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The missing anatomy of the living coelacanth, *Latimeria chalumnae* (Smith, 1939)

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Abstract

Anatomical features that have not been previously described in *Latimeria* were sought in histological section series, tissue-stained microCT scans, MRI scans, and synchrotron scan series. The spiracular organ, ultimobranchial endocrine gland, and m. cucullaris were identified in the expected locations. In addition, a muscle arising on the medial side of the pectoral girdle is identified and compared with a muscle in a similar location that attaches to the cranial rib in lungfish; these are proposed as homologues of the tetrapod m. omohyoideus.

These findings are placed in evolutionary context by comparison with selected other groups of fish, lungfish and tetrapods. The position of *Latimeria* as a key taxon in the fish-to-tetrapod transition is emphasised by these findings, and the findings have potential to inform research on cranial structure in extinct taxa.

Keywords

Introduction

The anatomy of *Latimeria chalumnae* has been covered comprehensively in the three books of Millot and Anthony (1958, 1965) and Millot, Anthony and Robineau (Millot et al. 1978), who described mainly gross anatomy and a limited range of histological information. Anthony (1980) provided some further information on anatomical issues derived from these Paris studies. The detailed overview of coelacanth morphology and evolution of Forey

(1998) summarises much previous work on *Latimeria*. Notable subsequent contributions in macroscopic anatomy included details on the lateral line system from Hensel and Ballon (2001). The serial histological sectioning of *Latimeria* fetal specimens retrieved from pregnant female adults initiated in San Francisco by Michael D. Lagios and in Tübingen by Wolfgang Maier led to the next major advances: W. E. Bemis collated the Lagios slides,

following which Northcutt and Bemis (1993) published a landmark account of the cranial nerves, and Bernstein (2003) produced reconstructions of the inner ear from the Tübingen slides.

The advent of cross-sectional imaging with CT and MRI scanning led to a new range of work with 3D reconstructions of structures such as the intracranial joint (Johnston 2011), hyoid apparatus (Dutel et al. 2015), the rostral organ (Berquist et al. 2015), and in combination with histological material, the vestigial lung (Cupello et al. 2017). Recent interest in the fin-to-limb transition in the evolution of tetrapods resulted in new dissection and 3D reconstruction approaches to the skeleton and musculature of *Latimeria* appendages and attempts at defining homologies among fin and limb muscles (Diogo et al. 2016; Huby et al. 2021; Miyake et al. 2016). *Latimeria* stands in a key position here: lungfish are generally accepted as closer to the tetrapod stem, but the appendicular morphology of modern lungfish has evolved in a unique autapomorphic fashion that is less clearly applicable to any tetrapod pattern than that of *Latimeria* (Diogo et al. 2016). The recent availability of synchrotron tomography imaging with its enhanced ability to define different tissues with a phase contrast method, and identification of *Latimeria* specimens of suitable size for this imaging, have offered another quantum step forward in morphological studies of *Latimeria* (Dutel et al. 2019; Mansuit et al. 2020, 2021) and also of taxa useful for comparative work (Dearden et al. 2021).

As these studies have progressed there remains some 'missing' anatomy: structures that would be expected to be found in a sarcopterygian fish, given the position of coelacanths in vertebrate phylogeny (Fig. 1). These include (a) the spiracular organ – a neuromast-like structure within the spiracular canal, or within a diverticulum or closed remnant of the spiracular canal; (b) the ultimobranchial endocrine gland, which is a derivative of the last branchial arch and is concerned with calcium metabolism; and (c) the m. cucullaris or m. protractor pectoralis, which passes between the caudal end of the cranium and the pectoral girdle, and is an important component of the head-trunk interface in terms of understanding the development of that domain (Ericsson et al. 2013; Sefton et al. 2016).

The spiracle is the dorsally placed remnant of the first gill cleft, between mandibular and hyoid arches (Gai et al. 2022). A patent spiracle is found in many elasmobranchs; in others the external opening is absent but the spiracular cleft remains as a diverticulum of the oropharynx. In most operculate fishes the spiracular cleft is closed in the course of development – holocephalans, teleosts, and lungfish – but in non-teleost actinopterygians – bichirs, bowfin, sturgeons and gars – both a patent spiracle and an operculum are present. The spiracular organ (SO) is a neuromast sensory organ that is present in extant chondrichthyans, lungfish, non-teleost actinopterygian fish apart from bichirs, but not teleosts. The SO arises from a discrete placode that is separate from the lateral line placodes (Agar 1906; O' Neill et al. 2012). The SO presumably migrates into the spiracle from the surface of the embryo, and can persist in a small enclosed remnant of the spiracular cavity in taxa where the spiracle otherwise completely closes, such as holocephalans and lungfish. A similar and presumably homologous structure is found in birds, where it is known as the paratympanic organ, in some non-avian reptiles including *Sphenodon*, and in some mammals (O' Neill et al. 2012), as shown on the phylogeny in Fig. 1. *Latimeria* has a large spiracular cavity without an external opening (Millot and Anthony 1958); there is close approximation to the exterior separated by a thin lamina at the confluence of the otic, supraorbital and infraorbital lateral line canals, but this may not be the site of the original spiracular opening (Millot and Anthony 1958). The SO has not been previously defined in *Latimeria*: Northcutt and Bemis (1993) identified a branch of the otic lateral line nerve (the expected innervation of a spiracular organ) that headed along the medial wall of the spiracular chamber, but could not find a spiracular organ. Here I will describe the spiracular organ of *Latimeria*, and briefly review the presence and morphology of a spiracular organ of notable taxa in the Fig. 1 phylogeny, where material is available: *Callorhinchus*, *Polypterus*, *Neoceratodus* and *Sphenodon*.

The ultimobranchial bodies (or glands) (UB), or suprapericardial bodies of early authors, are endocrine organs presumed to contribute to calcium metabolism by calcitonin secretion. They are found in all fish, amphibia, reptilians and monotremes; in therian mammals the ultimobranchial gland fuses with the thyroid during development and its calcitonin or 'C' cells disseminate throughout the thyroid (Kameda 2017). In chondrichthyans and non-teleost actinopterygians the UB is found in close association with the last branchial arch, as its name suggests; in teleosts the UB migrates from its embryological position and in the adult is found in the septum transversum, between heart and liver, or closely associated with the venous inflow of the heart (ducts of Cuvier) (Sasayama et al. 1995). No previous investigation appears to have looked specifically for the UB in *Latimeria*; Millot and Anthony (1958) did look for C cells within the thyroid gland, but this would not have been expected in a fish.

Musculus cucullaris passes between the lateral and caudal edge of the otic part of the braincase and the pectoral girdle (Greenwood and Lauder 1981) and would also have been expected in a fish or indeed at any point along the fish-tetrapod transition; Ericsson et al (2013) expressed surprise that it had not been discovered in *Latimeria*. Millot and Anthony (1958) and Greenwood and Lauder (1981) did not find it on dissection, and Northcutt and Bemis (1993) noted its absence in their serial section study of the cranial nerves. Sefton et al. (2016) examined the branchial area with MRI scans and came up with the hypothesis that a muscle body passing between pectoral girdle and the dorsal ends of the last branchial arches, identified as m. levator arcus branchialis 5 by Millot and Anthony (1958), was actually the m. cucullaris, in spite of its having a fibre direction at about 90° to that of the m. cucullaris in other fishes. Here I will demonstrate a thin body of muscle in the expected position, direction
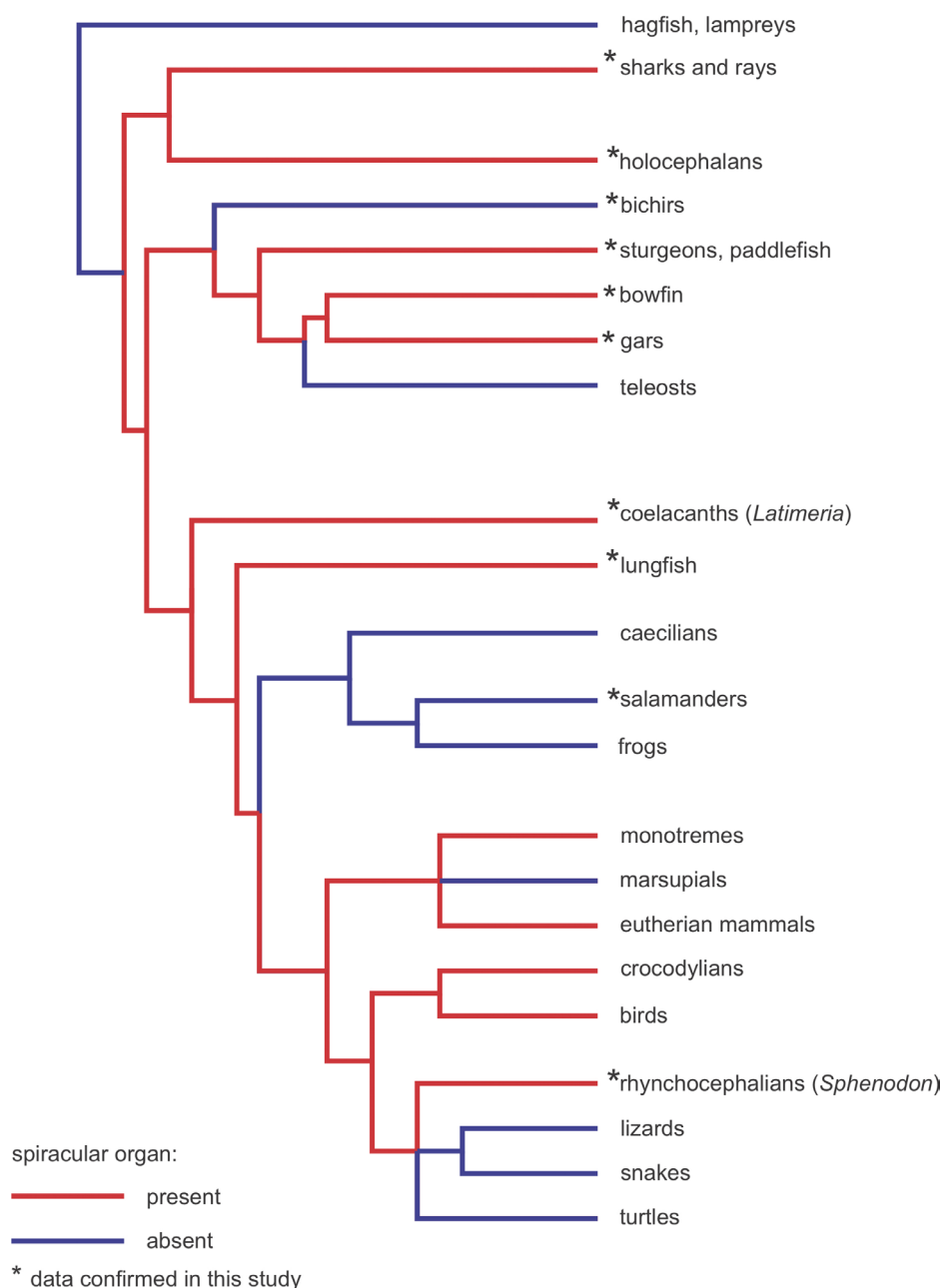
Figure 1. Vertebrate phylogeny showing presence of the spiracular organ, indicating taxa confirmed in this study. Branch lengths are approximate. Modified after O'Neill et al. (2012), with bony fish phylogeny based on Betancur-R et al. (2017).

and plane of a typical m. cucullaris, and agree with Millot and Anthony's original designation of m. levator arcus branchialis 5.

Additionally, muscles connecting the axial musculature with the fins are examined; a point of difference between the myological accounts of Diogo et al. (2016) and Huby et al. (2021) is the identification by Diogo et al. (2016) of a lateral elevator of the pelvic fin in *Latimeria*, passing from the hypaxial muscle to the proximal fin, similar to the a lateral elevator in *Neoceratodus*, but not identified by Millot and Anthony (1958) or Huby et al. (2021) in their dissection studies in *Latimeria*; I will revisit this issue.

A muscle connecting the hypaxial muscle in the proximal trunk with the pectoral girdle more dorsally was suggested in terms of an attachment to the cleithrum by Mil-

lot and Anthony (1958) but not further defined, and not mentioned by Diogo et al. (2016) in their dissection and MRI account. Here I will describe such a muscle, which corresponds with a similarly placed muscle in *Neoceratodus* that connects the pectoral girdle dorsally with the ventral tip of the cranial rib. Homology with the tetrapod m. omohyoideus is proposed given a corresponding muscle in lungfish, described below, and in both anurans and urodeles.

This study aims to fill in these 'missing' items in recorded *Latimeria* anatomy, and to discuss the significance of these structures in this survivor of a once dominant marine group, in light of *Latimeria*'s importance as one of the very limited number of extant members of the sarcopterygian grade in the transition between fish and tetrapods.

Methods

Material of *Latimeria* was examined in histological sections, contrast-enhanced CT and microCT scans, MRI scans, and synchrotron tomography series. Other taxa reviewed for comparative purposes were examined using these same methods, and by dissection in some instances. *Latimeria* specimens are identified by CCC (Coelacanth Control Council) numbers from the inventory of Nulens et al. (2011). Specimens acquired for this study were obtained and managed in accordance with CITES and New Zealand biosecurity regulations. Terminology for *Latimeria* generally follows Millot and Anthony (1958) except for the appendicular muscles, which follow Diogo et al. (2016). Terminology for lungfish cranial muscles follows Ziermann et al. (2018). For the longitudinal ventral muscles of the head and body wall, I use 'rectus cervicis' for the head and 'rectus abdominis' for the body, as terms encompassing discrete muscles variously described in those territories.

Image series were reconstructed into virtual 3D models using Amira 5.2.1 (Visage Imaging), generally after modifying size, contrast and brightness in ImageJ (NIH).

The material used is listed below; the microCT scan of *Polypterus delhezi* and the dissection of the pelvic fin of *Neoceratodus forsteri* were done specifically for this study. All other material was accessed by visiting institutions (Tübingen, New York) and sourcing imaging from public domain resources or by personal request to institutions or individual researchers; those specimens accessed by personal request are indicated in the list by an asterisk (*).

Serial histological sections

Latimeria chalumnae: University of Tübingen, Lehrstuhl für Spezielle Zoologie: CCC 162.11, 351mm pup, 40μm sections, Heidenhahn's Azan stain. Slides are numbered rostral to caudal and referred to with the prefix T.

American Museum of Natural History: 32949. CCC 29.1, 303 mm pup, 50 μm sections, Weigert's haematoxylin and van Gieson's picro-fuchsin stain. An account of the history and organisation of this section series is provided by Northcutt and Bemis (1993). Slides are numbered caudal to rostral with the prefix RC.
Sphenodon punctatus: specimen and staining details as in Ung and Molteno (2005): sequences from a serially sectioned hatchling head *.

Neoceratodus forsteri: photographs of multiple section series as listed in Ziermann et al. (2018)*.

Dissection material

Neoceratodus forsteri: formalin fixed specimens, details in Diogo et al. (2016).

Necturus maculosus: alcohol specimen, commercial supplier; author's collection, SVL 90mm.

MRI series

Latimeria chalumnae: Scripps institute of Oceanography, CCC 88, MRI scan accessed from digitalfishlibrary. org, scan details at * http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=124

Muséum Nationale d'Histoire Naturelle (MNHN), Paris. CCC94. Scan details in Mansuit et al. (2021), scan accessed at https://www.morphosource.org/concern/media/000113919?locale=en.

Neoceratodus forsteri: California Academy of Sciences SU 18139, specimen and scan details * http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=271.

Protopterus aethiopicus: California Academy of Sciences CAS 46377, specimen and scan details * http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=237.

Tissue-stained CT scan

Latimeria chalumnae: MNHN CCC27, phosphomolybdic acid staining, staining and scan details in Dutel et al. (2013) *.

Tissue stained microCT scan

Polypterus delhezi: phosphotungstic acid (PTA) staining, specimen, staining and scan details in Molnar et al. (2017).

Amia calva: stage 29 larva, PTA staining; specimen, staining and scanning details in (Funk et al. 2021)*.

Salamandra salamandra: https://www.morphosource.org/media/000345888 *

Necturus maculatus: https://www.morphosource.org/concern/media/000346019?locale=en *

Plethodon cinereus: https://www.morphosource.org/concern/media/000345972?locale=en *

Chiloscyllium punctatum: PTA staining, specimen and scan details in Coates et al. (2019). *

Polyodon spatula: PTA staining, specimen and scan details in Metscher (2009). *

Synchrotron tomography series

Latimeria chalumnae: fetus, South African Institute for Aquatic Biodiversity, CCC 202.
pup, MNHN, CCC 29.5.
pup, Zoologische Staatssammlung München (ZSM) CCC 162.21.
Scan details for the above 3 *Latimeria* series are given in Dutel et al. (2019), and scans were downloaded from http://paleo.esrf.eu.

Latimeria chalumnae: ZSM, CCC 162.21, pelvic girdle and fin, scan details in Mansuit et al. (2021). *

Scyliorhinus canicula: specimen and scan details in Dearden et al. (2021). *

Callorhinchus milii: specimen and scan details in Dearden et al. (2021). *

Data availability

The PTA stained microCT of *Polypterus delhezi* is available on request to the author.

Results

Spiracular organ in Latimeria

The spiracular organ is found on the medial side of the spiracular chamber, close to the orbital artery and enclosed in a recess in in the 'afacial' or 'affacial' eminence of Jarvik (1980) on the otic shelf of the oto-occipital moiety of the braincase (Fig.2), in which a surface impression is evident in skeletal preparations (Fig. 3). The afacial process bears a toothplate in *Latimeria*, and Jarvik (1965) refers to it as dermal bone applied to the endochondrally ossified otic shelf; the afacial process does have a more delicate trabecular structure than the ossification in the otic shelf. Histologically the spiracular organ conforms with the appearances in elasmobranchs (Barry and Boord 1984), in which it has elongated cells with basal nuclei and pale-staining cytoplasm (on haematoxylin and eosin staining), with an amorphous material occupying part of the lumen, formed by the cilia. Cytological detail is lacking in the relatively thick sections and aging staining of both the *Latimeria* section series, together with some loss of structure from delayed fixation after death, but the residua of these features remain visible (Fig. 3). The spiracular organ forms a narrow tube that is measured at 0.75mm length in CCC 29.1. With knowledge of the spiracular organ's location, its nerve, a branch of the otic lateral line nerve, can be traced further than was evident to Northcutt and Bemis (1993). The nerve passes out from the braincase alongside the combined facial and anteroventral lateral line trunk, passes ventral to the jugular vein and then traverses the bony interface between the otic shelf and the afacial process, as does the nearby orbital artery. The last, intraosseus, section of the course of the nerve cannot be confidently seen on the histological sections or synchrotron CT series, but its line of passage can be inferred (Fig. 4). On one side of one of the specimens used here (CCC 29.5), the nerve follows an alternative or aberrant course, which actually makes it easier to follow: it leaves the cranial cavity through a separate foramen lying dorsal to the exit of the facial nerve, passes dorsal to the jugular vein and then ventrally on the lateral side of the vein the enter the dorsal aspect of the otic

shelf-afacial process interface and travels directly ventrally through this to the spiracular organ. Fig.4 includes both paths of the SO nerve, and the whole distribution of the otic lateral line nerve.

The tissue-stained CT series of CCC27 was made after phosphomolybdic acid staining; this is very similar to the effects produced by phosphotungstic acid (PTA), which allows neuromast and related sensory structures such as the inner ear to become selectively enhanced on microCT scans (Schulz-Mirbach et al. 2013). In the CCC27 series the neuromast tissue of the SO is evident as an intense plaque of enhancement at the identical position to that noted histologically (Fig. 5 A, B). On the synchrotron series the indentation (or fossa) for the SO on the rostral surface of the afacial process can be identified; the slice image of the fetus CCC 202 is shown in Fig. 5 C, D: the afacial process is not fully developed at this stage and the opening of the SO faces laterally rather than rostrally.

Spiracular organ in other taxa

Scyliorhinus cannicula and *Chiloscyllium punctatum*: in these image series, the SO is seen at the base of the spiracular chamber on its medial wall (*Chiloscyllium*, Fig. 6 A, B). The orbital artery and its lateral branch are in close apposition to the SO.

Polypterus: In the PTA microCT series of the head of *Polypterus delhezi*, no neuromast tissue could be found within the spiracular chamber, but the expected enhancement of superficial and canal neuromasts, and the sensory structures on the snout known as the ampullae of Lorenzini, is confirmed, indicating absence of the SO. However, in the developing *Polypterus senegalus* a placode is seen within the spiracle in sections of stage 26 and at the location of the spiracle in whole-mount preparations of stage 29 (Diedhiou and Bartsch 2009), suggesting that the SO originally forms and is lost in later development (Robert Cerny, personal communication). In microCT series of *Amia* the SO is present on the medial wall of the spiracular chamber dorsally (Fig. 6C), consistent with previous reports (Goodrich 1930).

In *Polyodon* the spiracular organ is evident within a lateral diverticulum of the main spiracular chamber.

Callorhinchus and *Sphenodon* are mentioned by O'Neill et al. (2012) as unconfirmed instances of the SO, based on single reports. In the *Callorhinchus* synchrotron CT series, the residual, enclosed spiracular cavity is seen in a position identical to that reported by de Beer and Moy-Thomas (1935), immediately ventral to the lateral edge of the palatoquadrate at the level of the dorsal elements of the first branchial arch. In *Sphenodon*, a cavity lined by cuboidal epithelium is seen in a position identical to that described by Werner (1963), dorsal to the columella auris and adjacent to the stapedial artery. The section series available is incomplete in this area such that the whole cavity could not be followed, and the neuromast tissue demonstrated by Werner was not seen; all other features of Werner's de-
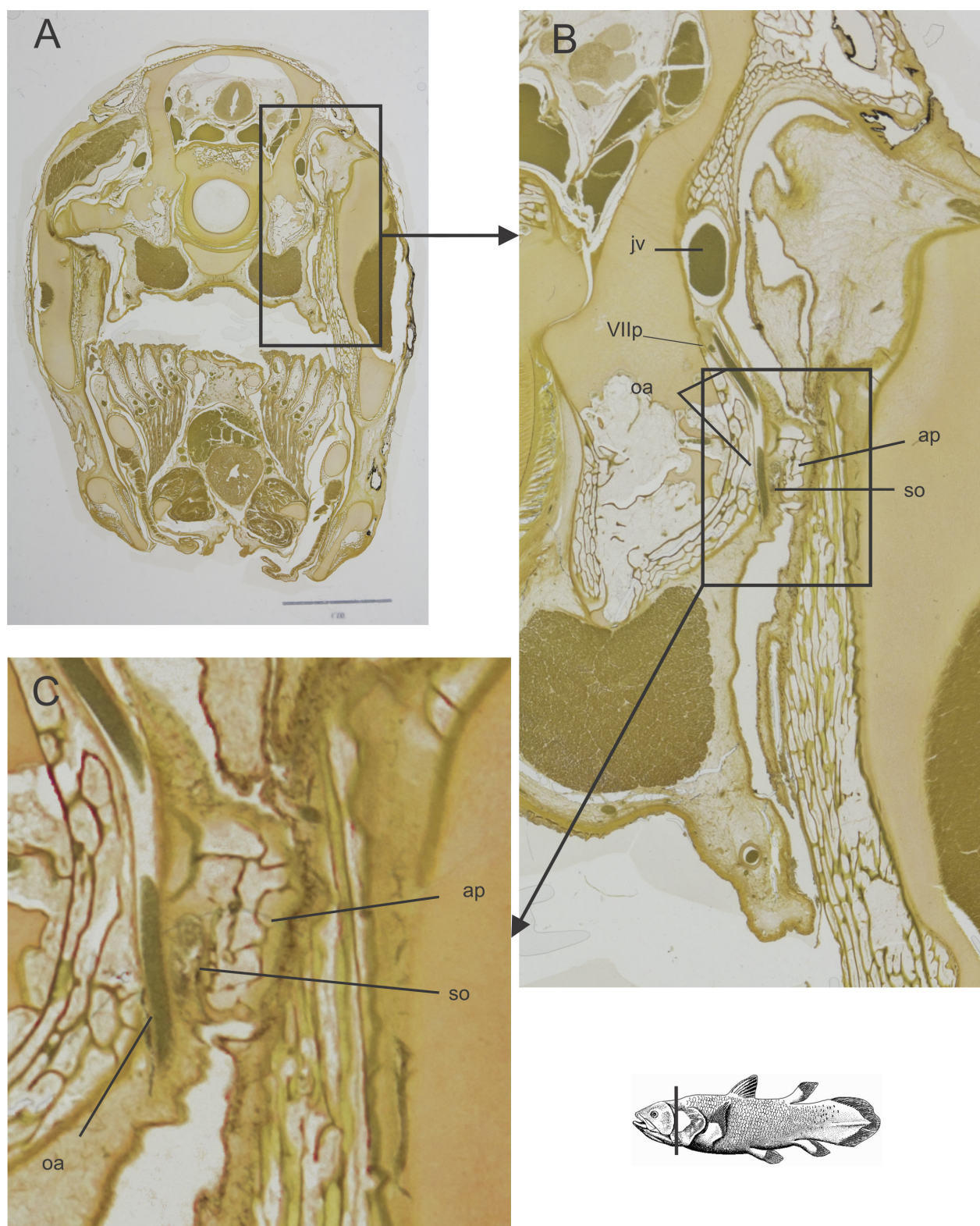
Figure 2. *Latimeria chalumnae* CCC29.1, spiracular organ in histological sections,. Slide RC 657 in progressive magnifications **A–C** Abbreviations: jv, jugular vein; VIIp, palatine ramus of facial nerve; oa, orbital artery; ap, afacial process; so, spiracular organ. Scale bar: A=10mm

scription are, however, confirmed. In the *Neoceratodus* section series the SO is as described by Bartsch (1994) as a neuromast structure within a very small, enclosed remnant of the spiracular canal; an artery wraps closely around the SO here, and is identified by Bartsch as the efferent mandibular artery.

Ultimobranchial body

The UB in *Latimeria* is seen as a shallow pouch or diverticulum from the ventral mucosa of the posterior pharynx, on the left side only. Findings are the same in both section series: a narrow lumen leads in from the
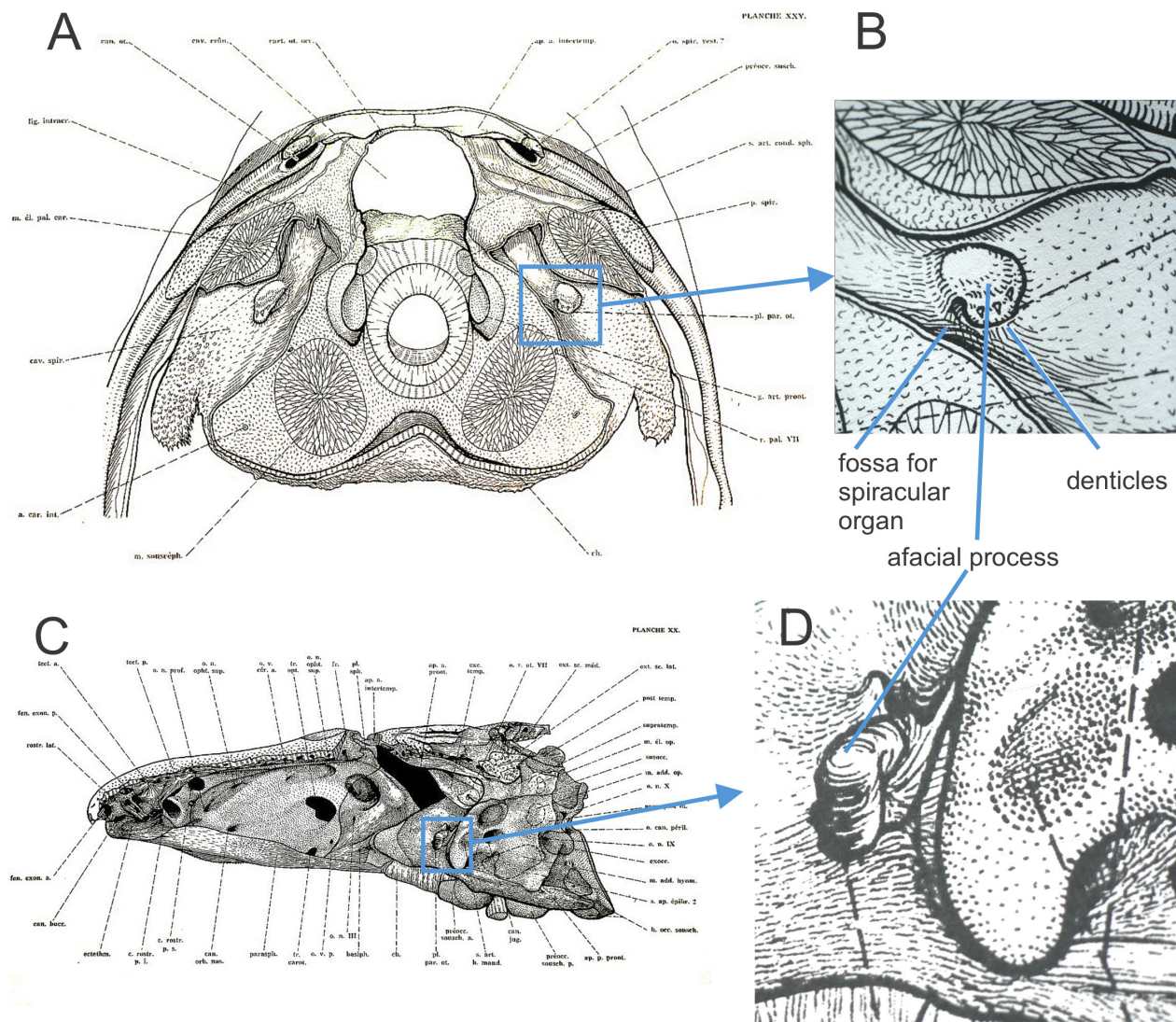
Figure 3. *Latimeria chalumnae*, drawings reproduced with permission from Millot and Anthony (1958). **A**, **B** (planche XXV) (rostral view of oto-occipital braincase) showing afacial process on otic shelf, and fossa for the spiracular organ. **C**, **D** (planche XX) lateral view showing afacial process.

mucosal surface to glands in small acini, mostly with their own lumen and not in obvious continuity with the main lumen of the pouch (Fig. 7). The total antero-posterior length of the UB in CCC 29.1 is 1.0cm, and in CCC 162.11is 0.84cm. In comparative material, the UB is seen as with very similar morphology in the posterior pharynx on the left side in the synchrotron series of *Scyliorhinus*.

M. cucullaris

In histological series (Fig. 8 A–E), m. cucullaris is found as a thin and discontinuous sheet in the expected position of this muscle, dorsal and exterior to the levatores arcuum branchialium; in its cranial end the muscle is represented by a thin fibrous sheet with a few muscle fibres radiating caudally from its origin on the 'processus post-oticus' of Millot and Anthony (1958) and Jarvik (1954) of the braincase, and the body of the muscle becomes more apparent toward its insertion on the pectoral girdle at the dorsal end of the cleithrum. The synchrotron CT images

at the same 'pup' stage have findings identical to the histological series (Fig. 8 F).

The MRI imaging used here is the same as that used by Sefton et al. (2016), but different conclusions are reached. Again, the m. cucullaris is seen (most clearly in horizontal plane image reconstruction, rather than axial or sagittal) as a narrow sheet in the typical position of this muscle, as just described in the histological slides. Muscle tissue on this imaging stops short of the bony pectoral girdle, implying a flat tendon connecting these, or insertion of the muscle into the epaxial fascia. 3D reconstructions from the fetus synchrotron imaging and the adult MRI are seen in Fig. 9.

In the synchrotron series the m. cucullaris is clearly seen again more prominent caudally, and is relatively larger in the 'fetus' (CCC202) (Fig. 9 A,B) than in the 'pup' (Fig. 8F) or adult (Fig. 9 C,D) specimens.

The nerve supply of m.cucullaris could be traced in the synchrotron scan of CCC29.5: a slender branch arises from the vagus just distal to the second branchial branch, and passes dorsally in the space between the epaxial mus-
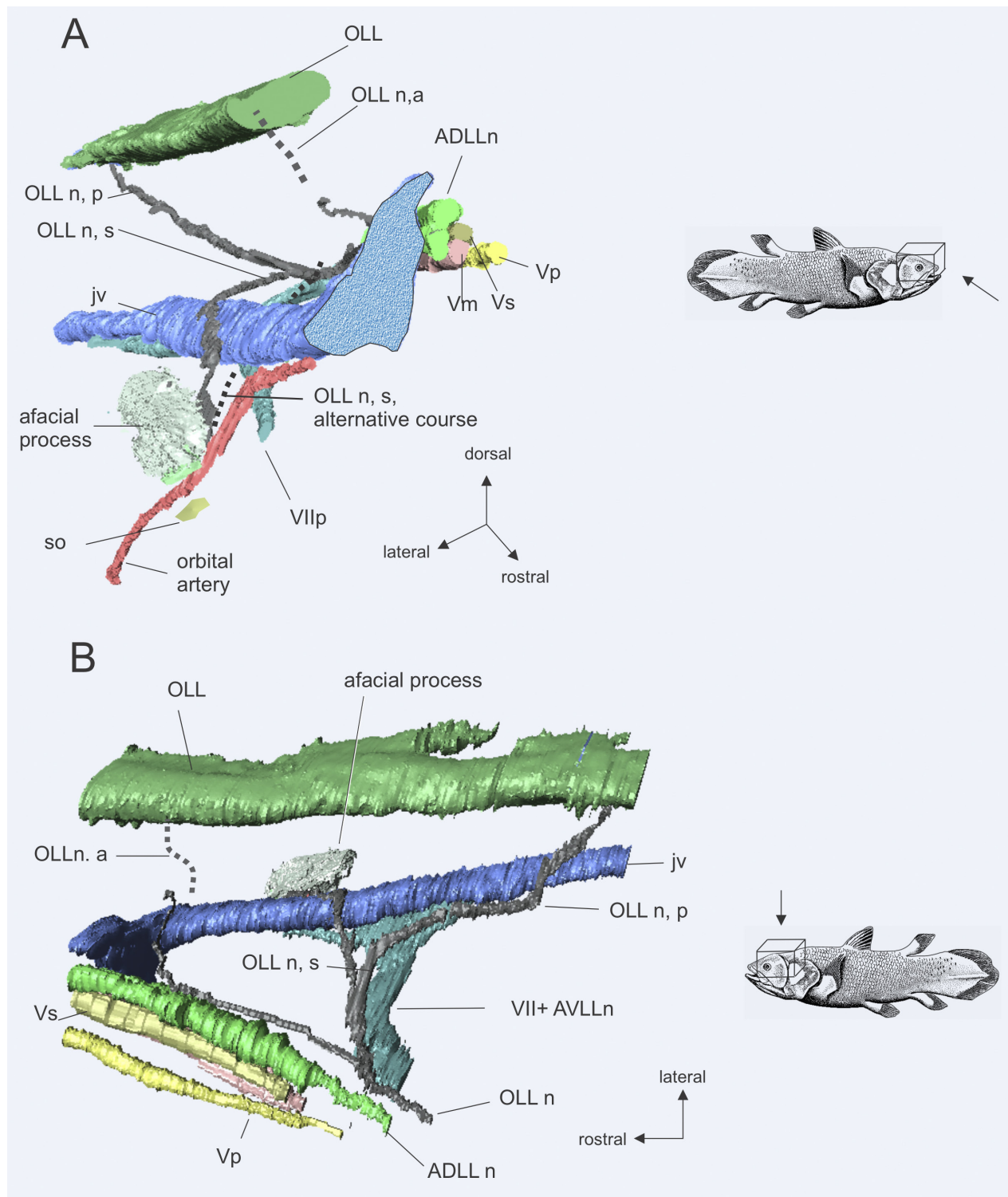
Figure 4. *Latimeria chalumnae* CCC 29.5, 3D reconstructions from synchrotron tomography, showing the otic lateral line nerve and its distribution. **A** anterolateral view; **B** dorsal view. Relationships among spiracular organ, orbital artery and palatine nerve are observed in **A**. Nerve branches too small to appear in the reconstruction are indicated with dashed lines. Abbreviations: OLL, otic lateral line; OLLn, otic lateral line nerve; OLLn,a, anterior ramus otic lateral line nerve; OLLn, p, posterior ramus; Olln, s, spiracular ramus of otic lateral line nerve; jv, jugular vein; so, spiracular organ; VIIp, palatine ramus of facial nerve; ADLLn, anterodorsal lateral line nerve; Vp, profundus ramus of Vth cranial nerve; Vs, sensory ramus of V nerve; Vm, motor ramus of V nerve; VII+ AVLLn, facial + anteroventral lateral line nerves. Terminology after Northcutt and Bemis (1993).

cles and the levatores arcuum, in parallel with slender branches from the posterior lateral line nerve to the posterior lateral line. Innervation of m. cucullaris in fishes is not well described, but the situation in *Latimeria* as described here is very similar to the innervation of m.cucullaris in ganoid fishes (Norris 1925).

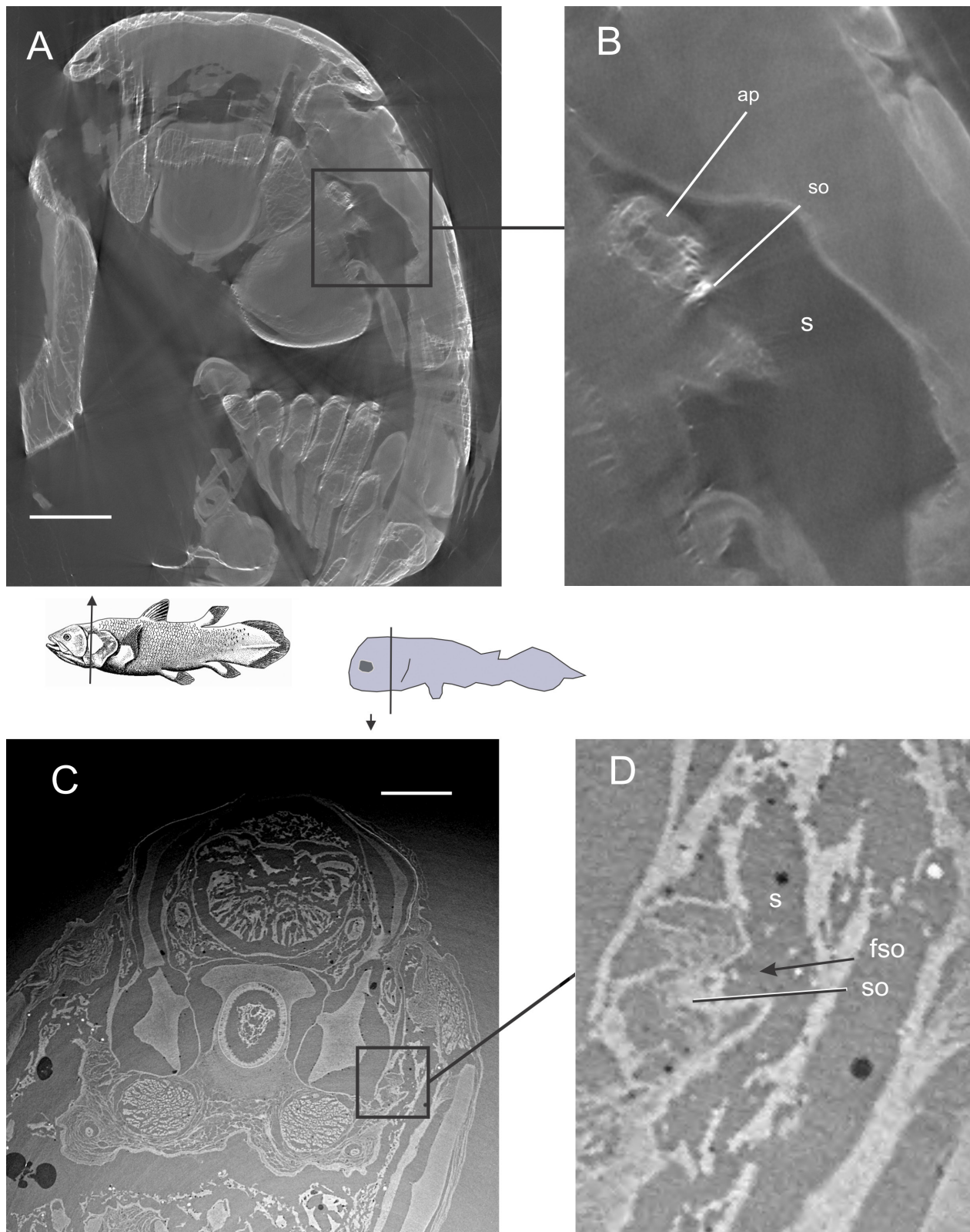The levatores arcuum are displayed in both fetus and adult reconstructions in Fig. 9: levator arcus branchialis

Figure 5. A, **B** *Latimeria chalumnae* adult, CCC 27, phosphomolybdic acid stained CT scan, demonstrating uptake of the stain in the spiracular organ. **C**, **D** *Latimeria chalumnae* fetus, CCC 202, showing spiracular organ. The afacial process is not yet developed. Abbreviations: ap, afacial process; fso, fossa for spiracular organ; s, spiracular cavity; so, spiracular organ. Scale bars: A=50mm, C=1mm.

5, as described by Millot and Anthony (1958), is a small muscle which arises from the pectoral girdle and meets and is partly enveloped by levator arcus branchialis 4 as both converge on the dorsal aspect of the epibranchial plate at the dorsal ends of ceratobranchialia 3+4. There is, however, a discrepancy between the text and figures of Millot and Anthony (1958): the text is as just described, but the figure (planche VII) shows levator arcus branchi-
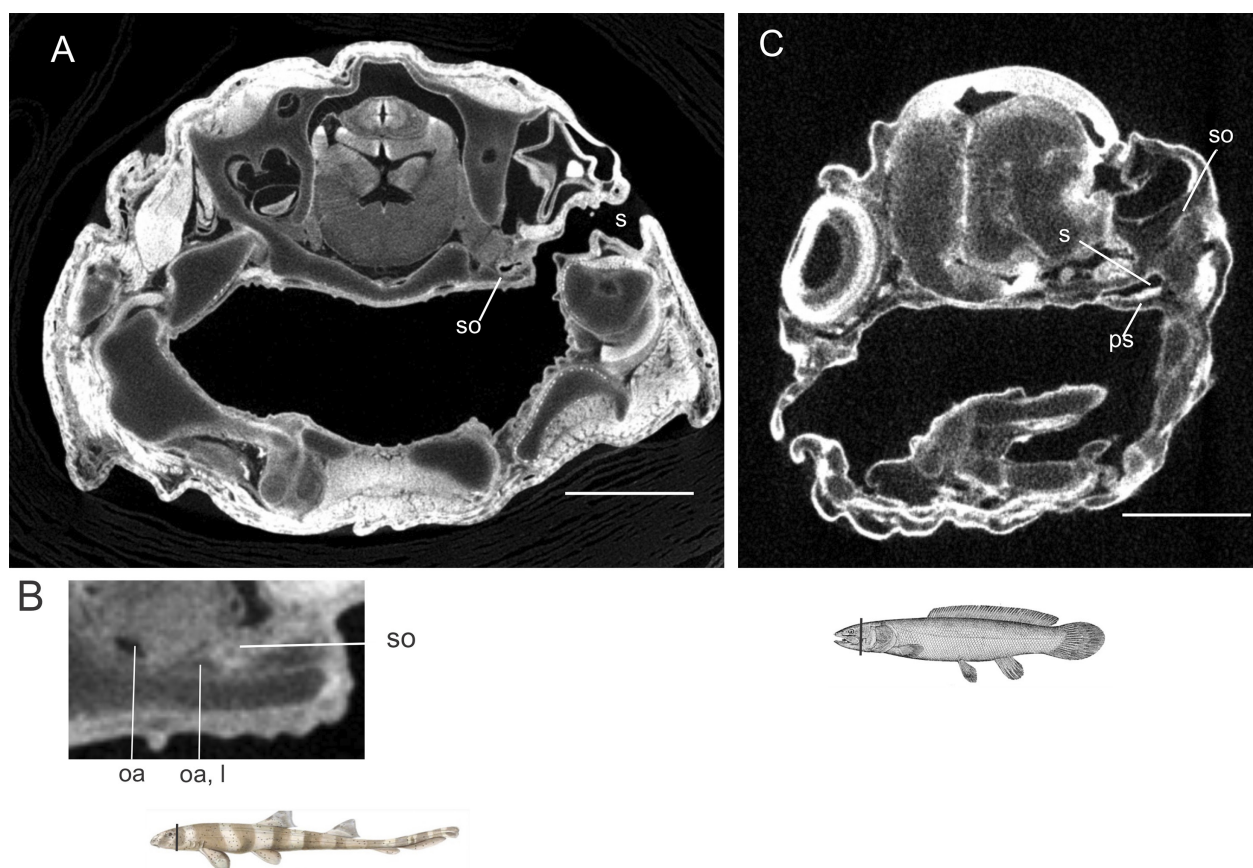
Figure 6. A *Chiloscyllium punctatum* (bamboo shark), phosphotungstic acid (PTA) stained microCT section with spiracular organ within a diverticulum close to the oral opening of the spiracular canal. **B** *Chiloscyllium punctatum*, adjacent section to **A**, showing orbital artery and its branch in apposition to spiracular organ. **C** *Amia calva* (bowfin), PTA stained microCT section, with pseudobranch ventrally and spiracular organ dorsally within the spiracular canal. Abbreviations: so, spiracular organ; oa, orbital artery; oa, l, lateral branch of orbital artery; ps, pseudobranch; s, spiracular canal. Scale bars: A=5mm, C=1mm.

alis 5 as a more elongated structure inserting on the dorsal tip of the 5th ceratobranchial. The latter situation was not seen in any of the specimens used here.

M. omohyoideus

In *Latimeria* this muscle is seen in the synchrotron and MRI imaging as a vertically directed body of muscle arising near the dorsal end of the cleithrum, and extending ventrally, medial to the levatores arcuum branchialium and the caudal part of the gill apparatus to meet the clavicle on its dorsal border close to the midline (Figs 9, 10E). In the adult images CCC88 (MRI), no continuity with the epaxial or hyaxial muscle can be determined, but in the 'fetus' CCC202 a medial extension within the epaxial muscle territory between the main trunks of vagus and posterior lateral line nerves is seen. This muscle lies with the contours of the epaxial and hypaxial muscle masses except at its ventral end, but has a quite different fibre direction to those muscles and lacks their close chevron intersections.

The equivalent muscle in *Neoceratodus*, passing between cleithrum and the ventral tip of the cranial rib, is obvious on dissection and shown here in reconstruction from MRI scan (Fig. 10 A–D). The cranial rib tip also forms an intersection in the longitudinal muscle of the ventral body wall, and gives attachment to a discrete muscle passing rostrally to meet the clavicle, which I have termed in Fig. 10 the deep lamina of the m. rectus abdominis. The situation in *Protopterus* is similar, although the m. sternohyoideus is considerably more bulky. In the absence of pleural ribs in *Protopterus*, another discrete muscle meets the tip of the cranial rib posteriorly, passing obliquely from the axial skeleton.

In the CT series of salamanders reviewed here, m. omohyoideus conforms with the descriptions of Francis (1934) and Özeti and Wake (1969): a muscle belly arises dorsally on the suprascapular or scapula and passes ventrally to lie close to the ventral end of the clavicle, where it meets the m. rectus cervicis via a tendinous intersection. This is similar to the plane and direction of the muscle I have proposed as m. omohyoideus in *Latimeria* and lungfish.

Lateral elevator of the pelvic fin

Musculus levator lateralis of the pelvic fin is a thin, short sheet of muscle passing between the hypaxial body muscle and the antero-lateral border of the proximal fin (Fig. 11A), and was identified in MRI sections of *Latimeria* as described by Diogo et al. (2016). This is almost identical to a muscle in this position in *Neoceratodus*, the abduc-
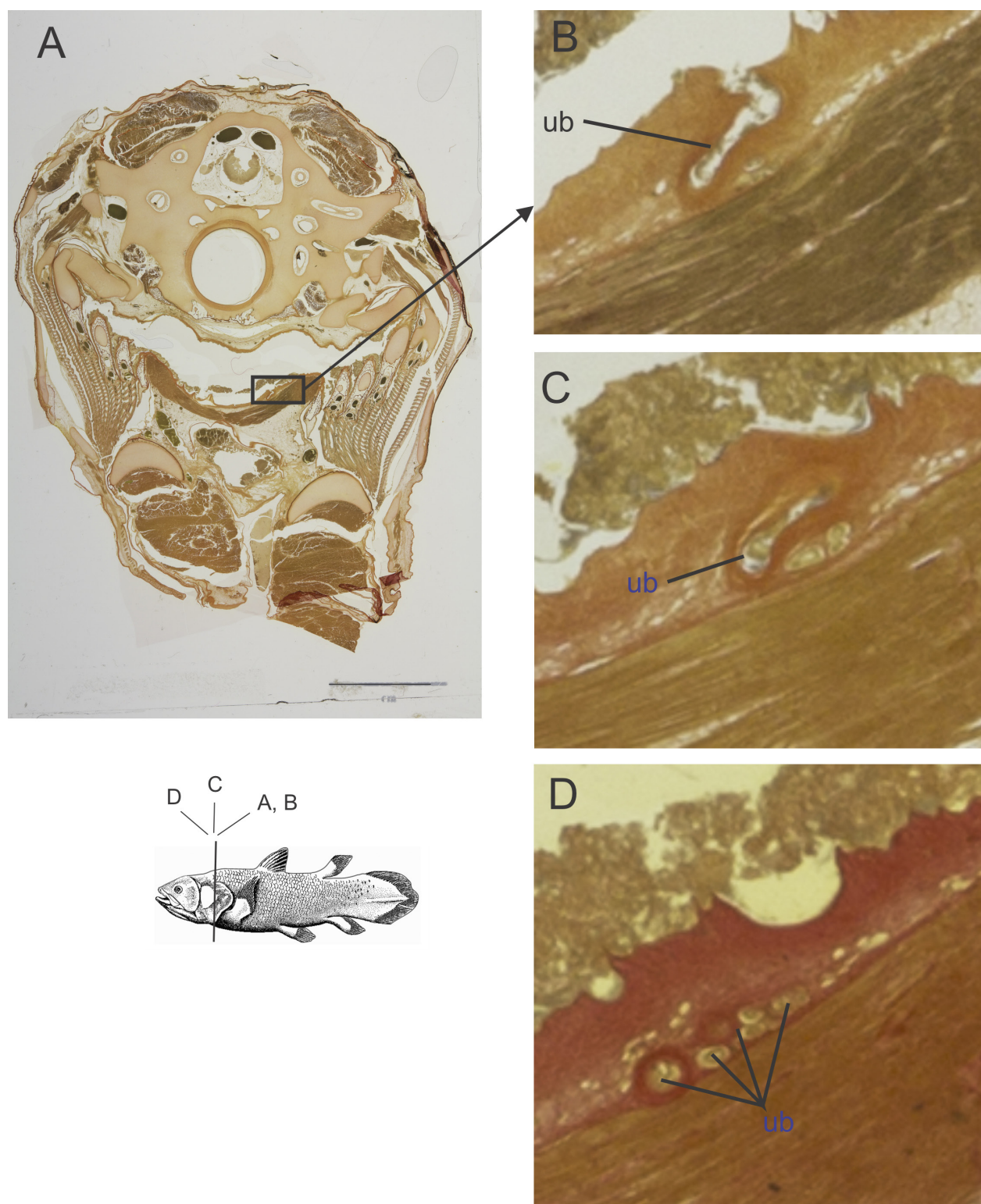
Figure 7. *Latimeria chalumnae*, CCC 29.1, histological sections. **A** slide RC893; **B** magnified location of ultimobranchial gland in A; **C** RC885; **D** RC 879 (more rostral sections), showing gland tissue as terminal branches of the central duct of the gland. Abbreviation: ub, ultimobranchial gland. Scale bar A=10mm.

tor dorsolateralis of Diogo et al. (2016), designated the superficial ventrolateral abductor of Young et al. (1989) and Boisvert et al. (2013). *Latimeria* dissection material available to us for Diogo et al. (2016) was damaged in this area, but Huby et al. (2021) could not confirm such a muscle in dissected material and doubted its existence. In the synchrotron series of the pelvic fin, a small sheet of muscle is clearly seen in this position (Fig. 11A) and it can also be identified on a different MRI series made available by Mansuit et al. (2021) (CCC162.21) from their study of skeletal elements of the fin. A specimen of *Neoceratodus* was dissected here and confirmed the presence of this small muscle (Fig. 11B).
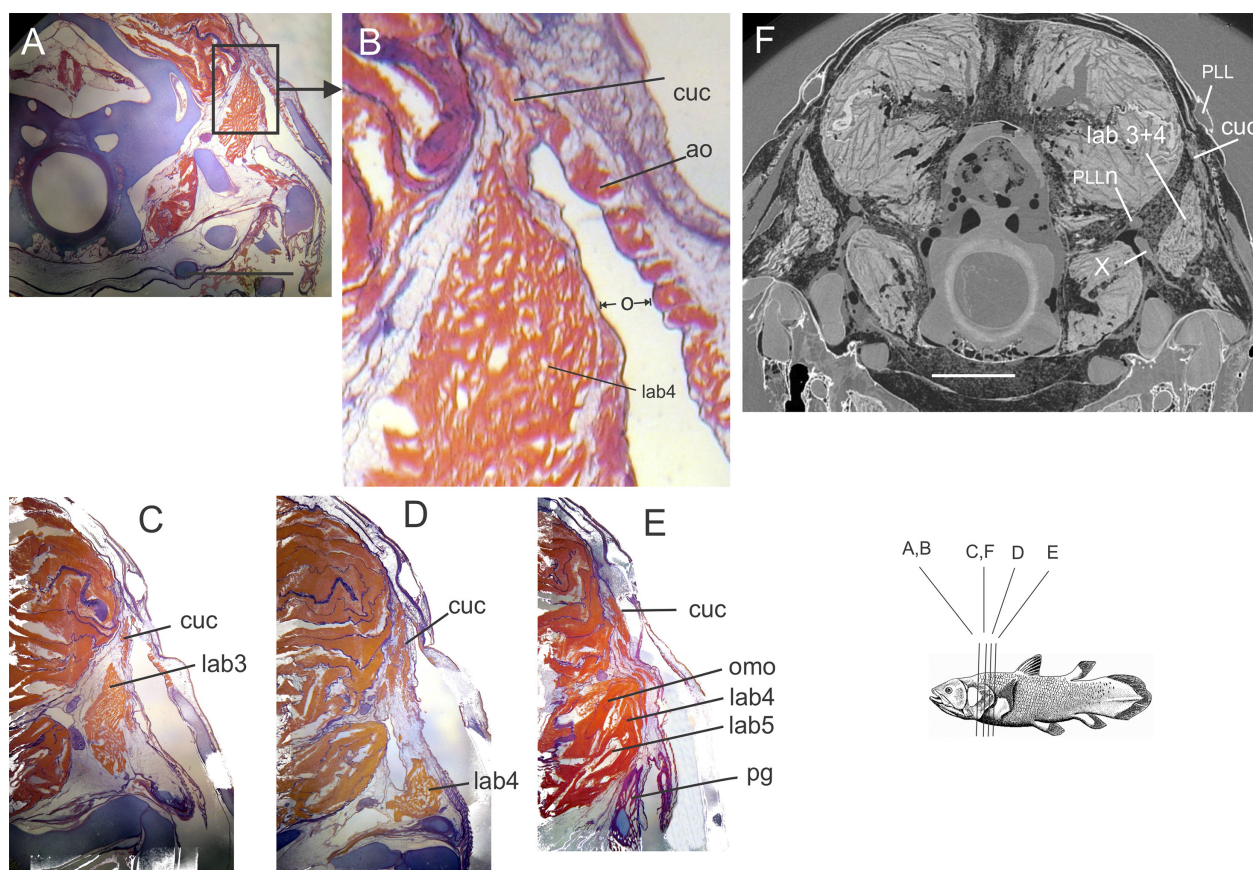
Figure 8. *Latimeria chalumnae* (**A–E**) CCC 162.11, histological sections. **A** (T971), overview; **B** magnified section of dorsal branchial region showing m. cucullaris. **C** (T1139); **D** (T1143); **E** (T1216): progressively more caudal representative sections, **E** being at the level of the pectoral girdle. **F** *Latimeria chalumnae* CCC 29.5, synchrotron CT section at a level similar to **C**. Abbreviations: cuc, m. cucullaris; ao, distal fibres of adductor operuli; lab 3+4, common belly of levator arcus branchialis 3 and 4; lab 3, 4, 5, levator arcus branchialis 2–5; o, opercular chamber; omo, m. omohyoideus; X, vagus nerve; PLLn, posterior lateral line nerve; PLL, posterior lateral line; pg, pectoral girdle. Scale bars: A=10mm; F=1mm.

Discussion

I have described here three structures which would be expected to be present in *Latimeria*, given their occurrence in chondrichthyans, non-teleost actinopterygians, and lungfish: the spiracular organ, ultimobranchial gland, and m. cucullaris. Each of these is found in the expected anatomical territory. Additionally, the muscle I am referring to as m. omohyoideus in lungfish is identified in *Latimeria*.

Spiracular organ

The identification of the spiracular organ of *Latimeria* has a number of interesting implications. The distribution of the SO across vertebrates is reviewed by O'Neill et al. (2012); a simple vertebrate phylogeny with distribution of the SO updated from O'Neill et al. (2012) with the data presented here is given in Fig. 1. Among fishes this structure is present in elasmobranchs, non-teleost actinopterygians with the exception of bichirs, and absent in teleosts. The SO is also present in holocephalans and lungfish,

is spite of the early closure of the spiracular cleft. The function of the SO (or paratympanic organ of tetrapods) is unknown, but is presumed to be mechanosensory in elasmobranchs, where its relation to the hyomandibula has suggested a function in positional information of that cartilage (Barry and Boord 1984). In avians a role in barometric or altimetric sensation has been proposed but not confirmed experimentally (Neeser and von Bartheld 2002). From the distribution of the SO across vertebrate phylogeny, it is reasonable and certainly more parsimonious to assume that the SO is a plesiomorphic feature among vertebrates (Gardiner 1984). The significance of the multiple losses of the SO is difficult to establish in the absence of functional information; investigative tools such as identification of placodes are now available, but have not yet been applied across a wide spectrum of vertebrates with focus on the SO placode, so the preliminary findings cited here in *Polypterus* are of interest.

A close spatial association between the SO and the orbital artery or its homologue, the stapedial artery of tetrapods, has not been noted previously. The orbital artery is a branch of the paired dorsal aorta, and passes lateral and inferior to supply the orbit and facial structures. Here this association is observed in *Latimeria*, *Chiloscyllium*, and
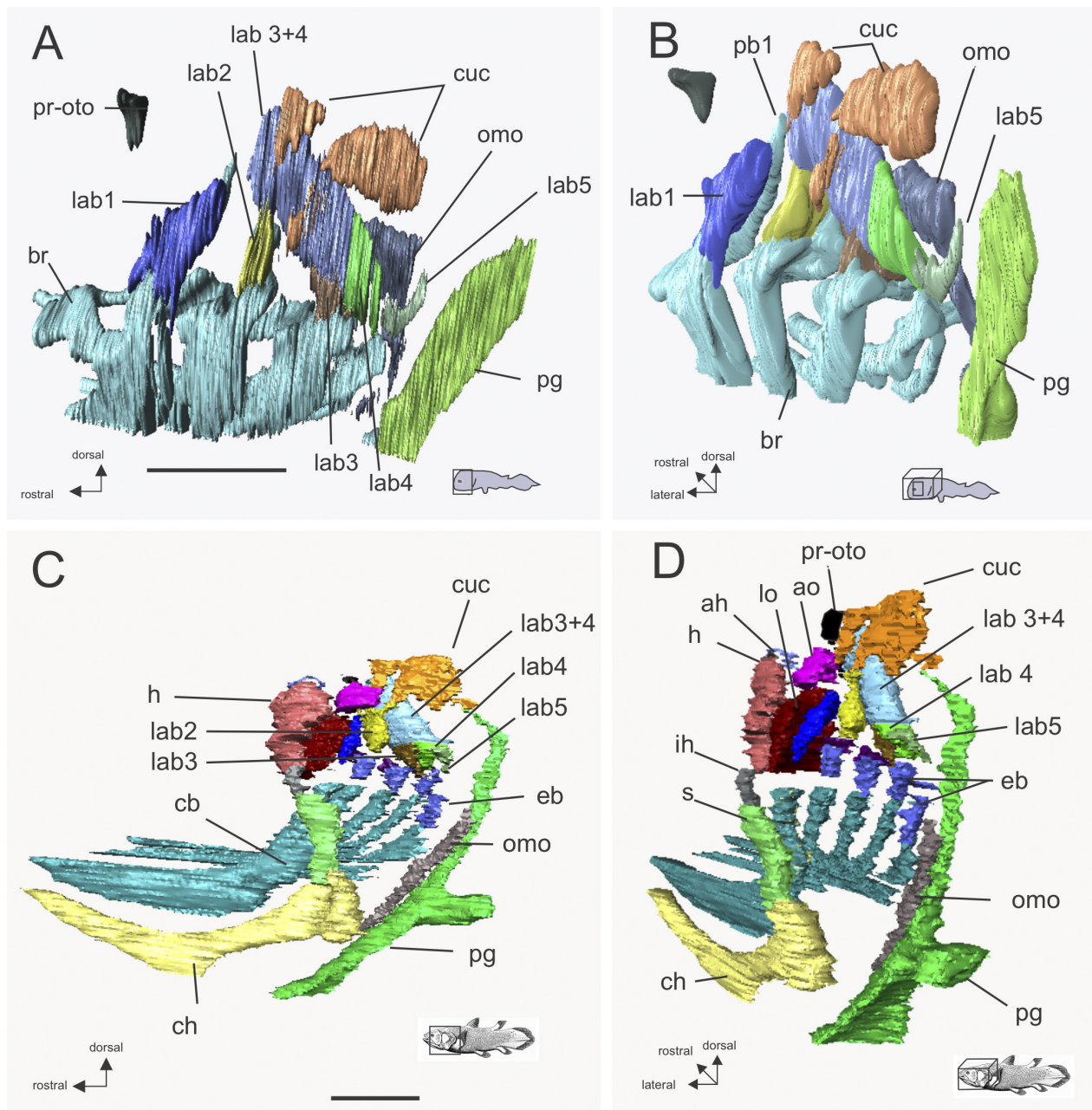
Figure 9. *Latimeria chalumnae*, 3D reconstructions: **A** and **B**: CCC 202 (fetus), synchrotron CT, and **C** and **D**, CCC 88 (adult), MRI scan, showing relationships of m. cucullaris to branchial levators and adjacent structures. Colours are similar in **A**, **B** and **C**, **D**; in **C**, **D** the epibranchial cartilages are segmented separately from the ceratobranchials, all are together in **A**, **B**. Nomenclature for the muscles of the hyomandibula and operculum in **C**, **D** as in Millot and Anthony (1958); in the scan images these three muscles form a continuous broad sheet. Abbreviations: pr-oto, location of the processus post-oticus of the braincase; br, branchial cartilages, cb, ceratobranchials, ch, ceratohyal; eb, epibranchials; pb1, suprapharyngobranchial 1; h, hyomandibula; ih, interhyale; s, sympletic; pg. pectoral girdle; cuc, m. cucullaris; omo, m. omohyoideus; lab 1–5, levator arcus branchialis 1–5 (lab 3 and 4 have a common dorsal belly which gives rise to separate muscles close to the dorsal arches); ah, m. adductor hyomandibulae; lo, m. levator operculi, ao, m. adductor operculi. Scale bars: A, B=1mm; C, D=50mm

Sphenodon; in *Neoceratodus*, the named orbital artery is a different structure to that of other fish, and the original territory of the typical orbital artery is taken over by branches from the ventral aorta. In neognathous birds, a close apposition of the paratympanic organ and stapedial artery is seen (Starck 1995). The situation in crocodylians is not as clear; combining the findings of Neeser and von Bartheld (2002) and Kundrát et al. (2009), the stapedial artery passes adjacent to the dorsal recess of the tympanic

cavity, where the paratympanic organ lies. The significance of this relationship across these disparate taxa is not clear, and could be examined more closely with further data. Whether there is a functional association is an interesting question: a role in cardiovascular regulation could be proposed.

Recognition of the SO in *Latimeria* could throw light on fossae and foramina in the spiracular area in extinct taxa. The afacial process of Jarvik (1980) is clearly pres-
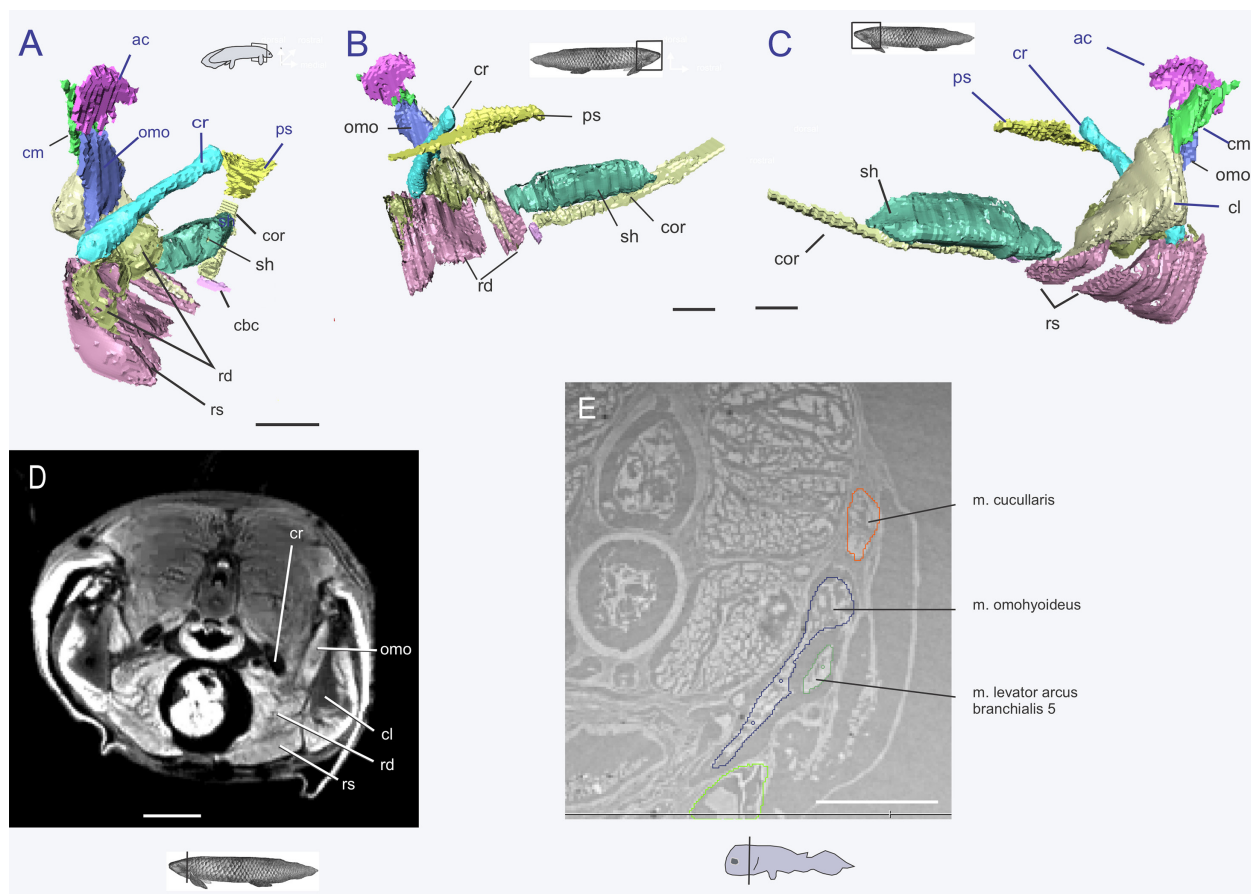
Figure 10. *Neoceratodus forsteri*, MRI scan: **A–C** reconstruction of muscles connected to the cranial rib in **A** caudomedial; **B** medial; and **C** lateral views. **D** slice image to demonstrate some of the structures segmented in A–C. **E** *Latimeria chalumnae* CCC 202, synchrotron tomography, slice image of segmentation to show muscle proposed as m. omohyoideus. Scale bars A–D=10mm, E=1mm. Abbreviations: ac, anocleithrum; cbc, cardiobranchial cartilage; cl, clavicle; cm, cleithrum; cor, m.coracomandibularis; cr, cranial rib; omo, m. omohyoideus; ps, parasphenoid; rs, m. rectus abdominis, superficial lamina; rd, m. rectus abdominis, deep lamina; sh, m. sternohyoideus.

ent in *Diplocercoides* (*Nesides*), a Devonian coelacanth that lies remote from *Latimeria* on all estimations of coelacanth phyogeny (Toriño et al. 2021). In *Diplocercoides*, Jarvik noted a groove or fossa in the anterior face of the afacial process and proposed this housed the orbital artery, which it may indeed have done, but perhaps more likely contained the spiracular organ. In *Eusthenopteron*, Jarvik (1980) proposed a spiracular organ in a much more dorsal position (Jarvik 1980; Fig. 206) than that identified here in *Latimeria*; Jarvik was using *Amia* as a baseline for a plesiomorphic osteichthyan fish, and in *Amia* and in other non-teleost actinopterygians the spiracular organ is found in a considerably more dorsal position than the *Latimeria* SO (Goodrich 1930; Fig. 6C). *Latimeria* would have been a better model, at least in this respect, for Jarvik's hypotheses about *Eusthenopteron*. *Latimeria* is closer phylogenetically to *Eusthenopteron* (in Fig. 1 it would appear on a branch from the stem leading to tetrapods), and the skull structure is much more similar, with the two-part cranium and intracranial persistence of the notochord. Jarvik (1980) was not clear about which phylogenetic hypothesis he was entertaining, but was clearly supporting *Amia* as a descendent of palaeoniscid fish and thus having arisen close to the base of the actinopterygian

tree. Several small fossae are seen in Jarvik's figures of *Eusthenopteron* on the medial and posterior aspect of the spiracular chamber at a similar level to the SO of *Latimeria*, and it would be interesting to investigate these with modern imaging.

The identification of the nerve to the SO in *Latimeria*, a discrete branch of the otic lateral line nerve, also has interest in the study of other taxa; I will offer some examples. As pointed out by Forey (1998) and Dutel et al. (2012), there are a number of foramina or fossae on the lateral face of the otic shelf in fossil coelacanths, and the identity of these can be difficult to work out. Among tetrapodomorphs, a foramen on the otic shelf in *Megalichthys* was designated by Romer (1937) as 'hypotic branch of the facial nerve', and this was followed for a similar foramen in the osteolepidid *Gogonasus andrewsae* by Long et al. (1997). However, the term 'hypotic nerve' is difficult to place among other taxa: none of the accounts of the cranial nerves in fish either by the classical anatomists or in more recent investigations have a facial nerve branch of this name or location. Allis (1934) considered 'hypotic' as an archaic term for 'otic' in the context of lateral line nerves; an otic lateral line nerve branch in this territory would presumably only be supply-
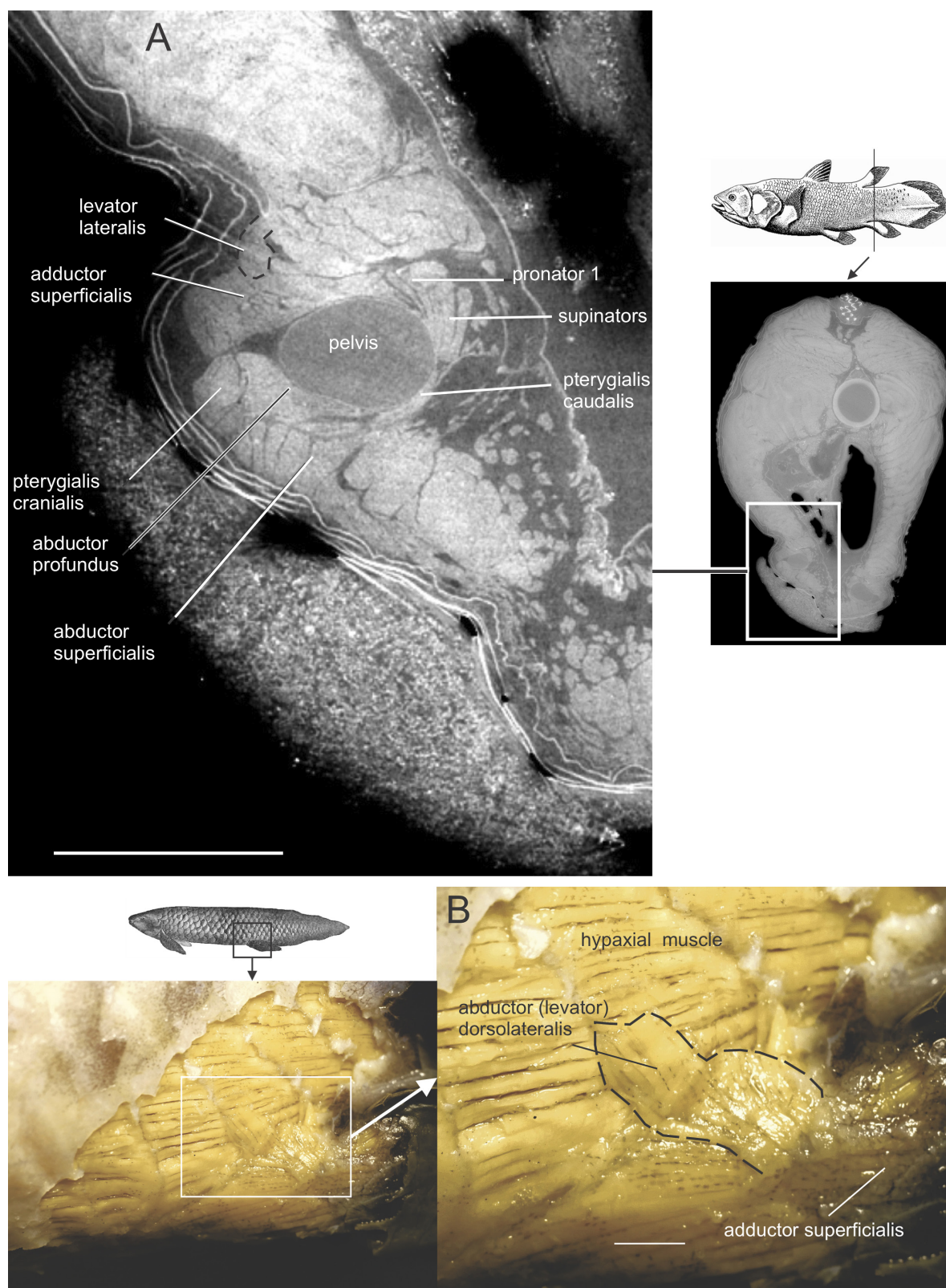
Figure 11. A *Latimeria chalumnae* CCC162.21, synchrotron CT section through base of pelvic fin with muscles labelled, confirming the presence of m. levator lateralis (Diogo et al. 2016), comparable to the m. abductor dorsolateralis of *Neoceratodus* (Diogo et al. 2016) ('superficial ventrolateral abductor' of Young et al. 1989, Boisvert et al. 2013). **B** *Neoceratodus forsteri*, superficial dissection of body wall at origin of pelvic fin, lateral view. Scale bars: A=5mm, B=1cm.

ing a spiracular organ, as the otic lateral line in these taxa is in the usual location, dorsolateral on the cranial roof. The 'hypotic' nerve foramen in these taxa could in fact be for the nerve to the SO, or even the SO itself. A search

for possible osteological correlates of the SO in other tetrapodomorphs and in osteichthyans may also be rewarding; just to give one further example, blind pits within the spiracular cavity of the early osteichthyan *Ligualepis*

(Basden and Young 2001; Clement et al. 2018) could be considered with the SO in mind.

Ultimobranchial body

The location and morphology of the ultimobranchial body (or organ) as demonstrated here in *Latimeria* is very similar to that described for chondrichthyans, and the structure and location of the gland closely resembles that of elasmobranchs in which the basibranchial copula has not occupied most of the space between the last hypobranchials, such as *Squalus* (Camp 1917) and *Torpedo* (van Bemmelen 1885), and as seen here in *Scyliorhinus*. Persistence of the UB on the left side only is characteristic of all groups of chondrichthyans and lungfish (Shinohara-Ohtani and Sasayama 1998). The location of the UB in *Latimeria* is as expected from its phylogenetic position: it originates as a small outpouching of the pharynx in relation to the afferent artery of the last branchial arch, and remains in that location after development, as is the case in chondrichthyans, non-teleost actinopterygians and lungfish. The location of the UB in a range of fish, including *Latimeria*, can be correlated with *hox* gene retentions and deletions that occur after the multiple whole-genome duplications that occur over the various fish lineages (author's observations, manuscript in preparation). No particular functional correlate of UB location can be deduced, and in fact the function of calcitonin itself is far from clear across the vertebrate spectrum (Hirsh and Baruch 2003).

M. cucullaris (M. protractor pectoralis)

M. cucullaris and its reported absence in *Latimeria* has given rise to comment in the literature, as it is an important component in the developmental discussion of the head-neck-trunk interfaces, where conflicting information about its mesoderm of origin has been discussed (Ericsson et al. 2013; Heude et al. 2018; Sefton et al. 2016), and the nature of its innervation has also been puzzling (Tada and Kuratani 2015). I am disagreeing here with the conclusion of Sefton et al. (2016) that the m. levator arcus branchialis 5 of Millot and Anthony (1958) is in fact the m. cucullaris, as a typical m. cucullaris is identified here, and the levator arcus branchialis 5 has quite a different fibre direction and attachments. The situation here is quite similar to that of *Lepisosteus* as described by Naumann et al. (2017): the m. cucullaris in *Latimeria* is relatively thin and developed only in its caudal half, thus escaping recognition. In *Lepisosteus*, Edgeworth (1935) had identified a branchial levator as m. cucullaris, in a similar way to the identification by Sefton et al. (2016) of m. cucullaris in *Latimeria*. M. cucullaris is identified here on histological, MRI and synchroton CT data; Sefton et al. (2016) used the same MRI data. It can be pointed out that these MRI series, made available by the Digital Fish Library of the University of California at San Diego, are made of a long-preserved adult specimen and there are many areas of signal loss; inspection of both sides of the specimen

in all of the axial, horizontal and sagittal projections are necessary to find structures that are known to be present, and finding 'new' structures is difficult.

The significance of m. cucullaris in *Latimeria* is probably mainly as an indicator of conserved developmental processes among gnathostomes; this thin, incomplete muscle is unlikely to be of any particular functional significance. In tetrapods m. cucullaris differentiates into or contributes to muscles which move the head and pectoral girdle independently of each other (Ericsson et al. 2013) and is part of the concept of a 'neck' — although 'neck' is not generally defined in morphological terms in the developmental literature. Separation of the pectoral girdle from the skull is one feature sometimes mentioned in this context, but this does not apply to chondrichthyans and coelacanths, where this connection is already lost. A more specific feature might be the loss of the gill chambers, in particular the loss of the need for the pectoral girdle to form the posterior wall of the gill chamber. Rostro-caudal movement of the pectoral girdle is an important part of ventilation and suction feeding in at least some chondrichthyans and actinopterygians (Camp et al. 2017), and this may be a plesiomorphic function of m. cucullaris which has been lost in *Latimeria* (pectoral girdle movement is still possible, powered by hypaxial muscles). An increased range of head movement on the axial column is another conceptual element in the function of a 'neck', although also less clearly defined. Mobility of the head on the axial column is present in fish in the absence of a neck, particularly dorso-ventral movements coupled with jaw and hyoid movements (Camp et al. 2014). Interestingly, *Latimeria* has other morphology that suggests adaptation to increasing head mobility: the most rostral part of the vertebral column is specialised with reduction in length of the neural spines dorsally and considerable reduction or absence of the vertebral elements ventral to the notochord in the first 5–8 segments (Andrews et al. 1977), suggesting increased freedom of movement of the notochord in this region. Regionalisation of the vertebral column has been demonstrated in some other fish, and in the little skate *Leucoraja erinacea* Criswell et al. (2021) have shown that morphological regions conform to *hox* expression boundaries, including a proximal region which conforms to what is described above for *Latimeria*; this may be the most specific definition of a neck region thus far established.

Also present in *Latimeria* are specialised, longitudinally oriented bundles of the hypaxial muscle connect the ventral aspect of the notochord in this region with the cranial base (the m. cervicis profundus of Dutel et al. 2015), suggesting a function in ventral flexion of the cranium on the axial column. These findings may point toward the evolution of the functions of a neck in *Latimeria*, but m. cucullaris is reduced and not a part of such a trend.

M. omohyoideus

The designation of the m. omohyoideus here as applied to lungfish and *Latimeria* is a new suggestion; in Diogo et al. (2016), homology was proposed with the slender bod-

ies of muscle connecting the epaxial trunk muscle with the pectoral girdle in chondrichthyans, the 'm. retractor ventralis lateralis pectoralis' of Shann (1919). Apart from being a cumbersome term, the various muscles of elasmobranch species grouped under that name are not anatomically consistent from species to species but all make contact with the epaxial muscle, not the hypaxial, as is the case in the muscle attaching to the tip of the cranial rib in lungfish. The situation in lungfish most resembles the m. omohyoideus in salamanders (Özeti and Wake 1969) (the m. pectori-scapularis of Francis [1934]), which passes ventrally from the anterior border of the pectoral girdle dorsally, medial to the gill chambers, where they are present, and inserts onto the superficial lamina of the m. rectus cervicis (also named as the m. sternohyoideus group). In *Latimeria* the muscle here designated m. omohyoideus lies in a similar plane to that of lungfish and salamanders, but does not make direct muscular contact with m. rectus cervicis; there may be a fibrous band linking the ventral end of this muscle with the medial end of the clavicle and the m. rectus cervicis, as in some tetrapods.

M. omohyoideus of tetrapods has the genetic signatures of a hypobranchial muscle (Heude et al. 2018), which is consistent with what is suggested here for sarcopterygians. More research could better define this anatomy, but we may be seeing here an evolutionary sequence *Latimeria*-lungfish-Lissamphibia-amniotes of an m. omohyoideus that originates in two parts, an oblique or vertically component arising from the pectoral girdle, and a horizontal component that is separated off from m. rectus cervicis.

Conclusions

A number of structures have been identified in *Latimeria* through focussed examination of traditional anatomical materials, and from exploration in the modern resources for 3D anatomy on a fine scale with tissue-enhanced microCT scanning, and synchrotron scans. These steps toward complete anatomical knowledge of the living coelacanth could help with the interpretation of structures in the fish-tetrapod transition, and in fossil taxa for which there is no direct extant model. A spatial association between the spiracular organ and the orbital artery in a variety of vertebrates is noted.

Acknowledgements

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