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Comparative Osteology of the Anal Fin Skeleton of Cyprinid Fishes from Northern Western Ghats

Raut Rupesh N.^{1*} and Kharat Sanjay S.²

¹Department of Zoology, Elphinstone College, Dr. Homi Bhabha State University, Mumbai 400032, India

²Modern College of Arts, Science and Commerce, Ganeshkhind, Pune 411053, Maharashtra, India

*Corresponding Author

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Abstract: The family Cyprinidae is the most diverse family of freshwater fishes in the world, with at least 3006 species. Osteological data is of importance in developing a more comprehensive understanding of the evolution within any taxon. The present study is the first endeavor to study osteological relationships within members of Sub-family- Labeoninae- tribe Garrini (*Garra mullya*), Sub-family Smiliogastrinae (*Puntius mahecola*, *Puntius sahyadriensis*, *Rohteeo gilbii* and *Systomus sarana*), Family Danionidae (*Barilius barna*, *Barilius bendelisis* and *Salmophasia balookee*), Family- Danionidae Sub-family Danioninae (*Devariao aequipinatus*) and Family Danionidae-Sub-family Rasborinae (*Rasbora daniconius* and *Rasbora labiosa*). Members of the above mentioned species were collected from local market or wild from Lonavala, Karjat, Patan, Pune, Bhor, and Malvan and were preserved in 4% buffered formalin or absolute alcohol. These preserved specimens were identified and were used for clearing and staining as per protocol by Taylor and Van Dyke (1985) with some modifications. These 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 anal fin were studied for variations.

Keywords: Cyprinidae, Osteology, Anal fin

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Introduction

The family Cyprinidae is the most diverse family of freshwater fishes in the world, with at least 3006 species (Nelson *et al.*, 2016). Recent evaluations of the phylogeny and classification of Cyprinidae have molecular phylogenetic analyses, sometimes combined with osteological data, to investigate the evolution of this complex family. Different molecular markers, such as RAPDs or sequences from mitochondrial genes (cytochrome b, 12S and 16S rRNA, control region) were used to estimate

relationships within the Cyprinidae. Molecular exemplars of resolving relationships of cyprinidae include studies by Briolay *et al.* (1998), Zardoya and Doadrio (1998), Gilles *et al.* (1998, 2001), Xiao *et al.* (2001), Cunha *et al.* (2002), Simons *et al.* (2003), He *et al.* (2004) and Wang *et al.* (2012).

The higher-level relationships of Cyprini-formes have been incompletely resolved by earlier morphological studies as they were based on study of too few species and study of too few

osteological characters. Howes (1981) attempted to resolve the phylogenetic position of Chinese major carps, *Ctenopharyngodon* and *Hypophthalmichthys* based on 22 morphological characters. Sawada (1982) has attempted to study cladistic relationships and zoogeography in members of Cobitoidea based on osteological features. Mabee *et al.* (2011) have studied the phylogenetic relationships between 53 cypriniform species based on 62 morphological characters. Conway and Mayden (2007) have attempted to resolve the position of *Psilorhynchus sucatio* and *P. balitora* based on gill arch osteology. Nelson (1969) has studied skeleton of gill arches in some tropical freshwater fishes. Katwate *et al.* (2020) in their study based on integrative taxonomic analysis using osteological study and cox1 and cyt b gene analysis have proposed a new genus *Waikhomia*.

Saxena and Chandy (1966) have studied osteology of pelvic girdle and fins in various genera of hill-stream cyprinid fishes. Mehta and Tandon (1984) have studied the osteology of Weberian apparatus, pelvic and pectoral girdles and caudal skeleton in Indian cyprinid fishes with their application in systematics. Shantakumar and Vishwanath (2006) have studied interrelationships within genus *Puntius* species from Manipur region of India. Vishwanath and Shantakumar (2007) have studied osteology of genus *Osteobrama* from Northeast India. Shangningam and Vishwanath (2012) have observed vertebral counts in *Garra namyaensis* from Manipur region. Pethiyagoda *et al.* (2012) have studied osteology of *Puntius* and *Systemus*. Katwate *et al.* (2013) have studied osteology of *Pethia setnai*. Raghavan *et al.* (2013) have studied osteology of new genus *Sahyadria*. Katwate *et al.* (2014) have studied osteology of *Pethia longicauda*. Katwate *et al.* (2016)) have observed osteology of a new species *Pethia sanjaymoluri*. Yadav *et al.* (2018) have studied osteology of caudal skeleton of some cyprinid fishes from Northeast India. Arunkumar *et al.* (2018) have studied the osteological characters of *Puntius*

species from 6 different river systems of southern Western Ghats.

Osteological data could be of importance in developing a more comprehensive understanding of the evolution within any taxon. The present study is the first endeavor to study osteology of anal fins within members of Sub-family- Labeoninae- tribe Garrini (*Garra mullya*), Sub-family- Smiliogastrinae (*Puntius mahecola*, *Puntius sahyadriensis*, *Rohteeo gilbii* and *Systemus sarana*), Family- Danionidae (*Barilius barna*, *Barilius bendelisis* and *Salmophasia balookee*), Family- Danionidae Sub-family- Danioninae (*Devariao aequipinatus*) and Family- Danionidae- Sub-family-Rasborinae (*Rasbora daniconius* and *Rasbora labiosa*).

Materials and Methods

Collection of fish specimen:

Members of Sub-family Labeoninae; tribe Garrini (*Garramullya*), Sub-family Smiliogastrinae (*Puntius mahecola*, *Puntius sahyadriensis*, *Rohteeo gilbii* and *Systemus sarana*), Family Danionidae (*Barilius barna*, *Barilius bendelisis* and *Salmophasia balookee*), Family Danionidae; Sub-family Danioninae (*Devariao aequipinatus*) and Family Danionidae; Sub-family Rasborinae (*Rasbora daniconius* and *Rasbora labiosa*) were collected from local market or wild from Lonavala, Karjat, Patan, Pune, Bhor, and Malvan and were preserved in 4% buffered formalin or absolute alcohol. These preserved specimens were identified and were used for clearing and staining.

Clearing and staining of fish specimens:

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

These cleared and stained specimens were observed under Olympus SZX stereo microscope, photography was done and illustrations or

Fig. 1: Anal pterygiophores of Sub-family Labeoninae; tribe Garrini (A) *Garra mullya* and Sub-family Smiliogastrinae (B) *Puntius mahecola*; (C) *Puntius sahyadriensis*; (D) *Rohteeo gilbii*; (E) *Systomus sarana*, left side in lateral view. Anterior to left. Abbreviations: AFS, anal fin stay; DR, distal radial; MR, middle radial; P-MR, proximal-middle radial; PR, proximal radial. Scale corresponds to 1mm.

drawings were made for further studies. Elements of Anal fin were studied for variations. Illustrations were made using Adobe Photoshop CC. Osteological nomenclature follows Conway (2011).

Results and Discussion

Anal fin skeleton of cyprinid fishes under study is illustrated in Figures 1 and 2. There are variable number of anal fin rays supported by series of anal fin pterygiophores which are inserted between hemal spines of vertebrae. The anal fin

pterygiophore comprises of large proximal – middle radial and a small distal radius. Remaining pterygiophores are composed of proximal, middle and distal radius. First pterygiophore supports first principal ray and smaller procurrent rays (unbranched). Last pterygiophore supports two branched rays. Remaining pterygiophore supports a single branched ray and distally bear the distal radials. Second anal pterygiophore supports unbranched ray. Pterygiophores gradually decrease in size posteriorly. The proximal middle radial exhibits a flange of membrane bone on its

Fig. 2: Anal pterygiophores of Family- Danionidae, Sub-family –Danioninae (A) *Barilius barna*; (B) *Barilius bendelisis*; (C) *Salmophasia balookei*; (D) *Devario aequipinatus* and Sub-family-Rasborinae; (E) *Amblypharyngodon mola*; (F) *Rasbora daniconius*; (G) *Rasbora labiosa*, left side in lateral view. Anterior to left. Abbreviations: AFS, anal fin stay; DR, distal radial; MR, middle radial; P-MR, proximal-middle radial; PR, proximal radial. Scale corresponds to 1mm.

anterior and posterior edge. These flanges are best developed on first proximal middle radial. All middle radials and distal radials are well ossified. Anal fin ray articulates with posteroventral edge of middle radial. Anal fin exhibits a large and horizontal flange of bone on its posteroventral surface. It is variable in shape and length among studied species.

There are few differences between anal fin skeleton of cyprinid fishes. The difference exists in terms of meristic counts and presence of bony flanges on proximal-middle radial and proximal radial. In *G. mullya* and *S. nukta*, each proximal-middle radial and proximal radial exhibit bony flanges on their anterior and posterior edges. In *P. mahecola*, *R. gilbii* and *S. sarana* bony flanges are present on all proximal-middle radial and proximal radials. In *P. sahyadriensis*, however, these bony flanges are more prominent in proximal-middle radial and anterior proximal radials.

In *B. barna* and *S. balookee*, bony flanges are more prominent in proximal-middle radial and anterior proximal radials. In *B. bendelisis* each proximal-middle radial and proximal radial exhibit bony flanges on their anterior and posterior edges.

In *D. aequipinatus*, bony flanges are more prominent in proximal-middle radial and anterior proximal radials. In *A. mola*, *R. daniconius* and *R. labiosa*, each proximal-middle radial and proximal radial exhibit bony flanges on their anterior and posterior edges. In *S. balookee* the first anal fin pterygiophore is present anterior to first hemal spine.

The phylogenetic analysis based on morphological, molecular and mixed data could certainly help to resolve relationships between genera of cyprinid fish. This is the first attempt to study osteological characters of these species. The identification of characters important for evolutionary hypothesis can be achieved by detailed osteological study within species of a particular genus as well as species of different

genera. Further investigation for such characters will certainly help us to resolve taxonomic as well as evolutionary uncertainties within this group and will need further efforts in this direction.

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