showed elements as central *(ce)*, fibulare *(fi)*, radiale *(rd)*, tibiale *(ti)*, ulnare and intermedium) at Stage XXX *(ul)*.

**Axial skeleton**

The vertebral column is composed of eight procoelous presacral vertebrae, the
sacrum, and the urostyle. The notochord *length* diminishes as the tadpoles grow,
reaching a *total* and is *complete resorption* resorbed at 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, included eight
presacral vertebrae, the sacrum, the urostyle *(u)* and the hypochord *(hy)*. The
sacral diapophyseal *(sd)* primordia are cylindrical. The last postsacral vertebra (first
coccygeal or *vertebra Vertebrar* X sensu Haas 1999) and the second cocygeal
vertebra *ossify* only in *D. labialis* at by GS45. Simultaneous to they with
the absorption of the ossification of presacral vertebrae, there is *notochord*
absorption—the cocygeal elements *fusiform* and form the urostyle *formation*.
The urostyle has a bicoudyr articulation with the sacral vertebra and the condyles
are widely separated *(Haas 1999)* in both species (Fig. 4).
The atlas *(a)* is concave at its point of articulation with the convex occipital
condyles at the base of the skull. Semicircular *procoelous* *(sensu Jolie 1962)*
vertebral centrum begins to develop as procoelous (sensu Jolie 1962) Jolie 1962) vertebras early as GS31 in D. labialis and at GS32 in S. ruber, increasing the thickness of both the neural arches and the transverse process. The neural arches appear as cartilaginous cartilage at GS33 in both species; these are complete 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). Both 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 of the two species was the GS25. Ossification timing in D. labialis begins appears at by GS34 and in S. ruber at GS35 (Fig. 1A). Ossification in D. labialis begins with the atlas and the transverse processes, whereas in S. ruber it begins with the parapophoid, the I-VII Transverse processes Processes I-VII and neural arches Arches I-III. The ossification sequence was constructed with the first appearance of bone. We perceived observed that the metamorphic climax (MC) begins at GS41 in D. labialis, and GS39-40 in S. ruber, at GS39-40. We identified seven ranks (I-VII) in D. labialis and five ranks (I-V) in S. ruber (Tables 1 and 2). The GS wherein which we found ossified elements (or bones) were perceptible in D. labialis were from GS35 to GS45, with 46 ossified elements, and from GS36 to GS43 in S. ruber from GS36 to GS43, with 26 ossified elements. S. ruber showed the number of ossified elements of each specimen according to the Gosner stage was more variable.
The MC Metamorphix climax in *D. labialis* was at GS 45 with 14 ossified elements, and in *S. ruber* at GS 39-40 with seven ossified elements. Of these, the structures in common were the femur, tibia, fibula, humerus, ilium, and radioulna (rad). The two species present both differences and similarities with respect to the ossification of the chondrocranium and postcranium.
Notwithstanding the fact that Colombia is housing the second richest country in the planet, after Brazil, concerning the largest number of hylid species on the planet, there are very few previous studies about have considered developmental ossification sequences of Colombian hylids. The species of the family Hylidae show variation in the has gone through a number of taxonomic re-arrangements, as it was showed by the variety of 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.

The Previous studies about of the cranial morphology in hylid tadpoles include being known in Acris crepitans (Maglia et al. 2007); Boana pulchella (Hoyos et al. 2012); Boana raniceps and Boana lanciformis (Alcalde and Rosset 2003); Dendropsophus nanus (Fabrezi and Lavilla 1992; Alcalde and Rosset 2003; Vera Candioti et al. 2004; Vera Candioti 2014); Boana pulchella (Hoyos et al. 2012); Julianus species: J. acuminatus (Fabrezi and Lavilla 1992; Faivovich 2002; Alcalde and Rosset 2003; Alcalde et al. 2011; Alcalde et al. 2011; Faivovich 2002); J. uruguayus. J. aff. pinimus (Alcalde et al. 2011; Rodrigues et al. 2017); Oloolygon species: Oloolygon
aromothyella and O. berthae (Rodrigues et al. 2017; Alcalde et al. 2011; Faivovich 2002); O. skuki (Rodrigues et al. 2017); Scinax species: Scinax granulatus and S. squalirostris (Rodrigues et al. 2017; Alcalde and Rosset 2003; Alcalde and Rosset 2003); S. boulengeri (Rodrigues et al. 2017; Vera Candioti 2007; Vera Candioti 2007); S. fuscovarius (Rodrigues et al. 2017; Fabrezi and Vera 1997;Fabrezi and Vera 1997); Ololygon orientalis (Vera 1997); S. nasicus (Rodrigues et al. 2017; Vera Candioti 2007; Fabrezi and Vera 1997); Scinax granulatus (Vera Candioti 2007); S. boulengeri (Rodrigues et al. 2017; Vera Candioti 2007; Fabrezi and Vera 1997); O. skuki (Rodrigues et al. 2017); Scinax species: Scinax granulatus and S. squalirostris (Alcalde and Rosset, 2003); S. boulengeri (Vera Candioti 2007); S. fuscovarius (Fabrezi and Vera 1997); S. nasicus (Fabrezi and Vera, 1997; Vera 1997); S. ruber (Haas 1996). For a complete overview of the findings of these studies, please see Appendix 1. Vera Candioti 2007). The studies about cranial morphology in hyloid tadpoles being known in Acris crepitans (Maglia et al., 2007); Boana raniceps (Alcalde and Rosset, 2003); Dendropsophus nanus (Fabrezi & Lavilla 1992; Vera Candioti et al., 2004); Hyla orientalis (Yildirim and Kaya, 2014); Boana pulchella (Hoyos et al., 2012); Julianus species: J. acuminatus (Fabrezi and Lavilla, 1992; Alcalde et al., 2011; Faivovich, 2002); J. uruguayus, J. aff. pinimus (Alcalde et al., 2011); Ololygon species: Ololygon aromothyella and O. berthae (Alcalde et al., 2011; Faivovich, 2002); O. skuki (Rodrigues et al., 2017); Scinax species: Scinax granulatus and S. squalirostris (Alcalde and Rosset, 2003); S. boulengeri (Vera Candioti 2007); S. fuscovarius (Fabrezi and Vera 1997); S. nasicus (Fabrezi and Vera, 1997; Vera Candioti et al., 2004; Vera Candioti, 2007).

The chondrocranial morphology and hyobranchial apparatus development is generally similar between both the species examined herein and those previously studied, but we did found identify several differences between the two species.
in: 1) the shape of the suprarostral, 2) the size and width of infrastral cartilages, 3) the length of processus articularis, 4) the thickness of palatoquadrate, 5) the size and width of infrarostral cartilages, 6) the presence of an operculum and processus posterolateralis of the auditory capsule, 7) the thickness of the processus muscularis quadrati, 8) the presence of a quadratoethmoid and pseudopterygoid, 9) the attachment of the ascending process to the braincase, 10) the thickness of the planum ethmoidale, 11) the development of the branchial apparatus, 12) the presence of copula I, and 13) the type of junction between the ceratobranchia and planum hypobranchiale (Fig. 1 and 2). These differences likely represent species-specific differences among the taxa examined. There are no admandibular cartilages attached to the anteroventral margin of the cartilago Meckeli as described by Hoyos et al. (2012) in Boana pulchella.

The overall similarities in the skeletal development skeleton in tadpoles of Scinax ruber (Scinaxinae) and Dendropsophus labialis (Dendropsophinae) relative to other hylids for which similar data are available could be an indication of a recent consequence of common origin, or— but also— could be a consequence of homoplasy with independent origins. However, the absence of additional morphologic data in combination with molecular data on other species, it is not possible to answer this question does not allow a clear comparison.

Several of the differences between the two species examined present interesting avenues for future examination. For example, the processus ethmoidalis of the
quadrato in *S. ruber* is wide, and it is not clearly distinct from the processus articularis, while *D. labialis*, the processes are easily distinguishable, and similar to that described by Alcalde and Rosset (2003), who also found the same similar features in *Hyla raniceps* comparing compared with to the *Scinax* species group (*S. squalirostris* and *S. granulatus*, *Scinax ruber* group). The palatoquadrate is similar between the species but the processus ascendens of the palatoquadrate in *D. labialis* is wider than in *S. ruber*, while and the distal side of the cornu trabeculae extends posteriorly toward the otic capsule, and the anterior region of the palatoquadrate is distinctively broader in *S. ruber* than in *D. labialis*, and in *S. ruber* the dorsomedial process is wider than the ventromedial process in *D. labialis*. 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 outstanding anatomical structures of the otic capsule are functionally related to To perception of vocalizations of their own (i.e., same species recognition) species in adult setages, but experiments must be conducted to check 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 the abundant chondrification of these parts or to the early developmental stages of larvae in this region, which allowed differentiation of craniopalatine carotid
foramina, contrary to what was found by Alcalde and Blotto (2006). Although the sample size for the *D. labialis* is very small in comparison with *S. ruber*, *Dendropsophus D. 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. Scinax ruber* showed ossification variations in various elements relative to total individuals ossified regarding. *D. labialis* presented uniformly stained (ossification ossified) elements in all individuals stained and cleared (ossification Table 3). This variation between *S. ruber* and *D. labialis* (Table 3). This variation suggests that may be caused by the intrinsic factors that determines the timing of development or because extrinsic factors may affect the osteogenesis (Vera and Ponsa 2014). It may not be a coincidence that On the other hand, *S. ruber* is a generalist species and *D. labialis* is an endemic species (Frost 2017) (Vera and Ponsa, 2014).

Haas (1996) identified (1996) observed reported that the Ceratohyalia II-IV are fused in *Scinax ruber* and *Megophrys montana nasuta*, characteristics that separating separate them from other species. This character has to be taken into account when taxonomic studies based on morphology are carried on. This character state was found confirmed in *S. ruber* but not in *D. labialis*. The ceratohyal in *D. labialis* presents a process on the articular condyle that is not present in *S. ruber*. Alcalde and Rosset (2003) (2003) found this process in both *S. granulatus* and *S. squamilostris*. Spicules I-III on the posterior margin of the
hypobranchial plate are present in *D. labialis* and *S. ruber*, but the spicule IV is not.

Copula II *cp* (II) is present in both species, but the Copula I *ca* (I) is present in *D. labialis*—absent in *S. ruber* and it is present in *D. labialis*, just as in *S. squalirostris*—but absent in *S. ruber* and *S. granulatus*, *Boana* *Hyla* *Hyla raniceps* (Alcalde and Rosset 2003) and *Tlalocophyla smithii* (Vera and Haas 2004). *Dendropsophus nanus* (Vera and Haas 2004) is absent. Although the presence of Copula I is extremely variable in hylids, and is shared by all non-hylid macrophages (Vera and Haas 2004), a relationship between this structure and the ecological function that it can perform (e.g., prey utilization), for example in relation to the type of food, has not been found/identified.

The Additional characteristics of the developmental morphology of these species align them with other hylids that have been studied previously. For example, the urostyle of *D. labialis* and *S. ruber* presents a bicondylar articulation with the sacral vertebra and the condyles are widely separated. The shoulder girdle of both tadpoles and adults presents 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 species of the family *Hylidae*: *Hypsiboas lanciformis* (De Sá 1988), *Boana pulchella* (Hoyos et al. 2012), *Pseudacris crucifer*, *Acris blanchardi*.
The variation of larval characters has been evaluated in the genera between *Scinax* and *Dendropsophus* in have been included in several phylogenetic studies between *Scinax* and *Dendropsophus* in 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 the fact that elements *Scinax ruber* exhibit more intraspecific variability than in *D. labialis* (see Table 3).

Regarding the ossification sequence, the first bones ossified in the cranium were the exoccipital, the frontoparietal and the parasphenoid after GS 36. Haas (1999) found that in *S. ruber*, and in other species of *Hyla* (some species named after *Dendropsophus*), this occurred one stage later, by at GS 37. Similar to those that Haas (1999) described for other species of Hylidae, 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, as Haas (2003) recorded for *Litoria nannotis*, similar to those that Haas (1999) described for other species of Hylidae. Haas (1999) also recorded the time of the first ossification of the transverse processes of the presacral vertebrae as the first to ossify—i.e., we found that it was the

Comment [F23]: Line 317: Regarding the ossification sequence, the first bones ossified in the cranium were the exoccipital, the frontoparietal and the parasphenoid after GS 36. I'm a bit confused—this doesn’t seem consistent with the tables. In fact, in Table 2, shouldn’t exoccipital be listed at GS38? There is little in terms of conclusion beyond the fact that the data might be useful in future studies. I think the paper might be more interesting if you used the conclusions to comment/speculate on why you think there might be differences among these two species. Just a thought.
ossification of Neural Arches I through IX in *D. labialis* (GS37) and I through III in *S. ruber* (GS36) identified observed these elements beginning at GS36 in both species. The ossification of the neural arches I to IX was in *D. labialis* (GS37) whereas in *S. ruber* (GS36) was I to III; the ossification of the arcs in these species is completed in the GS37. Interestingly, the first elements to ossify were those from the postcranium: the transverse process of the vertebral column and the atlas in *D. labialis* (Figure 4). The ossification process of the vertebra in the two species started ventrally from the centrum of each vertebra, and followed dorsally to close the vertebrae in advanced stages, as Haas (2003) recorded for *Litoria nannotis*. The differences between the ossification sequences of these two species are also evident with regard to the ossification ranks and number of ossified bones; in particular, *D. labialis* presented the highest number of ranks in the sequence and more elements that begin ossification prior to metamorphosis, the process of ossification. 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 changes, it is only a relative measure of timing and should not be used as a way to compare between species. Instead, we compared the occurrence of events in the development of each species is the relative timing of each event in the ossification sequence, allowing us to compare the relative timing of developmental events across different taxa. For this reason we did not take the developmental GS and we take the by examining the order of beginning of
the onset of ossification of each element, because these are based on the appearance of external morphological structures that are the same in both species studied. Moreover, Nunn and Smith (1998:86) considered, "that ontogeny may be ordered by age, size, or stage; none of these measures are useful for comparing ontogeny across significantly divergent taxa".

Analysis of the ossification sequences with different methodologies, for example Parsimov, could be found that the heterochronic cranial elements, or ossification timing appearing at different times, were the parasphenoid and prootic when we compared S. ruber with H. pulchellus (Hoyos et al. 2012), but in Dendropsophus labialis and Pseudis platensis were the frontoparietal, dentary and maxilla (Fabrezi and Goldberg 2009). Ossification sequences of D. labialis and S. ruber showed that the ossification timing of the parasphenoid was at the first rank. Meanwhile, the timing of ossification of the exoccipital, frontoparietal and prootic was at the second rank in both species. Cranial and postcranial characters described (Table 4 outlines the ossification sequences of different species of the family Hylidae. A) show that among these Hylid species, the number of ranks that includes elements of the skull and postcranium vary from one to five. The number of ranks increase when postcranial elements are included; the information lost in the ossification sequences, when the postcranial elements are excluded, showed postcranial elements demonstrating the importance of these structures, including not excluding them.

Weisbecker and Mitgutsch (2010), Harrington et al. (2013), and Sheil et al. (2014) used these similar ranked ossification sequence data for to
reconstruct phylogenetic trees of amphibians with sequences of cranial and postcranial elements of Leptodactylidae, Ranidae and Bufonidae. These researchers suggested to advocate for using cranial and post-cranium data, relating them to the type of development exhibited by the species, and to include ossification sequence information from fossils too, as far as possible when available.

Although the morphology and systematics of amphibians are organisms that 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), we need to include in a phylogeny the characters these researchers suggest is to deep additional comprehensive descriptions of the changes occurring in the appearance of skeletal development and ossifications. To employ all this include both cranial and post-cranion, relating them to contribute to the are needed to truly understand patterns of, of heterochrony in the groupies, development including sequences of fossils. Some of the biological implications of the heterochronies, which are well known in amphibians (Alberch 1985; Reilly et al. 1991), (Alberch 1985; Reilly et al. 1991) are to observe changes in the shape and size of anatomical structures in shape and size, and the evolution of changes in the relative rates of growth of developmental events among taxon and 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 reveal interactions or restrictions on the development with individual events of individual morphologies (Wagner, 1996). Studies that have used statistical methods (e.g., Parsimov) to analyze ossification sequences with different methodologies, for example Parsimov, could be found that have revealed heterochrony in the timing of onset of ossification in some cranial elements, such as the heterochronic cranium elements, or ossification timing appearing at different times, were the parasphenoid and prootic when we compared in S. ruber with H. pulchellus (Hoyos et al., 2012) or the frontoparietal, dentary and maxilla, but in Dendropsophus labialis and vs. Pseudis platensis were the frontoparietal, dentary and maxilla (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 showed that the ossification timing of the parasphenoid was at the first rank. Meanwhile, the timing of ossification of the exoccipital, frontoparietal and prootic was at the second second rank in 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 of the ossified elements between these species could be linked with the specific genes involved
We should note that it is possible that the difference in cartilage formation between the two species is due to paracrine factors induced in cells that express mesodermal transcription factors involved in the activation of gene-specific to cartilage (Gilbert 2000; Kozhemyakina et al. 2015); the present study did not account for these factors.

The detection of more intraspecific variability in *Scinax ruber* than in *Dendropsophus labialis* could also be due to the presence of the more great intrageneric diversity within their *Scinax ruber* clade. Within *Scinax ruber*, this issue has been poorly studied, then it could be studied in greater detail when comparing the adults of these species. (Fig. 5). Although amphibians are organisms that have been extensively studied (Canatella and Trueb 1988; de Sá and Hillis 1990; Canatella and Trueb 1988; de Sá and Hillis 1990; Haas 2001; Baez and Pugener 2003; Roelants and Bossuyt 2005; Faivovich et al. 2005, Frost et al. 2006; Pyron and Wiens 2011; Duellman et al. 2016) they would solve taxonomic and morphology voids.

However, the generality of this sentence makes it empty and the relationship with the previous sentence is not followed.

Comment [F34]: but why this would explain the variability within od D. labialis

Comment [AMM33]: I think this whole paragraph needs to be explained a little more. Not sure where you are trying to go with this.
Conclusions

The contribution of the ontogenetic data (development and ossification sequences of skeletal structures of skeleton and ossification sequences) provided herein is supported by the provides further information information to help knowledge that affords opportunity to understand the interactions between ontogeny and phylogeny in morphological and ecological diversity of frog genera. Likewise, ossification sequences data for the skeletal elements, combined with evolutionary hypotheses, may shed light about on patterns of development to support be used in future phylogenetic hypotheses. Aas Larson et al. (2003) claimed suggested, “the variation in chondrocranial morphology in larval anurans can be phylogenetically informative, even among closely related taxa”.

The great diversity that exists among tadpoles, especially for the type of food, are closely related to the type of maxilas they have, in addition larval forms could help predict characters in adults.

Acknowledgements

We thank to the Pontificia Universidad Javeriana and the Paläontologisches Institut und Museum, Universität Zürich, for partial funding of this work. We want to thank Timothy Sosa for help assistance with the English translation.

The authors declare that they lack have no conflicts of interest of any kind.

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https://doi.org/10.1046/j.1463-6395.2003.00140.x

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Laurenti JN (1768) Specimen medicum, exhibens synopsin reptilium emendatum cum experimentis circa venena et antidota reptilium austriacorum. Wien, Austria: Joan. Thom. nob. de Trattnern.


https://doi.org/10.1111/azo.12066


https://doi.org/10.1002/jmor.10362


https://doi.org/10.1002/jmor.10239


Comment [F36]: The figures could be much more detailed—for example, are the craniopalatine and carotid foramina in *D. labialis* visible? They aren't shown in Figure 1. You describe them in text but do not show them in the illustration.

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The discussion section should be focused to compare related species. In this sense, S. ruber should be compared with the information available for Scinax species (i.e. Alcalde and Rosset, 2003; Alcalde et al., 2011), and inferences (with caution by the absence of data in most taxa) with julianus and other Scinaxinae (like Ololygon), in the framework of Hylidae. In Dendropsophus, the authors omitted references useful to make comparisons (see above) and the result obtained should be discussed considering the complete framework of Hylidae. The similitudes observed between between taxa not closely related: Dendropsophus labials
(Dendropsophinae) and Scinax ruber (Scinaxinae) could be consequence of common origin, but also could be consequence of homoplasy with independent origin. So the absence of data not allow a clear comparison, and result obtained should be discussed considering the complete framework of Hylidae. The similitudes observed between between taxa not closely related:

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Validity of the findings

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