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Comparative Anatomy of the Caudal Skeleton of Cyprinid Fishes

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Abstract: With at least 3006 species, the family Cyprinidae is the most varied group of freshwater fishes in the entire globe. The development of a more thorough understanding of the evolution within any taxon can be assisted using osteological data. The current study is the first attempt to investigate the osteological relationships among members of the Subfamily- Labeoninae- tribe Garrini (*Garra mullya*), Subfamily Smiliogastrinae (*Puntius mahecola*, *Puntius sahyadriensis*, *Rohtee ogilbii* and *Systomus sarana*), Family Danionidae (*Barilius barna*, *Barilius bendelisis* and *Salmophasia balookee*), Family- Danionidae Subfamily Danioninae (*Devariao aequipinatus*) and Family Danionidae- Subfamily Rasborinae (*Rasbora daniconius* and *Rasbora labiosa*). Members of the aforementioned species were procured from local markets or collected from the wild from Lonavala, Karjat, Patan, Pune, Bhor, and Malvan, and they were then preserved in either absolute alcohol or 4% buffered formalin. These preserved specimens were identified and used for cleaning and staining with slight modifications to the Taylor and Van Dyke (1985) methodology. Under an Olympus SZX Stereo microscope, these cleaned and stained specimens were examined. Photos were taken, and pictures or drawings were created for further research. Variations in the caudal fin's components were studied.

Keywords: Cyprinidae, Osteology, Caudal fin, Labeoninae, Smiliogastrinae, Rasborinae, Danionidae

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Introduction

With 3006 species the family Cyprinidae is the most diverse family of freshwater fishes in the world (Nelson *et al*., 2016). Molecular phylogenetic analyses, sometimes combined with osteological data have been used 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) have been used to estimate relationships within the Cyprinidae. Molecular studies to investigate relationships of Cyprinidae include studies by Cunha *et al*. (2002), He *et al*. (2004), Xiao *et al.* (2001), Briolay *et al*. (1998), Gilles *et al*. (1998, 2001), Simons *et al.*

(2003), Wang *et al.* (2012) and Zardoya and Doadrio (1998).

The higher-level relationships of Cypriniformes 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) based on 22 morphological characters, attempted to resolve the phylogenetic position of Chinese major carps, *Ctenopharyngodon* and *Hypophthalmichthyes*. Sawada (1982) based on osteological features has attempted to study cladistic relationships and zoogeography in members of Cobitoidea. Mabee *et al.* (2011) based on 62 morphological characters have studied the phylogenetic relationships between 53 cypriniform species. 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. A new genus *Waikhomia* has been proposed by Katwate *et al*. (2020) in their study based on integrative taxonomic analysis using osteological study and cox1 and cyt b gene analysis.

Osteology of pelvic girdle and fins in various genera of hill stream cyprinid fishes has been studied by Saxena and Chandy (1966). 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* species 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 *Systomus*. 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 great assistance in understanding of the evolution within any taxon. The present study is the first attempt to document osteological features of caudal fins within the aforementioned members of Cyprinidae in this regard.

Materials and Methods

Collection of fish specimen:

Members of Subfamily Labeoninae; tribe Garrini (*Garra mullya*), Subfamily Smiliogastrinae (*Puntius mahecola*, *Puntius sahyadriensis*, *Rohtee ogilbii* and *Systomus sarana*), Family Danionidae (*Barilius barna*, *Barilius bendelisis* and *Salmophasia balookee*), Family Danionidae; Subfamily Danioninae (*Devariao aequipinatus*), Family Danionidae and Subfamily 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 the 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 was done 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 caudal fin were studied for variations. Illustrations were made using Adobe Photoshop CC. Osteological nomenclature follows Conway (2011).

Results

The caudal fin skeleton of fishes under study are depicted in Figures 1 to 3. There are principal rays, dorsal and ventral procurrent rays. Caudal fin rays are supported by the neural and haemal spines of the second and third preural caudal centra, the pleurostyle, a single epural, hypural elements, and the parhypural. The neural and haemal arches of the second and third preural centra bear large neural and haemal spines that form laminar flanges of membrane bone along their anterior edges. 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 preural centra have expanded tips which provide support for ventral procurrent rays. The tip of the neural spine of the second preural centrum is also expanded and provides support for all dorsal procurrent rays. The compound centrum bears a short neural process that is fused to the centrum. The anteriormost tips of the parhypural and first hypural, are fused and are firmly attached to the compound centrum but are autogenous from this element. The anterior tip of the second hypural is firmly fused to the posteroventral edge of the compound centrum. The pleurostyle is firmly fused to the compound centrum at the posteriodorsal edge. The third hypural, which is comparable in length but little narrow anteriorly and similar in width to the second, touches the compound centrum in the 'v' formed between the pleurostyle and second hypural. The remaining hypurals, which become successively smaller in size dorsally, are loosely bound to the pleurostyle. A paired uroneural is present just above hypural 6.

There are very few differences in the caudal skeleton. Some of them are variations in number of principal rays, dorsal and ventral procurrent ray and pattern of fusion of parhypural and hypural elements. In Subfamily- Labeoninae; tribe – Garrini (*Garra mullya*) (Fig. 1A) there are 10 plus 8 principal rays, 10 dorsal and 9 ventral procurrent rays. A paired uroneural is present just above hypural 6. Spine shaped hypurapophysis is present on parhypural.

Among Subfamily- Smiliogastrinae members, *Puntius mahecola* shows 10 plus 8 principal rays, 7 dorsal and 7 ventral procurrent rays. Posterior margin of hypural 3 is almost equal to the posterior margin of hypural 4. The posterior margin of parhypural is almost equal to the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Keel shaped hypurapophysis is present on parhypural (Fig. 1B). In *P. sahyadriensis* there are 11 dorsal plus 8 principal rays, 5 dorsal and 6 ventral procurrent rays. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is narrower than the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Keel shaped hypurapophysis is present on parhypural (Fig. 1C). In *Rohtee ogilbii* there are 11 dorsal plus 8 principal rays, 7 dorsal and 7 ventral procurrent rays. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is narrower than the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Keel shaped hypurapophysis is present on parhypural (Fig. 1D). In *Systomus sarana* there are 11 dorsal plus 8 principal rays, 7 dorsal and 8 ventral procurrent rays. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is narrower than the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Keel shaped hypurapophysis is present on parhypural (Fig. 1E).

In family Danionidae, *Barilius barna* there are 11 plus 8 principal rays, 8 dorsal and 10 ventral procurrent rays. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is almost equal to the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Blunt ended hypurapophysis is present on parhypural (Fig. 2A). In *B. bendelisis* there are 10 plus 8 principal rays, 8 dorsal and 10 ventral procurrent rays. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is narrower than the posterior margin of hypural 1. A paired

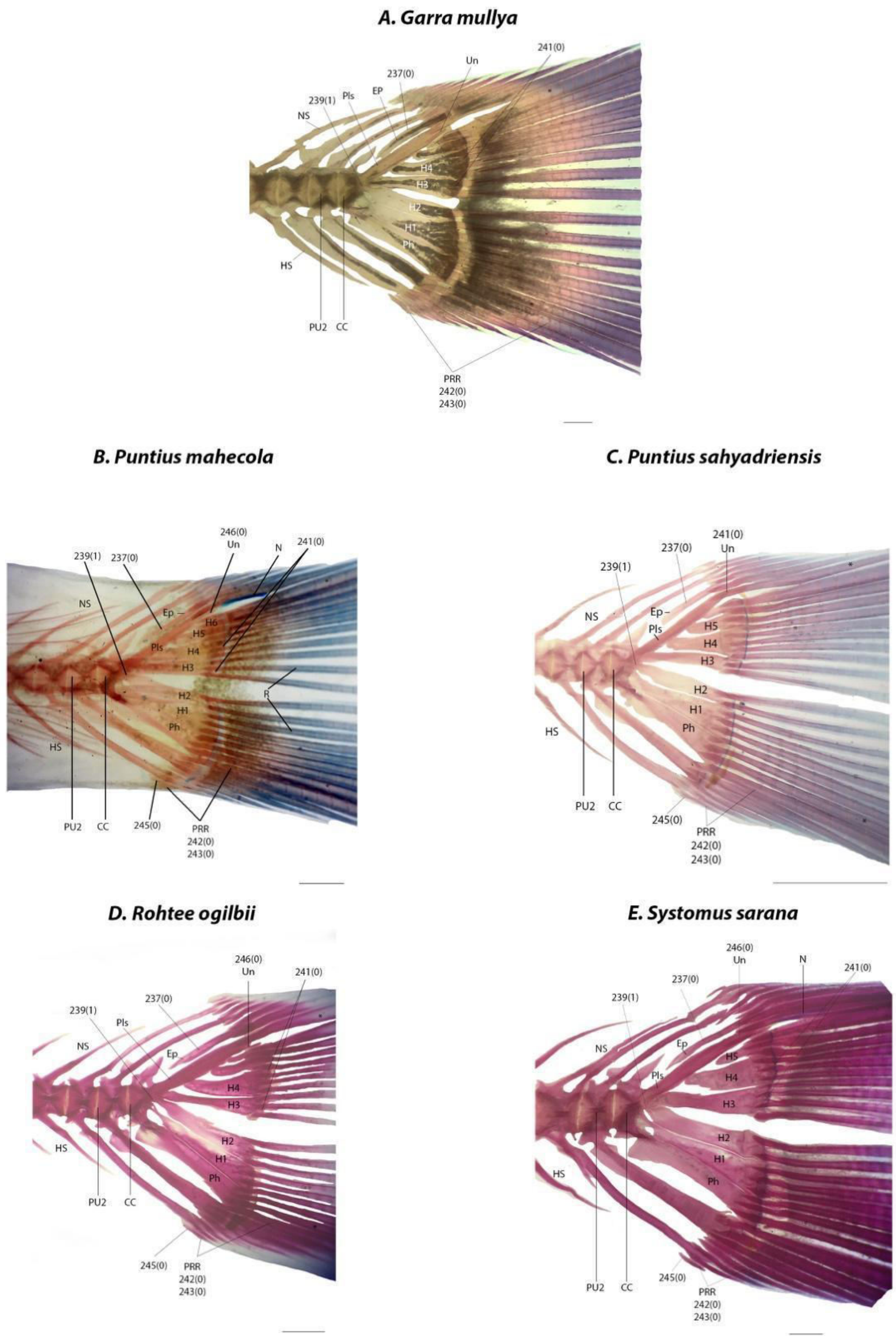

Fig. 1: Caudal fin skeletons of Subfamily Labeoninae; tribe *Garrini* (A. *Garra mullya*) and Subfamily Smiliogastrinae (B. *Puntius mahecola*; C. *Puntius sahyadriensis*; D. *Rohtee ogilbii*; E. *Systomus sarana*), left side in lateral view. Anterior to left. Abbreviations: CC, compound centrum; Ep, eplural; H1–5, hypurals 1–5; HS, haemal spine; NS, neural spine; Ph, parhypural; Pls, pleurostyle; PU2, preural centra 2; Un, uroneural. Upper- and lowermost principal caudal fin rays are indicated with *. Scale corresponds to 1mm.

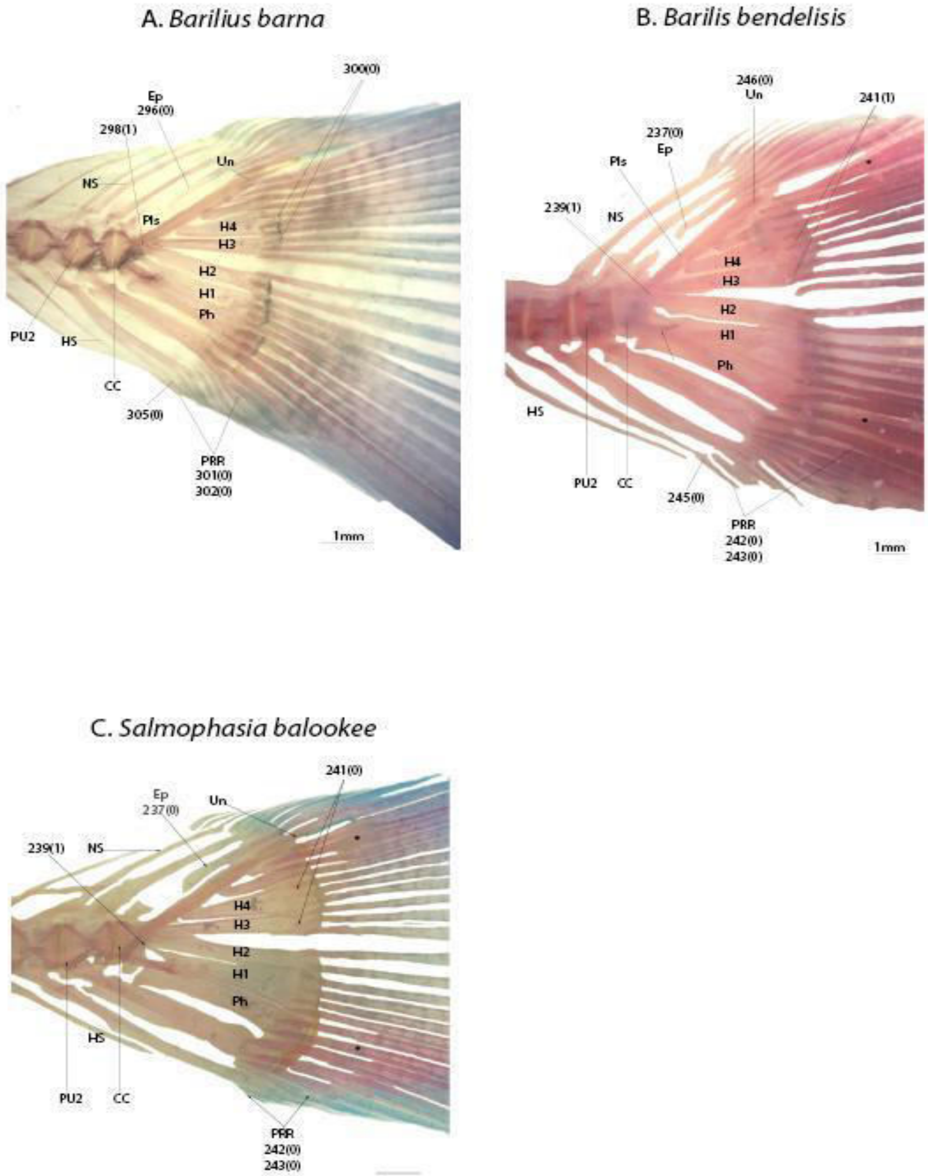

Fig. 2: Caudal fin skeletons of Family Danionidae (A. *Barilius barna*; B. *Barilius bendelisis*; C. *Salmophasia balookee*), left side in lateral view. Anterior to left. Abbreviations: CC, compound centrum; Ep, eplural; H1–5, hypurals 1–5; HS, haemal spine; NS, neural spine; Ph, parhypural; Pls, pleurostyle; PU2, preural centra 2; Un, uroneural. Upper- and lowermost principal caudal fin rays are indicated with *. Scale corresponds to 1mm.

uroneural is present just above hypural 6. Pointed hypurapophysis is present on parhypural (Fig. 2B). In *Salmophasia balookee* 9 plus 8 principal rays, 9 dorsal and 7 ventral procurrent rays are present. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is narrower than the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Pointed hypurapophysis is present on parhypural (Fig.2C).

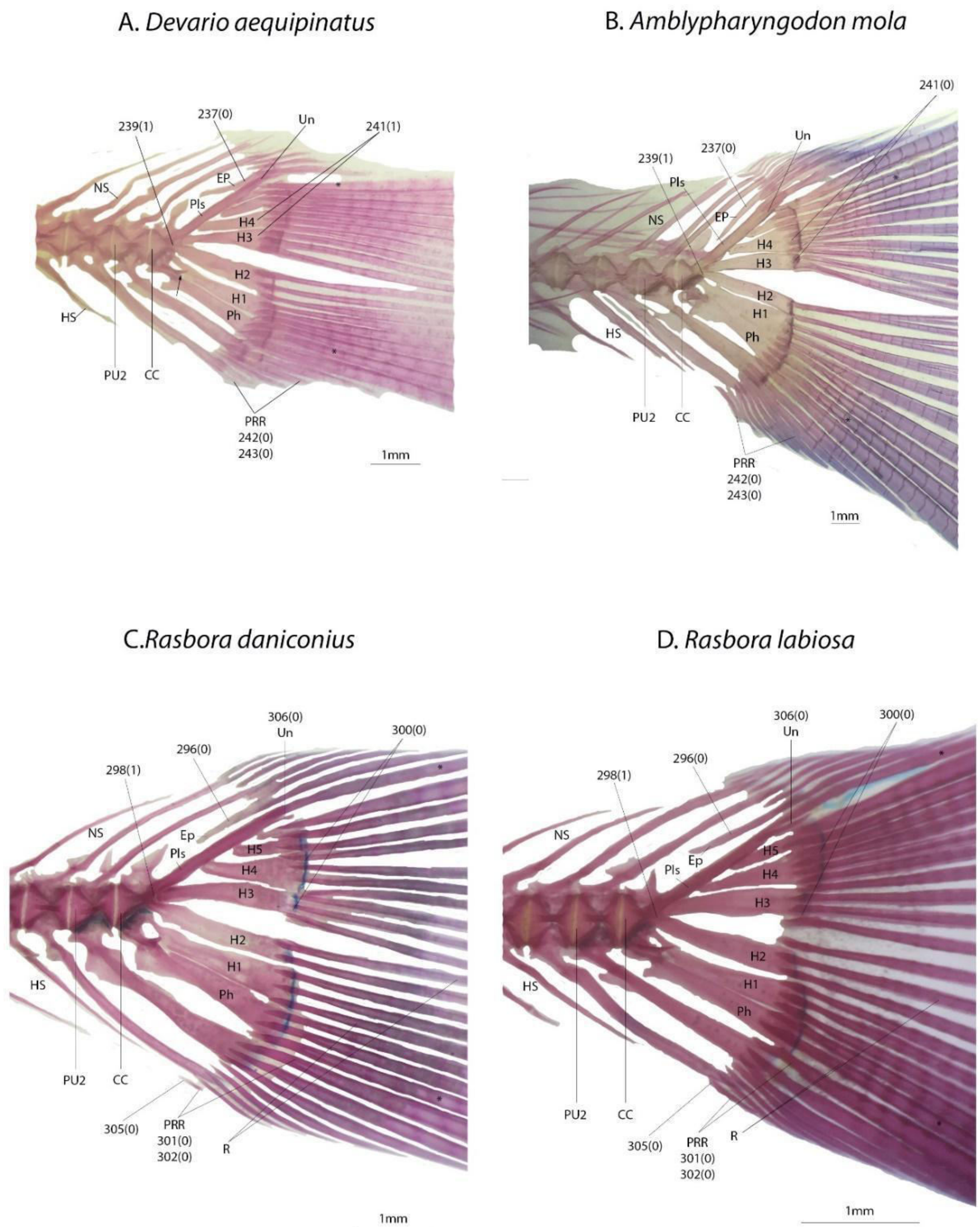

Fig. 3: Caudal fin skeletons of Family Danionidae, Subfamily Danioninae (A. *Devario aequipinatus*) and Subfamily-Rasborinae (B. *Amblypharyngodon mola*; C. *Rasbora daniconius*; D. *Rasbora labiosa*), left side in lateral view. Anterior to left. Abbreviations: CC, compound centrum; Ep, eplural; H1–5, hypurals 1–5; HS, haemal spine; NS, neural spine; Ph, parhypural; Pls, pleurostyle; PU2, preural centra 2; Un, uroneural. Upper- and lowermost principal caudal fin rays are indicated with *. Scale corresponds to 1mm.

In family- Danionidae; Subfamily –Danioninae, *Devariao aequipinatus* shows 10 plus 8 principal rays, 7 dorsal and 6 ventral procurrent rays are present. Posterior margin of hypural 3 is broader than the posterior margin of hypural 4. The posterior margin of parhypural is also broader than the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Pointed hypurapophysis is present on parhypural (Fig. 3A). In family- Danionidae; Subfamily –

Rasborinae, *Amblypharyngodon mola* shows 9 plus 8 principal rays, 9 dorsal and 9 ventral procurrent rays are present. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is also narrower than the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Keel shaped hypurapophysis is present on parhypural (Fig. 3B). In *Rasbora daniconius* 11 plus 8 principal rays, 6 dorsal and 8 ventral procurrent rays are present. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is almost equal to the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Pointed hypurapophysis is present on parhypural (Fig. 3C). In *R. labiosa* 11 plus 8 principal rays, 7 dorsal and 7 ventral procurrent rays are present. Posterior margin of hypural 3 is narrower than the posterior margin of hypural 4. The posterior margin of parhypural is almost equal to the posterior margin of hypural 1. A paired uroneural is present just above hypural 6. Pointed hypurapophysis is present on parhypural (Fig. 3D).

Discussion

The caudal fin skeleton of fishes under study shows principal rays, dorsal and ventral procurrent rays. Caudal fin rays are supported by the neural and haemal spines of the second and third preural caudal centra, the pleurostyle, a single epural, hypural elements, and the parhypural. Variations in caudal fin skeleton include number of principal rays, dorsal and ventral procurrent ray and pattern of fusion of parhypural and hypural elements.

Neural arches of posterior three vertebrae shows little variation. The neural arch of the compound centrum shows great deal of variation with respect to its shape, size and length. It may be stout, short or long. Hemal spines of preural centra 2 and 3 are long originating from the neural arches.

Doubling of neural spine on preural 2 vertebra has been recorded earlier in *Danio* (Sanger and McCune, 2002) and other cyprinids (Eastman, 1980). Accessory neural spines are observed in *Amblypharyngodon mola* on PU2, in *Barilius barna* on PU3, in *Devariao aequipinatus* on PU3, in *Systomus sarana* on PU3. Doubling of hemal spines on PU 3 is seen in *Amblypharyngodon mola*, *Barilius barna* and *Devariao aequipinatus*.

Parhypural bears a preural flange in all cyprinid species under study. In *Garra mullya* and *Salmophasia balookee* it is along the entire length of parhypural, In *Barilius barna* and *Rasbora labiosa* it is present on anterior 3/4th length of the parhypural approximately, In *Puntius mahecola* and *Puntius sahyadriensis* it is present along the anterior ½ length of the parhypural while in *Barilius bendelisis*, *Devariao aequipinatus*, *Rasbora daniconius* and *Rasbora ogilbii* it is present on anterior 1/3rd part of hypural.

The parhypural foramen is located between parhypural and hypural 1. It is very small in *Barilius bendelisis*, *Puntius mahecola* and *Puntius sahyadriensis*, small in *Garra mullya*, *Barilius barna*, *Amblypharyngodon mola* and *Devariao aequipinatus*, while it is large in *Rasbora ogilbii*, *Salmophasia balookee*, *Rasbora daniconius* and *Rasbora labiosa*.

The hypural foramen is located within Hypural 1 and hypural 2. In *Garra mullya*, *Puntius mahecola*, *Rasbora ogilbii*, *Barilius barna*, *Barilius bendelisis* and *Rasbora daniconius* it is small, it is very small in *Puntius sahyadriensis* and *Salmophasia balookee*, while in *Devariao aequipinatus* and *Rasbora labiosa* this foramen is not distinct.

Posterior margin of hypural 3 is approximately equal in width to the posterior margin of hypural 4 in *Puntius mahecola*. Posterior margin of hypural 3 is wider than the posterior margin of hypural 4 in *Devariao aequipinatus* while in remaining other cyprinid species it is narrower than posterior margin of hypural 4. Posterior margin of parhypural is approximately equal in width to the posterior margin of hypural 1 in *Puntius mahecola,* *Barilius barna*, *Rasbora daniconius* and *Rasbora labiosa*. Posterior margin of parhypural is wider than the posterior margin of hypural 1 in *Devariao aequipinatus* while in remaining other cyprinid species it is narrower than posterior margin of hypural 1.

Presence of four hypurals in the upper lobe of the caudal fin is typically observed in Cyprinidae (Sanger and McCune, 2002). *Systomus sarana* shows absence of hypural 6. It appears as if hypural 6 is fused with hypural 5. Hypurals might abut one another. Sometimes they can be seen to be fused at their tips or through their entire length along the margins.

The hypurapophysis of the parhypural is highly variable among different species under study. They may be blunt, keel shaped or pointed. Hypurapophysis is pointed in *Garra mullya*, *Barilius bendelisis*, *Salmophasia balookee*, *Devariao aequipinatus*, *Rasbora daniconius* and *Rasbora labiosa*. It is keel shaped in subfamily Smiliogastrinae members and *Amblypharyngodon mola*. In *Barilius barna* it is blunt.

Epural is separated from the neural arch in all species. A single uroneural is present in all species. It is in the form of a very thin bone along the side of pleurostyle.

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