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Descriptive Osteology of the Appendicular Skeleton of *Parambassis ranga* from Northern Western Ghats, India

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Abstract: Descriptive osteological study of appendicular skeleton was performed on *Parambassis ranga* collected from Northern Western Ghats. Osteology of dorsal, anal, caudal, pelvic and pectoral fins was studied. Cleared and stained specimens were observed for osteological structures and detailed descriptions of these structures have been documented.

Keywords: Ambassidae, Osteology, Fins, Appendicular skeleton, Western Ghats, *Parambassis ranga*

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Introduction

Glassfishes of the family Ambassidae are small to medium sized semitransparent fishes inhabiting marine, estuarine and freshwater of Indo Pacific region (Roberts, 1994; Nelson, 2016). Ambassidae is the only secondary freshwater family of percomorph fishes in tropical Asia in which freshwater forms are morphologically and biologically more diverse than marine ones.

In the Indian subcontinent these fishes are represented by *Ambassis*, *Chanda* and *Parambassis*. *Chanda* and *Parambassis* are freshwater inhabitants. Of these *Parambassis ranga* was described by Hamilton, 1922 as *Chanda ranga*. It was synonimised as *Parambassis ranga* by Kottelat (1989). These are moderately deep bodied fishes with 6-8 gill rakers, about 56-70 scales in lateral series, anal fin sheath with upto 3 scale rows. Dorsal soft fin rays are 14-16, anal soft fin rays are 14-17, pectoral fin rays are 12-13. They show presence of humeral spot. Vertebrae are around 25.

Body is elongated, compressed and abdomen

Fig. 1: Whole mount of *Parambassis ranga* after preservation (SL = 53.6 mm).

is rounded. Head is short and compressed. Snout is pointed. Mouth is large, eyes are large superior and are not visible from below ventral surface. Jaws are straight or slightly upturned. Lips are thin. Supraorbital Ridge is smooth or serrated with one or two spines posteriorly. Pre-orbit is serrated on both ridges. Suborbital is also serrated. Interoperculum is smooth. Tongue is without teeth. There are two dorsal fins with six or seven spines and 11 to 14 branched rays. Dorsal fins are closely placed with a notch in between. Anal fin is with three spines and with 11 to 16 branched rays. Lateral line is complete with 30 to 100 scales. (Jayaram, 2017).

The studies on *Parambassis ranga* are mostly focused on taxonomy of fishes. Verma *et al*. (2018) have carried out a molecular phylogenetic analysis of ambassid fishes, focusing on the species found in the freshwater, estuarine, and marine waters of the Indian subcontinent using a combined dataset of mitochondrial and nuclear markers. They used three genes- mitochondrial (mt) 16S rRNA gene (16S), the mt cytochrome c oxidase subunit 1 gene (cox1), and the nuclear rhodopsin gene (rho). They have also used hyopalatine arch of cleared and stained specimens for descriptive osteology.

The primary objective of this work is to study osteology of appendicular skeleton of *Parambassis ranga*. The study aims to describe the morphology and osteology of dorsal, anal, caudal, pelvic and pectoral fins. The work also aims to develop a database of additional osteological characters for these structures to the existing systematics of Ambassidae.

Materials and Methods

Collection of specimens

Specimens of *Parambassis ranga* were collected from the Indrayani river at Talegoan district Pune, Maharashtra, India (Fig. 1). Specimens were collected from local fishermen from the market and preserved in 4% buffer formalin. Later these specimens were processed for clearing and staining (Fig. 2).

Clearing and staining of fish specimens

Clearing and staining of fish specimens was done as per protocol by Taylor and Van Dyke (1985) with some modifications (Fig. 2). These clear specimens were stored in 60% glycerine and 40% KOH and later on in 100% glycerol with thymol crystals for final preservation.

Cleared and stained specimens were observed under Olympus SZX stereo microscope, photography was done, and illustrations or drawings were made for further studies. Elements of Pectoral girdle, Pelvic girdle, Dorsal fin, Anal fin and Caudal fin were studied for variations.

Fig. 2: Whole mount of *Parambassis ranga* after double staining and clearing (SL = 53.6 mm).

Illustrations were made using Adobe Photoshop software. Osteological nomenclature follows Fujita (1989); Conway (2011); Schultze and Arratia (2013) and Thieme *et al*. (2022).

Results and Discussion

Osteology of Anal fin

There are 18 anal-fin rays (iii, 15) supported by well ossified pterygiophores inserting between the hemal spines of V12–22. All pterygiophores comprise proximal-middle, middle and distal radials. First two anterior most proximal-middle radials are fused with each other. The first proximal-middle radial is the largest of the series. First three pterygiophores bears three spiny anal-fin rays, the two anterior most being shorter then rest. The remaining 13 pterygiophores each support a single branched anal-fin ray except for the posterior most, which supports two branched rays. There is a well-developed flange of membrane bone along the anterior edge of the first proximal-middle radial (Fig. 3). Smaller flanges of membrane bone are present on both the anterior and posterior margins of the proximal-middle or proximal radials of subsequent pterygiophores until the eleventh. All middle radials are well ossified endochondrally. Proximal radials are present on branched rays only. The last middle radial exhibits a small flange of bone, termed the end piece or anal-fin stay (Weitzman, 1962), on its dorsal edge. Distal radials are ossified. Three anterior most branched anal-fin rays are the longest, with rays gradually becoming shorter caudally.

Osteology of Dorsal fin

There are 22 dorsal-fin rays (viii, 14) supported by well ossified pterygiophores inserted between the neural spines of V5–22 (Fig. 4). First eight pterygiophores comprise a large proximal-middle radial and a small, rod like distal radial. The remaining pterygiophores are composed of proximal, middle and distal radials. First eight proximal-middle radials each support a spiny ray of which the second pterygiophore supports the longest spiny ray and sixth supports the smallest spiny ray. The last pterygiophore supports two branched rays. Remaining 9-19 pterygiophores support each a single branched ray, which embrace the distal radials distally and rest on the anterodorsal corner of the succeeding proximal-middle (the last unbranched ray only) or middle radials.

Each proximal-middle or proximal radial exhibits a flange of membrane bone on its anterior and posterior edge. Membrane bone flanges are best developed on first proximal-middle radial and subsequent eight proximal radials. All middle

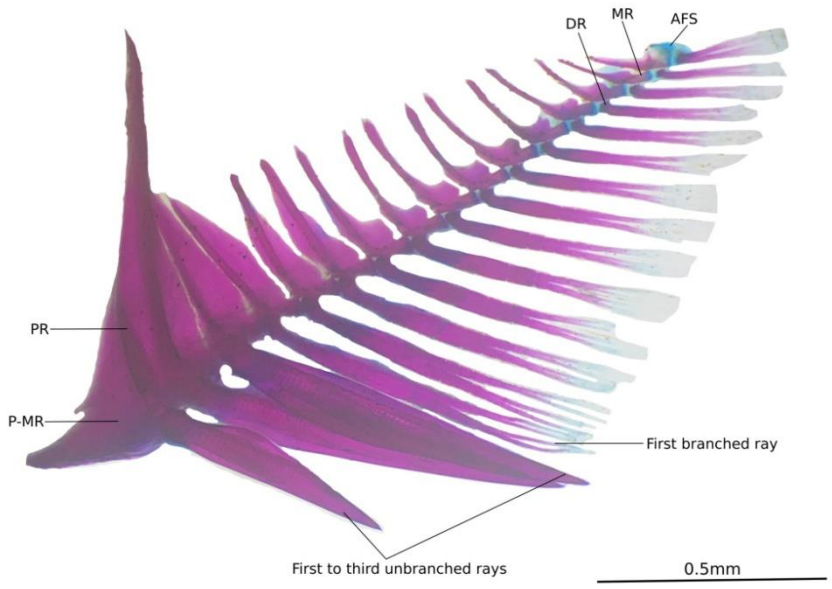

Fig. 3: Anal pterygiophores of *P. ranga* (SL = 53.6 mm), left side in lateral view. Anterior to the left. Abbreviations: AFS, anal fin stay; DR, distal radial; MR, middle radial; P–MR, proximal–middle radial; PR, proximal radial. Scale corresponds to 0.5 mm.

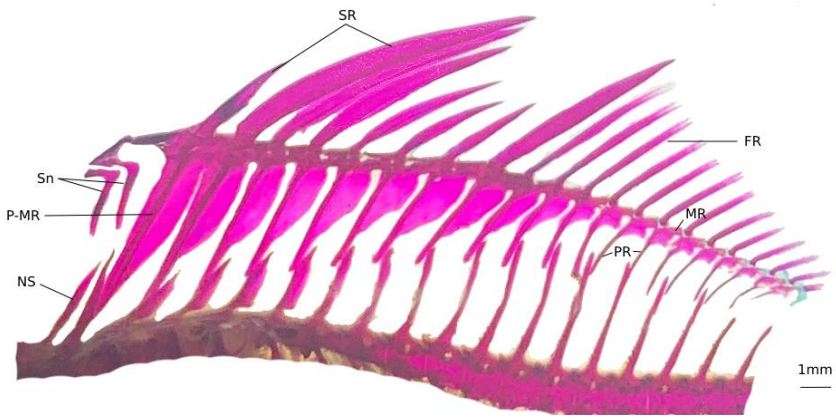

Fig. 4: Dorsal pterygiophores of *P. ranga* (SL = 53.6 mm), left side in lateral view. Anterior to the left. Abbreviations: DFS, dorsal fin stay; DR, distal radial; MR, middle radial; P–MR, proximal–middle radial; PR, proximal radial. Scale corresponds to 1 mm.

radials are well ossified endochondrally. The last middle radial exhibits a small flange of bone, termed the end piece or dorsal-fin stay (Weitzman, 1962), on its posteroventral edge. Branched anal-fin rays gradually become shorter caudally.

Osteology of Pelvic girdle

The pelvic girdle consists of a pair of triangular basipterygia. Each basipterygium is large, thick and well ossified, except for the anteriormost tip, which remains cartilaginous. Posteriorly, the base of each basipterygium supports a large pelvic splint, a single spine and 6 soft fin rays, and exhibits a long ischiac process on the posteromedial edge. First fin ray is osseous and spiny while the remaining ones are branched fin rays. A long subpelvic process is present on the posteromedial portion of the each basipterygium (Fig. 5).

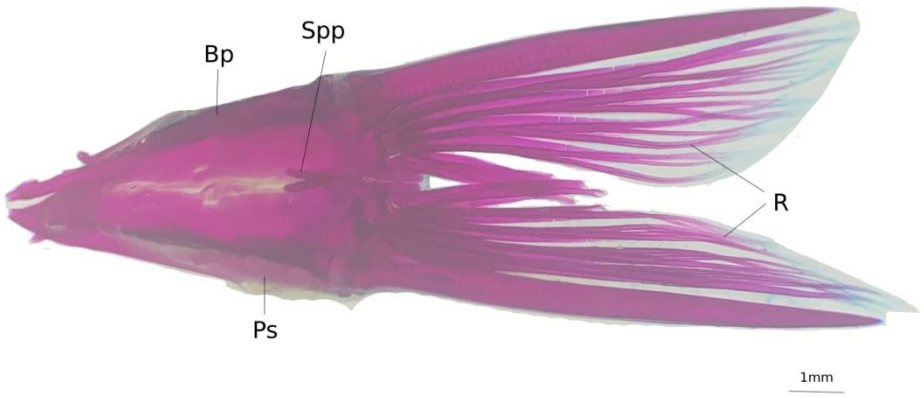

Fig. 5: Pelvic fin skeleton of P. ranga(SL = 53.6 mm) in ventral view. Anterior to left. Abbreviations: Bp, basipterygium; Is, ischiac process; Ps, pelvic splint; R, pelvic fin ray; Spp, sub pelvic process . Scale corresponds to 1 mm.

Osteology of Pectoral girdle

The pectoral girdle is a strong, broad bony structure consisting of the posttemporal, supracleithrum, cleithrum, scapula, coracoid, postcleithra and pectoral radials. The post-temporal is a plate like bone with dorsal and ventral limbs that articulate with the cranium anteriorly. The dorsal limb is elongate while the ventral limb is short. A short spine is present on the postero ventral portion of the posttemporal. The posttemporal is connected to the supra-cleithrum posteromedially and has a sensory canal that is continuous with that of the supracleithrum posteriorly. The supracleithrum is a plate-like bone, that is connected with the posttemporal anterolaterally and the dorsal limb of the cleithrum ventromedially. A short spine is present on the postero medial portion of the supracleithrum.

The cleithrum is a long, slightly curved bone with the dorsal and ventral limbs. The cleithral spine arises from the dorsal tip of the dorsal limb. It is long and covered by supracleithrum. The ventral limb is slightly longer compared to the dorsal limb. The cleithrum is connected to the supracleithrum dorsolaterally, the scapula posterolaterally and the coracoid postero-ventrally.

The postcleithrum is a single elongate element that attaches with the mesial surface of the cleithrum. It is pointed at both dorsal and ventral tips. The scapula is a rectangular bone, containing a large foramen anteriorly that is sutured with the cleithrum anterolaterally and separated from the coracoid by a narrow cartilaginous band ventrally. The scapula is connected with four pectoral radials posteriorly and supports two upper pectoral fin rays posterodorsally.

The coracoid is a 'L' shaped bone with a short and broad dorsal arm and a long and triangular ventral arm that attaches to the cleithrum anteroventrally. It is slightly connected to the lateral base of the lowest pectoral radial. There are four pectoral radials which are short and slightly broad arranged in decreasing order of size. Their one end is connected to the postero lateral surface of the scapula while the other ends are articulated with pelvic fin rays. Their bases towards attachment with pectoral fins rays are capped by cartilages (Fig. 6).

Osteology of Caudal skeleton

The terminology of the skeletal elements of the caudal fin generally follows Schultze and Arratia (2013) and Fujita (1989) and Thieme *et al.* (2022). The caudal fin skeleton of fishes under study are depicted in Figure 7. The caudal skeleton comprises a series of fan-like elements spreading out from the posteriormost centrum that includes the hypurals, parhypural, uroneural, epurals and urostyle, plus the second and third preuralcentra

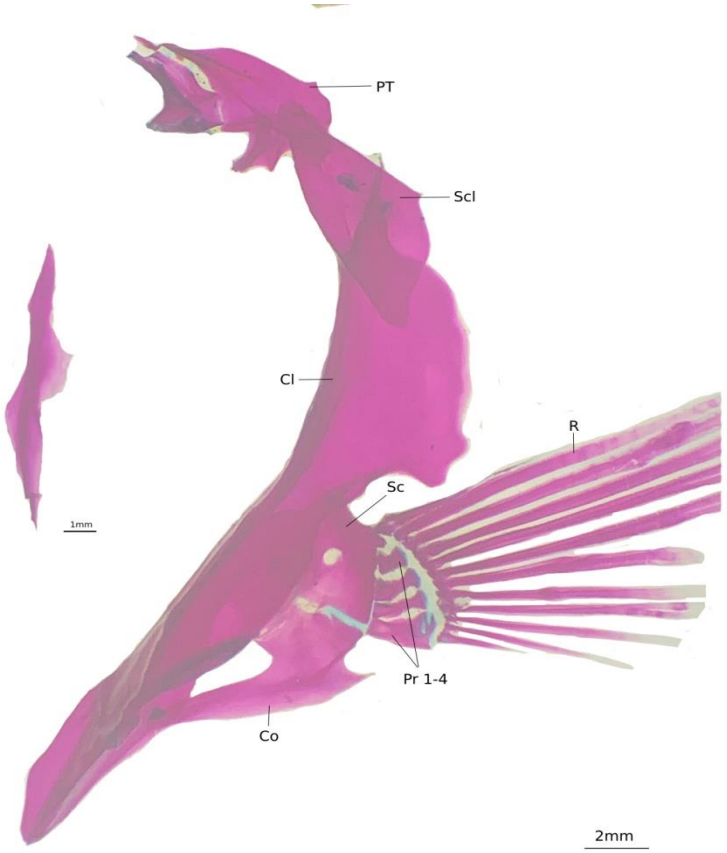

Fig. 6: Pectoral fin of *P. ranga*(SL = 53.6 mm), left side, in lateral view. Inset- Postcleithrum Anterior to the right. Abbreviations: Cl, cleithrum; Co, coracoid; Pcl, postcleithrum; Pr 1–4, pectoral radial 1–4; Sc, scapula; Scl, supracleithrum. Scale corresponds to 1 mm.

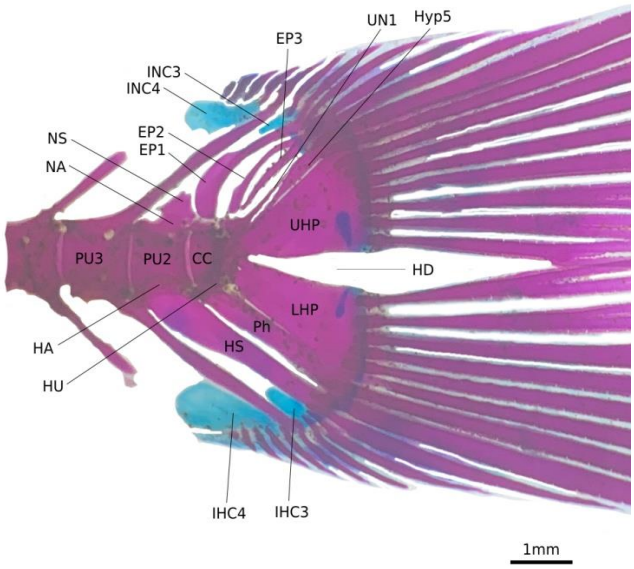

Fig. 7: Caudal fin skeleton of P. ranga (SL = 53.6 mm), left side in lateral view. Anterior to the left. Abbreviations: CC, compound centrum; HD, hypural diastema; Ep, epural; IHC, inter-haemal spine cartilage; HA, haemal arch; HS, haemal spine; HU, hypurapophysis; Hyp, hypural; LHP, lower hypural plate; NA, neural arch; NS, neural spine; PH, parhypural; PU, preural centrum; UHP, upper hypural plate; UN, uroneural. Scale corresponds to 1 mm.

and compound centrum. The posterior margin of the caudal skeleton supports the caudal rays and radial cartilages.

There are 9+7 principal rays and 10 dorsal and 9 ventral procurrent rays. Caudal fin rays are supported by the neural and haemal spines of the second and third preural caudal centra, the pleurostyle, three epurals, hypural elements, and the parhypural. The neural arch of the second preuralcentra bears a large neural spine on the posterior edge and the haemal arch of the second preuralcentra bears a large haemal spine forming laminar flanges of membrane bone along its edge.

The haemal arch of the second preural centrum, which exhibits an enlarged base, is autogenous from the centrum. Haemal spines of the second and third preuralcentra provide support for ventral procurrent rays. The compound centrum bears a short neural process that is fused to the centrum.

The posterior-most tips of the parhypural and first hypural, are fused and are autogenous from compound centrum.

The hypurals are situated on the posterior part of the compound centrum and consist of three plate-like bones: an autogenous fifth hypural, and the upper and lower hypural plates. The fifth hypural, a small and slender element, is attached to the upper hypural plate and uroneural. The upper hypural plate, formed by a fusion of the third and fourth hypurals, is attached to the uroneural and hypural 5 and is articulated with compound centrum. The lower hypural plate is formed by a fusion of the first and second hypurals and is attached to the parhypural. It is fused with compound centrum. The parhypural is plate-like and situated below the lower hypural plate. The bone has slightly curved hypurapophysis with blunt tip on its lateral surface. The parhypural is attached to the lower hypural plate posteriorly. The uroneural is a single rod-like bone (= first uroneural) lying above the fifth hypural. Three epurals are present. Epidural 1 is curved showing a flange of bone on its anterior edge. Epidural 2 is also slightly curved while epidural 3 is rod like. Epidural 2 and 3 are attached to each other towards their posteriormost tips. The hypural diastema is anteriorly restricted.

Radial cartilages are present on the distal tips of the neural spine of the third preural centrum, the epurals and the hemal spines of the second and third preuralcentra.

Only one study has provided information about the osteology of *P. ranga*. The study by Verma *et al*. (2018) describes osteology of hyoplalatine arch in Ambassis species found in *Chanda nama*, *P. ranga*, *P. lala*, *P. dayi*, P. *thomassi* and *Ambassis ambassis* from Indian subcontinent. They have also observed variation in serrations on interopercle and palatine and dentary regions of these species.

The present study is the first attempt to observe the osteology of fin elements in a member of family Ambassidae.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Thakur College of Science and Commerce, Kandiwali, Mumbai 400101, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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