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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 internal anatomy of *Seymouria* through specimens from the early Permian Richards Spur locality. The histological data indicate rapid metamorphosis, with the youngest specimen being definitively postmetamorphic despite being substantially younger than pre-metamorphic individuals of *Discosauriscus*, the only other seymouriamorph to have been histologically sampled. The data 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 evolution trajectory of terrestriality within the clade, but taken together, our analysis provides novel information regarding 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 internal anatomy of *Seymouria* through specimens from the early Permian Richards Spur locality. The histological data indicate rapid metamorphosis, 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 data 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, but taken together, our analysis provides novel information regarding the life history of *Seymouria* and highlights the need for further study of seymouriamorph ontogeny.

Introduction

Seymouriamorphs are among the best-known stem amniotes and are known primarily from Lower Permian deposits in North America, Europe, and Russia (Broili, 1904; Amalitzky, 1921; Vaughn, 1966; Berman, Reisz, & Eberth, 1987; Berman & Martens, 1993; Sullivan & Reisz, 1999; Bulanov, 2014). Within seymouriamorphs, only the monotypic family Seymouriidae is known from both North America and Eurasia (Broili, 1904; Vaughn, 1966; Berman & Martens, 1993). Of the North American localities, the materials of *Seymouria* with the greatest detail of preservation are known from the Dolese Brothers Limestone quarry near Richards Spur, Oklahoma (Sullivan & Reisz, 1999; Bazzana et al., in press). However, the only previously described postcranial material from this locality consists of a few isolated elements (Sullivan & Reisz, 1999). Furthermore, the most detailed description of the postcrania of *Seymouria* from any locality is arguably that completed by White (1939). Subsequent authors have provided focused descriptions of the atlas-axis complex (Berman, Reisz, & Eberth 1987; Sumida, Lombard, & Berman, 1992) and the manus and pes (Berman et al., 2000), but White's (1939) work remains the most thorough description of the postcranial skeleton in its entirety. While the interpretations made by White (1939) have been largely supported by subsequent authors, his illustrations were unlabelled, somewhat stylized, and from slightly angled, non-standard perspectives, which collectively limits their utility. As stem amniotes that are well-documented in the fossil record, seymouriamorphs provide a relatively accessible window through which to examine morphological changes associated with terrestrial lifestyles in Paleozoic tetrapods.

Here we describe new postcranial material of *Seymouria* from the early Permian Richards Spur locality, including several articulated vertebrae and a complete humerus, femora, and fibula, and provide updated descriptions, illustrations, and images of the postcrania. Analysis of several limb elements and vertebra using neutron computed tomography (nCT) and histological sampling provides important details regarding the development and internal anatomy of the postcrania of *Seymouria* and contributes to our understanding of the extent to which these stem amniotes were adapted to terrestrial lifestyles.

Materials & Methods

Neutron tomography. Neutron tomography measurements were performed at the DINGO thermal-neutron radiography/ tomography/imaging station (Garbe et al., 2015) at the Australian Nuclear Science and Technology Organisation's (ANSTO) 20 MW OPAL nuclear research reactor, Sydney, Australia. The instrument was equipped with an Iris 15TM Large Field of View sCMOS camera (5056 x 2968 pixel, 16-bit) and Zeiss Ikon 100 mm f/2.0 Makro Planar lens. Based on a desired spatial resolution of ~60 µm across the partially-embedded ROMVP 80915 and ROMVP 80917, a maximum sample width of 48.5 mm and minimum sample-to-detector distance of 28 mm, the DINGO instrument was configured with a 30 µm thick terbium-doped Gadox scintillator screen (Gd2O2S:Tb, RC Tritec AG) and 25.2 × 25.2 × 25.2 µm voxels for a Field-of-View of 100 x 74.5 mm. For OMNH 79348, the achieved voxel size was 72.0 x 72.0 x 72.0 µm. To maximise counting statistics and minimise subsequent noise in the tomographic reconstruction, a collimation ratio (*L/D*) of 500 was used, where *L* is the neutron aperture-to-sample length and *D* is the neutron aperture diameter. This high-flux configuration traditionally illuminates a 200 mm x 200 mm area around the sample area with 4.75×107 neutrons cm-2s-1, leading to high background radiation and zingers on the detector. A newly installed slit system was implemented to restrict the neutron-irradiated area about the specimen to achieve optimum scan conditions and a divergence-limited spatial resolution of 56 µm. A total of 900 equally-spaced angle shadow-radiographs were obtained every 0.20° as the sample was rotated 180°

about its vertical axis. Both dark (closed shutter) and beam profile (open shutter) images were obtained for calibration before initiating shadow-radiograph acquisition. To reduce anomalous noise, a total of three individual radiographs with an exposure length of 4.0 s were acquired at each angle (Mays, Bevitt, & Stilwell, 2017) for a total scan time of 4.6 h.

The individual radiographs were summed in post-acquisition processing using the 'Grouped ZProjector' plugin in ImageJ v.1.51h in accordance with our previous measurements; this plugin was developed by Holly (2004). Tomographic reconstruction of the 16-bit raw data was performed using commercially available Octopus Reconstruction v.8.8 software package and the filtered back-projection algorithm to yield virtual slices perpendicular to the rotation axis. When these slices are stacked in a sequence, they form a three-dimensional volume image of the sample. The reconstructed volume data were downsampled by a factor of 2 in ImageJ to reduce computation time, then rendered and segmented with Avizo Lite 9.3.0.

Histology. Histological preparation followed standard procedures (Padian & Lamm, 2013). All specimens were photographed prior to embedding in EP4101UV resin (Eager Polymers), which was allowed to cure for 24 hours. ROMVP 80916 (partial femur), ROMVP 81198 (vertebra), and ROMVP 81199 (vertebra) were prepared at the Royal Ontario Museum (ROMVP), Toronto, Canada. Specimens were cut on the IsoMet 1000 precision saw (Buehler) and mounted to frosted plexiglass slides with cyanoacrylate adhesive. For the femora, the cut was made at the approximate region of the minimum diaphyseal circumference; for the vertebra, the first cut was made sagittally (anteroposteriorly) down the midline, and the second cut was made transversely through one of the two halves of the block. For ROMVP 80916 (larger, partial femur), the section is taken slightly proximal to the inferred minimum circumference due to the incomplete specimen's nature.

Mounted blocks were trimmed using the IsoMet and ground on the Hillquist Thin Sectioning Machine lapidary wheel. Manual polishing using 1000-mesh grit on glass plates and a combination of 1-μm and 5-μm grit on polishing cloths was used to remove scratches. ROMVP 81200 (partial femur) was prepared in a similar fashion but with a different equipment setup at the University of Toronto Mississauga. Cutting was performed on the Metcut-5 low speed saw (MetLab), initial grinding on the Metcut-10 Geo (MetLab), and manual grinding on a cutlery whetstone block. Imaging was done on a Nikon AZ-100 microscope with a DS-Fi1 camera and NIS Elements-Basic Research software registered to David C. Evans.

Preparation of the specimens was performed by Diane Scott and Bryan M. Gee using pin vises and air scribes. Figures were prepared using Adobe Illustrator and Photoshop.

Systematic Paleontology

Order Seymouriamorpha Watson, 1917

Family Seymouriidae Williston, 1911

Genus *Seymouria* Broili, 1904

Horizon and locality. Dolese Brothers Limestone Quarry near Richards Spur, Oklahoma, USA. Early Permian (Artinskian).

Referred specimens. OMNH 74721, right humerus; OMNH 79346, complete vertebra; OMNH 79347, string of 11 articulated vertebrae; OMNH 79348, string of three articulated vertebrae;

OMNH 79349, complete vertebra; OMNH 79350, partial vertebra; OMNH 79351, complete vertebra; OMNH 79352, partial vertebra; OMNH 79353, partial vertebra; ROMVP 81198, complete vertebra; ROMVP 81199, complete vertebra; ROMVP 80915, left femur; ROMVP 80916, proximal left femur; ROMVP 80917, left fibula; ROMVP 81200, distal left femur.

Description

Vertebrae. 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).

All the vertebrae described here exhibit the swollen pre- and postzygapophyses that are characteristic of *Seymouria* (White, 1939). 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; the neural spines of the other specimens are either broken or missing. *Seymouria* has been previously described as having an irregular distribution of bifurcated and single spines along the vertebral column, from the tenth position to the twenty-second position (White, 1939). OMNH 79352 appears to be the first record of bifurcation of the neural spine in a vertebra from the anteriormost trunk. Although the seemingly complete co-ossification of the neural arch and centrum suggests skeletal maturity, the maturity of these vertebrae cannot be more definitively established given the known challenges of applying skeletochronology to vertebral elements (Danto et al., 2016).

ROMVP 81198, an isolated presacral neural arch of a small-bodied individual (Fig. 1E), and ROMVP 81199, a presacral neural arch with centrum of a larger individual (Fig. 1F), were histologically sectioned. The transverse profile reveals a similar microanatomy and histology to that of a previously sampled specimen (OMNH 73499) from Richards Spur (Danto et al., 2016). The pleurocentrum of ROMVP 81199 is formed by two domains (Fig. 4), a compact periosteal domain along the ventrolateral margin of the element that is formed by a thin layer of lamellar bone, and a less dense and more disorganized endochondral domain with trabeculae; this is captured in both sagittal and transverse sections. Although the suture between the pleurocentrum and the neural arch is not clearly defined externally, it is very apparent in the transverse section in which the separation was largely cartilaginous at the time of death. Neither the notochordal canal nor the neural canal were captured in the half-transverse section. The neural arches of ROMVP 81198 and 81199 are very similar in transverse section. Each arch is comprised of a spongy bone texture with a hollow interior. In the smaller ROMVP 81198, the ventral portion of the arch is poorly ossified, with a sparse network of trabecula (Fig. 5). The sagittal sections are also generally comparable, but a few differences may be noted. Indentations on the anterior and posterior surfaces representing the vestiges of the supraneural canal are present; these indentations are more prominent in the larger ROMVP 81199, and in each specimen, the anterior indentation is more pronounced. In both specimens, the neural arch is much thicker along the posterodorsal surface behind the neural spine and extending down to the posterior indentation of the supraneural canal when compared to the anterior surface. The thickened region is far more developed in the larger ROMVP 81199. The neural spines are very poorly ossified. The ventral portion of the arch, which roofs the neural canal, is thin in both specimens and with distinct remodelling (Figs. 4B, 5B). Remodelling is otherwise found mostly near the geometric center of the element (Fig. 5B). A pair of closely spaced, distinctive growth lines can be identified in the thickened region of ROMVP 81199 (Fig. 4A).

The three articulated vertebrae of OMNH 79348 were digitally sectioned using the neutron tomography data (Fig. 6). Viewed in transverse section, the data reveal pronounced differences in the compactness of the diapophyses; the diapophyses of the first sacral vertebra are not only greatly expanded, as is visible externally, but are also composed of substantially more compact bone than those of either the presacral or the possible second sacral, suggesting that the first sacral undergoes the majority of the stress loading. The identity of the vertebra immediately posterior to the first sacral has been historically debated; White (1939) and Berman et al. (2000) identified the element as a second sacral, while Williston (1911), Watson (1918), and Berman, Reisz, and Eberth (1987) identified it as the first caudal. White's (1939) argument was based on the presence of a rib extending anteriorly from the vertebra in question to contact the rib of the first sacral, stating unequivocally that it must have had a strong cartilaginous connection and was doubtlessly a functional second sacral vertebra (p. 354). In contrast, Berman, Reisz, and Eberth (1987) noted that the rib of the debated element curved posteriorly and thus could not contact the ilium or the rib of the first sacral, negating any possibility of it forming a true second sacral. In the absence of a preserved rib in OMNH 79348, we are unable to comment on whether the vertebra immediately following the first sacral represents a definitive second sacral or the first caudal vertebra.

Humerus. OMNH 74721 is a complete right humerus, exhibiting the tetrahedral shape typical of many reptiliomorphs (Fig. 7). The overall morphology matches that described by previous authors (White, 1939; Sullivan & Reisz, 1999) in being short and robust with the deltopectoral crest following an L-shaped path. The proximal and distal ends are broadly expanded and are set at an approximately 45-degree angle to each other with no distinct shaft separating the epiphyses. The supinator process is oval in cross-section and located just distal to the short arm of the deltopectoral crest (Fig. 7C). OMNH 74721 exhibits a keel that extends along the anteroventral surface from the deltopectoral crest to the entepicondylar foramen (Fig. 7D); a similar crest has been described by Sullivan & Reisz (1999) but was not mentioned by White (1939). The entepicondylar ridge runs along the ventral edge and expands proximally to form what may represent the insertion site for the subcoracoscapularis on the posterior surface of the humerus, near the glenoid articulation (Fig. 7B). Dorsal to this expansion is a pronounced tubercle for the insertion of the latissimus dorsi muscle. The absence of an ectepicondylar foramen, the proximal position of the insertions for the subcoracoscapularis and the latissimus dorsi muscles, and the position and shape of the supinator process all correspond exactly to the descriptions of *Seymouria* given by White (1939) and Sullivan and Reisz (1999).

Compared to the smaller and presumably more immature humerus (FMNH PR 2054) that was previously described from Richards Spur (Sullivan & Reisz, 1999: fig. 2), many features on OMNH 74721 are more developed. In FMNH PR 2054, the capitellum is indicated only by a slight swelling, whereas in OMNH 74721 it is a distinct hemispherical facet. Mirroring this disparity, the supinator process of FMNH PR 2054 remains connected to the capitellum by a ridge of unfinished bone, whereas the process of OMNH 74721 is clearly delineated from the distal articular surfaces by an emargination of smoothly finished bone.

Femur. ROMVP 80915 is a complete left femur (Fig. 8), the morphology of which corresponds to previous descriptions (White, 1939; Sullivan & Reisz, 1999). The element is short and robust. The insertion for the puboischiofemoralis muscle is visible on the dorsal surface of the head (Fig. 8B, D). As is known in *Seymouria* (White, 1939; Sullivan & Reisz, 1999), the adductor crest extends posterolaterally along the ventral surface of the femur from the trochanter to near the tibial condyle (Fig. 8F). The smaller femur that was previously described by Sullivan and Reisz (1999; FMNH PR 2053) was stated to have a prominent trochanter. This appears to remain constant throughout ontogeny, as little difference in the relative size of the trochanter is seen between the smallest (ROMVP 81200) and largest specimens (ROMVP 80915 and 80916). The distal articular surface has been described as following an M-shaped curve (White, 1939), but this may not be entirely accurate. In ROMVP 80915, ROMVP 81200, and a smaller, more immature femur (FMNH PR 2053; Sullivan & Reisz, 1999: fig. 3), the distal surface is V-shaped, with the tip of the anterior arm being bent ventrally (Fig. 8G), as opposed to the illustration given by White (1939: fig. 28) that reconstructs this surface with both ends curving ventrally. However, it is possible that the shape of the distal surface is ontogenetically variable, and disparities in length suggest that the specimen described by White (1939), measuring 6.4 cm in length, may be more mature than ROMVP 80915 (5.5 cm), the largest complete femur from Richards Spur.

ROMVP 80916 and ROMVP 81200 are partial left femora (Fig. 9); ROMVP 80916 is equivalent in size to ROMVP 80915 and is broken distal to the trochanter, while ROMVP 81200 is substantially smaller and is broken proximal to the trochanter. ROMVP 80916 was histologically sectioned slightly proximal to the minimum diaphyseal circumference (Fig. 10), while ROMVP 81200 was sectioned at this minimum region (Fig. 11). ROMVP 80915 was digitally sectioned at the minimum circumference (Fig. 12). All three femora are relatively similar in their microanatomical and histological features. The cortex is relatively compact, comprised primarily of lamellar bone; the tissue is distinctly less organized in the smaller ROMVP 82100, reflecting its immaturity and presumed faster growth rate. Secondary osteons and vascular canals are present in both specimens (Figs. 10-11); the latter decrease in abundance in the larger ROMVP 80916 (Fig. 11). Remodelling is found at the boundary of the medullary cavity in both specimens (Figs. 10C, 11C), but there is distinctly more parallel-fibered bone along the margin of the cavity in the larger ROMVP 80916 (Figs. 10-11). The medullary cavity is relatively open, although there is a network of trabeculae throughout; secondary remodelling is present within this network, but calcified cartilage is absent. The trabecular network appears to be less dense in the digital section of ROMVP 80915 than in the histological section of ROMVP 80916, but this may be a combination of a slightly more proximal plane of section in the latter and limitations on scanning resolution (25.2 µm). Histological features (e.g., lines of arrested growth) are not resolvable in ROMVP 80915. A confident determination of lines of arrested growth (LAGs) is complicated in these specimens; most lines appear not to extend for the full circumference or are disrupted by secondary remodelling. In the smaller ROMVP 81200, at least four lines of arrested growth are identifiable. In the larger ROMVP 80916, at least seven lines are identifiable.

Fibula. ROMVP 80917 is a complete left fibula (Fig. 13). Previous descriptions (White, 1939; Sullivan & Reisz, 1999) have commented only on the general shape of the element, to which ROMVP 80917 corresponds exactly in that the medial surface is deeply concave, the lateral surface is mostly straight with only a slight concavity, and the distal articular surface is more expanded than the proximal surface, which itself is crescentic with its dorsal margin being convex and its ventral margin being concave. Features that have not been described previously in *Seymouria* but that are present in ROMVP 80917 are the pronounced muscle scar along the proximal half of the lateral surface, which may represent the insertion site for the iliofibularis muscle (Fig. 13A-B, D; Romer, 1956), and the twisting of the fibular shaft such that the proximal and distal heads lie in planes that are at an approximately 45-degree angle to each other (Fig. 13E-F).

Discussion

Comparative external anatomy. The vertebrae of *Seymouria* bear a number of marked differences from those of other seymouriamorphs. In *Ariekanerpeton*, the presacral vertebrae differ in the level of ossification of the neural arch, with the arches of the third to fifth vertebrae remaining separate and the sixth to ninth arches being strongly co-ossified (Klembara & Ruta, 2005). The neural spines of *Kotlassia* are much higher than in *Seymouria* (Bystrow, 1944), whereas in *Discosauriscus* the spines are short (Klembara & Bartík, 2000), and in *Ariekanerpeton* the spines are either low or unossified altogether (Laurin 1996b). The vertebrae of *Utegenia* have been described as indistinguishable from those of *Discosauriscus* (Klembara & Ruta, 2004). The oval cross-section of the diapophyses is shared between *Seymouria* (White,

1939) and *Kotlassia* (Bystrow, 1944). However, in *Seymouria* the diapophyses remain the same size but transition in orientation from vertical to horizontal along the vertebral column (White, 1939), whereas the diapophyses of *Kotlassia* maintain the same orientation but decrease in size along the column (Bystrow, 1944). Unlike *Seymouria* in which the articular surfaces of the prezygapophyses and the postzygapophyses face directly dorsally and ventrally, respectively (White, 1939), the surfaces of *Discosauriscus* and *Ariekanerpeton* face dorsomedially and ventrolaterally (Klembara & Bartík, 2000; Klembara & Ruta, 2005). *Seymouria*, *Kotlassia*, and *Discosauriscus* retain an open notochordal canal in adulthood (White, 1939; Bystrow, 1944; Klembara & Bartík, 2000). It is unknown whether the notochordal canals were retained in the other seymouriamorphs (Klembara & Ruta, 2004; 2005; Klembara, 2005; 2009; Bulanov, 2014). *Discosauriscus* has only one sacral vertebra (Klembara & Bartík, 2000), as in OMNH 79348. It is unclear whether *Seymouria baylorensis* and *Seymouria sanjuanensis* differ in the number of sacral vertebrae (White, 1939; Berman, Reisz, & Eberth, 1987, 2000), so this character cannot be used to clarify the specific designation of this material.

The humerus of *Seymouria* is more extensively ossified than those of *Utegenia* or *Ariekanerpeton* (Laurin, 1996a, 1996b; Klembara & Ruta, 2005), in which the capitellum, trochlea, the supinator process, and the deltopectoral crest are indistinct in even the largest individuals. In particular, the humerus of *Utegenia* has been described as “little more than a stout, subcylindrical lump of bone” (Klembara & Ruta, 2004: 77). It has been noted, however, that the lack of ossification in *Utegenia* may represent ontogenetic immaturity (Klembara & Ruta, 2004). The humerus of *Kotlassia* is the slenderest of the seymouriamorphs and lacks an entepicondylar foramen (Bystrow, 1944). *Makowskia*, *Spinarerpeton*, and *Discosauriscus* exhibit the same massive, L-shaped deltopectoral crest extending from the proximal articular surface as that seen in *Seymouria* (Klembara, 2005, 2009; Klembara & Bartík, 2000). The humeri of *Makowskia* and *Spinarerpeton* are described as having a broad shaft, a proximodistally short entepicondyle, and an entepicondylar foramen that is open distally (Klembara, 2005, 2009); the last feature has been recognized as being likely ontogenetic. *Makowskia*, *Spinarerpeton*, and *Discosauriscus* differ substantially from *Seymouria* in having a well-developed insertion site for the subcoracoscapularis muscle and in lacking a supinator process (Klembara & Bartík, 2000; Klembara, 2005, 2009); in *Seymouria*, the former is represented by only a slight rugosity while the latter is quite pronounced (White, 1939). The humeri of *Discosauriscus* and *Ariekanerpeton* have been reported with a lesser degree of twisting, with the planes of the proximal and distal ends being at an approximately 38-degree angle (Klembara & Bartík, 2000; Klembara & Ruta, 2005). The positioning and degree of development of the insertions for the subcoracoscapularis and latissimus dorsi muscles are roughly equivalent in *Discosauriscus* as in *Seymouria* (Klembara & Bartík, 2000).

As with the humerus, the femur of *Seymouria* is more ossified than in *Utegenia* or *Ariekanerpeton* (Laurin 1996a, 1996b); the femur of *Utegenia* has been described as featureless (Klembara & Ruta, 2004). The femur of *Makowskia* is known but has only been described as having a crescentic proximal articular surface with a deep intertrochanteric fossa as in *Seymouria* (Klembara, 2005), and the only corresponding figure is a simplified line drawing. The femur of *Spinarerpeton* has been similarly described and figured in limited detail (Klembara, 2009). The femur of *Microphon* is much more gracile than in *Seymouria* with less pronounced expansion of the epiphyses and a proportionally longer, narrower shaft (Bulanov, 2014). *Microphon* also differs from *Seymouria* in that the adductor crest extends towards the fibular condyle (Bulanov, 2014), as opposed to the tibial condyle as in *Seymouria* and *Discosauriscus* (Klembara & Bartík, 2000). In addition, the intertrochanteric fossa of *Microphon* is approximately one-third the length of the femur (Bulanov, 2014), whereas in *Seymouria* it extends approximately halfway along the element. *Kotlassia* appears to be intermediate between these two conditions and exhibits a starker contrast between the slender shaft and the broadly expanded epiphyses (Bystrow, 1944: fig. 9). A feature found in the femora of *Microphon* and *Kotlassia* and in ROMVP 80915, though perhaps not all specimens of *Seymouria*, is the lesser development of the trochanter (Bystrow, 1944); in *Microphon*, the trochanter seems to not be developed at all (Bulanov, 2014). The femur of *Discosauriscus* is similar to that of *Seymouria* in overall morphology but is more gracile (Klembara & Bartík, 2000:fig. 25).

The fibulae of *Kotlassia* and *Utegenia* have not been illustrated or described in sufficient detail to allow for accurate comparison with *Seymouria*. The fibula of *Ariekanerpeton* has only been described as being poorly preserved with unfinished epiphyses (Klembara & Ruta, 2005). In *Makowskia* and *Discosauriscus*, the fibula is similar to *Seymouria* in being deeply concave medially with only a slight concavity on the lateral surface (Klembara & Bartík, 2000; Klembara, 2005). The fibula of *Spinarerpeton* is unknown (Klembara, 2009).

Histological interpretations and comparisons. In early tetrapods, interpretations of ecology (e.g., aquatic vs. terrestrial) are often based on the presence or absence of features such as lateral line grooves and the degree of development of external features of the limbs (Moodie, 1908). Histology has more recently been utilized as a means to further test these hypotheses by means of comparisons with extant taxa in which ecologies can be definitively observed and with the classically utilized external anatomical features (Germain & Laurin, 2005; Kriloff et al., 2008; Sanchez et al., 2010; Quemeneur, de Buffrénil & Laurin, 2013; Konietzko-Meier, Shelton, &

Sander, 2016). *Seymouria* is widely accepted to lack lateral line grooves, although they have been suggested by some to have been present in juveniles (Berman & Martens, 1993, but see Klembara et al., 2006), indicating a transition in ecology throughout ontogeny. Additionally, the limbs are well-developed, with prominent attachment sites for musculature and distinct processes (Figs. 7-8), and the neural arches are greatly expanded compared to other Paleozoic tetrapods and with prominent zygapophyses inferred to have supported the axial column. These features have been used to infer a terrestrial lifestyle (Sullivan & Reisz, 1999). This is further supported by the specific context of the Richards Spur locality, which is dominated by terrestrial fauna (MacDougall et al., 2017).

Contextualizing the histological data of the specimens of *Seymouria* is complicated by the paucity of work on other stem amniotes, let alone seymouriamorphs specifically. Limb elements of *Seymouria* have never been histologically analyzed. The only other seymouriamorph femur to be histologically sampled is that of the European *Discosauriscus* (Sanchez et al., 2008). Most individuals of *Discosauriscus* retain lateral line canals, but this may reflect a biased relative abundance of premetamorphic individuals in the fossil record (e.g., Klembara et al., 2006). Previous authors have inferred that *Discosauriscus* underwent metamorphosis (Klembara, 1995) but definitive adults, terrestrial or otherwise, are unknown (Klembara, 2001). Sanchez et al. (2008) defined the age bracket at which they believed metamorphosis to a terrestrial adult to have occurred. The general histology and microanatomy of *Discosauriscus* is similar to that of *Seymouria*, but the cortex of the former is more compact and with more organized lamellar bone, indicating a slower growth and a protracted aquatic larval stage in this taxon (Sanchez et al., 2008). In contrast, the individuals of *Seymouria* that were analyzed here are regarded as being definitively postmetamorphic in spite of at least one (ROMVP 81200) being distinctly younger than the onset of metamorphosis in *Discosauriscus* (Sanchez et al., 2008). This disparity may reflect the different environments in which these taxa are found, as *Seymouria* is primarily found in fluvial environments of North America that probably experienced marked seasonality, and *Discosauriscus* is primarily found in lacustrine settings in Europe that may have been more environmentally stable. Histological sections also indicate a less developed trabecular network in the medullary cavity of *Discosauriscus* (Sanchez et al., 2008:fig. 2); the significance of this is unclear in the absence of additional data.

Comparisons with other Paleozoic tetrapods are also limited by a paucity of comparative work. Of the major Paleozoic clades (e.g., pelycosaurian synapsids, ‘lepospondyls’), temnospondyls are the best-sampled (Sanchez et al., 2010a; Sanchez et al., 2010b; McHugh, 2014; Konietzko-Meier, Shelton & Sander, 2016). The relative thickness of the cortex and the development of the medullary spongiosa are most comparable to that of the co-occurring trematopid *Acheloma dunni*, a terrestrial taxon (Sanchez et al., 2010b; Quemeneur, de Buffrénil & Laurin, 2013). The spongiosa is less developed than in either definitively aquatic taxa such as the late Permian rhinesuchid *Rhinesuchus* (McHugh, 2014) or in controversially aquatic taxa such as the early Permian eryopid *Eryops* (Sanchez et al., 2010b; Quemeneur, de Buffrénil & Laurin, 2013; Konietzko-Meier, Shelton & Sander, 2016), and the cortex is not extensively thickened as in the definitively aquatic dvinosaur *Trimerorhachis* (Sanchez et al., 2010b; Quemeneuer et al., 2013). A large number of Mesozoic temnospondyls, which are predominantly aquatic, have also been sampled (Steyer et al., 2004; Konietzko-Meier & Sander, 2012; Sanchez & Schoch, 2013). Many of these taxa exhibit similar structure to that of *Trimerorhachis*, often with a high degree of pachyostotic development and with greatly reduced or nearly absent medullary cavities. The small-bodied Early Triassic lydekkerinid *Lydekkerina* and the amphibamiform *Micropholis*, both widely regarded as being terrestrial, were sampled by McHugh (2015) and exhibit a similar histological and microanatomical organization to that of terrestrial Paleozoic temnospondyls and to that of *Seymouria*. Collectively, the temnospondyl comparisons support an inferred terrestrial lifestyle of *Seymouria*. However, it is important to note that the spongiosa of *Seymouria* is more developed than in any of the co-occurring terrestrial temnospondyls at Richards Spur (Castanet et al., 2003; Quemeneur, de Buffrénil & Laurin, 2013; Richards, 2016) in which the spongiosa is either weakly developed (Trematopidae) or virtually non-existent (Dissorophidae, Amphibamiformes). The significance of this is uncertain at present and warrants further work to compare co-occurring taxa.

The vertebral histology is also difficult to compare with closely related taxa. For one, vertebrae are uncommon in histological studies compared to limb elements, and most studies that that have examined the vertebrae of Paleozoic tetrapods have focused on the inter- and pleurocentra (Konietzko-Meier, Danto & Gadek, 2014; Danto et al., 2017, 2019). However, both the centra and the neural arches contribute valuable information regarding the ecology of *Seymouria*. Previous workers have often suggested that the neural arch would have been subject to greater, more direct biomechanical constraints during locomotion in early tetrapods (Rockwell, Evans & Pheasant, 1938; Olson, 1976; Holmes, 1989). The prominent expansion of the neural arch and the development of the zygapophyses in *Seymouria* lends support to this hypothesis. *Discosauriscus* is the only other seymouriamorph to have its internal vertebral anatomy examined (Danto et al., 2016). The main difference is in the construction of the neural arch, which is comprised of thick, compact lamellar bone in *Discosauriscus*; in contrast, the neural arch of *Seymouria* is largely hollow. Based on the size of the sampled *Discosauriscus* material, the individual was likely premetamorphic and still aquatic, which would explain the higher degree of ossification. Whether this might have changed in later stages of ontogeny when individuals metamorphosed into a presumably terrestrial adult form remains unknown. Beyond seymouriamorphs, neural arches have not been sampled in many clades, which may be because most Paleozoic tetrapod clades have multipartite vertebrae in which the arch readily detaches from the centra during preservation. Furthermore, isolated neural arches have not traditionally been utilized as an ideal case study for exploring histological questions compared to either the centra or to other postcranial elements. Danto et al. (2016) sampled a number of Paleozoic lepospondyl taxa in which neural arches were preserved. Some of the aquatic taxa (e.g., an indeterminate nectridean) exhibit a similar spongy bone composition of the arch, but the interior of the arch is relatively well-ossified with little empty space.

The centra also contribute information through inferences on the skeletal mass of the element(s). The two traditionally utilized criteria are the thickness of the periosteal domain and the presence or absence of calcified cartilage. Greatly thickened domains (pachyostosis) and retention of calcified cartilage throughout ontogeny are frequently seen in large-bodied aquatic temnospondyls and probably served to increase the skeletal mass for buoyancy control (Danto et al., 2016). In both *Discosauriscus* and *Seymouria*, the periosteal domain is relatively thin, and calcified cartilage is primarily found around the notochordal canal (Danto et al., 2016). In *Seymouria*, this is the only location of this tissue, whereas calcified cartilage occurs sporadically in the endochondral domain of at least immature individuals of *Discosauriscus*.

What then can be concluded regarding the histological data from *Seymouria* postcrania and the ecology of the taxon? The femoral microanatomy, specifically the relatively thin cortex and the modest development of the medullary spongiosa, is more compatible with that of a terrestrial animal by comparison with other Paleozoic tetrapods (primarily temnospondyls) that have been inferred to be terrestrial. Based on studies of femoral and tibial microanatomy in extant tetrapods (Kriloff et al., 2008; Quemeneur, de Buffrénil & Laurin, 2013), these features also support a primarily terrestrial lifestyle. Collectively, this corroborates the conclusions of previous authors that *Seymouria* was most likely a terrestrial animal (White, 1939; Berman & Martens, 1993; Sullivan & Reisz, 1999; Marchetti, Mujal & Bernardi, 2017). The vertebral histology also confers support for a terrestrial lifestyle. The periosteal domain is thin, calcified cartilage is sparse and confined to the margin of the notochordal canal, and the neural arch is largely hollow. These data correspond favorably with the broad expansion of the arch and the zygapophyses, which Sullivan & Reisz (1999) interpreted to be for the stiffening of the axial column following White (1939).

Terrestriality in seymouriamorphs. Assessing the range of ecologies among seymouriamorphs from a macroevolutionary standpoint is important because the group has historically been regarded as being well-situated for understanding the associated skeletal modifications associated with terrestriality. *Seymouria* is one of the best seymouriamorphs for examining such modifications because complete, articulated skeletons are known (e.g., Berman, Reisz, & Eberth, 1987), but it then becomes important to assess whether a terrestrial or aquatic lifestyle is the plesiomorphic state among seymouriamorphs. Given that seymouriamorphs, and reptiliomorphs more broadly, are frequently used as exemplars for the skeletal changes associated with terrestrial adaptation, clarifying the primitive condition of this group is critical for informing accurate comparisons. A conceptual phylogeny is presented in Figure 14 which illustrates the distribution of terrestriality among seymouriamorphs.

Our data provide strong evidence at the histological and the microanatomical scales to support the longstanding hypothesis of terrestriality in *Seymouria.* This is not a particularly controversial idea; numerous aspects of the external morphology, such as the well-ossified limbs and the massively expanded vertebrae have long been cited as evidence for this ecology (Romer, 1956). Although Berman and Martens (1993) described a possible indication of a lateral line system in juvenile specimens of *S. sanjuanensis* from Germany, subsequent work (Klembara et al. 2006) on an early juvenile did not find any evidence for a lateral line system in other *S. sanjuanensis* from the same locality. As such, while it is often inferred that *Seymouria* underwent metamorphosis as with other seymouriamorphs and a number of other terrestrial tetrapods (e.g., some temnospondyls), definitive aquatic larval forms and morphological transitions associated with the presumed metamorphosis are unknown.

At least one other seymouriamorph, *Karpinskiosaurus*, is also represented only by specimens that lack lateral line grooves (Klembara, 2011). *Kotlassia* has also been historically regarded as lacking lateral line grooves (e.g., Bystrow, 1944), but the *Kotlassia* of most previous authors is actually a combination of material referable to the type species, *Kotlassia prima*, and material properly referable to *Karpinskiosaurus* (see Bulanov, 2002 and Klembara, 2011, for discussion). Whether these grooves are definitively absent in the holotype of *Ko. prima* is not apparent from previous works that accounted for this historical discrepancy. For *Karpinskiosaurus* and *Seymouria*, it has been proposed that these taxa underwent metamorphosis relatively early in their development and lived on land for the majority of their lives (Klembara, 2011).

In contrast, most other seymouriamorphs are known from individuals with lateral line grooves, including *Ariekanerpeton* (Klembara & Ruta, 2005), *Discosauriscus* (Klembara, 1996), *Spinarerpeton* (Klembara, 2009), and *Utegenia* (Malakhov, 2000). The most recent phylogenetic analysis that focused on seymouriamorph phylogeny is that of Klembara (2011), which followed a series of anatomical work that re-described virtually all known seymouriamorphs. Mapping the distribution of ecologies onto this topology suggests that seymouriamorphs are primitively aquatic (*Utegenia* being the earliest diverging taxon) and with two separate shifts to terrestriality, one in *Karpinskiosaurus* and one in *Seymouria* (Fig. 14). However, caution must be exercised in inferring the phylogeny of a clade in which metamorphosis is known to occur because biases in the record of premetamorphic larval forms versus that of postmetamorphic terrestrial adults can produce misleading data. As with *Seymouria* (Berman et al., 2000), it has been proposed that *Discosauriscus* transitioned from an aquatic to terrestrial lifestyle throughout its ontogeny, but even the largest known specimens of *Discosauriscus* are believed to be juveniles, and none have been recovered from the terrestrial environments that the adult individuals may have inhabited (Klembara, Martens & Bartik, 2001). This may relate to a relatively protracted larval stage recovered by the histological work of Sanchez et al. (2008) in which metamorphosis may not have begun until year six of an individual's life. The latest phylogenetic analyses (Klembara, 2011) do not bear out the slippage that is predicted when coding taxa based on immature specimens (i.e. *Discosauriscus* is a highly nested seymouriamorph), but this does not negate the potential for this disparity to affect the phylogeny. *Ariekanerpeton*, *Spinarerpeton*, and *Utegenia* are also likely represented only by juveniles (Klembara & Ruta, 2003, 2005; Klembara, 2009), which warrants consideration.

Histology offers one avenue for exploring the diversity of life histories within a clade through well-documented taxa (e.g., *Discosauriscus, Seymouria*) and for improving hypotheses and predictions regarding those of more poorly represented taxa with limited ontogenetic data.

Our histological data substantiate the hypothesis that *Seymouria* was a rapidly metamorphosing taxon that spent most of its life on land (Klembara, 2011), which may explain why it is predominantly found in fluvial deposits of south-southwestern North America and the upland Bromacker locality along with other highly terrestrial tetrapods whereas the slower growing *Discosauriscus* is restricted to lacustrine environments of Europe. Additionally, the Richards Spur locality preserves almost exclusively terrestrial taxa (MacDougall et al., 2017), and thus it is reasonable to infer that all known *Seymouria* material belonged to postmetamorphic individuals. The absence of larval forms of *Seymouria* may be explained by a short larval stage and the probable spatial separation of larvae from the fluvial environments that preserved the adults (niche partitioning between life stages).

In the sense that terrestriality in adults of *Seymouria* has not been widely questioned, our most novel data, the histological data, are not necessarily surprising. However, this should not diminish the value of these data; testing hypotheses using multiple approaches is important for assessing the rigor of such hypotheses. The existing histological framework and understanding of seymouriamorph development remains largely confined in traditional interpretations of relative maturity based on external features and their development, and there is an extensive precedent for the utility of histology (among other more recently accessible methods) to further explore paleobiological attributes of extinct taxa. Our interpretations of the data are somewhat limited, in part by sample size, but also in part by the absence of a substantive body of comparative data. It is unclear, for example, what to make of the persistence of a modestly developed medullary spongiosa in the femur of *Seymouria*, either compared to *Discosauriscus* or to other more distantly related terrestrial tetrapods. Our ontogenetic trends are more accurately stated as two partial points within the developmental trajectory, and the opportunity remains to explore ontogeny further within *Seymouria* across all regions of the skeleton.

Institutional Abbreviations

OMNH	Sam Noble Oklahoma Museum of Natural History, Norman, OK, USA
ROMVP	Royal Ontario Museum, Toronto, ON, Canada

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Figure 1

Posterior presacral vertebrae of *Seymouria*.

A, OMNH 79346; **B,** OMNH 79349; **C,** OMNH 79350; **D,** OMNH 79353; **E,** ROMVP 81198; and **F,** ROMVP 81199 in anterior, posterior, dorsal, ventral, and left lateral views. Scale bar equals 1 cm.

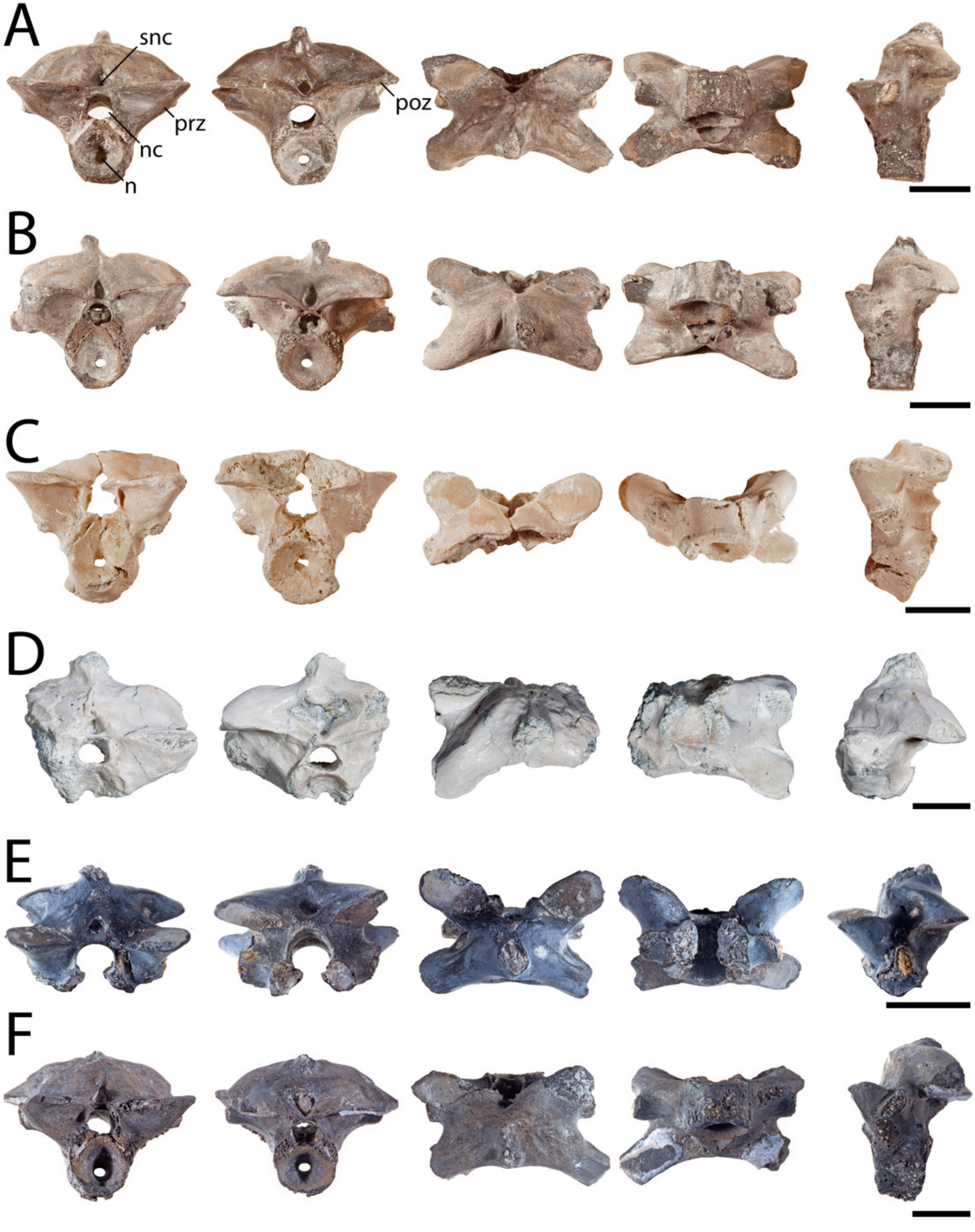
A
snc
prz
nc
n
poz
B
C
D
E
F

Figure 2

Anterior presacral vertebrae of *Seymouria*.

A, OMNH 79351 and **B,** OMNH 79352 in anterior, posterior, dorsal, ventral, and left lateral views. Scale bar equals 1 cm.

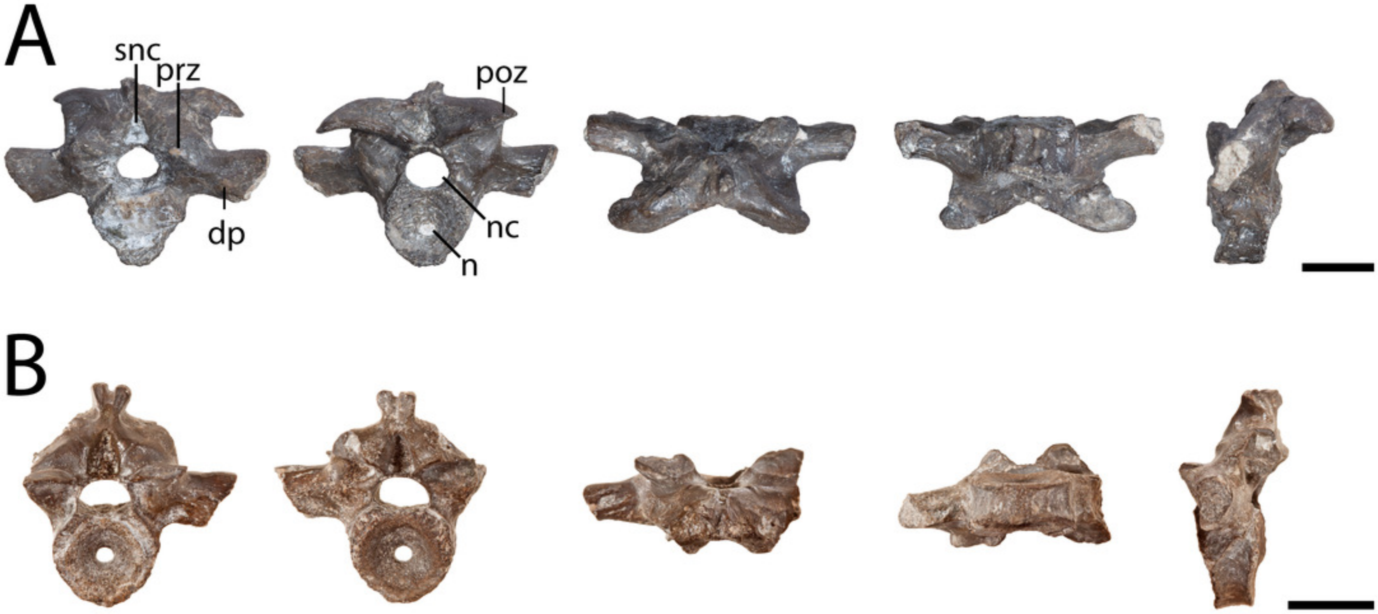

Figure 3

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.

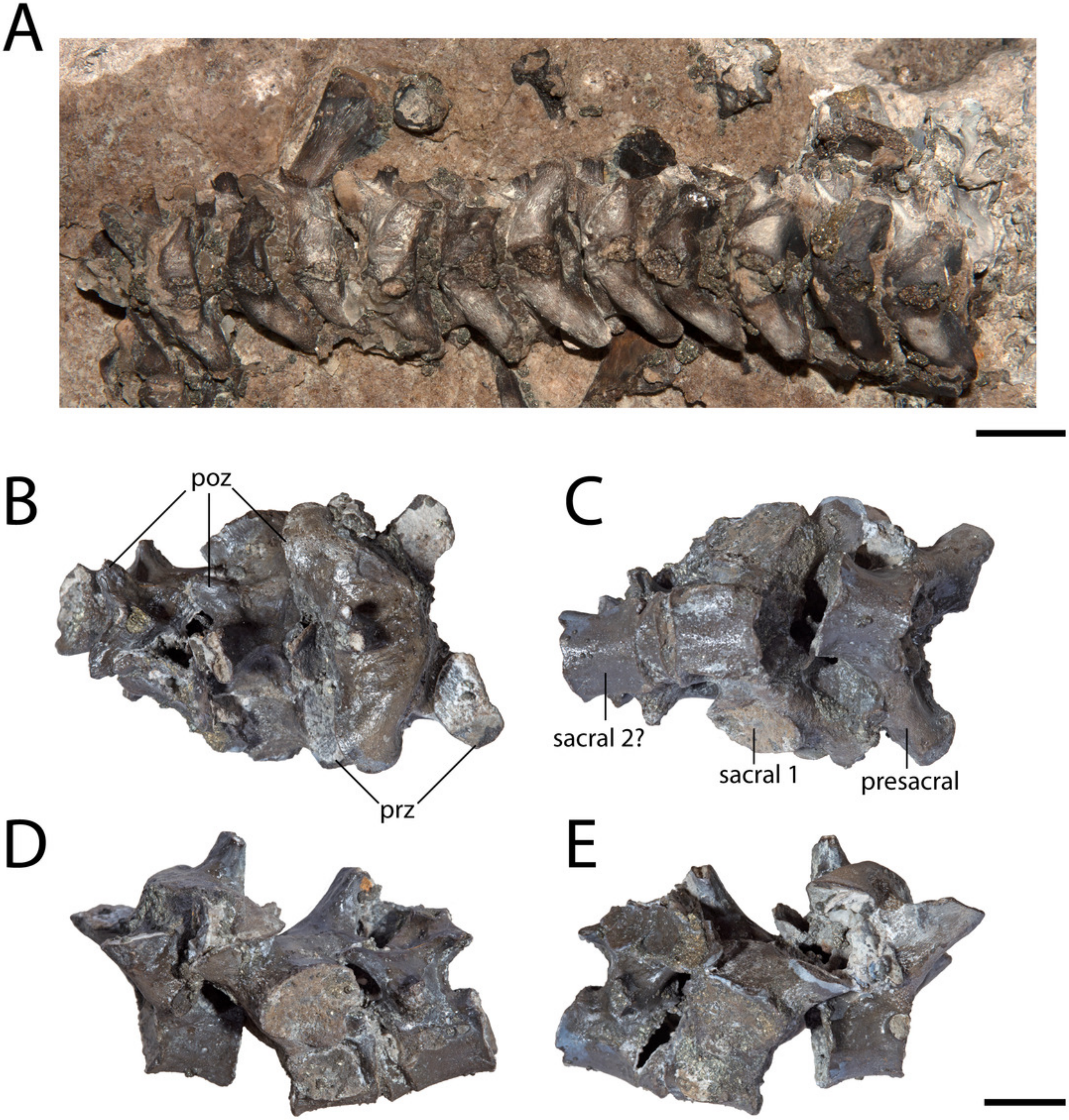
A
B
poz
prz
C
sacral 2?
sacral 1
presacral
D
E

Figure 4

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).

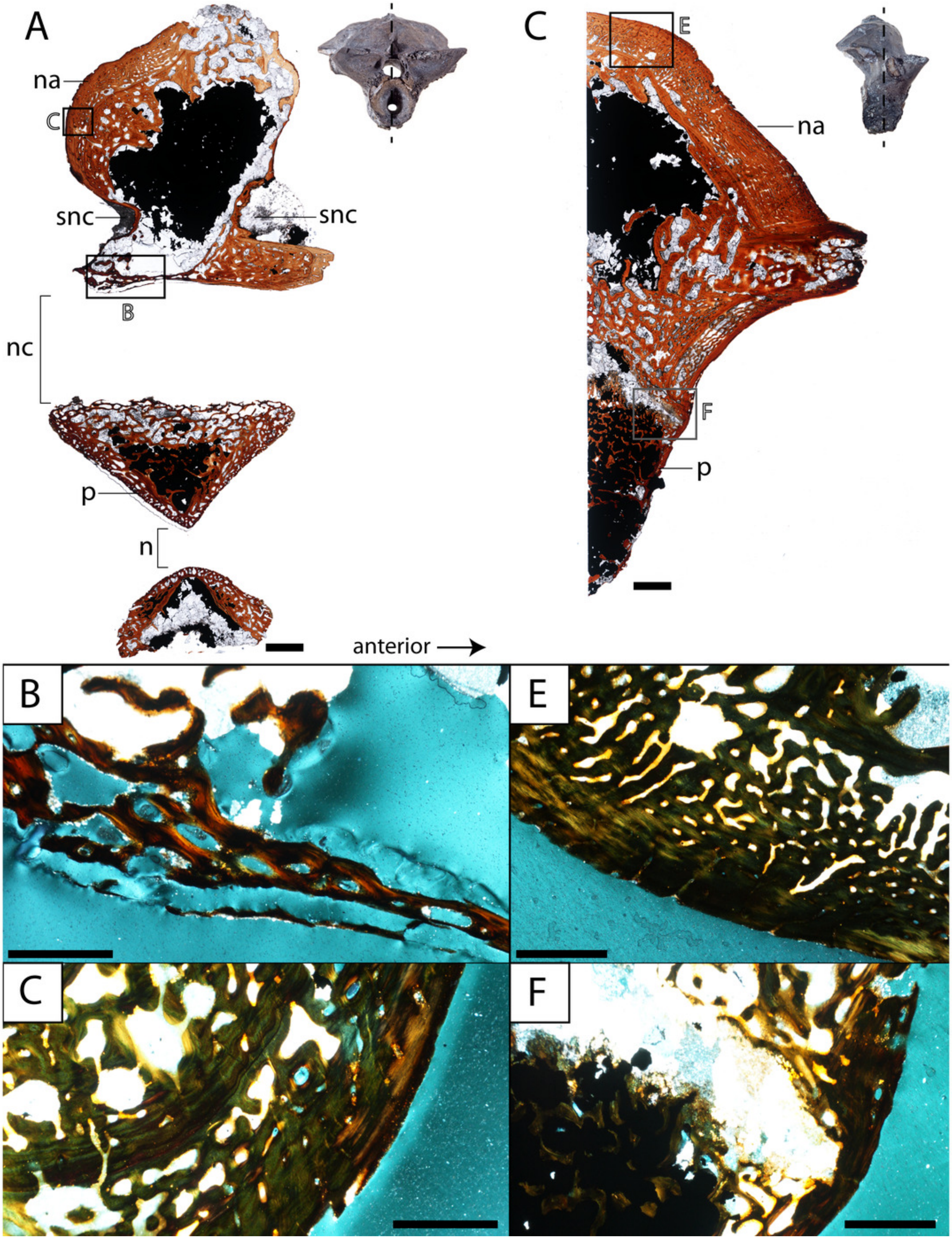
A
na
C
snc
snc
B
nc
p
n
anterior →
C
E
na
F
p
B
E
C
F

Figure 5

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).

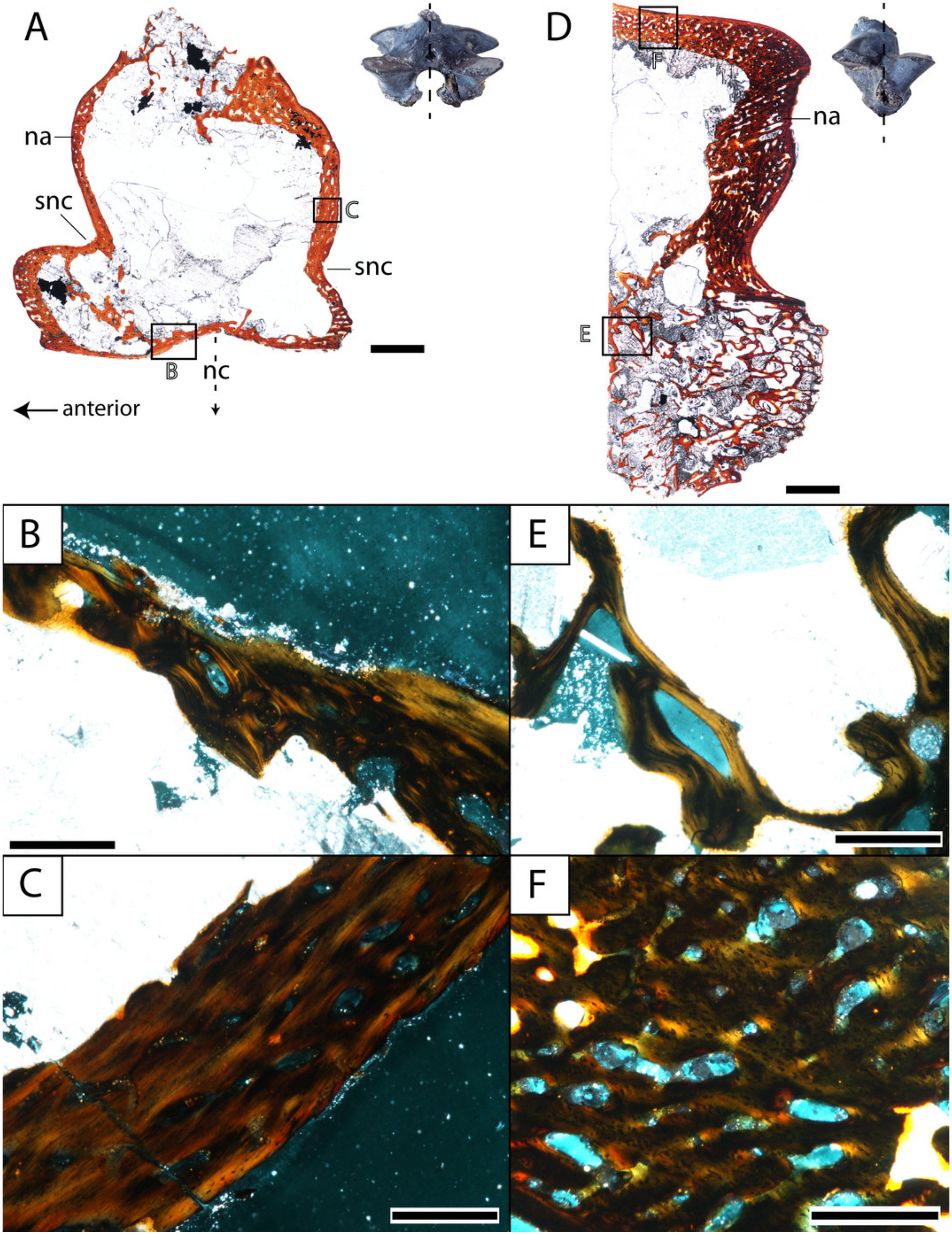
A
na
snc
C
snc
B
nc
anterior
D
F
na
E
B
E
C
F

Figure 6

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.

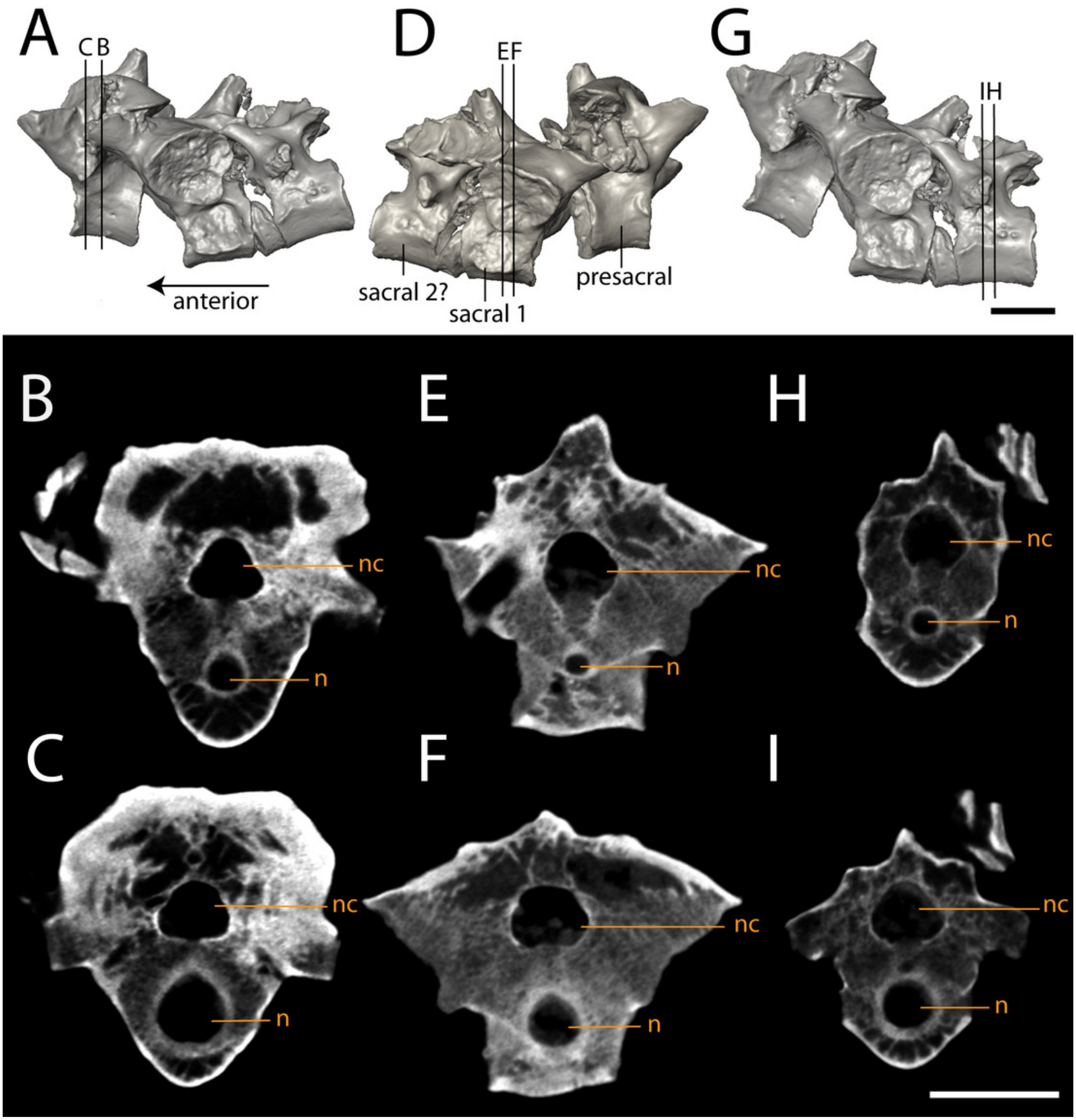
A
C B
anterior
D
E F
sacral 2?
sacral 1
presacral
G
I H
B
nc
n
C
nc
n
E
nc
n
F
nc
n
H
nc
n
I
nc
n

Figure 7

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.

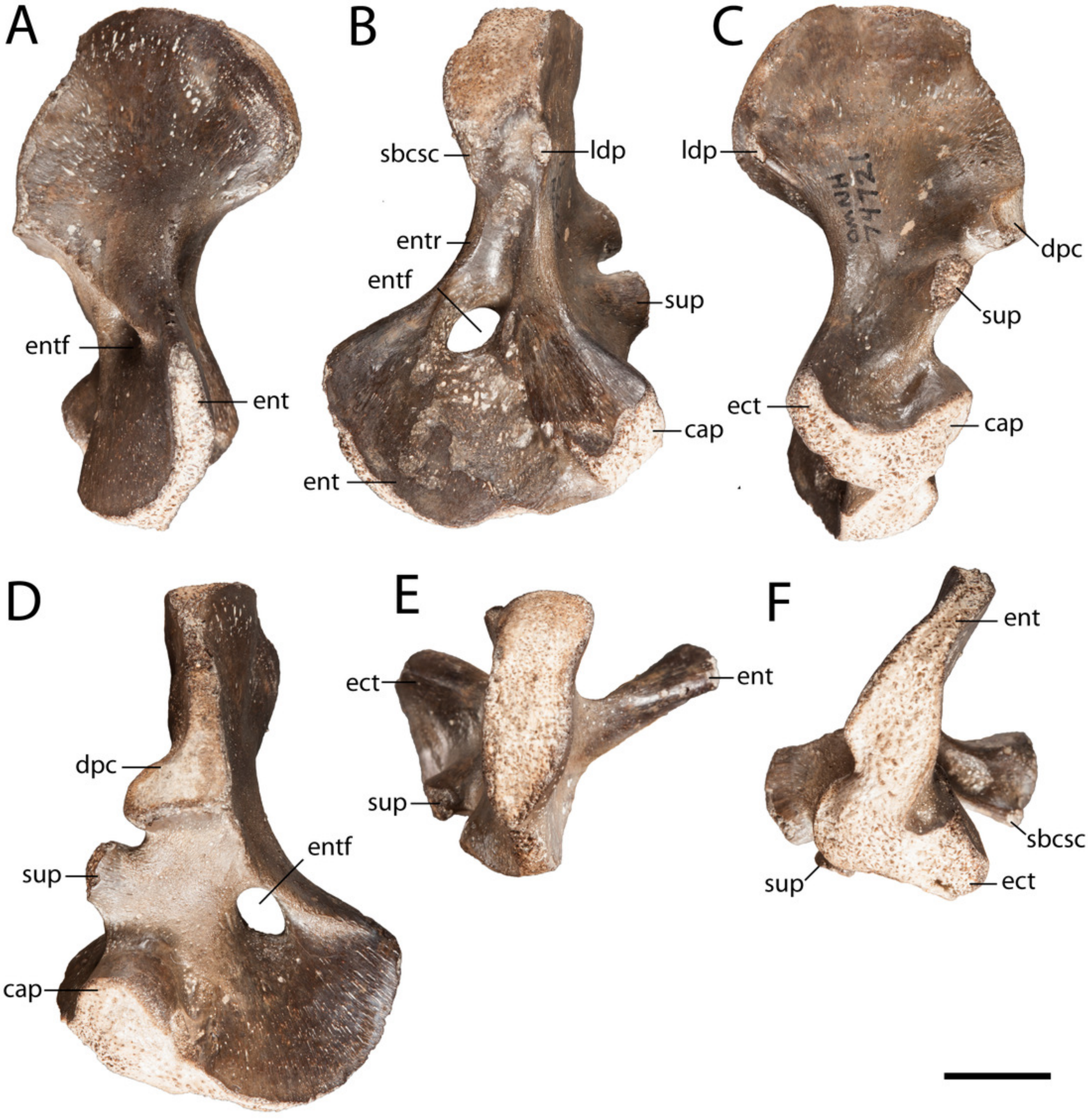
A
entf
ent
B
sbcsc
ldp
entr
entf
sup
cap
ent
C
ldp
OMNH 74721
dpc
sup
ect
cap
D
dpc
sup
entf
cap
E
ect
ent
sup
F
ent
sbcsc
sup
ect

Figure 8

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.

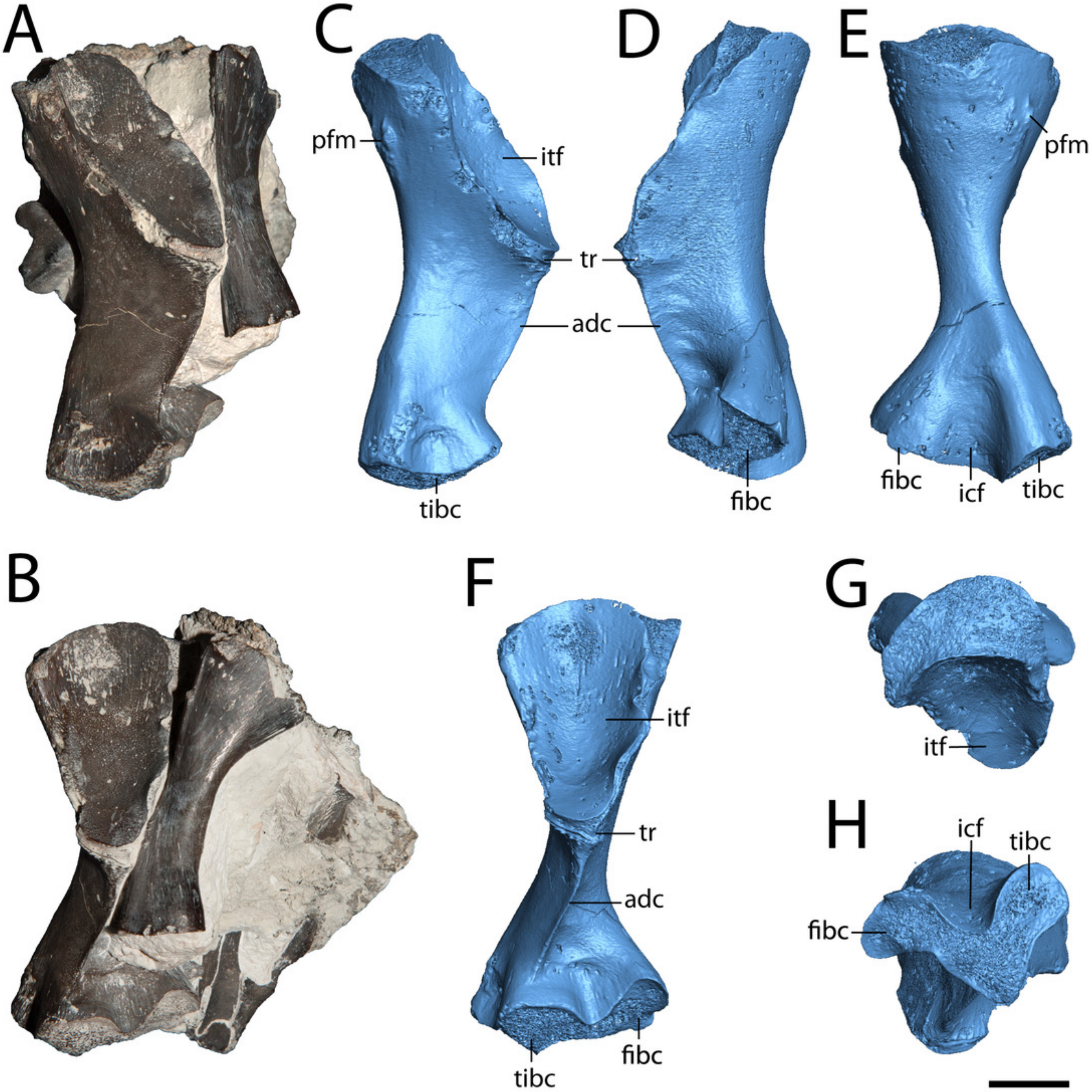
A
B
C
D
E
F
G
H
pfm
itf
tr
adc
tibc
fibc
icf
tr
adc
pfm
fibc
icf
tibc
itf
tr
adc
tibc
fibc
itf
icf
tibc
fibc

Figure 9

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.

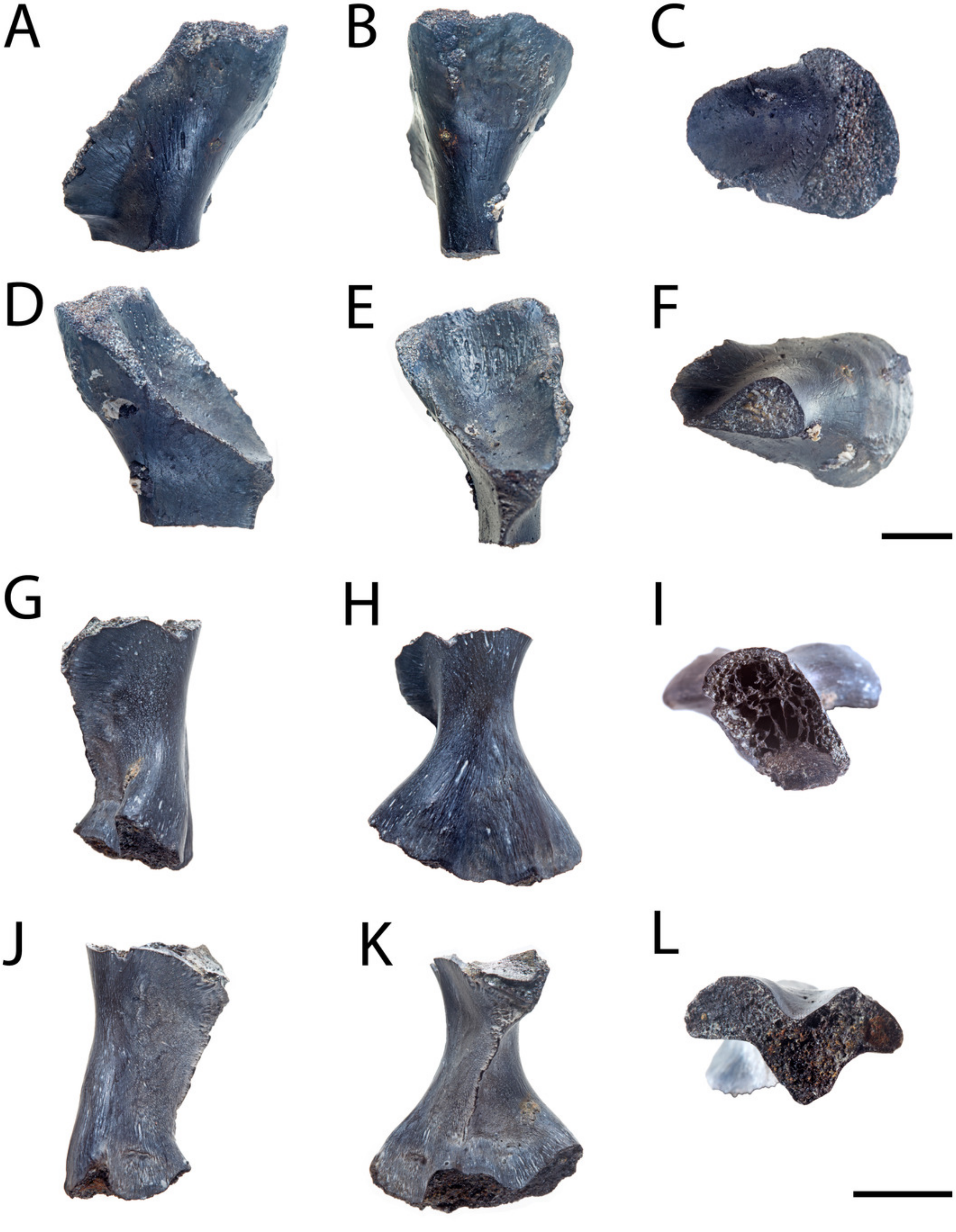
A
B
C
D
E
F
G
H
I
J
K
L

Figure 10

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 showing five lines of arrested growth (LAGs); **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).

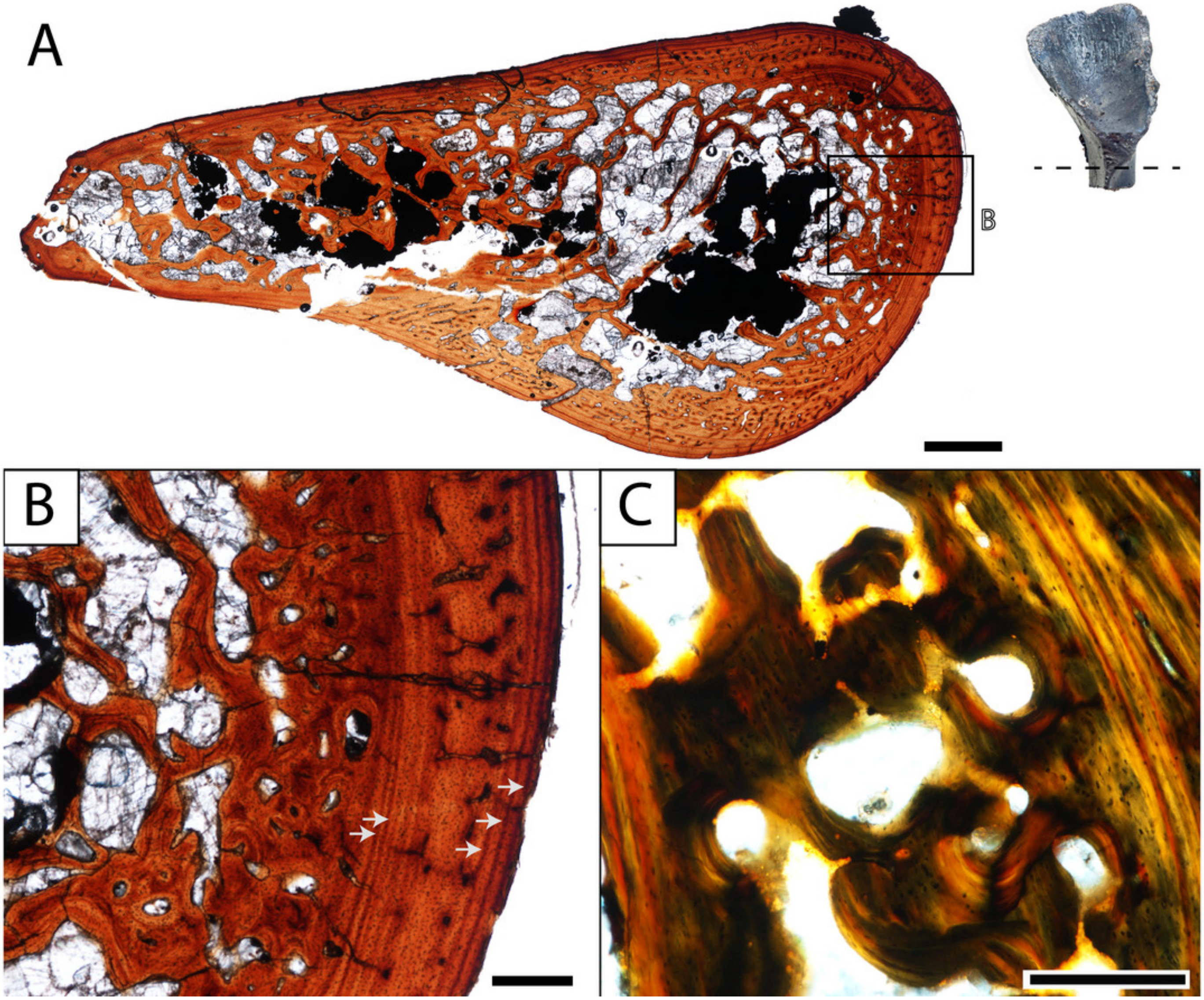

Figure 11

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).

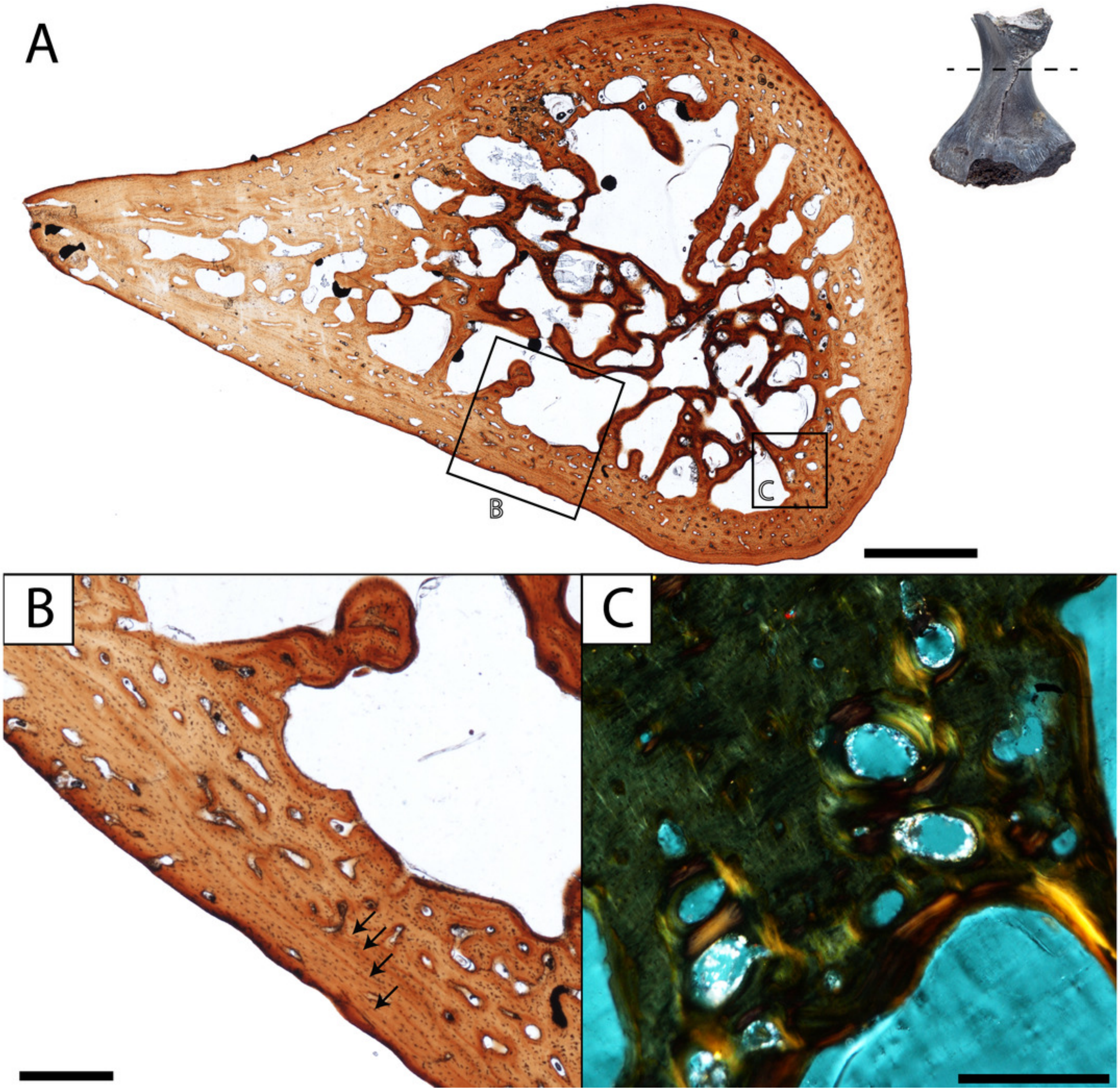
A
B
C
B
C

Figure 12

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.

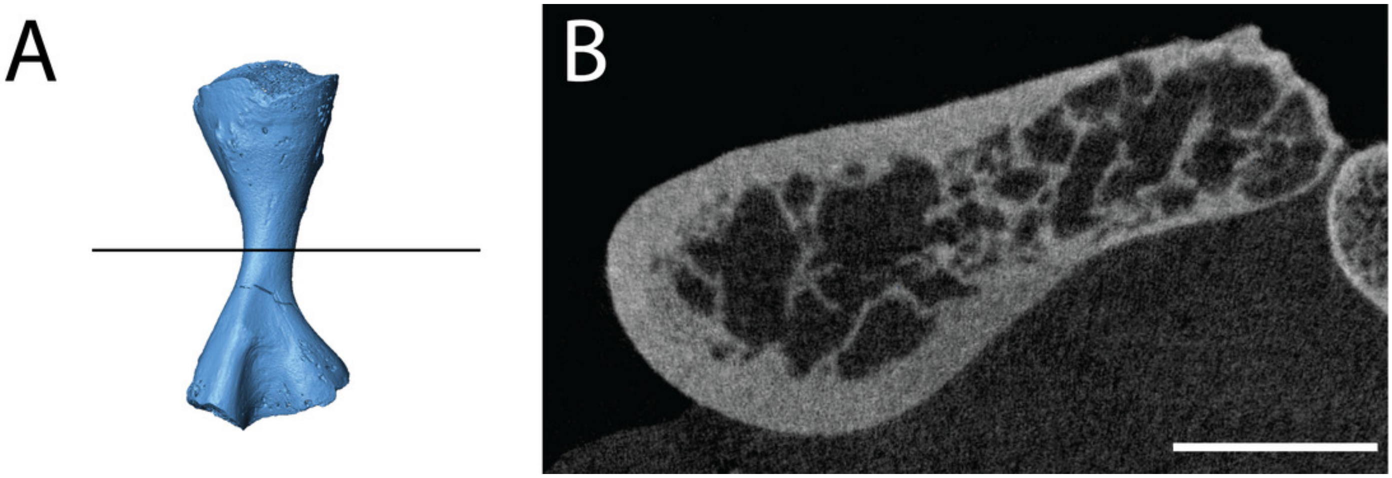

Figure 13

Digital renderings of ROMVP 80917.

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

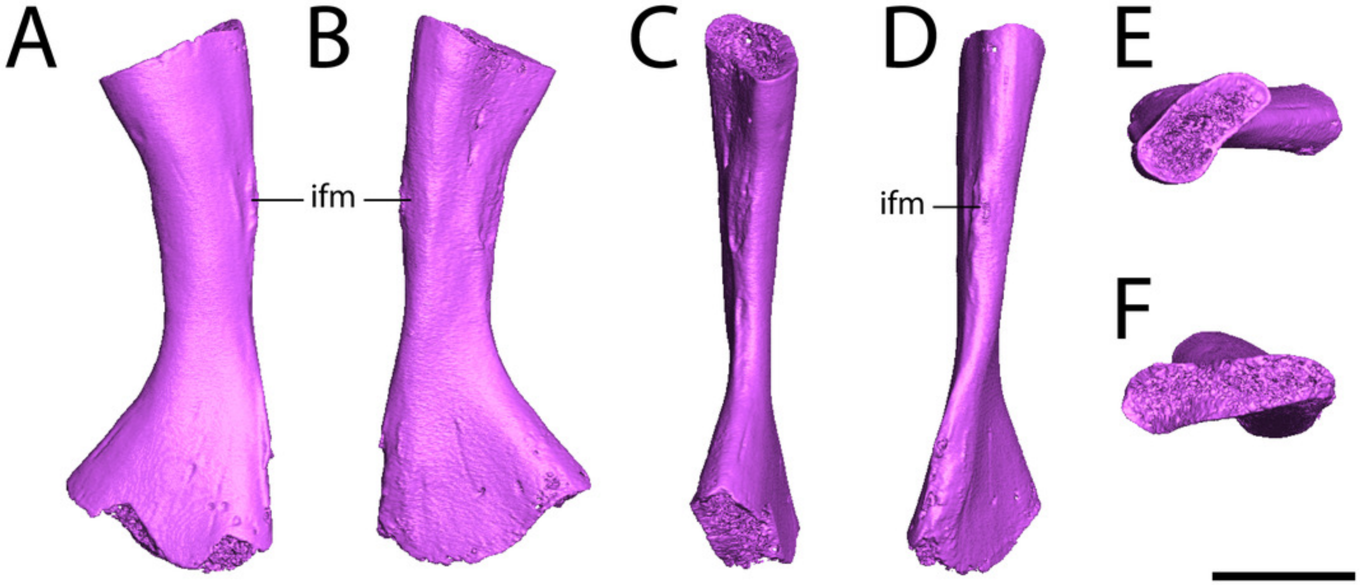

Figure 14

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.