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## BRIEF COMMUNICATION

### The occipital region in the basal bony fish *Erpetoichthys calabaricus* (Actinopterygii: Cladistia)

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*Erpetoichthys calabaricus* has unusual cranio-vertebral anatomy, with an occipital centrum forming a component part of the compound basiexoccipital bone, and a 'free-floating' occipital neural arch that differs from accessory arches found in some teleosts. The occipital neural arch bears autapomorphic lateral projections that articulate with small rod-like bones resembling the spatial relationship of parapophyses and ribs, a feature normally restricted to vertebral centra. Based on analyses of cleared and stained specimens, computed tomography and histology, it is hypothesized that the lateral projections and associated rod-shaped bones are structures that share developmental homologies to the unique 'dorsal ribs' of Polypteridae. © 2008 The Authors

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Key words: horizontal septum; intermusculars; myosepta; *Polypterus*.

Polypteridae is the basalmost extant lineage of Actinopterygii and includes the ropefish *Erpetoichthys calabaricus* Smith, 1865 and bichirs, *Polypterus* Lacepède, 1803 (Patterson, 1982; Schultze & Cumbaa, 2001; Venkatesh *et al.*, 2001; Nelson, 2006). Anatomical studies of ropefish and bichirs can offer important insights for the systematics and morphology of living and fossil bony fishes. Anatomical details of many living polypterids remain elusive, though hypotheses of a generalized bauplan for the family exist based on studies of the cranial anatomy of several species of *Polypterus* (Allis, 1922; de Beer, 1935; Jollie, 1984; Britz & Johnson, 2003) and *E. calabaricus* (Claeson *et al.*, 2007). By integrating observations in this study from clearing and double staining (C&S), X-radiographic microfocus computed tomography (CT), histology and dissection, an unusual aspect of neural arch morphology in *E. calabaricus* is recognized. Analysis of this neural arch morphology contributes to evolutionary interpretations of

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the occipital region of the skull and anteriormost portion of the vertebral column in Polypteridae and, thus, in bony fishes.

Specimens of *E. calabaricus* were acquired from the pet trade and deposited in the Massachusetts Natural History Collections (MNHC) at the University of Massachusetts after analyses. For comparison, museum specimens of three species of *Polypterus* were examined. Seven specimens of *E. calabaricus* were dry skeletal preparations. Four specimens were cleared and double stained according to the methods outlined by Dingerkus & Uhler (1977). Two specimens were histological preparations and one C&S specimen was dissected after staining but was not CT scanned. The remaining C&S specimens were overstained using alizarin red, to allow for greater contrast when scanning (see Table I). Next, these three overstained specimens were wedged in a matrix of 0.8% agarose gel and enclosed in thin plastic tubing to prevent dehydration. Specimens were CT scanned on a SkyScan 1072 X-ray microtomograph using X-ray settings of 51 kv and 196  $\mu$ A. Random vertical movements of 40 pixels during scanning were implemented to ameliorate ring artefacts. Slice volumes consisted of 1024 axial slices, each of which was 0.01890 mm thick. Post-processing of data was accomplished using contrast thresholding in the polygon modelling programme 'ANT' by SkyScan (Aartselaar, Belgium). Slices of the posterior limit of the skull were processed selecting for grayscale values between 0 and 190 on

TABLE I. Specimens studied

Specimen number	Species	Preparation	Orbit/ headlength (mm)
ANSP 66676	<i>Polypterus senegalus</i>	Wet	4/34
ANSP 66549	<i>Polypterus endlicheri</i>	Dry	18/126
ANSP 24236	<i>Polypterus bichir</i>	Dry	14/105
MNHC F20000	<i>Erpetoichthys calabaricus</i>	PD, Dry	2/17
MNHC F20001	<i>E. calabaricus</i>	Macerated, P	—
MNHC F20002	<i>E. calabaricus</i>	WD, Dry, P	2.5/21
MNHC F20003	<i>E. calabaricus</i>	Macerated, P	2.0/18
MNHC F20004	<i>E. calabaricus</i>	C&S, CT, P	2.5/17
MNHC F20005	<i>E. calabaricus</i>	P, Macerated	2.5/16
MNHC F20006	<i>E. calabaricus</i>	C&S, CT, P	3/18
MNHC F20007	<i>E. calabaricus</i>	P, Formalin Preserved	2.5/16.5
MNHC F20008	<i>E. calabaricus</i>	Dried for CT, no prep	—
MNHC F20009	<i>E. calabaricus</i>	C&S, CT, P	3/19
MNHC F20010	<i>E. calabaricus</i>	C&S, dissected	3/18
MNHC F20028	<i>E. calabaricus</i>	Histo	—
MNHC F20029	<i>E. calabaricus</i>	Histo	—

ANSP, Academy of Natural Sciences, Philadelphia; C&S, cleared and double stained; CT, computed tomography; Dry, dry skeletal preparation; Histo, histological cross-sections and tissue staining; Macerated, completely disarticulated; MNHC, Massachusetts Natural History Collections, Amherst; P, photographed; PD, partial dissection of occipital region of specimen; WD, whole dissection of cranium; Wet, preserved in alcohol.

a scale of 0–255 with a locality of five and a tolerance of one. Primary scan data were published by Claeson *et al.* (2007).

The posterior bone of the polypterid braincase is a compound element known as a basiexoccipital (Fig. 1). The basiexoccipital forms by the fusion of the median basioccipital with the paired exoccipitals (Allis, 1922; Bemis & Forey, 2001). The basiexoccipital in *E. calabaricus* is weakly ossified dorsally and in one specimen the exoccipital portions are not fully fused dorsally. Ventrally, the occipital centrum is a component of the basiexoccipital and is closely attached to the posterior flanges of the parasphenoid (Bartsch & Gemballa, 1992). Although clearly centrum-like, and presenting on its ventral surface

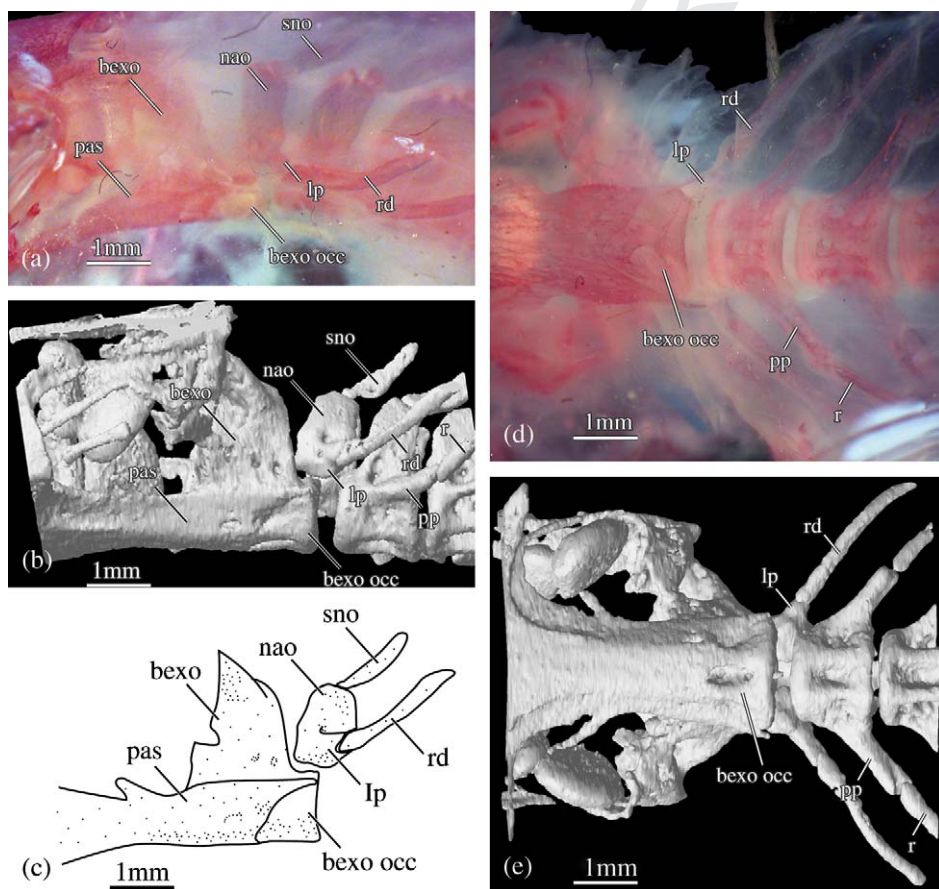


FIG. 1. Posterior limits of the braincase in *Erpetoichthys calabaricus*. (a) Ventrolateral view, specimen MNHC F20010, (b) volume rendering in ventrolateral view, specimen MNHC F20006, (c) line rendering of a portion of (b), (d) ventral view, specimen MNHC F20010 and (e) volume rendering in ventrolateral view, specimen MNHC F20006. bexo, basiexoccipital; bexo occ, occipital centrum of basiexoccipital; lp, lateral projection; nao, neural arch of the occipital; pas, parasphenoid; pp, parapophysis; r, rib; rd, rod-shaped bone; sno, neural spine of the occipital. Animation of a volume-rendered isosurface model of the posterior limit of the braincase of specimen MNHC F20006 is also available as supporting material at <<http://www.amherst.edu/~jwhagadorn/erpy>>.

Colour

a median pit identical to the pit on typical centra, the occipital centrum of the basiexoccipital lacks parapophyses and ribs (Fig. 1).

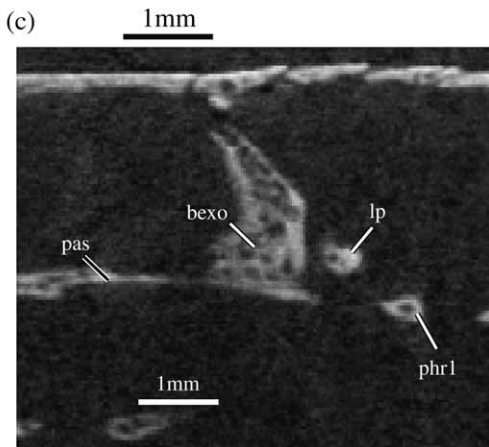
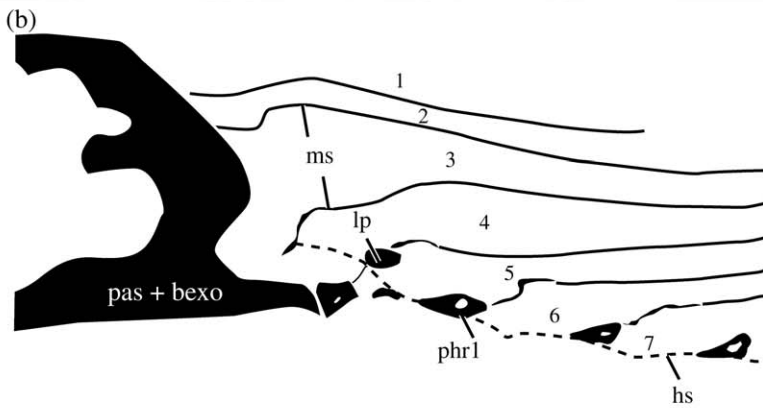
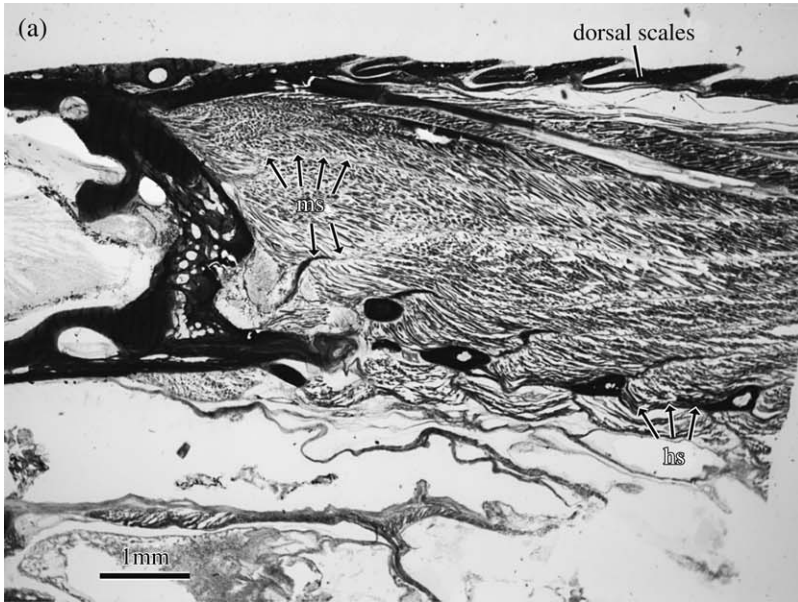
Posterodorsal to the occipital centrum of the basiexoccipital and anterodorsal to the first free vertebral centrum is the occipital neural arch. The dorsal tips of the occipital neural arch are weakly sutured together. Dorsal to the occipital neural arch is a supraneural spine [Fig. 1(a)–(c)]. In all specimens of *E. calabaricus* examined, the occipital neural arch is not fused to the basiexoccipital and is not associated with a typical centrum. Soft tissue (not cartilage) surrounds the occipital neural arch [Fig. 1(a)]. This tissue is not X-ray dense and thus appears as gaps in CT scans and volume renderings [Figs 1(b), 2(c)]. Therefore, the occipital neural arch is considered to be ‘free-floating’ osteologically. More unusual is that the occipital neural arch bears lateral projections that articulate with small rod-shaped bones. In CT scans and in C&S specimens, the projections and small bones look similar in morphology to the ribs associated with parapophyses of more posterior vertebral centra [Fig. 2(c)]. Cross-sectional CT slices and histological sections show that the rod-like process and the ‘dorsal’ ribs share the feature of a hollow shaft seen in *E. calabaricus*.

Parasagittal histological sections through the occipital region of *E. calabaricus* illustrate the relative position of muscle fibres to dorsal ribs and the lateral projections and rod-like bones in question (Fig. 2). Extending along the length of the body, adjacent myosepta are known to originate at the posterodorsal edge of the proximal heads of dorsal ribs [Fig. 2(a), (b)]. A horizontal septum also extends the length of the body and links each rib. Anterior to the proximal head of the first dorsal rib is the lateral process of the occipital neural arch (Fig. 2). Muscle fibres of segments 4 and 5 surround the lateral process of the occipital neural arch. The fibres meet each other at a myoseptum and flatten out at the horizontal septum ventrally. The lateral process lies dorsal to the horizontal septum.

Observations of the occipital neural arch in *E. calabaricus* are consistent with early written descriptions by Allis (1922) of an unnamed similar element in *Polypterus bichir* Lacepède, 1803 and with the ‘proatlas’ (de Beer, 1935) in *Polypterus senegalus* Cuvier, 1829. The occipital neural arch found in *Polypterus*, although once thought to be an accessory neural arch-like that found in teleosts, is not homologous to the bone found in those derived actinopterygians (e.g. clupeomorphs, elopomorphs and salmonoids; Fink & Fink, 1996; de Pinna & Grande, 2003). Instead, the arch in *Polypterus* is probably a remnant following the ontogenetic fusion of centra to the basiexoccipital, as demonstrated by Jollie (1984) using *P. senegalus*.

Previous authors addressing the ‘accessory neural arch’ or the ‘free-floating’ first neural arch of actinopterygians (Allis, 1922; Fink & Fink, 1996; Grande &

FIG. 2. Cross-section through posterior limits of the braincase in *Erpetoichthys calabaricus*. (a) Histological section, specimen MNHC F20028, (b) line rendering of (a) and (c) computed tomography slice of approximately same region in (a), specimen MNHC F20006. 1–7, muscle segments; bexo, basiexoccipital; hs, horizontal septa (---); lp, lateral projection; ms, myosepta; pas, parasphenoid; phr1, proximal head of first ‘dorsal’ rib.



Bemis, 1998) did not mention the association of any lateral projections. Instead, it was demonstrated that parapophyses and ribs are never found on a neural arch and are normally restricted to vertebral centra in other actinopterygians. For example, the first rib occurs on a free centrum in *Lepisosteus* (centrum 1), *Amia* (centrum 4) and teleosts (centrum 3; Grande & Bemis, 1998; Bemis & Forey, 2001). Additionally, Gemballa & Roeder (2004) argued for the presence of a dorsal rib on the occipital centrum in *Polypterus*, not the neural arch. Thus, the presence of the projections and rod-shaped bones on the occipital neural arch in *E. calabaricus* is apomorphic.

Parasagittal histological sections of the occipital region of *E. calabaricus* used in this study confirmed that dorsal ribs are present in the intersection of the horizontal septum and myosepta. The lateral projection of the occipital neural arch and the association of the rod-shaped bone, however, are found slightly dorsal to that same intersection [Fig. 2(b)]. Considered together, these observations suggest that the lateral projections and rod-shaped bones found are unique to *E. calabaricus*; however, they could represent a displaced parapophysis and 'dorsal' rib, respectively.

Even if the projections and rod-shaped bones are not parapophyses and ribs, respectively, they would not be homologous to intermusculars for several reasons. Parsimony argues against a homology because intermusculars were reported to occur only in teleosts among recent vertebrates, not basal actinopterygians including *Polypterus* (Patterson & Johnson, 1995; Gemballa & Roeder, 2004). Furthermore, previous examination of the development of ribs of polypterids as well as other actinopterygians and chondrichthyans indicated that the traditionally recognized ribs of polypterids differ structurally and developmentally from other gnathostome ribs (Britz & Bartsch, 2003). Although Britz & Bartsch (2003) did not discuss the occipital region, the rod-like bones observed in this region of *E. calabaricus* share the same hollow structure as the 'dorsal' ribs and were probably derived from cartilaginous precursors. If they were homologous to intermusculars, the rod-like bones would be membrane bones instead (Patterson & Johnson, 1995; Gemballa & Britz, 1998).

Previous hypotheses that at least one vertebral centrum was incorporated into the occiput of polypterids were poorly documented (Budgett, 1902; de Beer, 1935). Illustrations by de Beer (1935) of the early development of occipital region of *Polypterus* were misleading because they did not show the first neural arch as 'free-floating' or more specifically as unossified to a vertebral centrum. Present observations from C&S anatomy, histology and digital modelling reveal the presence of a 'free-floating' occipital neural arch in *E. calabaricus* and are consistent with the hypothesis that at least one vertebral centrum is incorporated into the occiput of polypterids. Taking into account the incorporation of the first free centrum into the occiput (Jollie, 1984), it is hypothesized here that the lateral projections and associated rod-shaped bones are new structures that share some developmental homologies to the unique 'ribs' of Polypteridae (Britz & Bartsch, 2003). The possibility that they are homologous with intermusculars, small bones within the myosepta, is not supported by this study. Future examination of the developmental pathway of the lateral projections in *E. calabaricus* could provide an independent test of this hypothesis.

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