Postcranial anatomy and histology of *Seymouria*, and the terrestriality of seymouriamorphs

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Seymouria is the best known of the seymouriamorphs, a group of Permo-Carboniferous reptiliomorphs with both terrestrial and aquatic taxa. The majority of research on *Seymouria* has focused on cranial anatomy, with few detailed descriptions or illustrations of the postcrania. We utilized neutron computed tomography (nCT) and histological sampling to provide updated, detailed illustrations that clarify details of the postcranial anatomy and to assess the development and histology of *Seymouria* through specimens from the early Permian Richards Spur locality. The correlation of morphological and histological data indicate rapid metamorphosis in this terrestrially capable stem amniote, with the youngest specimen being postmetamorphic despite being distinctly younger than premetamorphic individuals of *Discosauriscus*, the only other seymouriamorph to have been histologically sampled. The microanatomical data (e.g. semi-open medullary cavity) also substantiates the hypothesis that *Seymouria* was terrestrial based on interpretation of external features, although the persistence of a modestly developed medullary spongiosa in comparison to either *Discosauriscus* or to other co-occurring terrestrial tetrapods suggests additional nuances that require further exploration. In the absence of clearly recognizable postmetamorphic stages in several seymouriamorph taxa, it is difficult to determine the evolutionary trajectory of terrestriality within the clade. Our analysis provides the first histological characterization of the life history of *Seymouria* and highlights the need for further study of seymouriamorph ontogeny.

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Abstract

Seymouria is the best known of the seymouriamorphs, a group of Permo-Carboniferous reptiliomorphs with both terrestrial and aquatic taxa. The majority of research on *Seymouria* has focused on cranial anatomy, with few detailed descriptions or illustrations of the postcrania. We utilized neutron computed tomography (nCT) and histological sampling to provide updated, detailed illustrations that clarify details of the postcranial anatomy and to assess the development and histology of *Seymouria* through specimens from the early Permian Richards Spur locality. The correlation of morphological and histological data indicate rapid metamorphosis in this terrestrially capable stem amniote, with the youngest specimen being postmetamorphic despite being distinctly younger than premetamorphic individuals of *Discosauriscus*, the only other seymouriamorph to have been histologically sampled. The microanatomical data (e.g. semi-open medullary cavity) also substantiates the hypothesis that *Seymouria* was terrestrial based on interpretation of external features, although the persistence of a modestly developed medullary spongiosa in comparison to either *Discosauriscus* or to other co-occurring terrestrial tetrapods suggests additional nuances that require further exploration. In the absence of clearly recognizable postmetamorphic stages in several seymouriamorph taxa, it is difficult to determine the evolutionary trajectory of terrestriality within the clade. Our analysis provides the first histological characterization of the life history of *Seymouria* and highlights the need for further study of seymouriamorph ontogeny.

Introduction

40   Seymouriamorphs are among the best-known stem amniotes (but see Marjanović &

41   Laurin, 2019 for alternative phylogenetic placement) and are known primarily from Lower

42   Permian deposits in North America, Europe, and Russia (Broili, 1904; Amalitzky, 1921; White,

43   1939; Vaughn, 1966; Berman, Reisz, & Eberth, 1987; Berman & Martens, 1993; Sullivan &

44   Reisz, 1999; Bulanov, 2014). Within seymouriamorphs, only the monotypic family

45   Seymouriidae is known from both North America and Eurasia (Broili, 1904; White, 1939;

46   Vaughn, 1966; Berman & Martens, 1993). Of the North American localities, the materials of

47   *Seymouria* with the greatest detail of preservation are known from the Dolese Brothers

48   Limestone quarry near Richards Spur, Oklahoma (Sullivan & Reisz, 1999; Bazzana et al., 2020).

49   However, the only previously described postcranial material from this locality consists of a few

50   isolated elements (Sullivan & Reisz, 1999). Furthermore, the most detailed description and

51   figuring of the postcrania of *Seymouria* from any locality is that completed by White (1939).

52   Subsequent authors have provided focused descriptions of the atlas-axis complex (Berman,

53   Reisz, & Eberth 1987; Sumida, Lombard, & Berman, 1992) and the manus and pes (Berman et

54   al., 2000), but White's (1939) work remains the most thorough description of the postcranial

55   skeleton in its entirety. While the interpretations made by White (1939) have been largely

56   supported, or at least not overturned, by subsequent authors, his illustrations were unlabelled,

57   somewhat stylized, and from slightly angled, non-standard perspectives, which collectively

58   limits their utility. As stem amniotes that are well-documented in the fossil record,

59   seymouriamorphs provide a relatively accessible window through which to examine

60   morphological changes associated with terrestrial lifestyles in Paleozoic tetrapods, and updated

61   osteology of the postcranial skeleton with detailed figures and descriptions that can be readily

62   utilized by other workers is of great import.

63   Here we describe new postcranial material of *Seymouria* from the early Permian Richards

64   Spur locality, including several articulated vertebrae and a complete humerus, femora, and

65   fibula, and provide updated descriptions, illustrations, and images of the postcrania. Analysis of

66   several limb elements and vertebrae using neutron computed tomography (nCT) and histological

67   sampling provides important details regarding the development and internal anatomy of the

68   postcrania of *Seymouria* and contributes to our understanding of the extent to which these stem

69   amniotes were adapted to terrestrial lifestyles.

Materials & Methods

71   **Neutron tomography.** Neutron tomography measurements were performed at the DINGO

72   thermal-neutron radiography/ tomography/imaging station (Garbe et al., 2015) located at, and

73   tangentially facing, the 20 MW Open-Pool Australian Lightwater (OPAL) reactor housed at the

74   Australian Nuclear Science and Technology Organisation (ANSTO), Lucas Heights, New South

75   Wales, Australia. The DINGO facility utilises a quasi-parallel collimated beam of thermal

76   neutrons.

77   For the femur (ROMVP 80915) and the fibula (ROMVP 80917), the instrument was

78   equipped with an Iris 15TM Large Field of View sCMOS camera (5056 x 2968 pixel, 16-bit) and

79   Zeiss Ikon 100 mm f/2.0 Makro Planar lens. Based on a desired spatial resolution of ~60 μm

80   across the partially-embedded femur and fibula, a maximum sample width of 48.5 mm and

81   minimum sample-to-detector distance of 28 mm, the DINGO instrument was configured with a

82   30 μm thick terbium-doped Gadox scintillator screen (Gd2O2S:Tb, RC Tritec AG) and 25.2 ×

83   25.2 × 25.2 μm voxels for a Field-of-View of 100 x 74.5 mm.. To maximise counting statistics

84   and minimise subsequent noise in the tomographic reconstruction, a collimation ratio (*L/D*) of

85   500 was used, where *L* is the neutron aperture-to-sample length and *D* is the neutron aperture

86   diameter. This high-flux configuration traditionally illuminates a 200 mm x 200 mm area around

87   the sample area with 4.75×107 neutrons cm-2s-1, leading to high background radiation and

88   zingers on the detector. A newly installed slit system was implemented to restrict the neutron-

89   irradiated area about the specimen to achieve optimum scan conditions and a divergence-limited

90   spatial resolution of 56 μm. A total of 900 equally-spaced angle shadow-radiographs were

91   obtained every 0.20° as the sample was rotated 180° about its vertical axis. Both dark (closed

92   shutter) and beam profile (open shutter) images were obtained for calibration before initiating

93   shadow-radiograph acquisition. To reduce anomalous noise, a total of three individual

94   radiographs with an exposure length of 4.0 s were acquired at each angle (Mays, Bevitt, &

95   Stilwell, 2017) for a total scan time of 4.6 h.

96   For the sacral series (OMNH 79348), a collimation ratio (L/D) of 1000 (Garbe et al.,

97   2015) was used to ensure highest available spatial resolution. The field of view was set to 200 x

98   200 mm^2 with a voxel size of $73.3 \times 73.3 \times 73.3$ μm and sample to detector distance of 70 mm.

99   Neutrons were converted to photons with a 100 μm thick ZnS(Ag)/^{6}LiF scintillation screen (RC

100 Tritec AG); photons were then detected by an Iris 15 sCMOS camera (16-bit, 5056×2960

101 pixels) coupled with a Makro Planar 24 mm Carl Zeiss lens. The tomographic scan consisted of a

102 total of 720 equally-spaced angle shadow-radiographs obtained every 0.25° as the sample was

103 rotated 180° about its vertical axis. Both dark (closed shutter) and beam profile (open shutter)

104 images were obtained for calibration before initiating shadow-radiograph acquisition. To reduce

105 anomalous noise, a total of three individual radiographs with an exposure length of 8 s were

106 acquired at each angle (Mays, Bevitt, & Stilwell, 2017). These individual radiographs were

107 summed in post-acquisition processing using the Grouped ZProjector function in ImageJ v.1.51h.

108 Total scan time was 5.7 h.

109   The individual radiographs were summed in post-acquisition processing using the

110 'Grouped ZProjector' plugin in ImageJ v.1.51h in accordance with our previous measurements;

111 this plugin was developed by Holly (2004). Tomographic reconstruction of the 16-bit raw data

112 was performed using commercially available Octopus Reconstruction v.8.8 software package

113 and the filtered back-projection algorithm to yield virtual slices perpendicular to the rotation

114 axis. When these slices are stacked in a sequence, they form a three-dimensional volume image

115 of the sample. The reconstructed volume data were downsampled by a factor of 2 in ImageJ to

116 reduce computation time, then rendered and segmented with Avizo Lite 9.3.0.

117

118 **Histology.** Histological preparation followed standard procedures (Padian & Lamm, 2013). All

119 specimens were photographed prior to embedding in EP4101UV resin (Eager Polymers), which

120 was allowed to cure for 24 hours. ROMVP 80916 (partial femur), ROMVP 81198 (vertebra), and

121 ROMVP 81199 (vertebra) were prepared at the Royal Ontario Museum (ROMVP), Toronto,

122 Canada. Specimens were cut on the IsoMet 1000 precision saw (Buehler) and mounted to frosted

123 plexiglass slides with cyanoacrylate adhesive. For the femora, the cut was made at the

124 approximate region of the minimum diaphyseal circumference; for the vertebra, the first cut was

125 made sagittally (anteroposteriorly) down the midline, and the second cut was made transversely

126 through one of the two halves of the block. For ROMVP 80916 (larger, partial femur), the

127 section is taken slightly proximal to the inferred minimum circumference due to the incomplete

128 specimen's nature.

129   Mounted blocks were trimmed using the IsoMet and ground on the Hillquist Thin

130 Sectioning Machine lapidary wheel. Manual polishing using 1000-mesh grit on glass plates and a

131 combination of 1-μm and 5-μm grit on polishing cloths was used to remove scratches. ROMVP

132 81200 (partial femur) was prepared in a similar fashion but with a different equipment setup at

133 the University of Toronto Mississauga. Cutting was performed on the Metcut-5 low speed saw

134 (MetLab), initial grinding on the Metcut-10 Geo (MetLab), and manual grinding on a cutlery

135 whetstone block. Imaging was done on two Nikon AZ-100 microscopes, both fitted with a DS-

136 Fi1 camera and NIS Elements-Basic Research software registered to David C. Evans and to

137 Robert R. Reisz.

138 Preparation of the specimens was performed by Diane Scott and Bryan M. Gee using pin

139 vises and air scribes. Figures were prepared using Adobe Illustrator and Photoshop.

140

141 **Systematic Paleontology**

142 Order Seymouriamorpha Watson, 1917

143 Family Seymouriidae Williston, 1911

144 Genus *Seymouria* Broili, 1904

145

146 **Horizon and locality.** Dolese Brothers Limestone Quarry near Richards Spur, Oklahoma, USA.

147 Early Permian (Artinskian).

148

149 **Referred specimens.** OMNH 74721, right humerus; OMNH 79346, complete vertebra; OMNH

150 79347, string of 11 articulated vertebrae; OMNH 79348, string of three articulated vertebrae;

151 OMNH 79349, complete vertebra; OMNH 79350, partial vertebra; OMNH 79351, complete

152 vertebra; OMNH 79352, partial vertebra; OMNH 79353, partial vertebra; ROMVP 81198,

153 complete vertebra; ROMVP 81199, complete vertebra; ROMVP 80915, left femur; ROMVP

154 80916, proximal left femur; ROMVP 80917, left fibula; ROMVP 81200, distal left femur.

155

Anatomical Description

Vertebra. OMNH 79346, OMNH 79349, OMNH 79350, OMNH 79353, ROMVP 81198, and ROMVP 81199 are isolated presacral vertebrae between the tenth and twenty-second positions (Fig. 1). OMNH 79346 and OMNH 79349 are mostly complete, whereas OMNH 79350 and OMNH 79353 are incomplete. OMNH 79351 and OMNH 79352 are complete vertebrae from the third to ninth vertebral positions (Fig. 2). OMNH 79347 consists of 11 articulated presacral vertebrae, likely between the tenth and twenty-second positions (Fig. 3A). OMNH 79348 consists of the last presacral position, the first sacral vertebra, and a possible second sacral vertebra in articulation (Fig. 3B-E). Despite the isolated nature of the specimens, the position of the vertebrae can be inferred based on the morphology of the diapophyses, which change in length, cross-sectional profile, and anteroposterior orientation along the vertebral column (White, 1939: Fig. 13).

All the vertebrae described here exhibit the swollen pre- and postzygapophyses that are characteristic of *Seymouria* (White, 1939; Holmes, 1989). The first sacral vertebra in OMNH 79348 exhibits the transition from the expanded zygapophyses of the trunk series to the more transversely constricted morphology characteristic of the caudal series (White, 1939), with the prezygapophyses being slightly more than twice the width of the postzygapophyses (Fig. 3B). The neural spine of OMNH 79349 is strongly deflected to the right, as has been noted in other specimens of *Seymouria* (Sullivan & Reisz, 1999). Both OMNH 79351 and 79352 display the broadly expanded diapophyses characteristic of the anteriormost presacral vertebrae in *Seymouria* (Fig. 2). In OMNH 79352, the neural spine is bifurcated, with both projections directed dorsolaterally; the neural spines of the other specimens are either broken or missing.

178   *Seymouria* has been previously described as having an irregular distribution of bifurcated and

179   non-bifurcated spines along the presacral region between the tenth position and the twenty-

180   second position (White, 1939). OMNH 79352 appears to be the first record of bifurcation of the

181   neural spine in a vertebra from the anteriormost trunk. Although the seemingly complete co-

182   ossification of the neural arch and centrum suggests skeletal maturity, the maturity of these

183   vertebrae cannot be more definitively established given the known challenges of applying

184   skeletochronology to vertebral elements (Danto et al., 2016).

185

186   **Humerus.** OMNH 74721 is a complete right humerus, exhibiting the tetrahedral shape typical of

187   many reptiliomorphs (Fig. 4). The overall morphology matches that described by previous

188   authors (White, 1939; Sullivan & Reisz, 1999) in being short and robust with the deltopectoral

189   crest following an L-shaped path. The proximal and distal ends are broadly expanded and are set

190   at an approximately 45-degree angle to each other with no distinct shaft separating the epiphyses.

191   The supinator process is oval in cross-section and located just distal to the short arm of the

192   deltopectoral crest (Fig. 4C). OMNH 74721 exhibits a keel that extends along the anteroventral

193   surface from the deltopectoral crest to the entepicondylar foramen (Fig. 4D); a similar crest has

194   been described by Sullivan & Reisz (1999) but was not mentioned by White (1939). The

195   entepicondylar ridge runs along the ventral edge and expands proximally to form what may

196   represent the insertion site for the subcoracoscapularis on the posterior surface of the humerus,

197   near the glenoid articulation (Fig. 4B). Dorsal to this expansion is a pronounced tubercle for the

198   insertion of the latissimus dorsi muscle. The absence of an ectepicondylar foramen, the proximal

199   position of the insertions for the *m. subcoracoscapularis* and the *m. latissimus dorsi*, and the

200 position and shape of the supinator process all correspond exactly to the descriptions of

201 *Seymouria* given by White (1939) and Sullivan and Reisz (1999).

202

203 **Femur.** ROMVP 80915 is a complete left femur (Fig. 5), the morphology of which corresponds

204 to previous descriptions (White, 1939; Sullivan & Reisz, 1999). ROMVP 80916 and ROMVP

205 81200 are partial left femora (Fig. 6). The element is short and robust. The insertion for the

206 puboischiofemoralis muscle is visible on the dorsal surface of the head (Fig. 5B, D). As is known

207 in *Seymouria* (White, 1939; Sullivan & Reisz, 1999), the adductor crest extends posterolaterally

208 along the ventral surface of the femur from the trochanter to near the tibial condyle (Fig. 5F).

209 The smaller femur that was previously described by Sullivan and Reisz (1999; FMNH PR 2053)

210 was stated to have a prominent trochanter. This appears to remain constant throughout ontogeny,

211 as little difference in the relative size of the trochanter is seen between the smallest (ROMVP

212 81200) and largest specimens (ROMVP 80915 and 80916). The distal articular surface has been

213 described as following an M-shaped curve (White, 1939), but this may not be entirely accurate.

214 In ROMVP 80915, ROMVP 81200, and a smaller, more immature femur (FMNH PR 2053;

215 Sullivan & Reisz, 1999: fig. 3), the distal surface is V-shaped, with the tip of the anterior arm

216 being bent ventrally (Fig. 5G), as opposed to the illustration given by White (1939: fig. 28) that

217 reconstructs this surface with both ends curving ventrally. However, it is also possible that the

218 shape of the distal surface is ontogenetically variable, as disparities in length suggest that the

219 specimen described by White (1939), measuring 6.4 cm in length, may be more mature than

220 ROMVP 80915 (5.5 cm), the largest complete femur from Richards Spur.

221

222 **Fibula.** ROMVP 80917 is a complete left fibula (Fig. 7). Previous descriptions (White, 1939;

223 Sullivan & Reisz, 1999) have commented only on the general shape of the element, to which

224 ROMVP 80917 corresponds exactly in that the medial surface is deeply concave, the lateral

225 surface is mostly straight with only a slight concavity, and the distal articular surface is more

226 expanded than the proximal surface, which itself is crescentic with its dorsal margin being

227 convex and its ventral margin being concave. Features that have not been described previously in

228 *Seymouria* but that are present in ROMVP 80917 are the pronounced muscle scar along the

229 proximal half of the lateral surface, which may represent the insertion site for the *m. iliofibularis*

230 (Fig. 7A-B, D; Romer, 1956), and the twisting of the fibular shaft such that the proximal and

231 distal heads lie in planes that are at an approximately 45-degree angle to each other (Fig. 7E-F).

232

Histological Description

234 **Vertebra.** ROMVP 81198, an isolated presacral neural arch of a small-bodied individual (Fig.

235 1E), and ROMVP 81199, a presacral neural arch with centrum of a larger individual (Fig. 1F),

236 were histologically sectioned. The transverse profile reveals a similar microanatomy and

237 histology to that of a previously sampled specimen (OMNH 73499) from Richards Spur (Danto

238 et al., 2016). The pleurocentrum of ROMVP 81199 is formed by two domains (Fig. 8), an well-

239 ossified yet porous periosteal domain along the ventrolateral margin of the element that is

240 formed by a thin layer of lamellar bone, and a less dense and more disorganized endochondral

241 domain with trabeculae; this is captured in both sagittal and transverse sections. Although the

242 suture between the pleurocentrum and the neural arch is not clearly defined externally, it is very

243 apparent in the transverse section in which an oblique separation (Fig. 8F) demarcates a largely

244 cartilaginous connection at the time of death. Neither the notochordal canal nor the neural canal

245   were captured in the half-transverse section. The neural arches of ROMVP 81198 and 81199 are

246   very similar in transverse section. Each arch is comprised of a spongy bone texture with a hollow

247   interior. In the smaller ROMVP 81198, the ventral portion of the arch is poorly ossified, with a

248   sparse network of trabecula (Fig. 9). The sagittal sections are also generally comparable, but a

249   few differences may be noted. Indentations on the anterior and posterior surfaces representing

250   the vestiges of the supraneural canal are present; these indentations are more prominent in the

251   larger ROMVP 81199, and in each specimen, the anterior indentation is more pronounced (Figs.

252   8A, 9A). In both specimens, the cortex of the neural arch is much thicker along the posterodorsal

253   surface behind the neural spine and extending down to the posterior indentation of the

254   supraneural canal when compared to the anterior surface. The thickened region of cortex is far

255   more developed in the larger ROMVP 81199. The neural spines are very poorly ossified. The

256   ventral portion of the arch, which roofs the neural canal, is thin in both specimens and exhibits

257   distinct remodelling (Figs. 8B, 9B). Remodelling is otherwise found mostly near the geometric

258   center of the element (Fig. 9B). A pair of closely spaced, distinctive growth lines can be

259   identified in the thickened region of ROMVP 81199 (Fig. 8A).

260   The three articulated vertebrae of OMNH 79348 were digitally sectioned using the

261   neutron tomography data (Fig. 10). Viewed in transverse section, the data reveal pronounced

262   differences in the compactness of the diapophyses; the diapophyses of the first sacral vertebra are

263   not only greatly expanded, as is visible externally, but are also substantially less porous than

264   those of either the presacral or the possible second sacral. The scan resolution is not sufficient to

265   permit tissue identification.

266

267   **Femur.** ROMVP 80916 and ROMVP 81200 are partial left femora (Fig. 6); ROMVP 80916 is

268   equivalent in size to ROMVP 80915 and is broken distal to the trochanter, while ROMVP 81200

269   is substantially smaller (about 33% smaller) and is broken proximal to the trochanter. ROMVP

270   80916 was histologically sectioned slightly proximal to the minimum diaphyseal circumference

271   (Fig. 11), while ROMVP 81200 was sectioned at this minimum region (Fig. 12). ROMVP 80915

272   was digitally sectioned at the minimum circumference (Fig. 13). All three femora are relatively

273   similar in their microanatomical and histological features. The cortex is relatively compact and

274   well-vascularized, comprised primarily of lamellar bone and with a plexiform arrangement of

275   vascular canals. Primary osteons and vascular canals are abundant in the growth zones of both

276   specimens (Figs. 11-12), but ROMVP 81200 appears to have a denser concentration, implying a

277   greater immaturity and a faster growth rate at the time of death. Remodelling is found at the

278   boundary of the medullary cavity in both specimens (Figs. 11C, 12C), but there is distinctly more

279   along the margin of the cavity in the larger ROMVP 80916 (Figs. 11-12). Secondary osteons

280   associated with this remodelling are found in both histologically sectioned specimens. The

281   medullary cavity is relatively open, although there is a network of trabeculae throughout;

282   secondary remodelling is present within this network, but calcified cartilage is absent. The

283   trabecular network appears to be less dense in the digital section of ROMVP 80915 (Fig. 13)

284   than in the histological section of the comparably sized ROMVP 80916 (Fig. 11), but this may be

285   a combination of a slightly more proximal plane of section in the latter and limitations on

286   scanning resolution (25.2 μm) for the former.

287   In both histologically sectioned specimens, there are numerous circumferential lines;

288   none could be identified in the digital data for ROMVP 80195. In the smaller ROMVP 82100, at

289   least three lines are present (Fig. 12B). In the larger ROMVP 80916, at least seven lines are

290　present (Fig. 11B). Based on the distribution of these lines as distinct clusters of closely spaced

291　lines in the poorly vascularized regions, these probably represent multiple rest lines within an

292　annulus (the poorly vascularized region), rather than clusters of lines of arrested growth (LAGs)

293　with highly uneven growth between deposition of each LAG. In ROMVP 80916, there is an

294　annulus partially preserved toward the extensor surface (away from the adductor crest) with at

295　least five rest lines present (Fig. 11A). Most of this annulus has been obliterated by remodelling,

296　and the rest lines are not uniformly distinct around their partial circumference. There are a few

297　vascular canals and primary osteons in this region near the border of the medullary cavity that

298　likely represent a partially preserved growth zone. The partial annulus is followed by a thicker

299　region of well-vascularized tissue (Fig. 11B). The external cortex is then formed by a thinner

300　layer of poorly vascularized tissue with at least three distinct rest lines, one of which essentially

301　marks the surface of the femur. In ROMVP 81200, there appear to be two zones of growth, one

302　adjacent to the medullary cavity and one along the external cortex, and one annulus with at least

303　three continuous rest lines (Fig. 12B). The second growth zone is not fully continuous

304　throughout, but the annulus can be traced circumferentially such that it can be determined to

305　precede this zone. The observation that the most peripheral zone in ROMVP 80916 is an

306　annulus, whereas the corresponding zone in ROMVP 81200 is a growth zone, indicates that the

307　animals died during different seasons. For both specimens, a minimum age of two years can thus

308　be estimated.

309

310　**Discussion**

311　**Intraspecific variation.** Described specimens of *Seymouria* illustrate the intraspecific variation

312　that occurs across the postcranial skeleton. For example, the porosity of the diaphyses differs

313   between each vertebra in OMNH 79348, suggesting differential stress loading on each vertebra.

314   The identity of the vertebra immediately posterior to the first sacral has also been historically

315   debated; White (1939) and Berman et al. (2000) identified the element as a second sacral, while

316   Williston (1911), Watson (1918), and Berman, Reisz, and Eberth (1987) identified it as the first

317   caudal. White's (1939) argument was based on the presence of a rib extending anteriorly from

318   the vertebra in question to contact the rib of the first sacral, stating unequivocally that it must

319   have had a strong cartilaginous connection and was doubtlessly a functional second sacral

320   vertebra (p. 354). In contrast, Berman, Reisz, and Eberth (1987) noted that the rib of the debated

321   element curved posteriorly and thus could not contact the ilium or the rib of the first sacral,

322   negating any possibility of it forming a true second sacral. In the absence of a preserved rib in

323   OMNH 79348, we are unable to comment on whether the vertebra immediately following the

324   first sacral represents a definitive second sacral or the first caudal vertebra.

325   Compared to the smaller and presumably more immature humerus (FMNH PR 2054) that

326   was previously described from Richards Spur (Sullivan & Reisz, 1999: fig. 2), many features on

327   OMNH 74721 are more developed. In FMNH PR 2054, the capitellum is indicated only by a

328   slight swelling, whereas in OMNH 74721 it is a distinct hemispherical facet. Mirroring this

329   disparity, the supinator process of FMNH PR 2054 remains connected to the capitellum by a

330   ridge of unfinished bone, whereas the process of OMNH 74721 is clearly delineated from the

331   distal articular surfaces by an emargination of smoothly finished bone.

332

333   **Comparative external anatomy.** Comparisons of *Seymouria* with many other

334   seymouriamorphs, especially with the discosauriscids, are complicated by the absence of

335   definitively adult individuals of many seymouriamorph taxa. Further complications arise from

336   the evident taphonomic distortion and compaction of many seymouriamorph specimens. Any

337   discussion of comparative anatomy among seymouriamorphs, including the comparisons

338   presented here, must be viewed within the context of these limitations.

339   The vertebrae of *Seymouria* bear a number of marked differences from those of other

340   seymouriamorphs. In *Ariekanerpeton*, the presacral vertebrae differ in the level of ossification of

341   the neural arch, with the arches of the third to fifth vertebrae remaining separate and the sixth to

342   ninth arches being strongly co-ossified (Klembara & Ruta, 2005). The neural spines of *Kotlassia*

343   are much higher than in *Seymouria* (Bystrow, 1944), whereas in *Discosauriscus* the spines are

344   short (Klembara & Bartík, 2000), and in *Ariekanerpeton* the spines are either low or unossified

345   altogether (Laurin 1996b). The vertebrae of *Utegenia* have been described as indistinguishable

346   from those of *Discosauriscus* (Klembara & Ruta, 2004). The oval cross-section of the

347   diapophyses is shared between *Seymouria* (White, 1939) and *Kotlassia* (Bystrow, 1944).

348   However, in *Seymouria* the diapophyses remain the same size but transition in orientation from

349   vertical to horizontal along the vertebral column (White, 1939), whereas the diapophyses of

350   *Kotlassia* maintain the same orientation but decrease in size along the column (Bystrow, 1944).

351   Unlike *Seymouria* and *Kotlassia* in which the articular surfaces of the prezygapophyses and the

352   postzygapophyses face directly dorsally and ventrally, respectively (White, 1939; Bystrow,

353   1944; Holmes, 1989), the surfaces of *Discosauriscus* and *Ariekanerpeton* face dorsomedially and

354   ventrolaterally (Klembara & Bartík, 2000; Klembara & Ruta, 2005). *Seymouria*, *Kotlassia*, and

355   *Discosauriscus* retain an open notochordal canal in adulthood (White, 1939; Bystrow, 1944;

356   Klembara & Bartík, 2000). It is unknown whether the notochordal canals were retained in the

357   other seymouriamorphs (Klembara & Ruta, 2004; 2005; Klembara, 2005; 2009; Bulanov, 2014).

358   *Discosauriscus* has only one sacral vertebra (Klembara & Bartík, 2000), as in OMNH 79348. It

359   is unclear whether *Seymouria baylorensis* and *Seymouria sanjuanensis* differ in the number of

360   sacral vertebrae (White, 1939; Berman, Reisz, & Eberth, 1987, 2000), so this character cannot be

361   used to clarify the specific designation of this material.

362   The humerus of *Seymouria* is more extensively ossified than those of *Utegenia* or

363   *Ariekanerpeton* (Laurin, 1996a, 1996b; Klembara & Ruta, 2005), in which the capitellum, the

364   trochlea, the supinator process, and the deltopectoral crest are indistinct in even the largest

365   individuals. In particular, the humerus of *Utegenia* has been described as "little more than a

366   stout, subcylindrical lump of bone" (Klembara & Ruta, 2004: 77). It has been noted, however,

367   that the lack of ossification in *Utegenia* may represent ontogenetic immaturity (Klembara &

368   Ruta, 2004). The humerus of *Kotlassia* is the slenderest of the seymouriamorphs and lacks an

369   entepicondylar foramen (Bystrow, 1944). *Makowskia*, *Spinarerpeton*, and *Discosauriscus* exhibit

370   the same massive, L-shaped deltopectoral crest extending from the proximal articular surface as

371   that seen in *Seymouria* (Klembara, 2005, 2009; Klembara & Bartík, 2000). The humeri of

372   *Makowskia* and *Spinarerpeton* are described as having a broad shaft, a proximodistally short

373   entepicondyle, and an entepicondylar foramen that is open distally (Klembara, 2005, 2009); the

374   last feature has been recognized as likely being ontogenetic. *Makowskia*, *Spinarerpeton*, and

375   *Discosauriscus* differ substantially from *Seymouria* in having a well-developed insertion site for

376   the *m. subcoracoscapularis* and in lacking a supinator process (Klembara & Bartík, 2000;

377   Klembara, 2005, 2009); in *Seymouria*, the former is represented by only a slight rugosity while

378   the latter is quite pronounced (White, 1939). The humeri of *Discosauriscus* and *Ariekanerpeton*

379   have been reported with a lesser degree of twisting, with the planes of the proximal and distal

380   ends being at an approximately 38-degree angle (Klembara & Bartík, 2000; Klembara & Ruta,

381   2005). The positioning and degree of development of the insertions for the *m.*

382   *subcoracoscapularis* and *m. latissimus dorsi* are roughly equivalent in *Discosauriscus* as in

383   *Seymouria* (Klembara & Bartík, 2000).

384   As with the humerus, the femur of *Seymouria* is more ossified than in *Utegenia* or

385   *Ariekanerpeton* (Laurin 1996a, 1996b); the femur of *Utegenia* has been described as featureless

386   (Klembara & Ruta, 2004). The femur of *Makowskia* is known but has only been described as

387   having a crescentic proximal articular surface with a deep intertrochanteric fossa as in *Seymouria*

388   (Klembara, 2005), and the only corresponding figure is a simplified line drawing. The femur of

389   *Spinarerpeton* has been similarly described and figured in limited detail (Klembara, 2009). The

390   femur of *Microphon* is much more gracile than in *Seymouria* with less pronounced expansion of

391   the epiphyses and a proportionally longer, narrower shaft (Bulanov, 2014). *Microphon* also

392   differs from *Seymouria* in that the adductor crest extends towards the fibular condyle (Bulanov,

393   2014), as opposed to the tibial condyle as occurs in *Seymouria* and *Discosauriscus* (Klembara &

394   Bartík, 2000). In addition, the intertrochanteric fossa of *Microphon* is approximately one-third

395   the length of the femur (Bulanov, 2014), whereas in *Seymouria* it extends approximately halfway

396   along the element. *Kotlassia* appears to be intermediate between these two conditions and

397   exhibits a starker contrast between the slender shaft and the broadly expanded epiphyses

398   (Bystrow, 1944: fig. 9). A feature found in the femora of *Microphon* and *Kotlassia* and in

399   ROMVP 80915, though perhaps not all specimens of *Seymouria*, is the lesser development of the

400   trochanter (Bystrow, 1944); in *Microphon*, the trochanter seems to not be developed at all

401   (Bulanov, 2014). The femur of *Discosauriscus* is similar to that of *Seymouria* in overall

402   morphology but is more gracile (Klembara & Bartík, 2000: fig. 25).

403   The fibulae of *Kotlassia* and *Utegenia* have not been illustrated or described in sufficient

404   detail to allow for accurate comparison with *Seymouria*. The fibula of *Ariekanerpeton* has only

405   been described as being poorly preserved with unfinished epiphyses (Klembara & Ruta, 2005).

406   In *Makowskia* and *Discosauriscus*, the fibula is similar to *Seymouria* in being deeply concave

407   medially with only a slight concavity on the lateral surface (Klembara & Bartík, 2000; Klembara,

408   2005). The fibula of *Spinarerpeton* is unknown (Klembara, 2009).

409

410   **Histological interpretations and comparisons.**

411   Contextualizing the histological data of the specimens of *Seymouria* is complicated by

412   the paucity of work on other stem amniotes, let alone seymouriamorphs specifically. Limb

413   elements of *Seymouria* have never been histologically analyzed. The only other seymouriamorph

414   femur to be histologically sampled is that of the European *Discosauriscus*, the femur of which is

415   characterized by a parallel-fibered endosteal matrix with sparse vascularization comprised of

416   radially and longitudinally arranged canals in small individuals that shifts to being dominated by

417   radial vasculature in adults (Sanchez et al., 2008). *Seymouria* exhibits a lamellar matrix with a

418   plexiform arrangement of the vasculature in the femur, and the degree of vascularization far

419   exceeds that figured for *Discosauriscus*. The increased vascularization is indicative of relatively

420   fast growth at the time of death in the sampled specimens of *Seymouria*, which also suggests

421   relative immaturity. Skeletochronological markers also differ between the taxa. *Discosauriscus*

422   possesses numerous, well-defined and evenly spaced LAGs, whereas *Seymouria* is characterized

423   by distinctive growth zones and annuli bearing numerous closely spaced rest lines but without

424   clear LAGs. Similar variation in skeletochronological markers has been reported in the Late

425   Triassic metoposaurids *Dutuitosaurus* from Morocco and *Metoposaurus* from Poland; this

426   disparity was hypothesized to be the result of differing seasonal activity patterns associated with

427   climatic differences across paleolatitudinal gradients (Konietzko-Meier & Klein, 2013). Lastly,

428 the medullary spongiosa is distinctly less developed in *Discosauriscus* (Sanchez et al., 2008:fig.

429 2); the significance of this is unclear in the absence of additional data. It may relate to overall

430 body size, as extant lissamphibians, relatively small in comparison to *Seymouria*, typically lack

431 medullary spongiosa; however, the larger cryptobranchids possess a spongiosa (e.g., Laurin,

432 Canoville, & German, 2011). Similarly, small, semi-terrestrial to terrestrial temnospondyls also

433 have little to no spongiosa (e.g., McHugh, 2015) compared to the larger *Eryops* with a much

434 denser spongiosa (e.g., Konietzko-Meier, Shelton, & Sander, 2016).

435   Comparisons with other Paleozoic tetrapods are also limited by a paucity of comparative

436 work. Of the major Paleozoic clades (e.g., pelycosaurian synapsids, 'lepospondyls'),

437 temnospondyls are the best-sampled (Sanchez et al., 2010a; Sanchez et al., 2010b; McHugh,

438 2014; Konietzko-Meier, Shelton & Sander, 2016). The relative thickness of the cortex and the

439 development of the medullary spongiosa are most comparable to that of the co-occurring

440 trematopid *Acheloma dunni*, a terrestrial taxon (Sanchez et al., 2010b; Quemeneur, de Buffrénil

441 & Laurin, 2013). The spongiosa is less developed than in either definitively aquatic taxa such as

442 the late Permian rhinesuchid *Rhinesuchus* (McHugh, 2014) or in controversially aquatic taxa

443 such as the early Permian eryopid *Eryops* (Sanchez et al., 2010b; Quemeneur, de Buffrénil &

444 Laurin, 2013; Konietzko-Meier, Shelton & Sander, 2016), and the cortex is not extensively

445 thickened as in the definitively aquatic dvinosaur *Trimerorhachis* (Sanchez et al., 2010b;

446 Quemeneuer et al., 2013). A large number of Mesozoic temnospondyls, which are predominantly

447 aquatic, have also been sampled (Steyer et al., 2004; Konietzko-Meier & Sander, 2013; Sanchez

448 & Schoch, 2013). Many of these taxa exhibit similar structure to that of *Trimerorhachis*, often

449 with a high degree of pachyostotic development and with greatly reduced or nearly absent

450 medullary cavities. McHugh (2015) sampled the small-bodied Early Triassic lydekkerinid

451   *Lydekkerina*, a semi-aquatic taxon with terrestrial capabilities (e.g., Pawley & Warren, 2005;

452   Canoville & Chinsamy, 2015) and the amphibamiform *Micropholis*, a fully terrestrial taxon (e.g.,

453   Schoch & Rubidge, 2005). Both taxa exhibit a similar histological and microanatomical

454   organization to that of terrestrial Paleozoic temnospondyls and to that of *Seymouria*.

455   Collectively, the temnospondyl comparisons support an inferred terrestrial lifestyle of

456   *Seymouria*. However, it is important to note that the spongiosa of *Seymouria* is more developed

457   than in any of the co-occurring terrestrial temnospondyls at Richards Spur (Castanet et al., 2003;

458   Quemeneur, de Buffrénil & Laurin, 2013; Richards, 2016) in which the spongiosa is either

459   weakly developed (Trematopidae) or virtually non-existent (Dissorophidae, Amphibamiformes).

460   However, it is comparable in the developed medullary spongiosa to that of *Eryops*, the degree of

461   terrestriality of which has long been debated (Konietzko-Meier, Shelton, & Sander, 2016, and

462   references therein). The significance of the spongiosa in *Seymouria* is uncertain at present and

463   warrants further work to compare with other co-occurring taxa and with more closely related

464   stem amniotes.

465   The vertebral histology is also difficult to compare with coeval tetrapods, let alone with

466   closely related taxa. Vertebrae are uncommon in histological studies compared to limb elements,

467   and most studies that that have examined the vertebrae of Paleozoic tetrapods have focused on

468   the inter- and pleurocentra (Konietzko-Meier, Danto & Gadek, 2014; Danto et al., 2017, 2019).

469   However, both the centra and the neural arches contribute valuable information regarding the

470   lifestyle of *Seymouria*. Previous workers have often suggested that the neural arch would have

471   been subject to strong biomechanical constraints during locomotion in early tetrapods (Rockwell,

472   Evans & Pheasant, 1938; Olson, 1976; Holmes, 1989). The prominent expansion of the neural

473   arch and the development of the zygapophyses in *Seymouria* lends support to this hypothesis.

474   *Discosauriscus* is the only other seymouriamorph to have its internal vertebral anatomy

475   examined (Danto et al., 2016). The main difference is in the construction of the neural arch,

476   which is comprised of thick, compact lamellar bone in *Discosauriscus*; in contrast, the neural

477   arch of *Seymouria* is largely hollow. Based on the size of the sampled *Discosauriscus* material,

478   the individual was likely premetamorphic and still aquatic, which would explain the higher

479   degree of ossification. Whether this might have changed in later stages of ontogeny if or when

480   individuals metamorphosed into a terrestrial adult form remains unknown. Beyond

481   seymouriamorphs, neural arches have not been sampled in many clades, which may be because

482   most Paleozoic tetrapod clades have multipartite vertebrae in which the arch readily detaches

483   from the centra during preservation. Furthermore, isolated neural arches have not traditionally

484   been utilized as an ideal case study for exploring histological questions compared to either the

485   centra or to other postcranial elements. Danto et al. (2016) sampled a number of Paleozoic

486   lepospondyl taxa in which neural arches were preserved. Some of the aquatic taxa (e.g., an

487   indeterminate nectridean) exhibit a similar spongy bone composition of the arch, but the interior

488   of the arch is relatively well-ossified with little empty space.

489

490   **Skeletochronological interpretations.** In the absence of comparative histological data,

491   most inferences regarding the life history of seymouriamorphs have been based on external

492   anatomy of different skeletal regions. For example, most individuals of *Discosauriscus* retain

493   lateral line canals on the skull, indicating an aquatic lifestyle, but this may also reflect a biased

494   relative abundance of premetamorphic individuals in the fossil record (e.g., Klembara et al.,

495   2006). Although definitive adults of this taxon, terrestrial or otherwise, are unknown (Klembara,

496   2001), previous authors have inferred that *Discosauriscus* underwent metamorphosis (Klembara,

497   1995), or that if some species were paedomorphic, were derived from an ancestor that did

498   metamorphose into a terrestrial adult (e.g., Boy & Sues, 2000). For *Discosauriscus austriacus*,

499   Sanchez et al. (2008) reported that metamorphosis occurred around the sixth year of life.

500   Although not explicitly stated as such, determination of metamorphosis in that study was

501   rendered feasible through the sampling of limb material of *Discosauriscus* from articulated

502   skeletons that would permit correlation of the skeletochronological data from the histological

503   analysis with external osteological features traditionally used for relative age determination.

504   Characterizing ontogeny in *Seymouria* is complicated by a general paucity of associated

505   postcrania compared to *Discosauriscus* such that most characterizations are solely based on the

506   cranium (e.g., Klembara et al., 2006, 2007). Indeed, it is not known for certain that *Seymouria*

507   underwent metamorphosis, as no larval forms have been recovered. The smallest reported

508   specimen is one from the Bromacker quarry with a skull measuring 2.1 cm (Berman & Martens,

509   1993), but the preservation is too poor to be described for comparative anatomical purposes.

510   Most of the other well-described cranial specimens exceed 80 cm (e.g., Berman, Reisz, &

511   Eberth, 1987), and small specimens remain poorly represented (e.g., Klembara et al., 2006).

512   Simultaneously, the histological signals of metamorphosis in early tetrapods, if they exist,

513   remain poorly understood. In extant lissamphibians, which may be the most appropriate extant

514   analogue for seymouriamorphs, there is often a 'transformation mark' (Schroeder & Baskett,

515   1968) or a 'metamorphosis line' (Rozenblut & Ogielska, 2005) that demarcates overwintering

516   following metamorphosis. Unfortunately, the criteria for identifying this line are somewhat

517   ambiguous in the literature beyond it being the closest circumferential line to the medullary

518   cavity and likely being closely spaced to the first LAG. Identifying this feature may thus rely

519   mainly on an absence of endosteal resorption (present in our sectioned specimens) that would

520   obliterate this mark. The line has a similar appearance to LAGs (e.g., Tsiora & Kyriakopoulou-

521   Sklavounou, 2002), and there is some debate over whether there is a correlation of this line with

522   deposition of woven-fibered bone (e.g., see Castanet & Smirina, 1990; Guarino et al., 2003). To

523   the best of our knowledge, this feature has never been identified in a Paleozoic tetrapod, and the

524   applicability of crown lissamphibian life history to seymouriamorphs on the amniote stem is

525   unknown.

526   As a result, determining whether a specimen of *Seymouria* is 'postmetamorphic' is based

527   largely on an established precedent of using external features from which admittedly arbitrary

528   and gradational terms such as 'juvenile,' 'sub-adult,' and 'adult' are derived. It bears noting that

529   these terms may refer to different biological attributes (e.g., sexual maturation vs. the process of

530   metamorphosis) and may be used differently by various workers. With respect to ROMVP

531   80916, the large partial femur that was sectioned, there is a well-established precedent for

532   identifying the element as belonging to a probable 'adult' that had completed metamorphosis.

533   ROMVP 80916 is incomplete, but the preserved portion is the same size as the complete

534   ROMVP 80915, which measures 5.5 cm in length and which is on the larger end of previously

535   reported specimens (e.g., 6.4 cm; White, 1939). Based on comparisons with articulated

536   specimens of *Seymouria* (Berman, Reisz, & Eberth, 1987, 2000), this femur would belong to an

537   individual with a skull length exceeding 10 cm, which falls well within a range for which

538   specimens have been previously described as 'adults.' Femora of the previously described

539   articulated skeletons are smaller than ROMVP 80916 yet the skulls possess numerous features

540   accepted as evidence for both somatic maturity and terrestriality, such as the absence of lateral

541   line grooves, ossified carpals and tarsals, firmly interdigitated sutures, and pronounced

542   ornamentation (Boy & Sues, 2000). ROMVP 82100 is smaller, measuring only 2.6 cm as

543   preserved. By comparison with ROMVP 80915, assuming isometric scaling of the element,

544   ROMVP 81200 would have been around 4.2 cm in length when preserved. Using this estimate

545   and comparisons with articulated specimens, the skull of this individual would have been around

546   8 cm, which is only slightly below the lower size bound reported for most specimens of

547   *Seymouria* (e.g., Berman, Reisz, & Eberth, 1987) and which would at least represent a 'sub-

548   adult' based on previous designations. Further evidence for a postmetamorphic determination

549   may be found in the nature of the preservational environment of Richards Spur. Beyond the

550   enigmatic and extremely rare aïstopod *Sillerpeton permianum*, there is no evidence of aquatic

551   tetrapods, either larval forms of metamorphosing adults or obligately aquatic adult forms, even

552   though material of very small-bodied tetrapods is captured (MacDougall et al., 2017). Regardless

553   of whether this represents a biased sample, it is clear that the fissure fills were not conducive to

554   the capture of aquatic tetrapods.

555   Based on a postmetamorphic interpretation of the sectioned material, the onset of

556   metamorphosis in *Seymouria* may thus be constrained to probably occurring by the second year

557   of life. It may be inferred that the larger ROMVP 81096 was probably older than two years,

558   although this cannot be proven nor can the amount of growth cycles lost to remodeling

559   determined at present. Metamorphosis in *Seymouria* occurred much earlier than in

560   *Discosauriscus*, which was suggested to undergo a delayed metamorphosis around the sixth year

561   of life (Sanchez et al., 2008). The more compact and sparsely vascularized lamellar bone of

562   *Discosauriscus* also support interpretations of a slower growth and a protracted aquatic larval

563   stage in this taxon (Sanchez et al., 2008). This disparity among closely related taxa may reflect

564   the different environments that these taxa inhabited (correspondent with their differing

565   lifestyles), as *Seymouria* is primarily found in fluvial environments of North America, and

566 *Discosauriscus* is primarily found in lacustrine settings in Europe. Both taxa likely experienced

567 distinct seasonality, but the presence of distinct LAGs in *Discosauriscus* suggests a stronger

568 relative influence of environmental fluctuations on this taxon. Some seasonality at Richards

569 Spur, previously demonstrated through other lines of evidence (e.g., Woodhead et al., 2010;

570 MacDougall et al., 2017) is evident in our samples from the clearly defined annuli and growth

571 zones (Figs. 11-12), and it seems probable that these environmental conditions (particularly the

572 ephemerality of bodies of water) would have favored an early onset of metamorphosis in

573 *Seymouria*. Taxa living in the same habitat and experiencing similar conditions can exhibit

574 different responses to those conditions – amniotes are typically less constrained by water stress

575 relative to non-amniotes, for example. A hypothesis of variable responses to similar climate at

576 Richards Spur is supported by the differing skeletochronological records of co-occurring

577 tetrapods from the site. The dissorophoid temnospondyls exhibit clear LAG patterns (Richards,

578 2016; Gee, Haridy, & Reisz, in press), whereas the varanopid synapsids exhibit a mixture of

579 LAGs and annuli with growth zones (Huttenlocker & Shelton, 2020), and the captorhinid

580 eureptiles exhibit only annuli with growth zones (Peabody, 1961; Richards, 2016; Huttenlocker

581 & Shelton, 2020). Other hypotheses to explain the differing patterns include secondary

582 environmental factors that may themselves be influenced by climate but in more nuanced and

583 asymmetrical fashions, such as prey availability or limited spatial occupation within the habitat.

584   Future work will be necessary to identify histological markers of pre- versus

585 postmetamorphic life stages. The present study cannot inform further on this because of the

586 absence of definitive larval specimens of *Seymouria*, which in turn creates more uncertainty

587 regarding the nature of metamorphosis, if it occurred in this taxon, as well as the external

588 osteological changes associated with this process if it did. Even if our interpretation of the

589   sampled specimens as postmetamorphic is accepted, the timing of either the onset or completion

590   of metamorphosis relative to the time of death remains unclear. In order to more precisely

591   constrain histological markers of metamorphosis will require sampling of taxa, such as

592   *Discosauriscus*, for which metamorphosis is definitively known and for which osteological

593   changes associated with this process are well-documented.

594

595   **Lifestyle interpretations.** In early tetrapods, interpretations of lifestyle (e.g., aquatic vs.

596   terrestrial) are often based on the presence or absence of features such as lateral line grooves and

597   the degree of development of external features of the limbs (e.g., Moodie, 1908; Schoch, 2002;

598   Witzmann, 2016). Histology has more recently been utilized as a means to further test these

599   hypotheses by means of comparisons with extant taxa in which mode of life can be definitively

600   observed and with the classically utilized external anatomical features (Germain & Laurin, 2005;

601   Kriloff et al., 2008; Sanchez et al., 2010; Quemeneur, de Buffrénil & Laurin, 2013; Konietzko-

602   Meier, Shelton, & Sander, 2016). *Seymouria* is widely accepted to lack lateral line grooves,

603   although they have been suggested by some to have been present in juveniles (Berman &

604   Martens, 1993, but see Klembara et al., 2006), suggesting a transition in lifestyle throughout

605   ontogeny. Additionally, the limb bones are well-developed, with prominent attachment sites for

606   musculature and distinct processes (Figs. 4-5), and the neural arches are greatly expanded

607   compared to other Paleozoic tetrapods with prominent zygapophyses inferred to have supported

608   the axial column in a terrestrial animal (Sullivan & Reisz, 1999). Our data further corroborate the

609   interpretation of the Richards Spur locality as an assemblage dominated by terrestrial fauna

610   (MacDougall et al., 2017).

611   The centra also contribute information through inferences on the skeletal mass of the

612   element(s). The two traditionally utilized criteria are the thickness of the periosteal domain and

613   the presence or absence of calcified cartilage. Greatly thickened domains (pachyostosis) and

614   retention of calcified cartilage throughout ontogeny are frequently seen in large-bodied aquatic

615   temnospondyls and probably served to increase the skeletal mass for buoyancy control (Danto et

616   al., 2016). In both *Discosauriscus* and *Seymouria*, the periosteal domain is relatively thin, and

617   calcified cartilage is primarily found around the notochordal canal (Danto et al., 2016). In

618   *Seymouria*, this is the only location of this tissue, whereas calcified cartilage occurs sporadically

619   in the endochondral domain of at least immature individuals of *Discosauriscus*.

620   What then can be concluded regarding the histological data from *Seymouria* postcrania

621   and the lifestyle of the taxon? The femoral microanatomy, specifically the relatively thin cortex

622   and the modest development of the medullary spongiosa, is more compatible with that of a

623   terrestrial animal by comparison with other Paleozoic tetrapods (primarily temnospondyls) that

624   have been inferred to be terrestrial (e.g., Sanchez et al., 2010b; Quemeneur, de Buffrénil &

625   Laurin, 2013). Based on studies of femoral and tibial microanatomy in extant tetrapods (Kriloff

626   et al., 2008; Quemeneur, de Buffrénil & Laurin, 2013), these features also support a primarily

627   terrestrial lifestyle. Collectively, this corroborates the conclusions of previous authors that

628   *Seymouria* was most likely a terrestrial animal (White, 1939; Berman & Martens, 1993; Sullivan

629   & Reisz, 1999; Marchetti, Mujal & Bernardi, 2017). The vertebral histology also confers support

630   for a terrestrial lifestyle. The periosteal domain is thin, calcified cartilage is sparse and confined

631   to the margin of the notochordal canal, and the neural arch is largely hollow. These data

632   correspond favorably with the broad expansion of the arch and the zygapophyses, which Sullivan

633   & Reisz (1999) interpreted to be for the stiffening of the axial column following White (1939).

634

635   **Terrestriality in seymouriamorphs.** Assessing the range of ecologies among seymouriamorphs

636   from a macroevolutionary standpoint is important because the group has historically been

637   regarded as being well-situated for understanding the associated skeletal modifications

638   associated with terrestriality. *Seymouria* is one of the best seymouriamorphs for examining such

639   modifications because complete, articulated skeletons are known (e.g., Berman, Reisz, & Eberth,

640   1987), but it then becomes important to assess whether a terrestrial or aquatic lifestyle is the

641   plesiomorphic state among seymouriamorphs. Given that seymouriamorphs, and reptiliomorphs

642   more broadly, are frequently used as exemplars for the skeletal changes associated with

643   terrestrial adaptation, clarifying the primitive condition of this group is critical for informing

644   accurate comparisons. A conceptual phylogeny, adapted from Klembara (2011), is presented in

645   Figure 14, illustrating the distribution of terrestriality among seymouriamorphs.

646   Our data provide strong evidence at the histological and the microanatomical scales to

647   support the longstanding hypothesis of terrestriality in *Seymouria.* This is not a particularly

648   controversial idea; numerous aspects of the external morphology, such as the well-ossified limbs

649   and the massively expanded vertebrae have long been cited as evidence for this lifestyle (Romer,

650   1956). Although Berman and Martens (1993) described a possible indication of a lateral line

651   system in juvenile specimens of *S. sanjuanensis* from Germany, subsequent work (Klembara et

652   al. 2006) on an early juvenile did not find any evidence for a lateral line system in other *S.*

653   *sanjuanensis* from the same locality. As such, while it is often inferred that *Seymouria* underwent

654   metamorphosis as with other seymouriamorphs and a number of other terrestrial tetrapods (e.g.,

655   some temnospondyls), definitive aquatic larval forms and morphological transitions associated

656   with the presumed metamorphosis are unknown.

657   At least one other seymouriamorph, *Karpinskiosaurus*, is also represented only by

658   specimens that lack lateral line grooves (Klembara, 2011). *Kotlassia* has also been historically

659   regarded as lacking lateral line grooves (e.g., Bystrow, 1944), but the *Kotlassia* of most previous

660   authors is actually a combination of material referable to the type species, *Kotlassia prima*, and

661   material properly referable to *Karpinskiosaurus* (see Bulanov, 2002 and Klembara, 2011, for

662   discussion). Whether these grooves are definitively absent in the holotype of *Ko. prima* is not

663   apparent from previous works that accounted for this historical discrepancy. For

664   *Karpinskiosaurus* and *Seymouria*, it has been proposed that these taxa underwent metamorphosis

665   relatively early in their development and lived on land for the majority of their lives (Klembara,

666   2011).

667   In contrast, most other seymouriamorphs are known from individuals with lateral line

668   grooves, including *Ariekanerpeton* (Klembara & Ruta, 2005), *Discosauriscus* (Klembara, 1996),

669   *Spinarerpeton* (Klembara, 2009), and *Utegenia* (Malakhov, 2000). The most recent phylogenetic

670   analysis that focused on seymouriamorph phylogeny is that of Klembara (2011), which followed

671   a series of anatomical work that re-described virtually all known seymouriamorphs. Mapping the

672   distribution of ecologies onto this topology suggests that seymouriamorphs are primitively

673   aquatic (*Utegenia* being the earliest diverging taxon) and with two separate shifts to terrestriality,

674   one in *Karpinskiosaurus* and one in *Seymouria* (Fig. 14). However, caution must be exercised in

675   inferring the phylogeny of a clade in which metamorphosis is known to occur because biases in

676   the record of premetamorphic larval forms versus that of postmetamorphic terrestrial adults (if

677   such determinations can be made to begin with) can produce misleading data. As with *Seymouria*

678   (Berman et al., 2000), it has been proposed that *Discosauriscus* transitioned from an aquatic to

679   terrestrial lifestyle throughout its ontogeny, but even the largest known specimens of

680   *Discosauriscus* are believed to be juveniles, and none have been recovered from the terrestrial

681   environments that the adult individuals may have inhabited (Klembara, Martens & Bartik, 2001).

682   This may relate to a relatively protracted larval stage that was recovered through the histological

683   work of Sanchez et al. (2008) in which metamorphosis may not have begun until year six of an

684   individual's life. The latest phylogenetic analyses (Klembara, 2011) do not bear out the slippage

685   that is predicted when coding taxa based on immature specimens (i.e. *Discosauriscus* is a highly

686   nested seymouriamorph), but this does not negate the potential for this disparity to affect the

687   phylogeny. *Ariekanerpeton*, *Spinarerpeton*, and *Utegenia* are also likely represented only by

688   juveniles (Klembara & Ruta, 2003, 2005; Klembara, 2009), which warrants consideration.

689

690   **Conclusions**

691   Histology offers one avenue for exploring the diversity of life histories within a clade

692   through well-documented taxa (e.g., *Discosauriscus, Seymouria*) and for improving hypotheses

693   and predictions regarding those of more poorly represented taxa with limited ontogenetic data.

694   The correlation of the skeletochronological data from our histological analysis with the relative

695   size and development of external features of the sampled femora substantiate the hypothesis that

696   *Seymouria* was a rapidly metamorphosing taxon that spent most of its life on land (Klembara,

697   2011). This life history may explain why it is predominantly found in fluvial deposits of south-

698   southwestern North America and the upland Bromacker locality along with other highly

699   terrestrial tetrapods whereas the slower growing *Discosauriscus* is restricted to lacustrine

700   environments of Europe. A relatively early onset of metamorphosis may also account for the

701   absence of larval forms of *Seymouria* (i.e. a short aquatic larval stage) and the probable spatial

702   separation of larvae from the fluvial environments that preserved the adults (niche partitioning

703   between life stages). Recovery of small and presumably immature specimens of *Seymouria* will

704   be required to further explore the taxon's life history and to contextualize it with other terrestrial

705   tetrapods.

706   In the sense that terrestriality in adults of *Seymouria* has not been widely questioned, our

707   most novel data, the histological data, are not necessarily surprising. However, this should not

708   diminish the value of these data; testing hypotheses using multiple approaches is important for

709   assessing the rigor of such hypotheses. The existing histological framework and understanding of

710   seymouriamorph development remains largely confined in traditional interpretations of relative

711   maturity based on external features and their development, and there is an extensive precedent

712   for the utility of histology (among other more recently accessible methods) to further explore

713   paleobiological attributes of extinct taxa. Our interpretations of the data are somewhat limited, in

714   part by sample size, but also in part by the absence of a substantive body of comparative data. It

715   is unclear, for example, what to make of the persistence of a modestly developed medullary

716   spongiosa in the femur of *Seymouria*, either compared to *Discosauriscus* or to other more

717   distantly related terrestrial tetrapods (though see our previous comment regarding the potential

718   for there to be a size correlation). Taken in isolation, the presence of this spongiosa could

719   indicate that *Seymouria* was more semi-terrestrial than previously believed, at least at the

720   captured stages of its life history, but it bears reiterating that microanatomy captures more than

721   just a signal of ecological lifestyle, such as signals from phylogeny or from life history. Lifestyle

722   is also gradational, both between and within taxa, such that binning taxa into discrete categories

723   can prove challenging. Our ontogenetic trends are more accurately stated as two partial points

724   within the developmental trajectory, and the opportunity remains to explore ontogeny further

725   within *Seymouria* across all regions of the skeleton.

726

Institutional Abbreviations

728 **FMNH**   Field Museum of Natural History, Chicago, IL, USA

729 **OMNH**   Sam Noble Oklahoma Museum of Natural History, Norman, OK, USA

730 **ROMVP**   Royal Ontario Museum, Toronto, ON, Canada

731

Acknowledgements

738

739 *Note to reviewers/editor: embargoed data during review can be viewed on MorphoBank using a*

740 *special log-in: username "3549" and password "ROMVP80915".*

741

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Figure 1

Posterior presacral vertebrae of *Seymouria*.

A, OMNH 79346 in anterior view; **B,** the same in posterior view; **C**, the same in dorsal view; **D,** the same in ventral view; **E,** the same in left lateral view; **F,** OMNH 79349 in anterior view; **G,** the same in posterior view; **H,** the same in dorsal view; **I,** the same in ventral view; **J,** the same in left lateral view; **K,** OMNH 79350 in anterior view; **L,** the same in posterior view; **M,** the same in dorsal view; **N,** the same in ventral view; **O,** the same in left lateral view; **P,** OMNH 79353 in anterior view; **Q,** the same in posterior view; **R,** the same in dorsal view; **S,** the same in ventral view; **T,** the same in left lateral view; **U,** ROMVP 81198 in anterior view; **V,** the same in posterior view; **W,** the same in dorsal view; **X,** the same in ventral view; **Y,** the same in left lateral view; **Z,** ROMVP 81199 in anterior view; **AA,** the same in posterior view; **BB,** the same in dorsal view; **CC,** the same in ventral view; **DD,** the same in left lateral view. Scale bar equals 1 cm. **n**, notochordal canal; **nc**, neural canal; **poz**, postzygapophysis; **prz**, prezygapophysis; **snc**, supraneural canal
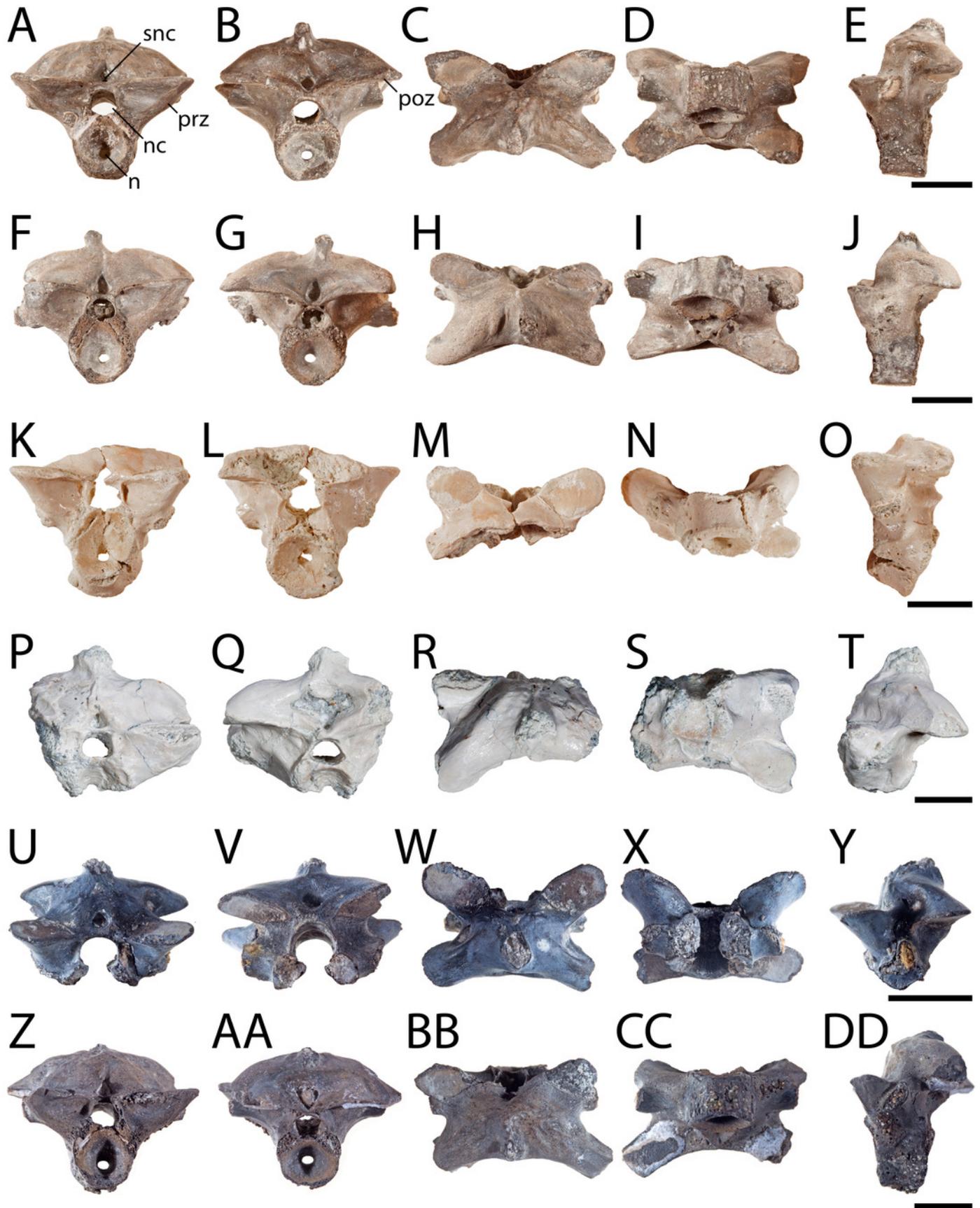
Figure 2

Anterior presacral vertebrae of *Seymouria*.

A, OMNH 79351 in anterior view; **B,** the same in posterior view; **C,** the same in dorsal view; **D,** the same in ventral view; **E,** the same in left lateral view; **F,** OMNH 79352 in anterior view; **G,** the same in posterior view; **H,** the same in dorsal view; **I,** the same in ventral view; **J,** the same in left lateral view. Scale bar equals 1 cm. **dp**, diapophysis; **n**, notochordal canal; **nc**, neural canal; **poz**, postzygapophysis; **prz**, prezygapophysis; **snc**, supraneural canal
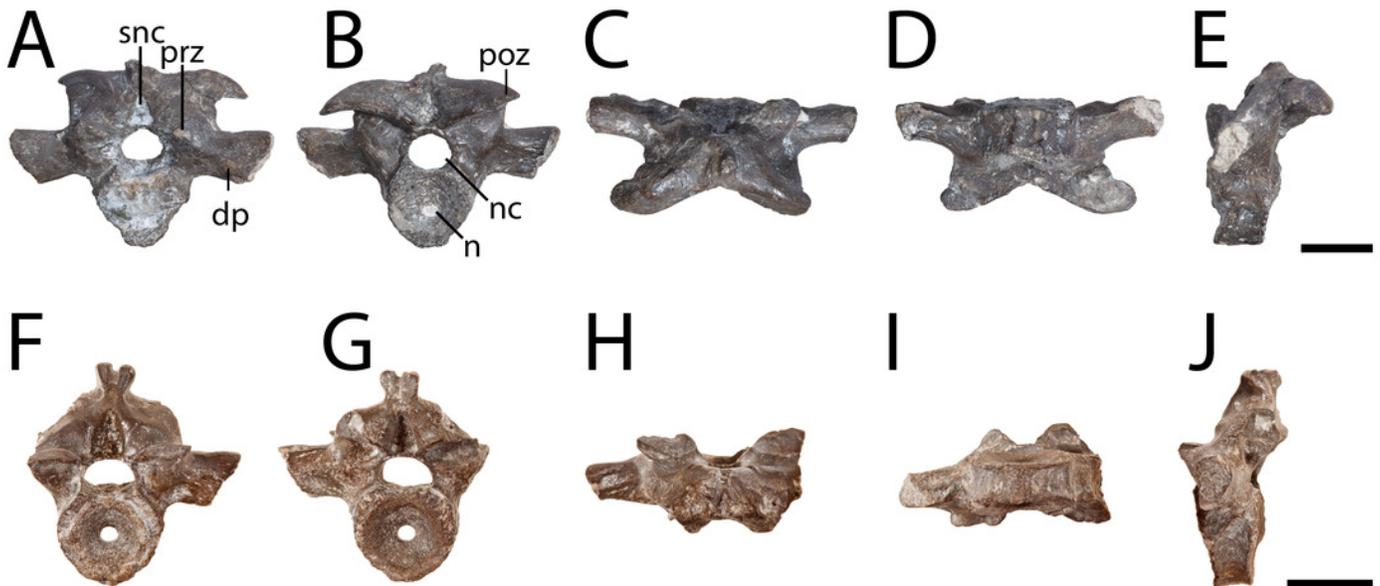
Figure 3

Articulated vertebrae of *Seymouria*.

A, OMNH 79347 in dorsal view; **B,** OMNH 79348 in dorsal view; **C,** the same in ventral view; **D,** the same in left lateral view; **E,** the same in right lateral view. Scale bar equals 1 cm. **poz**, postzygapophysis; **prz**, prezygapophysis

A

B

poz

prz

C

sacral 2?

sacral 1

presacral
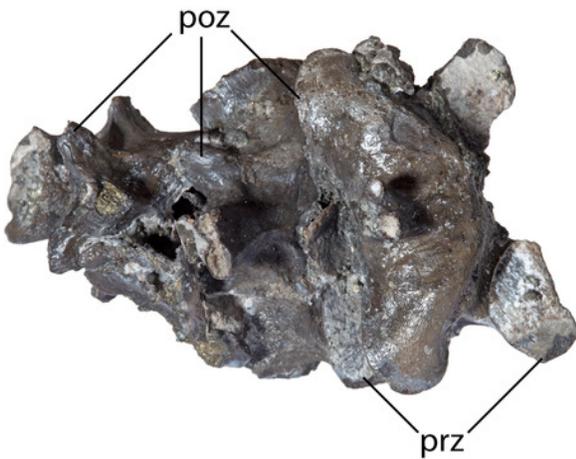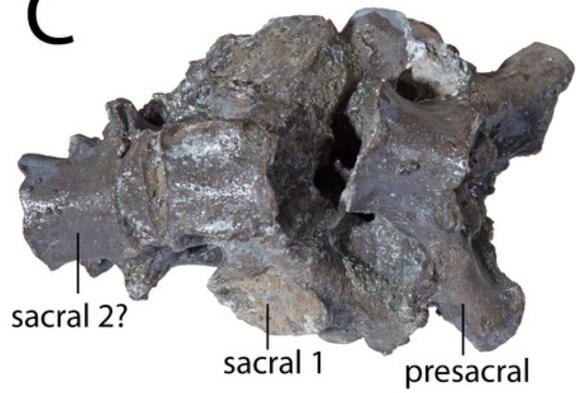
D

E

Figure 4

OMNH 74721.

A, anterior view, **B,** posterior view, **C,** dorsal view, **D,** ventralview, **E,** proximal view, and **F,** distal view. Scale bar equals 1 cm. **cap**, capitellum; **dpc**, deltopectoral crest; **ect**, ectepicondyle; **ent**, entepicondyle; **entf**, entepicondylar foramen; **entr**, entepicondylar ridge; **sbcsc**, supcoracoscapularis; **sup**, supinator process
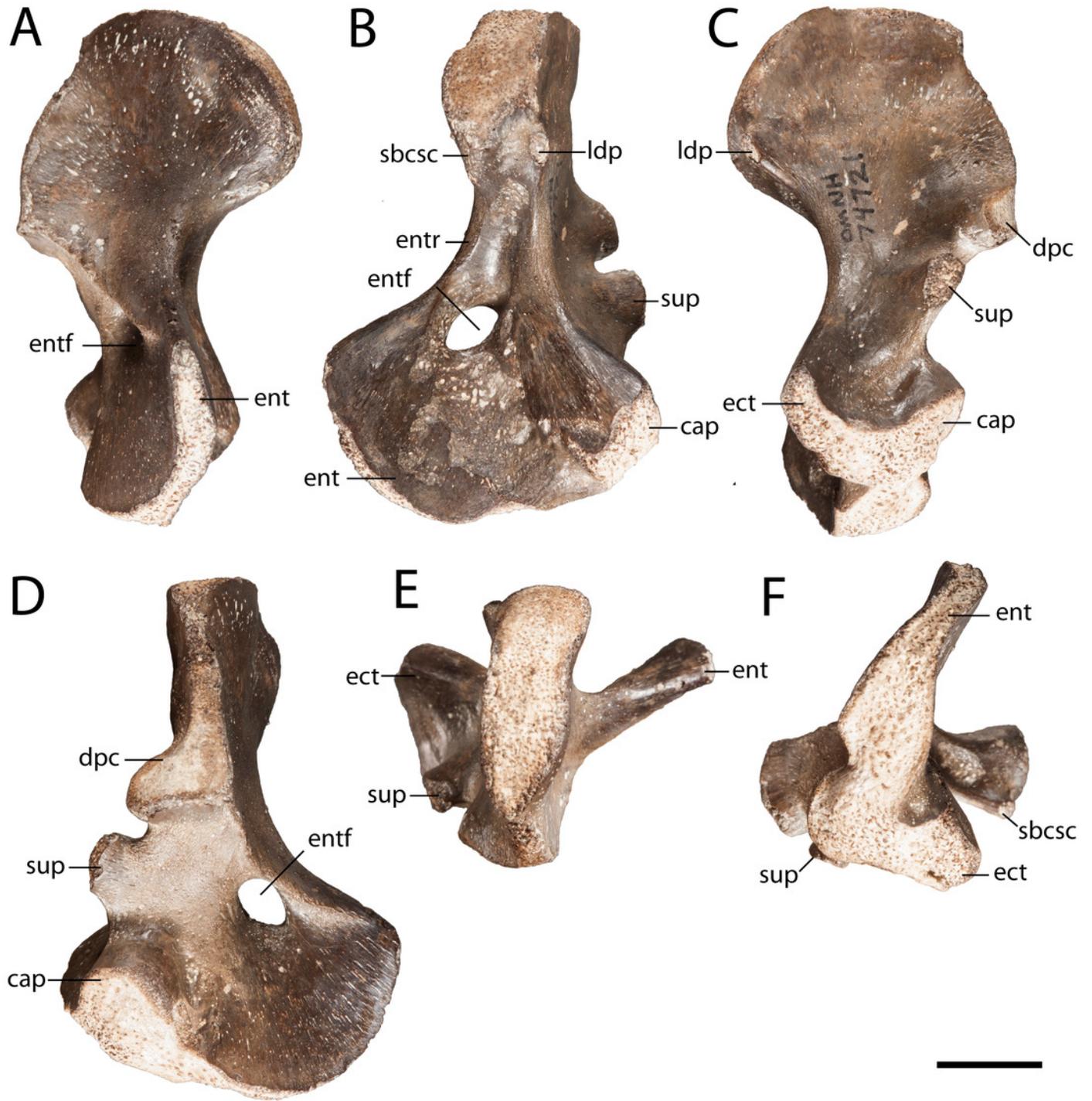
Figure 5

Hindlimb elements of *Seymouria*.

A, photograph of ROMVP 80915 and ROMVP 80917 in posterior view; **B,** the same in ventral view; **C,** digital rendering of ROMVP 80916 in posterior view; **D,** the same in anterior view; **E,** the same in dorsal view; **F,** the same in ventral view; **G,** the same in proximal view; **H,** the same in distal view. Scale bar equals 1 cm. **adc**, adductor crest; **fibc**, fibular condyle; **icf**, intercondylar fossa; **itf**, intertrochanteric fossa; **pfm**, *m. puboischiofemoralis*; **tibc**, tibial condyle; **tr**, trochanter
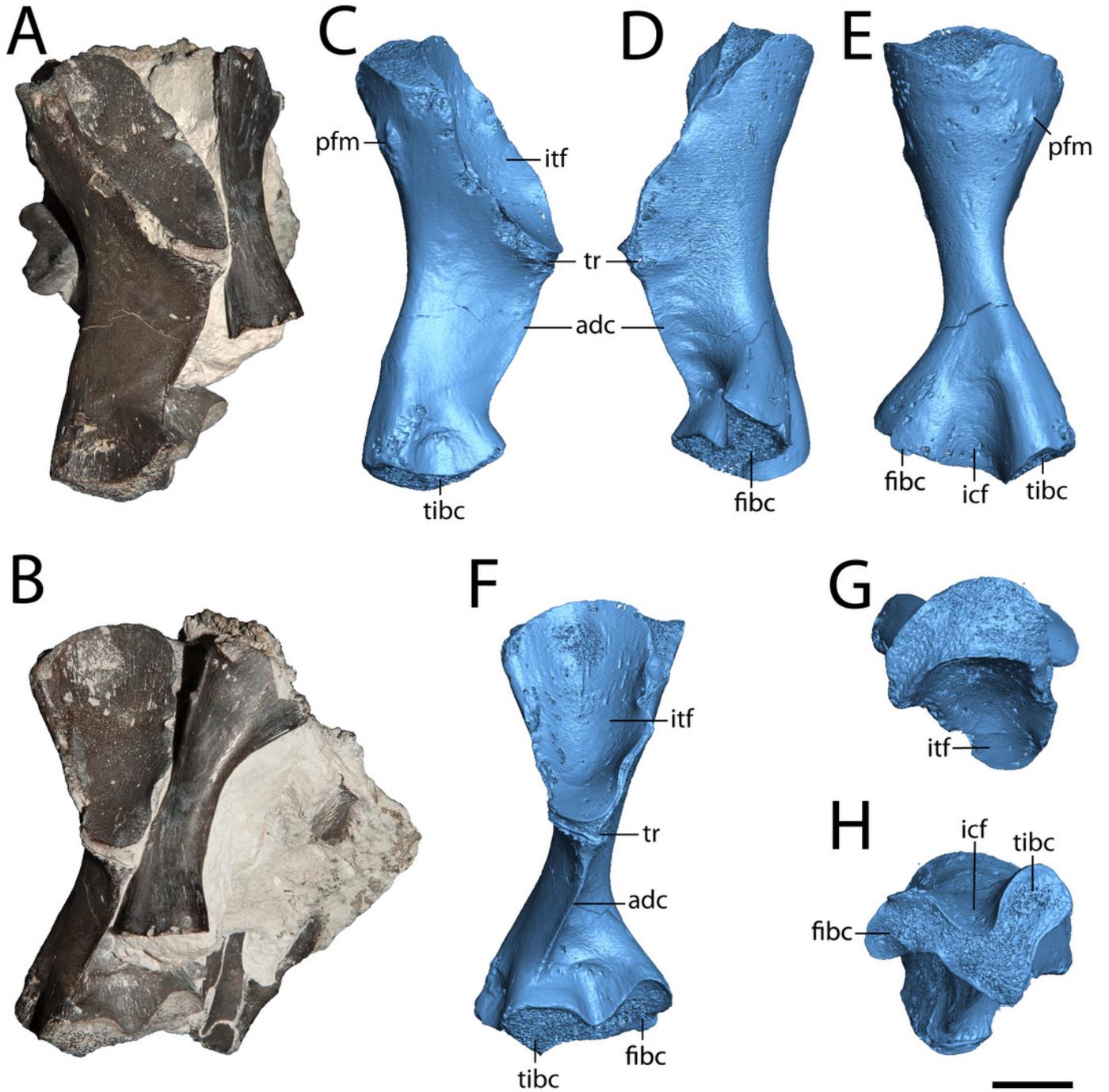
Figure 6

Partial femora of *Seymouria*.

A-F, ROMVP 80916 and **G-L,** ROMVP 81200 in anterior, posterior, dorsal, ventral, proximal, and distal views. Scale bar equals 1 cm.

A

B

C

D

E

F

G

H

I

J

K

L

Figure 7

Digital renderings of ROMVP 80917.

A, anterior; **B,** posterior; **C,** medial; **D,** lateral; **E,** proximal; **F,** distal views. Scale bar equals 1 cm. **ifm**, *m. iliofibularis*
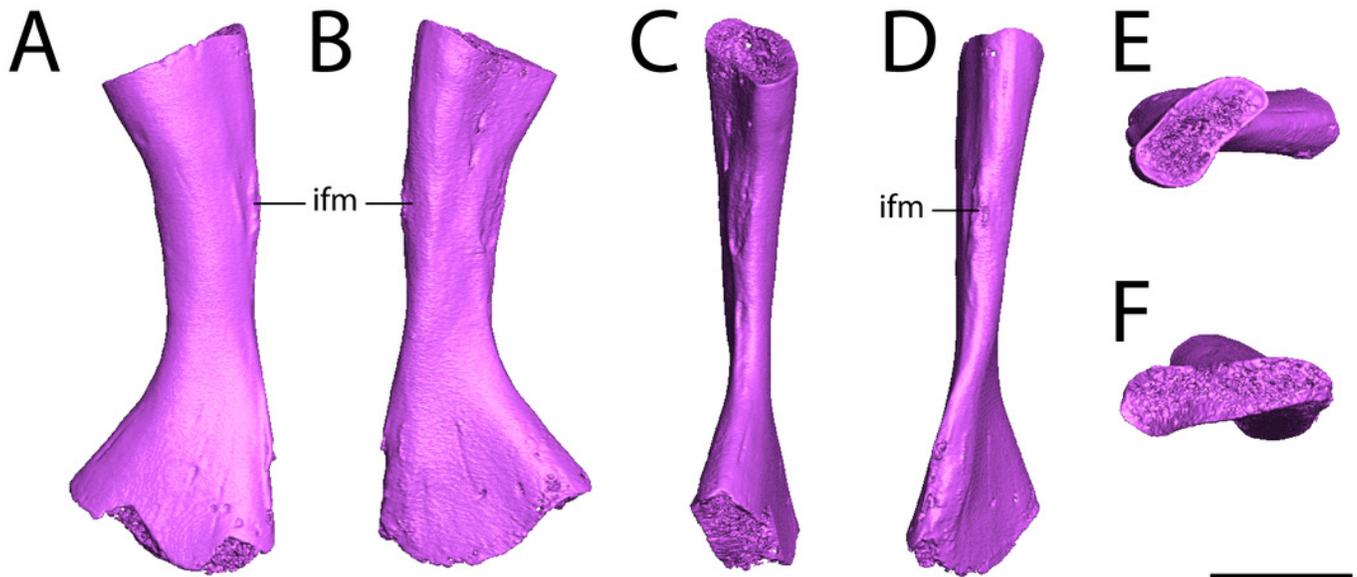
Figure 8

Histological sections of a presacral vertebra (ROMVP 81199) of *Seymouria.*

A, Full sagittal section; **B,** close-up under cross-polarized light of the ventral border of the neural arch showing remodelling; **C,** close-up under cross-polarized light of the thickened posterior surface; **D,** half transverse section; **E,** close-up under cross-polarized light of the dorsal surface; **F,** close-up under cross-polarized light of the cartilaginous separation between the neural arch and the centrum. Scale bars equal to 1 mm (A, D); 250 μm (B-C, E-F). **n**, notochordal canal; **na**, neural arch; **nc**, neural canal; **ns**, neural spine; **p**, pleurocentrum; **snc**, supraneural canal

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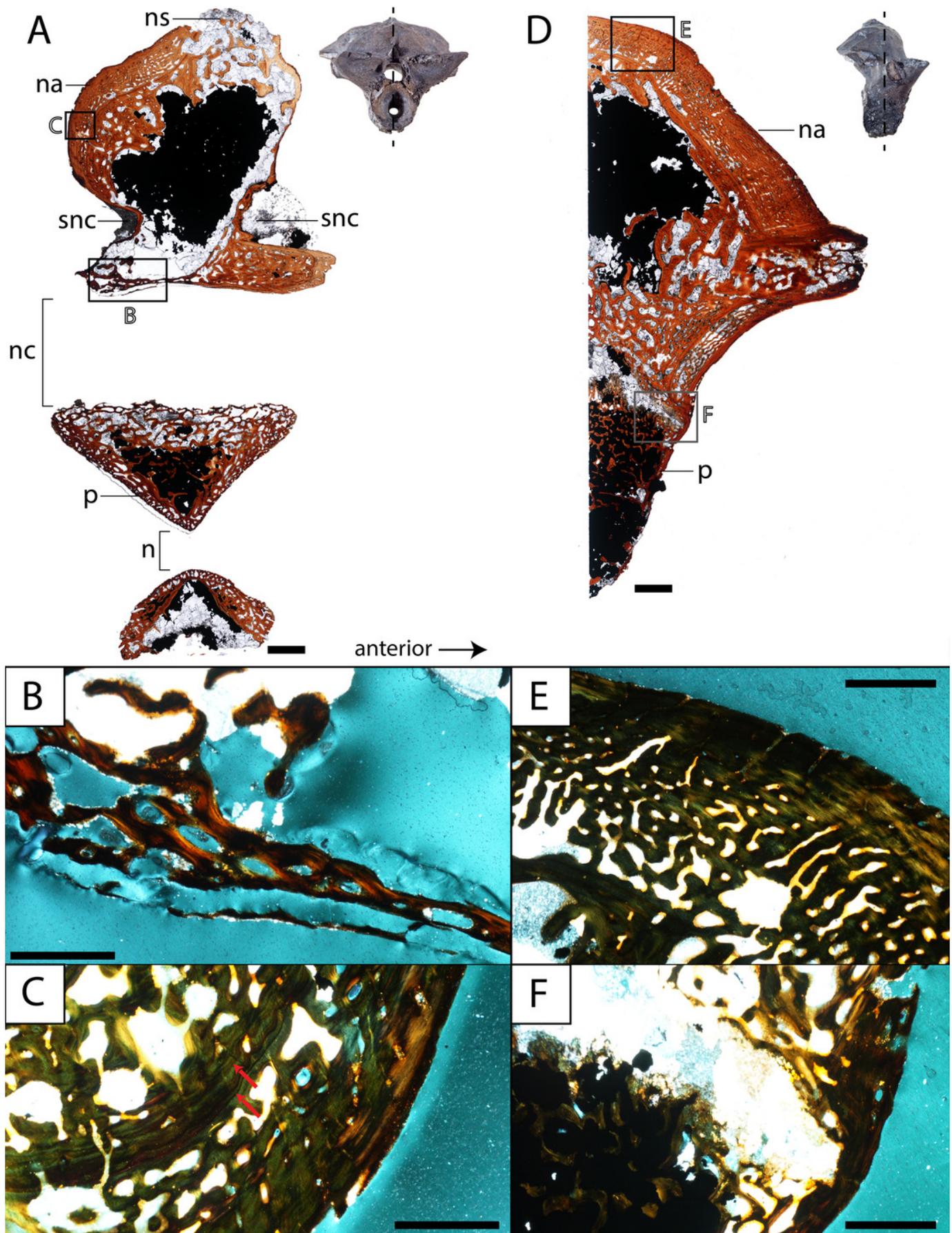
Figure 9

Histological sections of a presacral vertebra (ROMVP 81198) of *Seymouria*.

A, Full sagittal section; **B,** close-up under cross-polarized light of the ventral border of the neural arch showing remodelling; **C,** close-up under cross-polarized light of the thickened posterior surface; **D,** half transverse section; **E,** close-up under cross-polarized light of the dorsal surface; **F,** close-up under cross-polarized light of the geometric center of the neural arch. Scale bars equal to 1 mm (A, D); 250 µm (B-C, E-F). **na**, neural arch; **nc**, neural canal; **ns**, neural spine; **snc**, supraneural canal
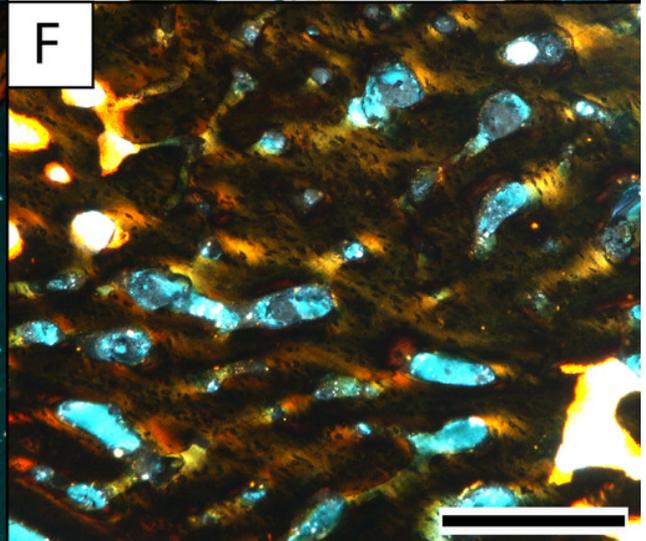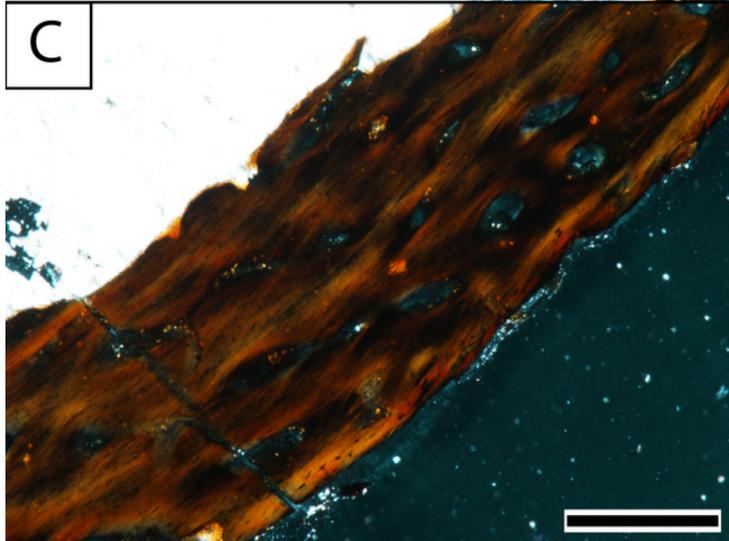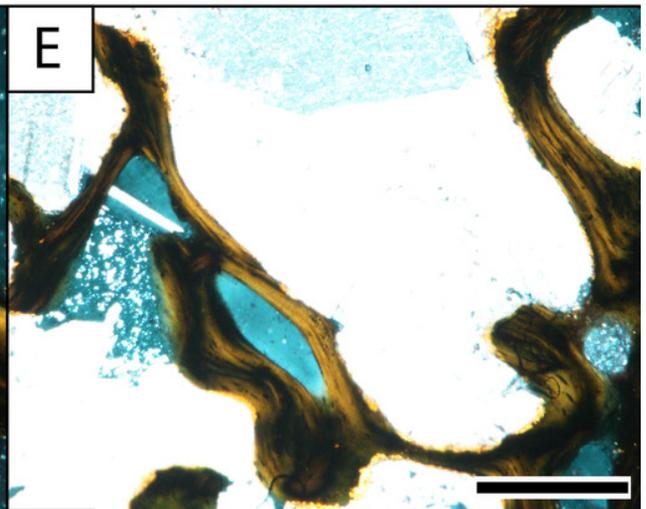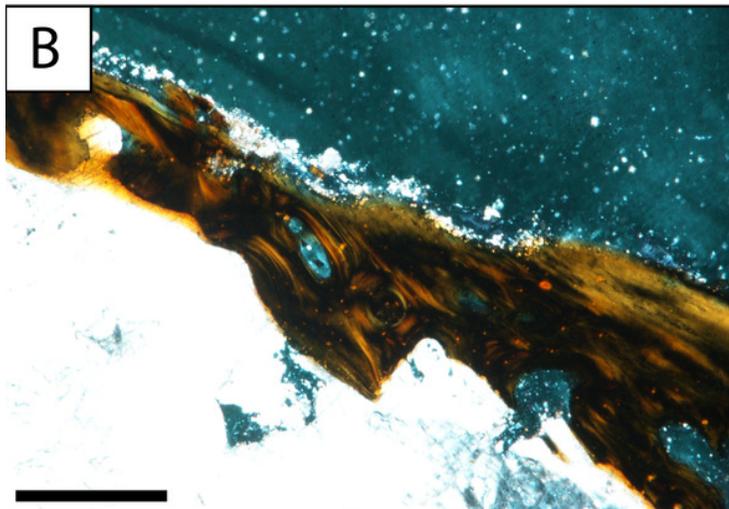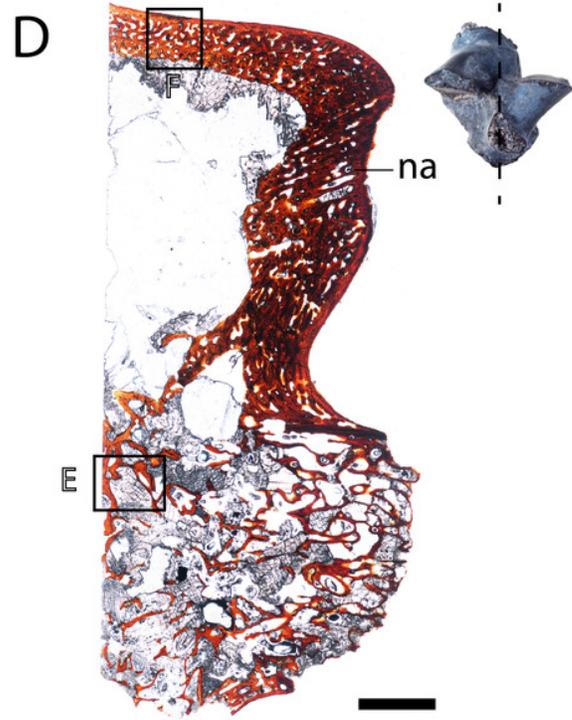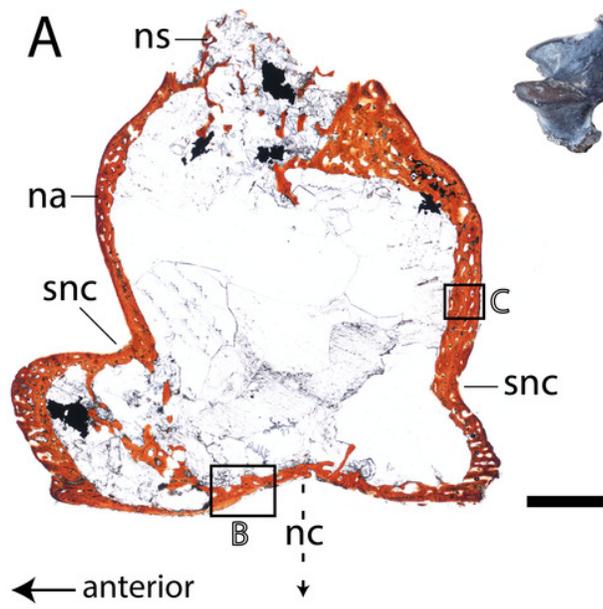
Figure 10

Isolated profiles of OMNH 79348.

A, D, G, digital renderings indicating location of digital sections; **B-C,** sagittal sections of presacral vertebra at mid-centrum (B) and mid-diapophysis (C); **E-F,** sagittal sections of sacral vertebra at mid-centrum (E) and mid-diapophysis (F); **H-G,** sagittal sections of caudal vertebra at mid-centrum (H) and mid-diapophysis (I). Scale bar equals 1 cm. **n,** notochordal canal; **nc,** neural canal

anterior

sacral 2?   sacral 1   presacral

nc

n
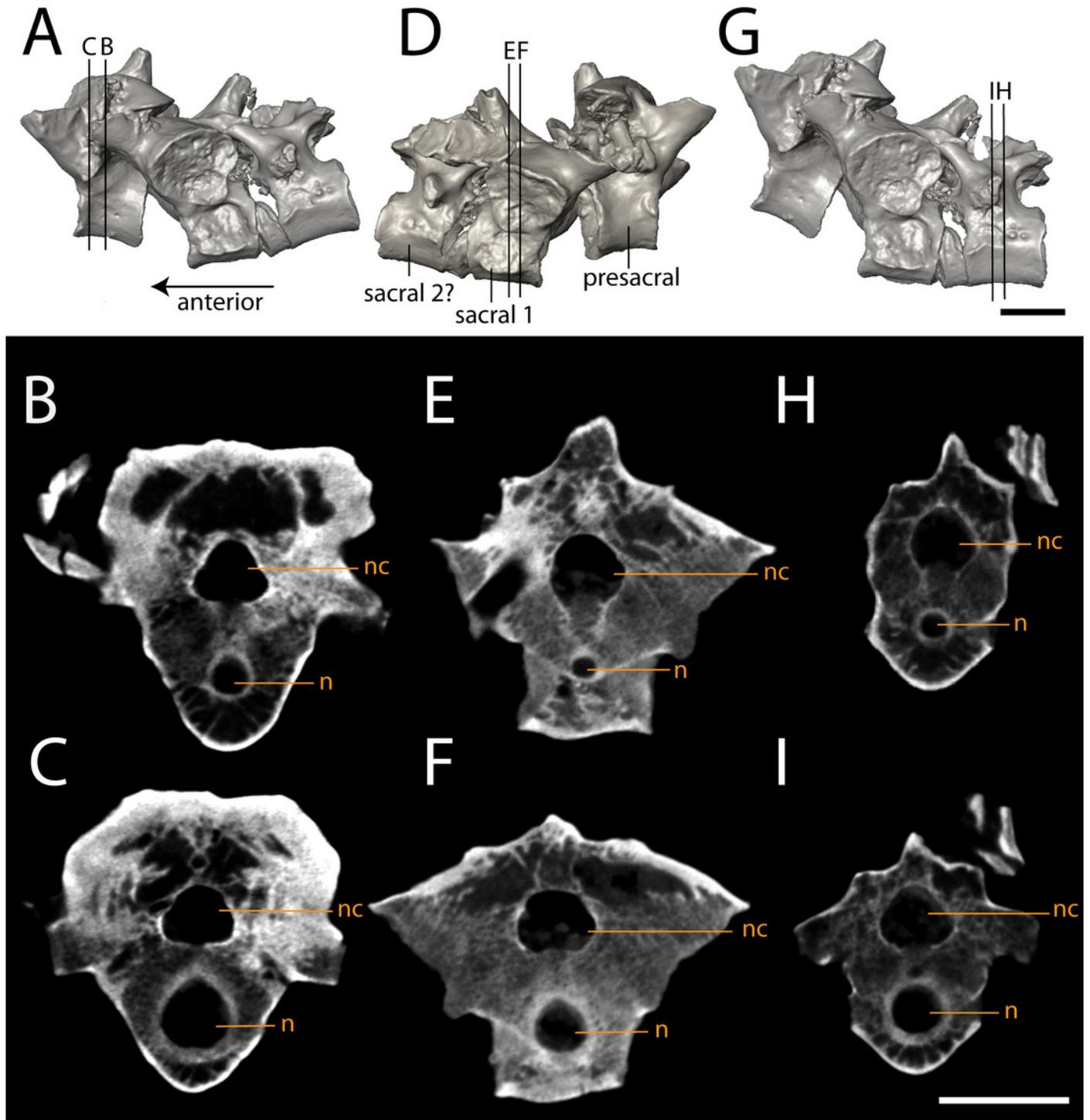
Figure 11

Histological section of a partial femur (ROMVP 80916) of *Seymouria*.

A, full cross-section near the minimum diaphyseal circumference; **B,** close-up of the cortical bone; **C,** close-up under cross-polarized light of the remodelled bone interface between the cortical bone and the medullary cavity. Note that part C is from a different thin section than in part A and thus an inset magnification box is not marked. Scale bars equal to 1 mm (A); 250 μm (B-C). **a**, annulus; **gz**, growth zone; **po**, primary osteon; **so**, secondary osteon; **vc**, vascular canal. Arrows mark rest lines.

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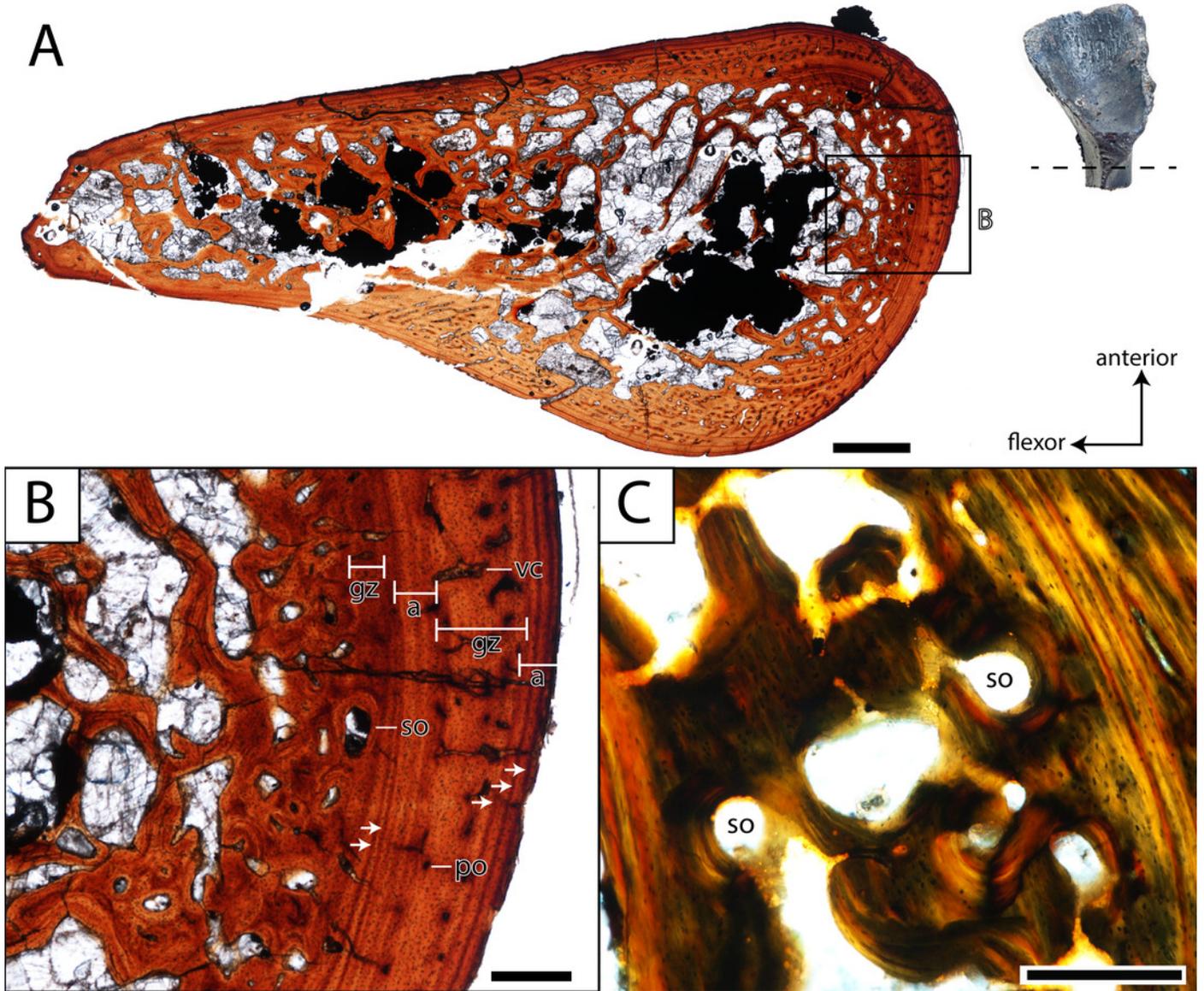
Figure 12

Histological section of a partial femur (ROMVP 81200) of *Seymouria*.

A, full cross-section near the minimum diaphyseal circumference; **B,** close-up of the cortical bone showing four lines of arrested growth (LAGs) marked by black arrows; **C,** close-up under cross-polarized light of the remodelled bone interface between the cortical bone and the medullary cavity. Scale bars equal to 1 mm (A); 250 μm (B-C). **a**, annulus; **gz**, growth zone; **po**, primary osteon; **so**, secondary osteon; **vc**, vascular canal. Arrows mark rest lines.

A

B

gz a gz

vc po

C

so

so

so
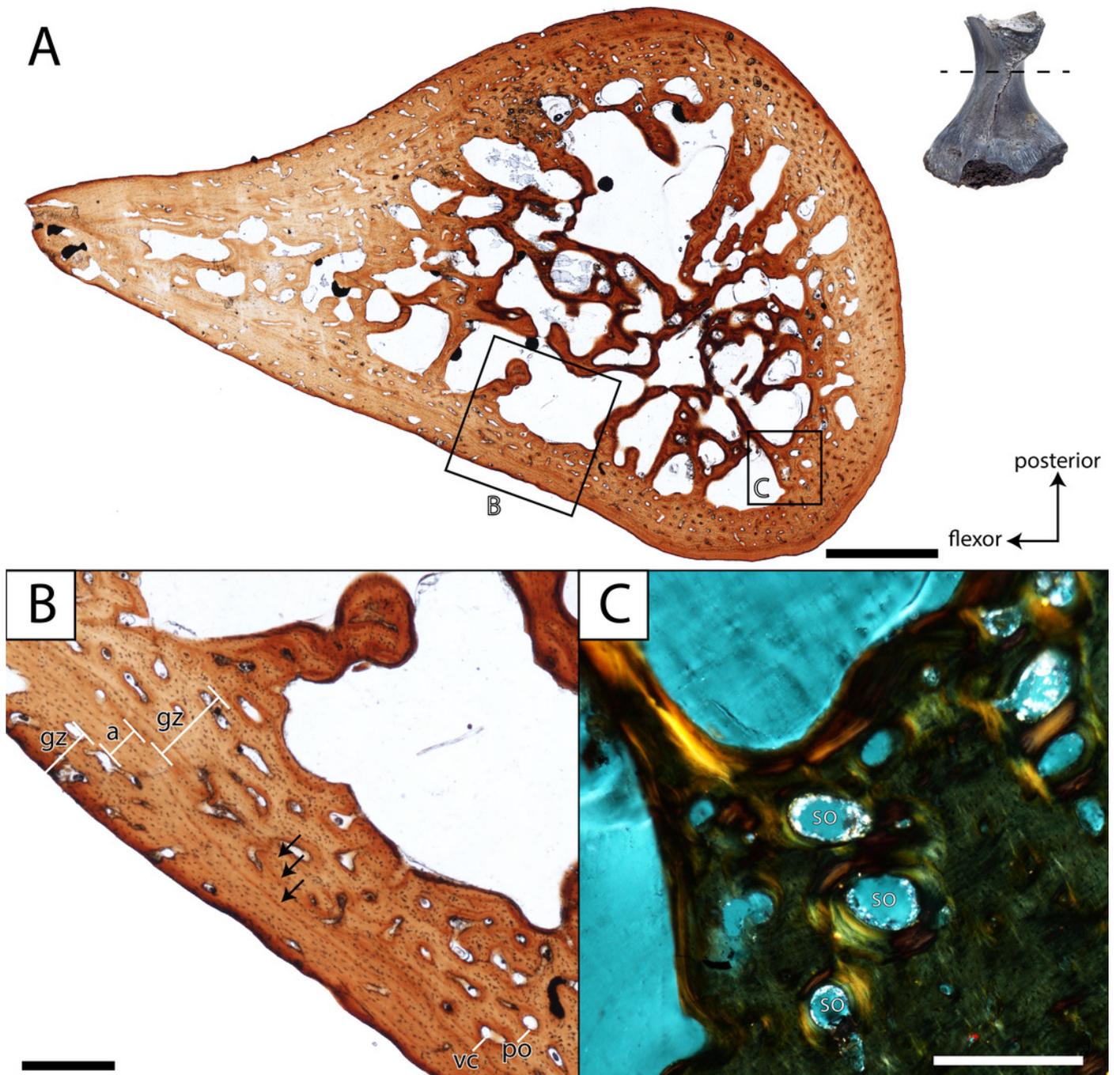
Figure 13

Digital section of a femur (ROMVP 80915) of *Seymouria*.

A, digital rendering showing location of section; **B,** transverse digital section. Scale bar equals 5 mm.
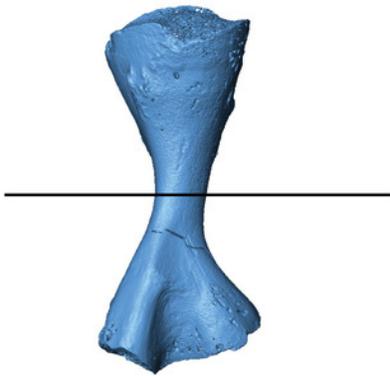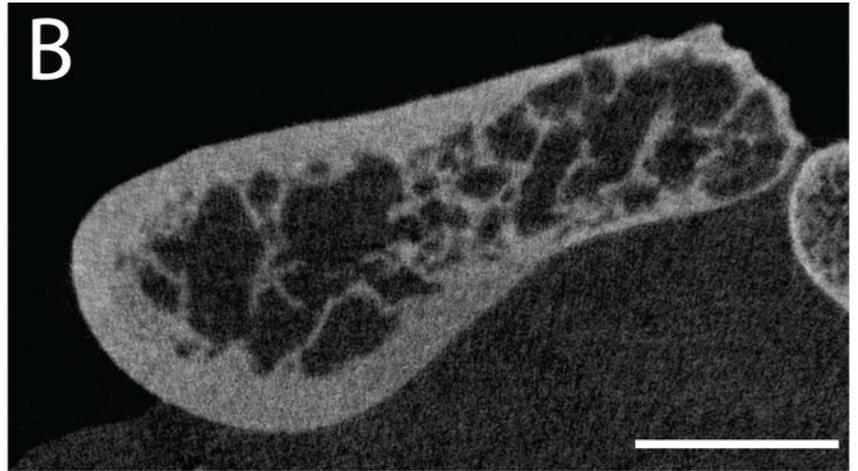
Figure 14

Conceptual phylogeny adapted from Klembara (2011), illustrating the distribution of aquatic and terrestrial taxa among seymouriamorphs.

Blue boxes represent taxa interpreted as being aquatic. Green boxes represent taxa interpreted as terrestrial. Grey boxes represent taxa for which no interpretation has been made. Boxes in pale blue or green containing question marks indicate taxa for which there have been some suggestions regarding the possible ecology of a given ontogenetic stage, but for which there are no known specimens that definitively verify the suggested interpretations.