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

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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.

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9 **Short Title:** Skeletal development of two hylids

11 **Abstract** Although comparative studies of anuran ontogeny have provided new data

12 on heterochrony in the life cycles of frogs, most of them have not included ossification

13 sequences. Using differential staining techniques, we observe and describe differences

14 and similarities of cranial and postcranial development in two hylid species, *Scinax*

15 *ruber* (Scinaxinae) and *Dendropsophus labialis* (Hylinae), providing new data of

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17 Gosner Stages 25 to 45. We found differences between species in the infrarostral and

18 suprarostral cartilages, optic foramen, planum ethmoidale, and gill apparatus. In both

19 species, the first elements to ossify were the atlas and transverse processes of

20 vertebral column and the parasphenoid. Both species exhibited suprascapular

21 processes as described in other hylids. Although the hylids comprise a large group

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24 provided herein will be useful for future analyses of heterochrony in the group.

Introduction

26 Comparative morphological descriptions for a specific group of frogs have provided

27 useful systematic characters since the 1960 (e.g. Canatella, 1999; Duellman, Marion

28 and Hedges, 2016). However, most studies of frog morphological characters focus on

29 adults (Faivovich, 2002; Faivovich et al., 2005; Maglia et al. 2007; Wiens et al, 2010;

30 Pyron and Wiens, 2011; Yıldırım and Kaya, 2014; Duellman et al., 2016), and tadpoles

31 have often been overlooked (Alcalde et al., 2011). Of those comparative studies that

32 examine tadpoles, most consider external morphological characters and skeletal

33 characters are often neglected (Fabrezi and Lavilla, 1992; Faivovich, 2002; Maglia et

34 al., 2007; Hoyos et al., 2012; Yıldırım and Kaya, 2014). When skeletal features are

35 considered, the chondrocranium is most often described, while the postcranium is

36 frequently ignored (e.g. Orton, 1953; Starrett, 1973; Wassersug, 1980; Wassersug and

37 Heyer, 1988; Haas, 2003). However, as with other groups, relatively few detailed

38 comparative morphological studies of hylid tadpole skeletal development have been

39 completed. Given the diversity and recent taxonomic re-arrangements of the hylids

40 (Duellman et al., 2016; Jungfer, 2017) is important to amass as much comparative

41 information about the group as possible. Thus, there continues to be a pressing need to

42 conduct comprehensive comparative studies of hylids developmental morphology.

43   Interspecific variations in morphology help to clarify taxonomic groups within in the

44 Hylidae. The family is predominantly distributed across the Neotropical region (Frost,

45 2018; Duellman et al., 2016) and comprises 706 species grouped into seven

46 subfamilies: Acridinae, Cophomantinae, Dendropsophinae, Hylinae, Lophyophylinae,

47 Pseudinae, and Scinaxinae (Faivovich et al., 2005; Wiens et al., 2010; Duellman et al.,

48   2016; Frost, 2018). Ossification sequences are known for only 15 species, and only

49   eight of those include the postcranial skeleton: *Acris blanchardi* (Havens, 2010; Maglia

50   et al., 2007), *Boana lanciformis* (former *Hyla lanciformis,* De Sá, 1988), *Boana pulchella*

51   (former *Hypsiboas pulchellus* Hoyos et al., 2012), *Dryophytes chrysoscelis* (former *Hyla*

52   *chrysoscelis,* Sherman and Maglia, 2014), *Dryophytes versicolor* (former *Hyla*

53   *chrysoscelis,* Sheil et al., 2014), *Hyla orientalis* (Yıldırım and Kaya, 2014), *Osteopilus*

54   *septentrionalis* (Sheil et al., 2014), *Pseudacris crucifer* (Havens, 2010).

55   Because identifying variations in developmental morphology and ossification sequence

56   can lead to informative phylogenetic characters (Weisbecker and Mitgutsch, 2010;

57   Harrington, Harrison, Sheil, 2013), we provide a detailed anatomical comparison of the

58   cranial and postcranial development (including the sequence of onset of ossification)

59   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

83   Larsen (1998), Maglia and Pugener (1998), Cannatella (1999), Haas (1999), Sheil and

84   Alamillo (2005), Pugener and Maglia (2007), Bowatte and Meegaskumbura (2011),

85   Hoyos et al. (2012), adult nomenclature is based on Avilán and Hoyos (2006), using the

86   Latin names given by the ICVAN (1973).

87   The ossification sequence was determined by the first appearance of ossified bone.

88   We refer to metamorphic climax (MC) *sensu* Banbury and Maglia (2006) as the Gosner

89   stages (GS) at which major modifications and fundamental structural changes occur,

90   resulting in the loss of most of the larval characters. We also used the term "rank" to

91   refer to the ordinal number within an ossification sequence at which an element begins

92   to ossify. We note the first time any specimen at that stage showed stain. If two or more

93   elements begin ossifying at the same time Gosner stage, they were assigned the same

94   rank (i.e. a tie) as per Nunn and Smith (1998).

95 **Results**

96 Skeletal development and sequence of onset of ossification of the cranial and

97 postcranial elements of *Dendropsophus labialis* and *Scinax ruber* are showed in Table 1

98 and 2. Many young specimens (between Stages 26 and 35) stained poorly, however we

99 had sufficient sample size assess ossification sequence. Specimens older than Stage

100 35 stained more reliably.

101 **Chondrocranium**

102 We observed similar changes in the shape, size, and modification of structures in the

103 development of chondrocranium in the two species. The elements of the skeleton were

104 compared according to the initiation of ossification and not with a specific stage,

105 because in the two study species the ossification occurred in different Gosner stages

106 (Table 3). The overall width of the chondrocranium in *Dendropsophus labialis* and

107 *Scinax ruber* is *roughly* 80-90% of this total length (Fig 1). The chondrocranium in *D.*

108 *labialis* is wider (dorsal view) and lower (lateral view) than *S. ruber* (Fig 1A, 1B, 1C).

109 Basicranial fenestrae did not differentiate with Alcian Blue in either species. We

110 perceived a stronger blue coloration in *D. labialis*, and the jugular, prootic, and

111 oculomotor foramen were clearly defined, whereas in *S. ruber* we could not see the

112 oculomotor foramen.

113 The cartilaginous regions of the taenia tecti medialis and tectum sinoticum both

114 represent a quarter of the basis cranii, extending from the frontoparietal fontanelle in

115 both species. The tectum nasi roofs the nasal region, and the ethmoid plate forms the

116 floor. The tectum nasi is separated from the orbit by a wall, the lamina orbitonasalis

117 (=planum antorbitale *sensu* Canatella, 1999). Because these regions are weakly

118 chondrified, the lamina orbitonasalis is not observable in the tadpole stages, and the

119 nasal capsules become visible after metamorphic climax (stage 42 and beyond). The

120 taenia tecti marginalis is evident and clearly differentiated by GS37 in *D. labialis* and by

121 GS35 in *S. ruber*. In neither species did we observe a frontoparietal fenestra, nor was a

122 taenia tecti transversalis visible on the edge of the frontoparietal fontanelle (Fig. 1A).

123 *Suprarostral cartilage.* In both species, the suprarostral cartilage is composed of a

124 discontinuous cartilaginous plate divided into a corpus suprarostralis and a pars alaris;

125 posterolaterally we observed a distal syndesmotic junction between the corpus and the

126 ala. The ala has three processes: two rounded anterolateral processes that join

127 syndesmotically with the cornu trabecula, and one process posterolaterally (Fig. 1C).

128 Fenestrations were not observed in the suprarostral cartilage nor, in the adrostral

129 cartilage near the processus posterodorsalis (=processus dorsalis posterior, *sensu*

130 Bowatte and Meegaskumbura, 2011). In *D. labialis* the corpus suprarostral is curved,

131 while in *S. ruber* it is straighter and wider distally, articulating proximally with the cornu

132 trabecula (trabecular horn, *sensu* Cannatella, 1999). The cornua trabecula are

133 approximately 35% of the total length of chondrocranium (lateral view) in both species,

134 but they are shorter and narrower in *D. labialis* than in *S. ruber*. The cornua trabeculae

135 articulate anteriorly with the corpus rostrale and laterally with the pars alaris of the

136 suprarostral cartilage.

137 *Cartilago Meckeli.* The cartilago Meckeli (= Meckel's cartilage, *sensu* Cannatella,

138 1999) has three processes: the retroarticular (short and blunt), the dorsomedial, and the

139 ventromedial. These processes articulate with the infrarostral cartilage (commissura

140 intramandibularis, *sensu* Cannatella, 1999) which is composed of two syndesmotically,

141   joined flat plates, and the processus muscularis quadrati. The shape of the processus

142   dorsomedialis and the processus ventromedialis are the same in both species. The

143   palatoquadrate cartilage and the commissura quadratocranialis are joined anteriorly to

144   the base cranii. Laterally, the palatoquadrate cartilage forms the arcus subocularis. The

145   process muscularis quadrati is joined to the processus antorbitalis (= pars plana *sensu*

146   Parker, 1876; = lamina externa *sensu* Higgins, 1921; = processus antorbitalis *sensu*

147   Roček, 1981; = triangular plane *sensu* Hall and Larsen, 1998 = cartilaginous planum

148   triangulare *sensu* Pugener and Maglia, 2007) anterolaterally, projecting above the cornu

149   trabecula. The processus hyoquadrati of the palatoquadrate cartilage articulates

150   ventrally with the ceratohyalia of the hyobranchial apparatus (Fig. 1D).

151   *Otic capsule*. This structure is longer and higher than wide, occupying about a fifth of

152   the total length of the skull. The crista parotica exhibits a more pronounced lateral

153   projection in *D. labialis* than in *S. ruber*. The crista parotica is laterally developed,

154   forming a small processus posterolateralis (= processus lateralis posterior *sensu*

155   Bowatte and Meegaskumbura, 2011) and a small processus anterolateralis (more

156   developed in *D. labialis*). The processus anterolateralis projects vertically, descending

157   obliquely and overlapping the ventral posterolateral margin of the palatoquadrate

158   cartilage. The otic capsule is perforated by the fenestra ovalis, which occupies about

159   20% of the otic capsule.

160   *Hyobranchial apparatus*. The large ceratohyal has a processus anterioris hyalis, a

161   processus posterioris hyalis, and a processus anterolateralis hyalis. The first two

162   processes are longer than the third, which extends to meet the transverse crease of the

163   processus lateralis hyalis.

164 The basihyal plate is oval and extends proximally to the copula anterior (= Basibranchial

165 I *sensu* Duellman and Trueb, 1986; = basihyale *sensu* Haas, 1995 and Haas, 1997; =

166 copula I *sensu* Maglia and Pugener, 1998; Sheil and Alamillo, 2005) in *D. labialis*, but is

167 absent in *S. ruber*. The basibranchial plate is semi-oval and located between the two

168 hypobranchial plates (= planum hypobranchiale *sensu* Haas, 1999; = *plate hyoid sensu*

169 Maglia and Pugener, 1998; = *hyobranchial plate sensu* Sheil and Alamillo, 2005), and a

170 branchial bridge is present in both species, being wider in *S. ruber* than in *D. labialis*.

171 The junction between each ceratobranchium and the planum hypobranchiale is

172 syndesmotic. The ceratobranchia are united posteriorly by the commissura terminalis

173 and bear three spicules anteriorly (Fig. 1D).

174   The chondrocranial morphology and hyobranchial apparatus is generally similar

175 between the species examined here and those previously studied. However, we did

176 identify several differences between *S. ruber* and *D. labialis*, including: 1) the shape of

177 the suprarostral, 2) the size and width of infrarostral cartilages, 3) the length of

178 processus articularis, 4) the thickness of palatoquadrate, 5) the size of optic foramen, 6)

179 the presence of an operculum and processus posterolateralis of the otic capsule, 7) the

180 thickness of the processus muscularis quadrati, 8) the attachment of the ascending

181 process to the braincase, 9) the thickness of the planum ethmoidale, 10) the

182 development of the branchial apparatus,11) the presence of Copula I, and 12) the type

183 of junction between the ceratobranchia and planum hypobranchiale (Fig. 1 and 2).

184 These differences likely represent species-specific differences between the two taxa

185 examined.

186   **Appendicular skeleton**

187   *Shoulder girdle.* The pectoral girdle is arciferal in both species. The earliest

188   ossification of the clavicle, coracoid, and scapula appears at GS36 (Fig. 3A). The

189   clavicle and the cleithrum are distinct, and an epicoracoid cartilage is prominent

190   between the clavicle and the coracoid. The epicoracoids are not mineralized. In *D.*

191   *labialis* the omosternum is elongated, and the sternum has two projections. The

192   omosternum and the sternum are oval in *S. ruber*. The clavicle articulates with the

193   coracoid, which is ossified in *D. labialis* at GS41 and in *S. ruber* at GS46. The sternum

194   is formed by the epicoracoid and the mesosternum, which joins the medial junction of

195   the epicoracoids (Fig. 2B).

196   *Pelvic girdle.* In both species, the primordium of the ilium appears at GS34 and is fully

197   developed by GS41. The ilium begins to ossify by GS41 in *D. labialis* and by GS39/40 in

198   *S. ruber* and articulates anteriorly with the ventral surface of the lateral margin of the

199   sacral diapophyses by GS42. The iliac crest appears dorsally prominent. The primordia

200   of the pubis and the ischium appear at GS36, and are synchondrotically fused by GS38

201   in both species. The sacral diapophyses is wider in *D. labialis* that in *S. ruber*. The pubis

202   is completely fused by GS40. The pelvic girdle is completely ossified with the halves

203   fused at the midline, extending anterodorsally forming an angle of 55º with the head of

204   the femur by GS45 (Fig. 3).

205   *Fore limb and hind limb.* The first cartilaginous elements of the forelimbs (radius, ulna,

206   and humerus) appear at GS32, and those of the hindlimbs at GS33 (femur, tibia, and

207   fibula). The tibia and fibula are fused in *D. labialis* by GS41 and in *S. ruber* by GS38.

208   We observed ossification of the radius and ulna in *D. labialis* (GS41) and *S. ruber* (post

209   metamorphic). The radius and ulna are fused in both species. Primordia of the four

210   carpal and five tarsal elements appear by GS33 and complete development by GS41.

211   The phalangeal carpal formula is 3-3-4-4 and the phalangeal tarsal formula is 3-3-4-5-4

212   in both species. Metacarpals are curved and phalanges are cylindrical, having a conical

213   shape at the tip of the terminal phalanges. Digits IV (manus and pes) and V (pes) begin

214   to ossify by GS42 in *D. labialis*, although all phalanges are ossified at GS45 in both

215   species (Fig. 3). The carpals were cartilaginous in all specimens and stages examined,

216   and the distal tarsals were cartilaginous in *S. ruber*. The relative size of carpal elements

217   is 3 < 4 < 2 < 1< prehallux and the tarsal elements is 4 < 5 < 3 < 2 < 1 < prepollex.

218   Sesamoids are absent from GS25 to GS45. Figure 3A shows the limb elements

219   (central, fibulare, radiale, tibiale, ulnare, and intermedium) at Stage 45.

220   **Axial skeleton**

221   The vertebral column is composed of eight procoelous presacral vertebrae, the sacrum,

222   and the urostyle. The notochord diminishes as the tadpoles grow and is completely

223   resorbed by GS44 in both species (Fig. 4). We found that the axial skeleton was more

224   chondrified in *D. labialis* than in *S. ruber*. The first postcranial skeletal elements to

225   develop in both species were the nine pairs of semicircular cartilaginous primordia of

226   neural arches, including eight presacral vertebrae, the sacrum, the urostyle and the

227   hypochord. The sacral diapophyseal primordia are cylindrical. The last postsacral

228   vertebra (first coccygeal or Vertebra X *sensu* Haas, 1999) and the second coccygeal

229   vertebra ossify only in *D. labialis* by GS45. Simultaneously to the ossification of

230   presacral vertebrae, there is notochord absorption, fusion of the coccygeal elements,

231   and urostyle formation. The urostyle has a bicondylar articulation with the sacral

232   vertebra and the condyles are widely separated in both species (Fig. 4).

233   The atlas is concave at its point of articulation with the convex occipital condyles at

234   the base of the skull. Semicircular procoelous (*sensu* Jolie, 1962) vertebral centra begin

235   to develop as early as GS31 in *D. labialis* and GS32 in *S. ruber*, increasing the

236   thickness of both the neural arches and the transverse process. The neural arches

237   appear as cartilage at GS33 in both species, completing at GS34 in *D. labialis* and at

238   GS38 in *S. ruber*. The arches are fused dorsally at the midline at GS38 in *S. ruber* and

239   at GS38 in *D. labialis*. The transverse processes are the first elements to ossify in both

240   species (Tables 1 and 2). Postzygapophyses and prezygapophyses are conspicuous in

241   presacral vertebrae II, III, and IV in both species. Sesamoids are absent from GS25 to

242   GS45.

243   **Ossification sequence**

244   The earliest stage examined in both species was GS25. Ossification in *D. labialis*

245   appears by GS34 and in *S. ruber* by GS35 (Fig. 1A and Fig. 4). Ossification in *D.*

246   *labialis* begins with the atlas and the transverse processes, whereas in *S. ruber* it

247   begins with the parasphenoid, the transverse processes I-VII and neural arches I-III.

248   Metamorphic climax (MC) begins at GS41 in *D. labialis* and GS39-40 in *S. ruber*. We

249   identified seven ranks (I–VII) in *D. labialis* and five ranks (I–V) in *S. ruber* (Tables 1 and

250   2). Ossified elements were perceptible in *D. labialis* from GS35 to GS45, with 46

251   ossified elements, and from GS36 to GS43 in *S. ruber*, with 26 ossified elements.

252   Metamorphic climax in *D. labialis* was at GS45 with 14 ossified elements and in *S. ruber*

253   at GS39-40 with seven ossified elements. Of these, the structures in common are the

254   femur, tibia, fibula, humerus, ilium, and radioulna.

Discussion

256   Despite Colombia housing the second greaest number of hylid species on the planet,

257   few previous studies have considered developmental ossification of Colombian hylids.

258   The family Hylidae has gone through a number of taxonomic re-arrangements, as

259   elucidated by various phylogenetic hypotheses based on molecular, chromosomal, and

260   morphological data from both larvae and adults (Faivovich, 2002; Faivovich et al., 2005;

261   Wiens et al., 2010; Pyron and Wiens, 2011; Duellman et al., 2016). Data from additional

262   morphological studies of Colombia hylids may help to support or refute these

263   hypotheses.

264   Previous studies of the cranial morphology in hylid tadpoles include *Acris crepitans*

265   (Maglia et al., 2007), *Boana lanciformis* (de Sá, 1988; Alcalde and Rosset, 2003),

266   *Boana pulchella* (Hoyos et al., 2012), *Boana raniceps* and *Dendropsophus nanus*

267   (former *Hyla raniceps* and *Hyla nana* Fabrezi and Lavilla 1992; Vera Candioti and Haas.

268   2004), *Dryophytes versicolor* (former *Hyla versicolor,* Sheil et al.2014), *Hyla orientalis*

269   (Yıldırım and Kaya 2014), *Julianus acuminatus* (former *Scinax acuminatus*, Fabrezi and

270   Lavilla 1992; Faivovich 2002; Alcalde and Rosset 2003; Alcalde et al. 2011), *J.*

271   *uruguayus, J. aff. pinimus* (former *Scinax uruguayus* and *Scinax aff. pinima*, Alcalde et

272   al., 2011; Rodrigues et al., 2017), *Ololygon aromothyella* and *O. berthae* (former *Scinax*

273   *berthae*, Rodrigues et al., 2017; Alcalde et al., 2011; Faivovich, 2002), *O. skuki*

274   (Rodrigues et al., 2017); *Scinax granulatus* and *S. squalirostris* (Rodrigues et al., 2017;

275   Alcalde and Rosset, 2003), *S. boulengeri* (Rodrigues et al., 2017; Vera Candioti, 2007),

276   *S. fuscovariatus* (Fabrezi and Vera, 1997), *S. nasicus* (Rodrigues et al., 2017; Vera

277 Candioti, 2007; Vera Candioti et al., 2004; Fabrezi and Vera, 1997), *S. ruber* (Haas,

278 1996). For a complete overview of the findings of these studies please see Appendix 1.

279   Several of the differences between the two species examined here present interesting

280 avenues for future examination. For example, processus ethmoidalis of the quadrate in

281 *S. ruber* is wide, and it is not clearly distinct from the processus articularis. In contrast,

282 the processes of *D. labialis* are easily distinguishable and similar to that described by

283 Alcalde and Rosset (2003), who found similar features in *Boana raniceps* compared

284 with to the *Scinax* group (*S. squalirostris* and *S. granulatus, Scinax ruber* group). The

285 palatoquadrate is similar but the processus ascendens of the palatoquadrate in *D.*

286 *labialis* is wider than in *S. ruber*, and the distal side of the cornu trabecula extends

287 posteriorly toward the otic capsule. The anterior region of the palatoquadrate is

288 distinctively broader in *S. ruber* than in *D. labialis,* and in *S. ruber* the dorsomedial

289 process is wider than the ventromedial process in *D. labialis*.

290   When comparing the development of *D. labilis* with *D. nanus* (Vera Candioti et al.,

291 2004 and Alcalde and Rosset, 2003), we found that *D. labialis* can be differentiated by

292 the reduction of the buccopharyngeal and branchial basket structures and a missing

293 sinus posterior hypobranchialis and processus quadrato-ethmoidale. On the other hand,

294 the information that is available for *Scinax* species (Fabrezi and Lavilla, 1992; Haas,

295 1996; Fabrezi and Vera, 1997; Faivovich, 2002; Alcalde and Rosset, 2003; Vera

296 Candioti et al., 2004; Vera and Haas, 2007; Alcalde et al., 2011; Rodrigues et al., 2017)

297 revel that there are a numerous variations that requires extending the morphological

298 studies in tadpoles.

299 *Scinax ruber* presents alae and corpus of suprarostral cartilage with deeper notches.

300   The chondrocranium, hyobranchial apparatus, and the suprarostral body are joined

301   syndesmotically as found by Vera and Haas (2007) in microphagous tadpoles of *S.*

302   *nasicus* and *S. boulengeri*. *Dendropsophus labialis* like *Dendropsophus nanus* shows a

303   suprarostral cartilage with corpus and alae forming a continuous structure, which is

304   evidently, associated with a deviation of the macrophogous mechanisms described by

305   Alcalde and Rosset (2003).

306   The lateral development of the crista parotica is more prominent in *S. ruber* than in *D.*

307   *labialis*. It is possible that some of the variation in the anatomical structures of the otic

308   capsule are functionally related to perception of vocalizations (i.e. same species

309   recognition) in adult stages, but experiments must be conducted to confirm the

310   relationship of these anatomical structures with hearing physiological functions

311   (Ruggero and Temchin, 2002; Boistel et al., 2013).

312   The chondrification of skull in *S. ruber* is faint when viewed laterally, and foramina are

313   not clearly visualized. By contrast, in *D. labialis*, much more blue coloration was

314   observed. This could be due to abundant chondrification of these parts or to the early

315   developmental stages of this anatomical area in which allowed differentiation of

316   craniopalatine carotid foramina.

317   Although the sample size for the *Dendropsophus labialis* is very small in comparison

318   with *S. ruber*, *Dendropsophus labialis* exhibited more ossified elements with stronger

319   chondrification and less intraspecific variation, while *S. ruber* showed more intraspecific

320   variation and less overall chondrification in the samples (Fig. 1). *D. labialis* presented

321   uniformly stained (ossified) elements in all individuals (Table 3).

322   This variation between *S. ruber* and *D. labialis* could be caused by intrinsic factors that

323   determine the timing of development or extrinsic factors affecting osteogenesis (Vera

324   and Ponssa, 2014). It may not be a coincidence that *S. ruber* is a generalist species and

325   *D. labialis* is an endemic one (Frost 2018).

326   Haas (1996) reported that the ceratohyalia II-IV are fused in *Scinax ruber* and

327   *Megophrys montana nasuta*, characteristics that separate them from other species. We

328   confirmed this observation in *S. ruber* but not in *D. labialis*. The ceratohyal in *D. labialis*

329   has a process on the articular condyle that is not present in *S. ruber*. Alcalde and

330   Rosset (2003) found this process in both *S. granulatus* and *S. squalirostris.* Spicules I-III

331   on the posterior margin of the hypobranchial plate are present in *D. labialis* and *S.*

332   *ruber*, but spicule IV is not.

333   Copula II is present in both species. Copula I is present in *D. labialis* as in *S.*

334   *squalirostris*, but absent in *S. ruber* as in *S. granulatus, Boana raniceps* (Alcalde and

335   Rosset, 2003), and *Tlalocohyla smithii* (Vera and Haas, 2004). Although the presence of

336   copula I is extremely variable in hylids and is shared by all non-hylids (Vera and Haas,

337   2004), a relationship between this structure and the ecological function that it performs

338   (e.g. prey utilization), has not been identified.

339   Additional characteristics of the developmental morphology of these species places

340   align them with other hylids that have been studied previously. For example, the

341   urostyle of *D. labialis* and *S. ruber* forms a bicondylar articulation with the sacral

342   vertebra, and the condyles are widely separated. The shoulder girdle of both species

343   present differences in the shape of the omosternum and sternum at GS 45.

344   *Dendropsophus labialis* and *S. ruber* present suprascapular processes in tadpoles and

345   adults similar to those in other hylids (*Hypsiboas lanciformis,* De Sá, 1988; *Boana*

346   *pulchella,* Hoyos et al., 2012; *Pseudacris crucifer* and *Acris blanchardi,* Havens, 2010

347   and *A. crepitans,* Maglia et al., 2007) but is absent in *Scinax catharinae* clade

348   (Faivovich et al., 2005).

349   Variations of larval characters between *Scinax* and *Dendropsophus* have been

350   included in several phylogenetic studies (Fabrezi and Vera, 1997; Haas, 1996; 1999;

351   2003; Alcalde and Rosset, 2003; Vera, 2007). In our study, the skeleton shows

352   significant differences between the species *Scinax ruber* and *Dendropsophus labialis*,

353   beginning with fact that elements ossified in *S. ruber* exhibit more intraspecific variability

354   than in *D. labialis* (see Table 3)*.*

355   Regarding ossification sequence, the first bones ossified in the cranium were the

356   exoccipital, the frontoparietal, and the parasphenoid by GS 36. Haas (1999) found that

357   in *S. ruber* this occurred one stage later by GS37. Similar to those that Haas (1999)

358   described for other hylids, the ossification of the vertebrae begins from the centra of the

359   presacral vertebrae and continues ventrally along the notochord, forming osseous rings

360   around the notochord in both species. We found that the ossification of the centra in

361   both species we studied begins ventrally and proceeds dorsally. Haas (1999) recorded

362   the transverse processes of presacral vertebrae II- III as the first to ossify, while we

363   found that it was the ossification or neural arches proceeds from I to IX in *D. labialis*

364   (GS37) and from I to III in *S. ruber* (GS36) (Fig 4).

365   The detection of more intraspecific variability in *Scinax ruber* than in *Dendropsophus*

366   *labialis* could also be due to the presence of more intra-generic diversity in the *Scinax*

367   *ruber* clade. Alcalde and Rosset (2003) associated the type of feeding with the

368   development of the lateral anterohial process of the ceratohial, between species with

369   macrophage larvae (*Dendropsophus nanus*), and scraping microphages (*Boana*

370   *pulchellus, Julianus acuminatus* and *S. nasicus*). This may indicate that morphological

371   characteristics of the jaw may be involved in the particular feeding behaviors of these

372   tadpoles, and therefore these traits would help to distinguish the species (Appendix 1).

373   Differences between the ossification sequences of these two species are also evident

374   when examining the ossification ranks and number of ossified bones. In particular, *D.*

375   *labialis* has more ranks in the sequence and more elements that begin ossification prior

376   to metamorphosis. With respect to the postcranium, the number of elements ossified

377   appears earlier in *D. labialis* than in *S. ruber.* Because Gosner stages are based on

378   external characteristics that rely on underlying skeletal change, it is only a relative

379   measure of timing and should not be used as a way to compare species. Instead, we

380   compared the relative timing of events in the ossification sequence by examining the

381   order of onset of ossification of each element. Nunn and Smith (1998:86) considered

382   "ontogeny may be ordered by age, size, or stage; none of these measures are useful for

383   comparing ontogeny across significantly divergent taxa".

384   Table 4 outlines the ossification sequences of different species of the family Hylidae.

385   The number of ranks that include elements of the skull and postcranium vary from one

386   to five. The number of ranks increases when postcranial elements are included.

387   Weisbecker and Mitgutsch (2010), Harrington et al. (2013), and Sheil et al. (2014) used

388   similar ranked ossification sequence data to reconstruct phylogenetic trees of

389   amphibians in the families of Leptodactylidae, Ranidae and Bufonidae. These

390   researchers suggested using cranium and post-cranium data, relating them to the type

391   of development, and to include sequences of fossils, as far as possible.

392 Although the morphology and systematics of amphibians have been extensively

393 studied (Canatella and Trueb, 1988; de Sá and Hillis, 1990; Baez and Pugener, 2003;

394 Roelants and Bossuyt, 2005; Faivovich et al., 2005, Frost et al., 2006; Pyron and

395 Wiens, 2011; Duellman et al., 2016), additional comprehensive descriptions of skeletal

396 development and ossification sequences are needed to truly understand patterns of

397 heterochrony in the group. Some of the biological implications of heterochrony, which

398 are well known in amphibians (Alberch, 1985; Reilly, Wiley and Meinhardt, 1991),

399 include changes in structure, and changes in the rate of growth of entire organisms

400 (Raff, 1996; Smith, 2001; 2002; 2003). Some scholars have recognized that

401 heterochrony may work as modules of developmental events with evolutionary

402 implications that can promote or restrict the development of individual morphologies

403 (Wagner 1996).

404 Studies that have used statistical methods (e.g. Parsimov) to analyze ossification

405 sequences have revealed heterochrony in the timing of onset of ossification in some

406 cranial elements such as parasphenoid and prootic in *S. ruber* vs. *H. pulchellus* (Hoyos

407 et al*., 2012), or the frontoparietal, dentary, and maxilla in *D. labialis* vs. *Pseudis*

408 *platensis* (Fabrezi and Goldberg, 2009). In our study, we found that the parasphenoid

409 was the first element to ossify in both *D. labialis* and *S. ruber*, and the exoccipital,

410 frontoparietal, and prootic were the second elements to ossify in both species.

411 It is possible that the difference in cartilage formation between the two species

412 examined herein is due to paracrine factors induced in cells that express mesodermal

413 transcription factors involved in the activation of genes specific to cartilage (Gilbert,

414 2000; Kozhemyakina et al., 2015); however, we did not account for these factors.

415   Additionally, the intraspecific variation in the ossified elements between these species

416   could be linked to specific genes (Raff, 1996).

Conclusions

418   The contribution of ontogenetic data (development and ossification sequences of

419   skeletal structures) provides further information to help understand the interactions

420   between ontogeny and phylogeny in morphological and ecological diversity of frogs.

421   Ossification sequence data combined with evolutionary hypotheses may shed light on

422   patterns of development to be used in future phylogenetic hypotheses. As Larson de Sá

423   and Arrieta (2003) suggested, "variation in chondrocranial morphology in larval anurans

424   can be phylogenetically informative, even among closely related taxa".

Acknowledgements

426   We want to thank Timothy Sosa for help with the English translation. We appreciate

427   the contributions of the anonymous reviewers.

References

429   Alberch P (1985) Problems with the interpretation of developmental sequences.

430   *Systematic Zoology* 34:46–58.

431   Alcalde L, Rosset SD (2003) Descripción y comparación del condrocráneo en larvas de

432   *Hyla raniceps* (Cope, 1862), *Scinax granulatus* (Peters, 1863) y *Scinax*

433   *squalirostris* (Lutz, 1925) (Anura: Hylidae). *Cuadernos de Herpetología* 17:33–49.

434   Alcalde L, Vera Candioti MF, Kolenc F, Borteiro C, Baldo D (2011) Cranial anatomy of

435   tadpoles of five species of *Scinax* (Hylidae, Hylinae). *Zootaxa* 2787:19–36.

436 Avilán P, Hoyos JM (2006) Osteology of *Eleutherodactylus bogotensis* (Amphibia,

437   Anura, Leptodactylidae) from the Parque Nacional Natural Chingaza

438   (Cundinamarca, Colombia). *Caldasia 28*: 89-109.

439 Báez AM, Pugener LA (2003) Ontogeny of a new Palaeogene pipid frog from southern

440   South American and xenopodinomorph evolution*. Zoological Journal of the*

441   *Linnean Society* 139:439–476. DOI: 10.1046/j.1096-3642.2003.00085.x

442 Banbury B, Maglia AM (2006) Skeletal development of the Mexican spade foot, *Spea*

443   *multiplicata* (Anura: Pelobatidae). *Journal of Morpology* 267:803–821.

444 Boistel R, Aubin T, Cloetens P, Peyrin F, Scotti T, Herzog P, Gerlach J, Pollet N, Aubry

445   JF (2013) How minute sooglossid frogs hear without a middle ear. *Proceedings of*

446   *the National Academy of Sciences of the United States of America* 2:15360–

447   15364. DOI: 10.1073/pnas.1302218110

448 Bowatte G, Meegaskumbura M (2011) Morphology and ecology of tadpoles of

449   *Ramanella obscura* (Anura: Microhylidae). *Ceylon Journal of Science* 40: 109-120.

450   DOI: 10.4038/cjsbs.v40i2.3927

451 Cannatella DC (1999) Architecture: Cranial and axial musculoskeleton. In R. W.

452   McDiarmid, and R. Altig (Eds.), *Tadpoles: The biology of anuran larvae* (pp. 52–

453   91). United States of America: University of Chicago Press.

454 Cannatella DC, Trueb L (1988) Evolution of pipoid frogs: Intergeneric relationships of

455   the aquatic frog family Pipidae (Anura). *Zoological Journal of the Linnean Society*

456   94:1–38. DOI: 10.1111/j.1096-3642.1988.tb00880.x

457 de Sá RO (1988) Chondrocranium and ossification sequence of *Hyla lanciformis.*

458   *Journal of Morphology* 195:345–355. DOI: 10.1002/jmor.1051950308

459 de Sá RO, Hillis DM (1990) Phylogenetic relationships of the pipid frogs *Xenopus* and

460   *Silurana*: an integration of ribosomal DNA and morphology. Molecular *Biology and*

461   *Evolution* 7:365–376.DOI:10.1093/oxfordjournals.molbev.a040612

462 Dingerkus G, Uhler LD (1977) Enzyme clearing of Alcian blue stained whole small

463   vertebrates for demonstration of cartilage. *Stain Technology* 52:229–232.

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

465 Duellman WE, Marion AB, Hedges SB (2016) Phylogenetics, classification, and

466   biogeography of the tree frogs (Amphibia: Anura: Arboranae). *Zootaxa* 4104:1-

467   109.

468 Fabrezi M, Goldberg J (2009) Heterochrony during skeletal development of *Pseudis*

469   *platensis* (Anura, Hylidae) and the early offset of skeleton development and

470   growth*. Journal of Morphology* 270:205–220. DOI: 10.1002/jmor.10680.

471 Fabrezi M, Lavilla EO (1992) Estructura del condrocráneo y esqueleto hiobranquial en

472   larvas de algunos hílidos neotropicales (Anura: Hylidae). *Acta Zoologica Lilloana*

473   41: 155−164.

474 Fabrezi M, Vera R (1997) Caracterización morfológica de larvas de anuros del

475   Noroeste argentino. *Cuadernos de Herpetología* 11:37−49.

476 Faivovich JC (2002) A cladistics analysis of *Scinax* (Anura: Hylidae). *Cladistics* 18:367–

477   393. DOI: 10.1111/j.1096-0031.2002.tb00157.x

478 Faivovich JC, Haddad CFB, García P, Frost DR, Campbell JA, Wheeler WC (2005)

479   Systematic review of the frog family Hylidae, with special reference to Hylinae:

480   phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of*

481   *Natural History* 294:1–240. DOI: 10.5531/sd.sp.12

482 Frost DR (2018) Amphibian Species of the World: an Online Reference. New York,

483   USA: American Museum of Natural History New York, USA. Available at

484   http://research.amnh.org/herpetology/amphibia/index.html (accessed 12

485   December 2017)

486 Frost DR, Grant T, Faivovich JC, Haddad CFB, Wheeler WC, et al. (2006) The

487   amphibian tree of life. *Bulletin of the American Museum of Natural History*

488   297:1−370. DOI: 10.5531/sd.sp.13

489 Gilbert SF (2000) *The anatomical tradition: Evolutionary Embryology: Embryonic*

490   *homologies.* Chapter 1 Developmental Biology. Sunderland (MA): Sinauer

491   Associates, Inc.

492 Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes

493   on identification. *Herpetologica* 16:183–190.

494 Haas A (1995) Cranial features of dendrobatid larvae (amphibia: Anura:

495   Dendrobatidae). *Journal of Morphology* 224: 241-264. DOI:

496   10.1002/jmor.1052240302

497 Haas A (1996) Das larvale cranium von *Gastrotheca riobambae* und seine

498   metamorphose (Amphibia, Anura, Hylidae). *Verhandlungen des*

499   *Naturwissenschaftlichen Vereins in Hamburg* 36:33–162.

500 Haas A (1997) The larval hyobranchial apparatus of discoglossoid frogs: Its structure

501   and bearing on the systematics of the Anura (Amphibia: Anura). *Journal of Zoological*

502   *Systematics and Evolutionary Research 35*: 179-197. DOI: 10.1111/j.1439-

503   0469.1997.tb00422.xh

504   Haas A (1999) Larval and metamorphic skeletal development in the fast-developing frog

505   *Pyxicephalus adspersus* (Anura, Ranidae). *Zoomorphology* 119:23-35.

506   Haas A (2003) Phylogeny of frogs as inferred from primarily larval characters

507   (Amphibia: Anura). *Cladistics* 19:23–89. DOI: 10.1111/j.1096-0031.2003.tb00405.x

508   Hall JA, Larsen JH (1998) Postembryonic ontogeny of the spadefoot toad, *Scaphiopus*

509   *intermontanus* (Anura: Pelobatidae): Skeletal morphology. *Journal of Morphology*

510   238: 179–244.

511   Harrington SM, Harrison LB, Sheil CA (2013) Ossification sequence heterochrony

512   among amphibians. *Evolution and Development* 15: 344–364. DOI:

513   10.1111/ede.12043.

514   Havens SB (2010) The role of skeletal development in body size evolution of two North

515   American frogs. Master thesis, Missouri University of science and technology.

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

517   Hoyos JM, Sánchez-Villagra MR, Carlini AA, Mitgutsch C (2012) Skeletal development

518   and adult osteology of *Hypsiboas pulchellus* (Anura: Hylidae). *Acta Herpetologica*

519   7: 119–138.

520   ICVAN, International committee on veterinary anatomical nomenclature (1973) *Nomina*

521   *anatomica veterinaria*. 2nd edition. Vienna, Austria: International Committee on

522   Veterinary Anatomical Nomenclature under the financial responsibility of the World

523   Association of Veterinary Anatomists.

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

525   Jungfer KH (2017) On Warszewicz's trail: the identity of *Hyla molitor* O. Schmidt, 1857.

526   *Salamandra* 53:18–24.

527   Kozhemyakina E, Lassar AB, Zelzer E (2015) A pathway to bone: Signaling molecules

528   and transcription factors involved in chondrocyte development and maturation.

529   *Development* 142:817–831. DOI: 10.1242/dev.105536.Larson, P. M.; de Sá, R. O.;

530   Arrieta, D. (2003) Chondrocranial, hyobranchial and internal oral morphology in

531   larvae of the basal bufonid genus *Melanophryniscus* (Amphibia: Anura). *Acta*

532   *Zoologica Stockholm* 84(2): 145–154. DOI: 10.1046/j.1463-6395.2003.00140.x

533   Maglia AM, Pugener LA (1998) Skeletal development and adult osteology of *Bombina*

534   *orientalis* (Anura: Bombinatoridae). *Herpetology* 54: 344-363.

535   Maglia AM, Pugener LA, Mueller JM (2007) Skeletal morphology and postmetamorphic

536   ontogeny of *Acris crepitans* (Anura: Hylidae): a case of miniaturization in frogs.

537   *Journal of Morphology* 268: 194–223. DOI: 10.1002/jmor.10508

538   Nunn CL, Smith KK (1998) Statistical analyses of developmental sequences: the

539   craniofacial region in marsupial and placental mammals. American Naturalist

540   152:82–101.

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

542   Parker WK (1876) On the structure and development of the skull in the Batrachia. Part

543   II. *Philosophical Transactions of the Royal* Society 166:648–699.

544   Pugener LA, Maglia AM (2007) Skeletal morphology and development of the olfactory

545   region of *Spea* (Anura: Scaphiopodidae). *Journal of Anatomy* 211:754–768. DOI:

546   10.1111/j.1469-7580.2007.00826.x

547   Pyron A, Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2800

548   species, and a revised classification of extant frogs, salamanders, and caecilians.

549   *Molecular Phylogenetic Evoltion* 61:543–583. DOI: 10.1016/j.ympev.2011.06.012

550 Raff RA (1996) *The shape of life: genes, development and the evolution of animal form*.

551   Chicago: University of Chicago Press.

552 Reilly SM, Wiley EO, Meinhardt DJ (1991) An integrative approach to heterochrony: the

553   distinction between interspecific and intraspecific phenomena. *Biological Journal* of

554   the *Linnean Society* 60:119–43.DOI: 10.1111/j.1095-8312.1997.tb01487.x

555 Roček Z. (1981) Cranial anatomy of frogs of the family Pelobatidae Stannius, 1856, with

556   outlines of their phylogeny and systematics. *Acta Universitatis Carolinae Biologica*

557   1980: 1–164.

558 Rodrigues GDV, do Nascimento FAC, de Almeida JPFA, Mott T (2017) The tadpole of

559   *Scinax skuki* (Anura: Hylidae) from the type locality, with a description of its larval

560   skeleton. *Studies on Neotropical Fauna and Environment* 52: 204-215. DOI:

561   10.1080/01650521.2017.1342485

562 Roelants K, Bossuyt F (2005) Archaeobatrachian paraphyly and pangaean

563   diversification of crown-group frogs. *Systematc Bology* 54:111-26. DOI:

564   10.1080/10635150590905894

565 Ruggero MA, Temchin AN (2002) The roles of the external, middle, and inner ears in

566   determining the bandwidth of hearing. *Proceedings of the National Academy of*

567   *Sciences of the United States of America* 99:13206–13210. DOI:

568   10.1073/pnas.202492699

569 Shearman RM, Maglia AM (2014) Osteological development of Cope's gray treefrog,

570   *Hyla chrysoscelis. Acta Zoologica Stockholm* 96: 181–198. DOI:

571   10.1111/azo.120662

572   Sheil CA, Jorgensen M, Tulenko F, Harrington S (2014) Variation in timing of

573   ossification affects inferred heterochrony of cranial bones in Lissamphibia.

574   *Evolution and Development* 16:292–305. DOI: 10.1111/ede.1209

575   Sheil CA, Alamillo H (2005) Osteology and skeletal development of *Phyllomedusa*

576   *vaillantii* (Anura: Hylidae: Phyllomedusinae) and a comparison of this arboreal

577   species with a terrestrial member of the genus. *Journal of Morphology* 265: 343–

578   368..DOI: 10.1002/jmor.10362

579   Smith KK (2001) Heterochrony revisited: the evolution of developmental sequences.

580   *Biological Journal* of the *Linnean Society* 73:169–86. DOI: 10.1006/bijl.2001.0535

581   Smith KK (2002) Sequence heterochrony and the evolution of development. *Journal of*

582   *Morphology* 252:82–97. DOI: 10.1002/jmor.10014

583   Smith KK (2003) Time's arrow: heterochrony and the evolution of development.

584   *International Journal of Biomedical Research* 47:613–21.

585   Starrett PH (1973) *Evolutionary patterns in larval morphology*. In J. L. Vial, editor,

586   Evolutionary Biology of the Anurans: Contemporary Research on Major Problems.

587   Columbia: University of Missouri Press. p 251–271.

588   Vera Candioti MF (2007) Anatomy of anuran tadpoles from lentic water bodies:

589   Systematic relevance and correlation with feeding habits. *Zootaxa* 1600:1175–

590   5326.

591   Vera Candioti MF, Haas A (2004) Hyobranchial Apparatus of *Hyla nana. Cuadernos de*

592   *herpetología* 18:3–15.

593   Vera MC, Ponssa ML. (2014) Skeletogenesis in anurans: cranial and postcranial

594   development in metamorphic and postmetamorphic stages of *Leptodactylus*

595   *bufonius* (Anura: Leptodactylidae). *Acta Zoologica* 95:44-62. DOI:

596   10.1111/azo.12007

597 Wagner GP (1996) Homologues, natural kinds and the evolution of modularity.

598   *American Zoology* 36:36–43.

599 Wassersug RJ (1980) Internal oral features of larvae from eight families: Functional,

600   systematic, evolutionary and ecological considerations. *Miscellaneous publication -*

601   *University of Kansas, Museum of Natural History* 68:1−146. DOI:

602   10.5962/bhl.title.16230

603 Wassersug RJ. and Heyer, W. R (1988) A survey of internal oral features of

604   leptodactyloid larvae (Amphibia: Anura). *Smithsonian Contributions to*

605   *Zoology*457:1−99. Weisbecker V, Mitgutsch C (2010) A large-scale survey of

606   heterochrony in anuran cranial ossification patterns. *Journal of Zoological*

607   *Systematics and Evolutionary Research* 48:332–347. DOI: 10.1111/j.1439-

608   0469.2010.00570.x

609 Wiens JJ, Kuczynski CA, Hua X, Moen DS (2010) An expanded phylogeny of tree frogs

610   (Hylidae) based on nuclear and mitochondrial sequence data. *Molecular*

611   *Phylogenetic Evolution* 55:871–82. DOI: 10.1016/j.ympev.2010.03.013

612 Yıldırım E, Kaya U (2014) Comparative skeletogenesis of the oriental tree frog *Hyla*

613   *orientalis* (Anura: Hylidae). *Zoologischer Anzeiger* 253:361-71. DOI:

614   10.1016/j.jcz.2014.04.002

615

Ossification sequence of cranial and postcranial elements in *Dendropsophus labialis* (Peters, 1863)

Rank	Gosner stage (Number of specimens)	Elements ossified	
		Cranium	Postcranium
I	26(3), 27(3), 28(2), 29(2), 31(3), 32(1), 33(1), 34(1)		
	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).

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, tibiofibula, 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 (No. Specimens with ossified elements)			35 (1)	36 (2)	37 (3)	38 (1)	41 (3)	42 (3)	43 (1)	45 (1)	46 (1)	36 (6)	37 (9)	38 (3)	39/40 (3)	41 (4)	42 (5)	43 (3)	44 (4)	45 (1)	46 (1)
Cranium	Paraesfenoids			1	1	1	3	3	1	1	1	3	5	2	3	3	3	3	3	1	1
	Exoccipital			1	1	1	3	3	1	1	1			3	3	3	4	3	3	1	1
	Frontopariental				1		2	3	1	1	1			2	2	2	1	3	3	1	1
Pos-cranium	Transverse Process	I			1							6	9	3	3	3	4	3	3	1	1
		II	1	1	1	1	3	3	1	1	1	5	6	3	3	3	4	3	3	1	1
		III	1	1	1	1	3	3	1	1	1	5	6	3	3	3	4	3	3	1	1
		IV	1	1	1	1	3	3	1	1	1	5	6	3	3	3	4	3	3	1	1
		V	1	1	1	1	3	3	1	1	1	3	6	3	3	3	4	3	3	1	1
		VI	1	1	1	1	3	3	1	1	1	3	6	3	3	3	4	3	2	1	1
		VII	1	1	1	1	3	3	1	1	1	1	4	2	3	3	3	3	2	1	1
		VIII		1	1	1	3	3	1	1	1	1	2	2	3	3	2	3	1		
		IX			1									1	3	1	1	3			
	Neural arch	I		1	1	1	3	3	1	1				3	3	3	4	3	3	1	1
		II			1	1	3	3	1	1				3	3	3	3	3	3	1	1
		III			1									2	3	3	3	3	3	1	1
		IV			1									3	3	3	3	3	2	1	1
		V			1									2	3	3	3	3	2		
		VI			1									2	3	3	1	3			
		VII			1									1	3	2		3			
		VIII			1									1	3	1		3			
		IX																			
	Man...	Metacarpal I			1					1											

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Metacarpal II			1					1											
	Metacarpal III			1					2	1										
	Metacarpal IV			1			1		2	1										
	Metacarpal V			1					2	1										
	Metatarsal distal tarsal IV			1					2	1										
	Metatarsal distal tarsal IV			1					1											
	Prepollex			1					1											
Pes	**Metatarsal I**			1					1											
	Metatarsal II			1			1		1	1										
	Metatarsal III			1		1	2		1	1										
	Metatarsal IV			1		1	2		1	1										
	Metatarsal V			1		1	2		1	1										
Atlas		1	1	1	1	3	3	1	1	1			3	3	3	4	3	3	1	1
Femur				1		2	3	1	1	1				3	1		3	1		
Fibula				1		2	3	1	1	1										
Tibia				1		2	3	1	1	1										
Tibiofibula				1		2	3	1	1	1				3	1	1	3	1	1	
Scapula				1		2	2		1	1				3	1		3			
Suprascapula				1		2	2		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		

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	
	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, tl] [cl, co, mt, pf, po] [mc, ph] is [ma, pm, sm] ns [an, vo] [me, pt, qj, qu, sq] sp	
	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]	
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, tl] pf [pm, sm] [ns, ma][an, de, is, sq][ap, me, pa, ph, pt, qj, sp, vo]	
	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]	
Hylinae	*Dryophytes chrysoscelis*	C	8	ps [ex][fe][fp][sm, pm, po][ma][de, ns, an, sq][vo]	Sherman and Maglia, 2014

		C, P	10	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]	
	Dryophytes versicolor	C	6	ps [ex, fp] [ma, pm, po] [an, de, sq] [pa, pt, qj]	Sheil et al., 2014
		C, P	7	ps [cl, co, fe, fi, fr, hu, il, na, ra, sc, ti, tl, ul] [ex, fp, mc, ph, pf, ve] [ma, pm, po] [an, de, sq] [ns, me] [pa, pt, qj]	
	Hyla orientalis	C	5	ps [ex, po] fp [ns, sm] [an, de, ma, pm, pt, sq, vo]	Yıldırım and Kaya, 2014
		C, P	6	ps [ex, hu, na, po, ve][ct, fe, fr, il, mc, tf, tl, ru][fp, cl, co, mt, pf, ph][ns, sm][an, de, is, ma, pm, pt, sq, pu, vo] qj	
	Smilisca baudinii	C	7	fp [ex, ps, sm] [ag, de, ma, pm, sq] [ns, pt, qj] [me, pa, pv][cm, sp] po	Trueb, 1966
				[ex, fp, ps, sm][ma, pm, sq][ns, pt][pa, qj, vo][cm, et] so, po	Gaudin, 1973
	Triprion petasatus	C	6	fp, ns [an, de, ex, ma, me, pa, pm, ps, pt, qj, sm, sq][cm, pv] sp, po	Trueb, 1970
Lophyohylinae	*Osteopilus septentrionalis*	C	3	fp, sm [ag, cm, de, et, ex, ma, me, ns, pa, pm, po, ps, pt, qj, sq, vo]	Trueb, 1966
			7	fp, sm [an, de, ex, ma, ns, pm, ps, pt, pv, sq] pa, qj [me, po, sp] cm	Trueb, 1970
			5	[fp, sm][ag, de, ex, ns, ma, pm, ps, pt, pv, sq] pa, qj [po, sp]	Gaudin, 1973
		C, P	13	ps, ve, fe [hu, il, ra, su, ul] [ct, ex, fp, fr, sc, tf, tl] [cl, co, mt, pf, po] [mc, ph] is [ma, pm, sm] ns [an, vo] [me, pt, qj, qu, sq] sp	Sheil et al., 2014
Pseudinae	*Pseudis platensis*	C	5	[fp, ps] [ex, po] [ns, pm, sq] ma [de, pt, vo]	Fabrezi and Goldberg, 2009
Scinaxinae	*Scinax ruber*	C	3	ps, [fp, po] [an, de, ma, me, np, pc, pm, sm, sq, vo]	**This study**
		C, P	7	ps, ve [ex, fp, po][fe, hu, il, ru, sc, tf, tl] is [an, de, ma, me, pm, pc] [np, sm, sq, vo]	

1 C, cranium; P, poscranium

2 ag, angular; an, angulosplenial; ap, plectral apparatus; cl, clavicle; cu, columella; co, coracoid; ct, cleithrum; de, dentary;

3 et, ethmoid; ex, exoccipital; fe, femur; fi, fibula; fp, frontoparietalis; fr, fibulare; ha, hyoid apparatus; hu, humerus; hy,

4   hypochord; il, ilium; is, ischium; ma, maxilla; mc, metacarpals; me, mentomeckelian; mt, metatarsals; na, neural arches;

5   nc, neural center; np, neopalatine; ns, nasal; pa, palatine; pc, coronoid process; pr, presacral vertebrae; pf, phalanges of

6   feet; ph, phalanges of manus; pm, premaxilla; po, prootic; ps, parasphenoid; pt, pterygoid; pv, pre vomer; qj,

7   quadratojugal; qu, quadrate; ra, radius; ru, radioulna; sc, scapula; sm, septomaxilla; sp, sphenethmoid; sq, squamosal; su,

8   suprascapula; ti, tibia; tf, tibiofibula; tl, tibiale; tp, transverse process; ul, ulna; ve, vertebra (including na, nc, pr, tp); vo,

9   vomer.

10   C = Cranial elements; P = Postcranial elements.

11   [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; commisura terminalis; pah, processus anterioris hyalis; palh, processus anteriolateralis hyalis; phb, planum hypobranchiale; plh, processus lateralis hyalis; pph, processus posterioris hyalis; pr, pars reuniens; sp, spicula. Blue: cartilage, light blue: fontanella.

Dendropsophus labialis   **Scinax ruber**

A

cs
ci
ct
cm
ts
pqu
pmq
cqc
pq
pof
oc

C   **D. labialis**

opf of
ct oc fo
cs cm pmq pof
ci

S. ruber

B

cs
ci
cm
pqu
pq

oc
jf

D

pah
ca (I)
palh
pr
plh

pph
cp (II)
sp
cb I - IV
ct

phb
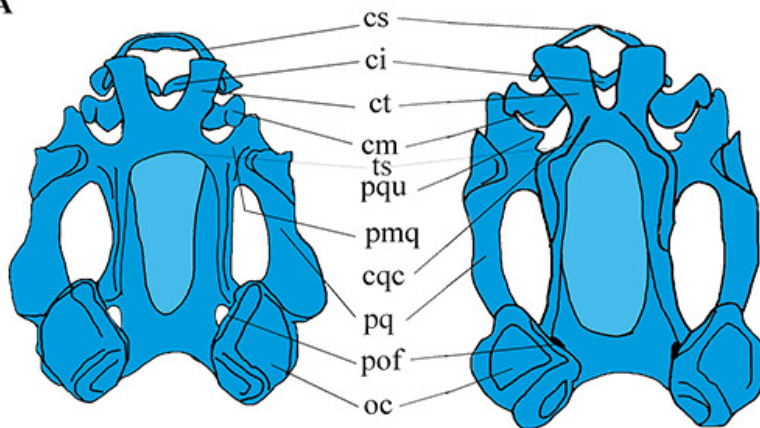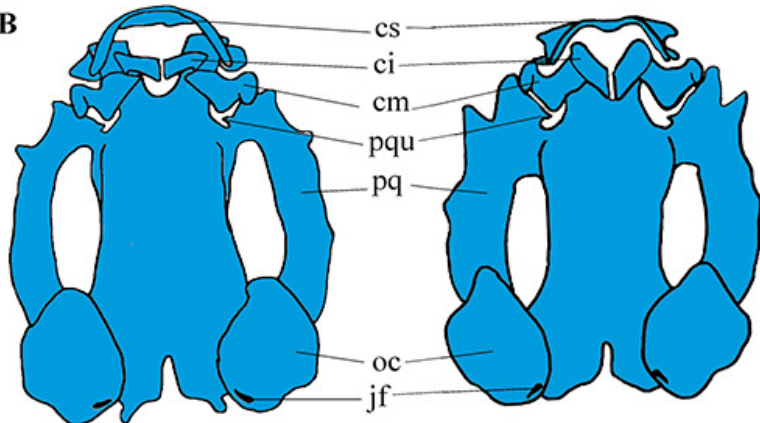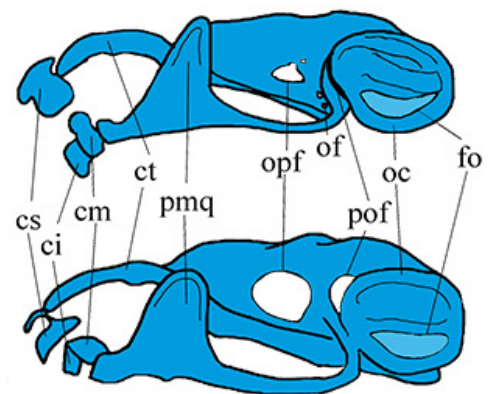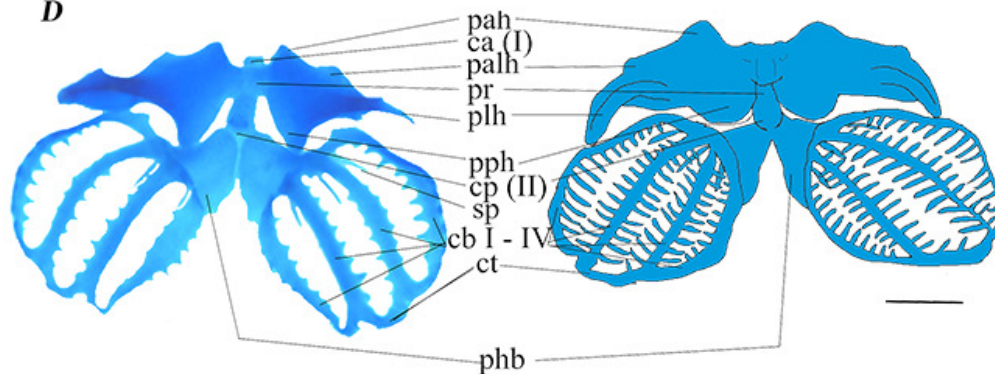
Figure 2

Appendicular skeleton of *Dendropsophus 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.

A *Dendropsophus labialis*   *Scinax ruber*

ps

ps

B

o

c

e

co

st

C

il

is
pu
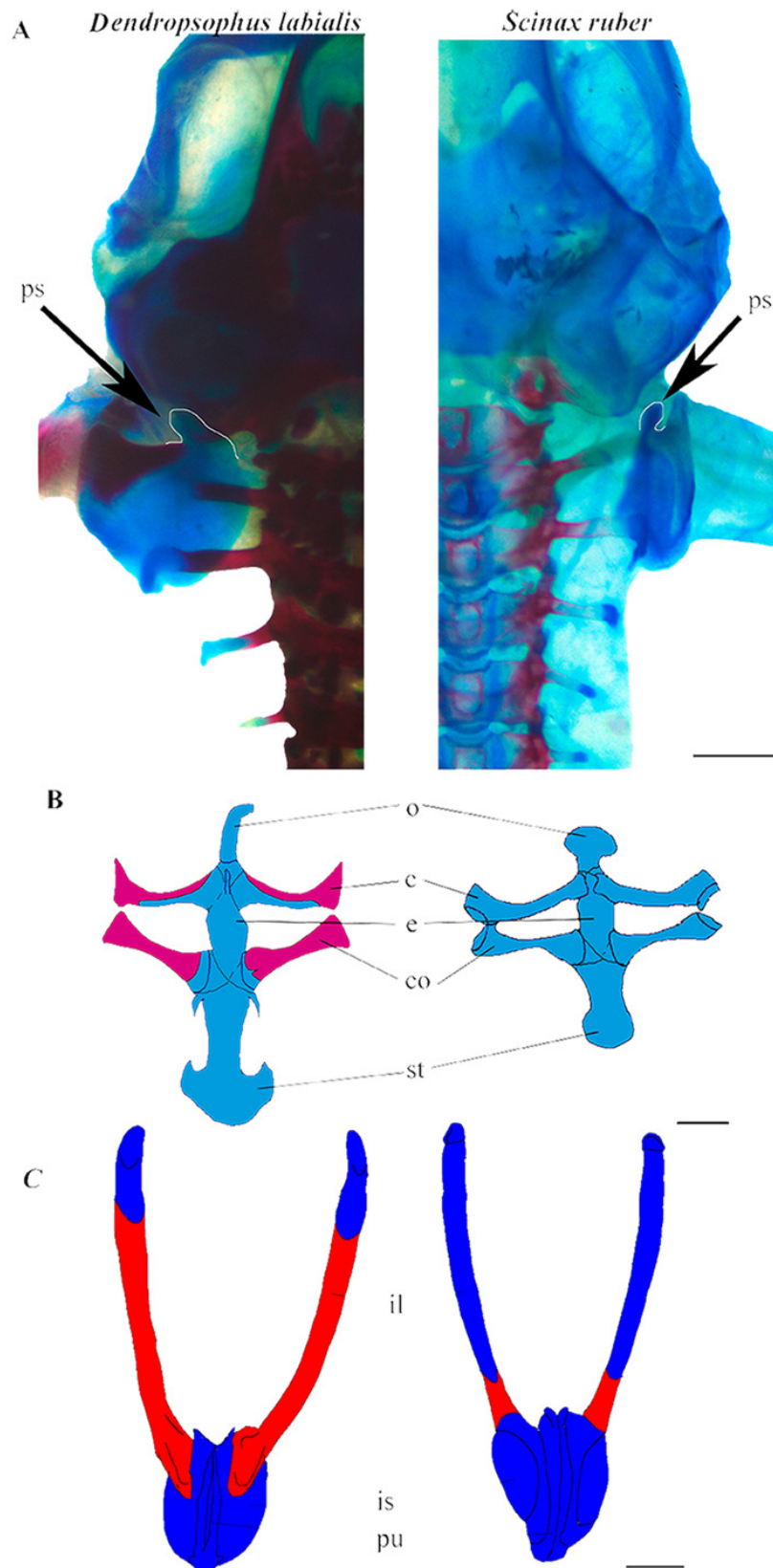
Figure 3

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, condrificated.
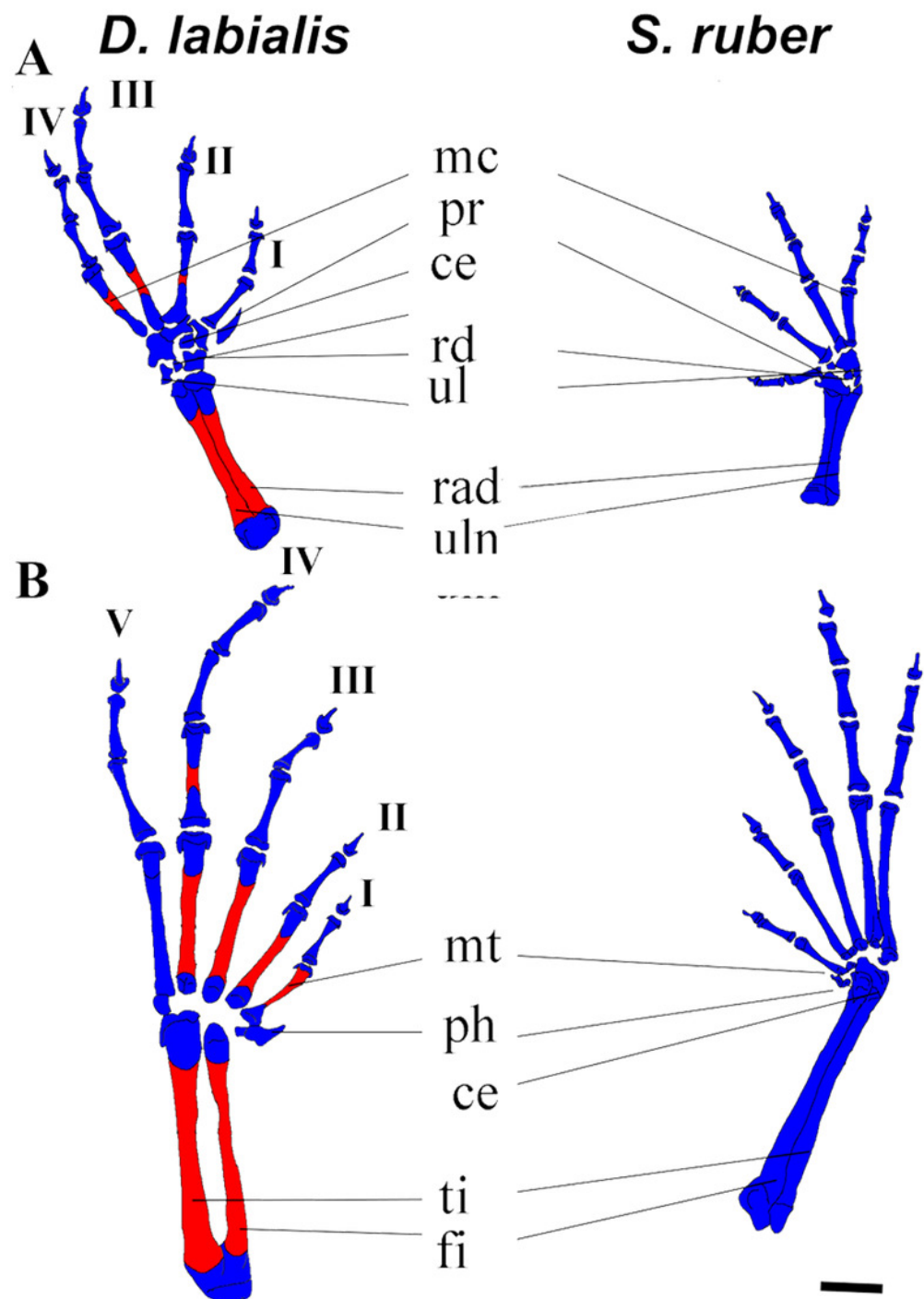
Figure 4

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.