International Journal of Fisheries and Aquatic Studies 2016; 4(1): 442-447

ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(1): 442-447

www.fisheriesjournal.com
Received: 12-12-2015
Accepted: 15-01-2016

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The significance of osteology in identification of *Schizothorax* species

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Abstract
Osteology is one of the important taxonomic characters for identification and classification of the fishes. In present study, 40 specimens of *Schizothorax richardsonii* (Gray) were divided into 4 groups based upon the standard length (SL) to determine vertebral count and position of different fins. The test of significance for correlation 'r' was employed. The various skeletal structures of *Schizothorax richardsonii* (Gray) studied in detail and were compared with other Schizothoracid fishes. It has been found that the osteological characters play very important role in identification of Schizothoracid fishes at specific as well as generic level.

Keywords: Osteology, Systematic, *Schizothorax*, Taxonomist

1. Introduction
The osteology is the study of skeletal structure of fish which helps in understanding the impressive and rapid adaptive variation in bone-forms among different species Dhanze, (1980) [2]. This helps in understanding the important taxonomic characters for identification and classification of fish, either at the species, generic, family, or at higher levels. The osteology is gaining immense importance in the fish systematic because of the pattern of the bone in the animals which is most conservative and is not much influenced by environment. So it is being regarded as the most reliable tool for the taxonomists and is being used in tracing out the interrelationship between and within the group Shukla and Verma, (1973) [10]. Further, a number of workers reported that some osteological characters play a very important role in identification of fish at generic as well as specific level and to draw the phylogenetic tree Gosline, (1955)[3]; Swada, (1982)[8]; Springer, (1983)[11]; Howes, (1990)[4]; Ashiwa and Hosoya, (1998)[1]; Norris, (2001)[6]; Sanger and Mccune, (2002)[7]; Shantakumar and Vishwanath, (2006)[9]. The present work is an attempt to identify the Schizothoracid species compressively based on the osteological attributes.

2. Materials and Methods
The specimens of *Schizothorax richardsonii* (Gray) were collected from tributaries of River Beas at different reaches during October 2011 to December 2012. The sampling sites *i.e.* Tanda (Latitude: 32.088083, Longitude: 76.537204) on the Mole stream; Bhatuu (Latitude: 32.084184, Longitude: 76.493876) and Nagni (Latitude: 31.979104, Longitude: 76.46705) on Neugal stream was selected. In the present study 40 number of fish species of *Schizothorax richardsonii* (Gray) ranging from 124.18$\pm$2.5 to 328.19$\pm$2.7; 105.51$\pm$3.2 to 272.32$\pm$2.9 in standard lengths and 20.45$\pm$2.3 to 292.70$\pm$3.1 in body weight were subjected for detail analysis. The total length and body weight of the fishes to the nearest millimetres (0.1mm) and grams (0.1g) respectively were recorded and preserved in 10% formalin for morphometric study and in 2% KOH solution for osteological preparation. The regression method has been employed for constant a and b. The different body parameters accessed for relative growth patterns were standard length (SL), head length (HL), head width (HW), body depth (BD), body weight (BW), dorsal fin length (DFL), pectoral fin length (PFL), ventral fin length (VFL), mouth width (MW), snout length (LS), eye diameter (ED), pre dorsal length (PDL), post dorsal length (PstDL), pre pectoral length (PPL), post pectoral length (PstPL), pre anal length (PAL) and post anal length (PstAL). The test of significance for correlation 'r' was employed. A total number of 40 specimens were divided into 4 groups based upon the

Length (SL) to determine vertebral count and position of different fins.

3. Result and Discussion

The skull of *Schizothorax richardsonii* (Gray) is almost well ossified and vault shaped (Figure i). It is elongated and narrow without suspensorium. The hyomandibular and the opercular series when attached to skull, gave it a massive appearance. The braincase is longer than wide, laterally compressed and tapering towards snout region. The neurocranium comprises of four parts constituting the major portion of the skull. It provided a protective covering to the brain and major sense organs as discussed here under.

3.1 Olfactory region

1. *Ethmoid (ETH)* (Fig. i): It is a triangular shaped, median dorsal bone of olfactory region and situated anterior to the frontals. Laterally it is flanked by two nasals, which are completely fused with it. Anteriorly, it is expended into two knobs like structures marked by a deep median groove. Yousuf *et al.*, (1988) [12] have also noted similar observation in *Schizothoraichthys Niger* (Heckel). The knob provides attachment to the palatine bone and the cartilaginous disc of maxilla. The groove itself is used for articulation of kinethmoid, which is an ossified ligament. Mid-ventrally the ethmoid is produced into a septum which partially separates the two nasal chambers.
2. *Lateral ethmoids (LETH)* (Fig. i): Lateral ethmoids are the paired bones, attached to the frontals on the ventral side. Each lateral ethmoid comprises of three parts (i) dorsal vertical plate attached with frontals and ethmoid (ii) basal region forming the floor of the ethmoid region and (iii) wing like outgrowth forming the partition between the olfactory region and the orbit. The is bone described by Yousuf *et al.*, (1988) [12] in *S. Niger* (Heckel) as parethmoid but in confirmations of the present observations.
3. *Nasals (N)* (Fig. i): The two nasals located on either side of the ethmoid bone and are completely fused with the ethmoid from which they are differentiated by faint demarcation. Yousuf *et al.*, (1988) [12] have recorded similar observation in *S. Niger* (Heckel) whereas this bone is comparatively broad but sickle shaped in *S. richardsonii* (Gray).
4. *Vomer (VO)* (Fig. ii): It is a median, triangular shaped bone located at the anterior end of the floor of neurocranium. It is placed in close contact with the basal plate and the wing like out-growth of lateral ethmoid and the knob like process of the ethmoid. Posteriorly, its tapering end fitted into the forked end of the parasphenoid.

3.2 Orbital region

1. *Orbitosphenoids (ORPS)* - (Fig. ii): These are irregular bones, located ventral to the frontals and join alisphenoids posteriorly. It is attached with the wings of lateral ethmoid anteriorly. Each bone is divided into (i) horizontal basal plate which meets its counterpart from the other side to form the floor of cranium and part of the inner roof of orbit and (ii) the longitudinal vertical process which is in close contact with its counterpart and forms the inter-orbital septum. The shape and location of this bone resembles to that of *S. niger* (Heckel) Yousuf *et al*., (1988) [12]. However, Mehta and Tandon (1984) did not notice vertical process of this bone.
2. *Parasphenoid (PS)* - (Fig. ii): This is an elongated median bone forming the floor of the cranium and extends from the vomer in anterior to basioccipital in posterior. The vertical process of orbito-sphenoid rests on dorsal apex of middle conical region of this bone. Posteriorly, the bone is flattened and formed sutures with respective prootic to enclose a large cranial space within, whereas Yousuf *et al*., (1988) [12] have reported that it formed suture with respective alisphenoid posteriorly to enclose large cranial space but in present study the alisphenoid is located ventrally to parasphenoid and encloses small cranial space.
3. *Alisphenoid (AS)* - (Fig. ii): These are paired irregular bone bordered ventrally by the frontals and posteriorly by sphenotic. Anteriorly, these bones join with the horizontal basal plates of respective orbito-sphenoids to enclose a cranial space. Dorsally these bones join above the parasphenoid and enclose a cavity in conjunction with the two orbits. The present observation is in conformity with the findings of Yousuf *et al*., (1988) [12].
4. *Lachrymal (LA)* - (Fig. i): These are paired flat, somewhat triangular shaped bones similar to that of *S. niger* (Heckel) Yousuf *et al.*, (1988) [12]. Lachrymal is attached to the lateral wing of lateral ethmoid ventrally and to the maxilla anteriorly. It forms the ventral rim of nasal capsule and antero-ventral rim of orbit.
5. *Orbital (OB)* - (Fig. i): The orbital bone consists of paired pre-orbitals, sub-orbitals, post-orbitals, sphenotics and supra-orbitals. All the bony elements of orbital are rod shaped except lachrymals. The supra orbital restricts to anterior median part of the orbit only.
6. *Frontals (FR)* - (Fig. i): The frontals are rectangular and most elongated bone of the neurocranium. They form the roof of the cranium and the posterior part of the dorsal border of the orbit. The two bones are sutured with each other on the mid -dorsal line and with the ethmoid as well as nasal anteriorly, with parietals posteriorly and sphenotic posterolaterally. It resembles in shape and location with that of *S. niger* (Heckel) Yousuf *et al.*, (1988) [12].

3.3 Otic region

1. *Sphenotics (SPH)* - (Fig. i): These are paired almost square shaped bones, located on the lateral wings of frontals and attached with it anteriorly. Posteriorly, they are joined with pterotics and postero-dorsally parietals. These bones enclose an elongated concavity with pterotics on ventral side for the condyle of hyomandibular bone. However, Mehta and Tandon, (1984) [5] reported that this bone has no attachment with parietal.
2. *Pterotics (PTR)* - (Fig. i): Pterotics are the paired narrow bones. Anteriorly, it articulates with the sphenotics, posteriorly with the epiotic and dorsally with the parietals on either side. This bone provides groove for the articulation of hyomandibular condyle.
3. *Prootics (PRO)* - (Fig. ii): These are paired and large size bones, sutured anteriorly with alisphenoid, antero-ventrally with sphenotics, dorsally with the parasphenoid and ventrally with pterotic. They form the lateral wall of cranium and inner wall of otic chamber. Mehta and Tandon, (1984) [5] have reported that this bone takes part in the formation of facet for hyomandibular in some Schizothoracines but as per their diagram, the present

observation are not in agreement.

4. *Epiotics (EP)* - (Fig. i): Epiotics are also paired bones. These are 'Q' shaped having spine on posterior side known as epiotic lamellae. Anteriorly, these are articulated with the parietals and pterotics, dorsally with the supra-occipital and posteriorly with the ex-occipitals. The lamellae of the two epiotics are equidistant from the median spine of supra-occipital. Epiotics form the posterior wall of otic chamber. The epiotic lamellae are well developed in *S. richardsonii* (Gray) and *Schizothoraichthys labiatus* (McClellend) as compared to other Schizothoracines Mehta and Tandon, (1984) [5].
5. *Parietals (PR)* - (Fig. i): These are large, somewhat square shaped flat bones and connected to each other mid-dorsally by sutures. Ventrally, they are articulated with pterotics and sphenotics, anteriorly by frontals and posteriorly by supraoccipital and epiotic. They form the postero-lateral roof of the cranium. However, this bone is longer in *Diptychus maculatus* Steindachner and *Schizopygopsis stoliczkae* Steindachner Mehta and Tandon, (1984) [5].

3.4 Occipital region

1. *Supraoccipital (SO)* - (Fig. i): A median bone located on the posterior extremity of the cranium. This is articulated with the parietals on anterior side and with the epiotic on lateral side. Posteriorly, it is attached with ex-occipital. This bone had a median spine that is supraoccipital spine which provides attachment to trunk muscles. Mehta and Tandon, 1984 [5] have stated that the length of this spine is species specific and is high in *S. richardsonii* (Gray) and *Schizothoriachthys* esocinus (Heckel). This bone takes part in the formation of foramen magnum but in *Schizoraichthys micropogon* (Heckel) and *S. labiatus* (McClelland) it does not take part in the formation of foramen magnum Mehta and Tandon, (1984) [5].
2. *Exoccipitals (EO)* - (Fig. i): These are paired bones lying posterior to supra-occipital and epiotics. Each exoccipital is divided into three regions: (a) ventral flat basal plate which meet its counterpart in mid-ventral line to form the floor of cranium, (b) dorsal ridge, which together with that of the other bone enclose the foramen magnum whose ventral rim is formed by the ventral basal plate and (c) lateral process similar to that of *S. niger* (Heckel) Yousuf *et al*., (1988) [12]. The two lateral processes form the base for the attachment of supra-temporal bone of pectoral girdle.
3. *Basioccipital (BO)* - (Figure i, ii): It is a large median bone forming the ventro-posterior portion of cranial box. Posteriorly, it is produced into an occipital condyle having the shape of opisthocoelous vertebral centrum. It also has a foramen for the blood vessel. The basioccipital is anteriorly attached to parasphenoid, antero-ventrally with ex-occipital and posteriorly drawn out into a keel like structure for attachment of muscles.

3.5 Branchiocranium

3.5.1 The Mandibular Region

1. *Kinethmoid (KE)* - (Fig. i): Kinethmoid is a single small, ossified ligament. It is attached to the anterior process of premaxilla ligamentously on ventral and dorsal to the groove formed by ethmoid at its anterior end. Mehta and Tandon, (1984) [5] stated that this bone is well developed in all the Schizothoracines except *S. richardsonii* (Gray).
2. *Premaxillaries (PMX)* - (Fig. i): The premaxillaries are paired long curved bones; meet each other in the middle line. Each bone is broad anteriorly and slightly tapering posteriorly, thick near the anterior end with an ascending process known as rostral process. The edge fitted into the anterior forked cartilaginous part of the maxilla, while the two cartilaginous pieces of the latter lied firm in the two facets. The rostral process is absent in *Schizopygopsis stoliczkae* Steindachner and *Diptychus maculatus* Steindachner, Mehta and Tandon, (1984) [5].
3. *Maxillaries (MX)* - (Fig. iii): The maxillaries are also paired, long and curved bones. These are larger in size than premaxillaries. Each maxillary bone lies over the dorsal surface of premaxilla behind the ascending process of the latter in the anterior end and extended beyond its posterior end. Anteriorly, each maxilla terminate in to a bifurcated cartilaginous piece, the two portions of which lodge the premaxillary process and joins its counterpart of other side. The maxilla is laterally compressed and relatively broader at its anterior end and attached ligamentously with ethmoid. Posterior end of this bone is attached with the dentary and dorsally it provides ligamentous attachment to the palatine.
4. *Palatine (P)*- (Fig. iii): The palatine run forward on the external side of the vomer and hooked over the dorsal end of the maxilla, immediately ventral to the nasal. Posteriorly the palatine is broader and connected with the endopterygoid and ectopterygoid. It is the anterior most element of the pterygoquadrate bar.
5. *Endopterygoid (ENPT)* - (Fig. iii): The endopterygoid is a long typically thin papery bone which connects anteriorly with the palatine and ventrally with ectopterygoid and metapterygoid.
6. *Ectopterygoid (ECPT)* - (Fig. iii): The ectopterygoid is a small square shaped bone and joins the endopterygoid dorsally. Anteriorly, it is attached with the palatine and posteriorly with the metapterygoid and quadrate.
7. *Metapterygoid (MPT)* - (Fig. iii): The metapterygoid is a flat, triangular bone and anteriorly attached with ectopterygoid. Its postero-dorsal margin is fringed for the articulation of the stem of the hyomandibular whereas antero-dorsal margin is smooth. Ventrally it is attached to the quadrate and symplectic.
8. *Quadrate (Q)* - (Fig. iii): The quadrate is angular in structure and is connected with ectopterygoid anteriorly. It articulates dorsally with the metapterygoid and postero-dorsally with symplectic, posteriorly with the preopercular and ventrally with the angular.
9. *Symplectic (S)* - (Fig. iii): The groove on the posterior surface of the quadrate lodged the symplectic bone which is a small and narrow bone. The dorsal projecting portion of symplectic articulates with the lower end of hyomandibular. Ventrally it provides attachment to preopercular bone and anteriorly to the quadrate.
10. *Angular (A)* - (Fig. iii): The angular is a small paired bone, concave anteriorly and convex posteriorly. Anteriorly, it provides the attachment to the dentary and posteriorly to the quadrate.
11. *Dentary (D)*- (Fig. iii): The dentary is a large paired irregular bone, which forms the basis of the lower jaw, and like the premaxilla and maxilla it is laterally flattened. The anterior end of dentary joins its counterpart of opposite side by means of a symphysis. At the symphysial region, the lower jaw is tipped with a small conical piece

of cartilage. Posteriorly it is wider and has two projections; the postero-dorsal projection is attached with the maxilla by ligament and posterior one attached with the angular bone. The shape of these projections differs in different species as per report of Mehta and Tandon, (1984) [5].

3.6 Hyoid Region

1. *Hyomandibular (HM)*- (Fig. iii): The hyomandibular is a triangular shaped bone with three condyles, two of them for articulating with the neurocranium through two facets, one in the postero-ventral face of the sphenotic and the other on the ventral surface of the pterotic. The third condyle articulated with the opercular dorsally. Anteriorly, the ventral surface of the hyomandibular is connected with the metapterygoid and symplectic. The posterior surface of the hyomandibular is attached with the vertical limb of the preopercular.
2. *Opercular(OP)* - (Fig. iii): The opercular is a thin bone, roughly triangular in shape. Dorsally it is articulated with the hyomandibular with opercular process. The posterior margin of opercular is finely fringed. Its anterior margin is plain and attached with the posterior surface of preopercular, ventrally attached with the subopercular and antero-ventrally with the interopercular. The opercular process is small and pointed in *S. richardsonii* (Gray) but broad in *Diptychys maculatus* Steindachner, *Ptycobarbus conirostris* Steindachner and *Schozothoraichthys spp.* Mehta and Tandon, (1984) [5].
3. *Preopercular* (POP) - (Fig. iii): The preopercular is an elongated crescent shaped, strongest of all the opercular bones and located dorsal to interopercular. The posterior surface of preopercular overlaps the opercular and its anterior surface is attached with the hyomandibular, metapterygoid, symplectic and the quadrate. Its dorsal end along with its ridge and groove is attached to the hyomandibular and ventrally with the angular.
4. *Subopercular (SOP)* - (Fig. iii): The subopercular is a long, narrow, thin bone with narrow anterior and broad posterior end. Anteriorly, it is overlapped by the interopercular and dorsally with opercular.
5. *Interopercular (IOP)* - (Fig. iii): The interopercular is almost spear shaped thin bone present just posterior to the preopercular bone. Anteriorly, it is attached with the angular. The dorsal margin of interopercular is overlapped by the posterior margin of the preopercular and the posterior margin joins the subopercular and opercular.

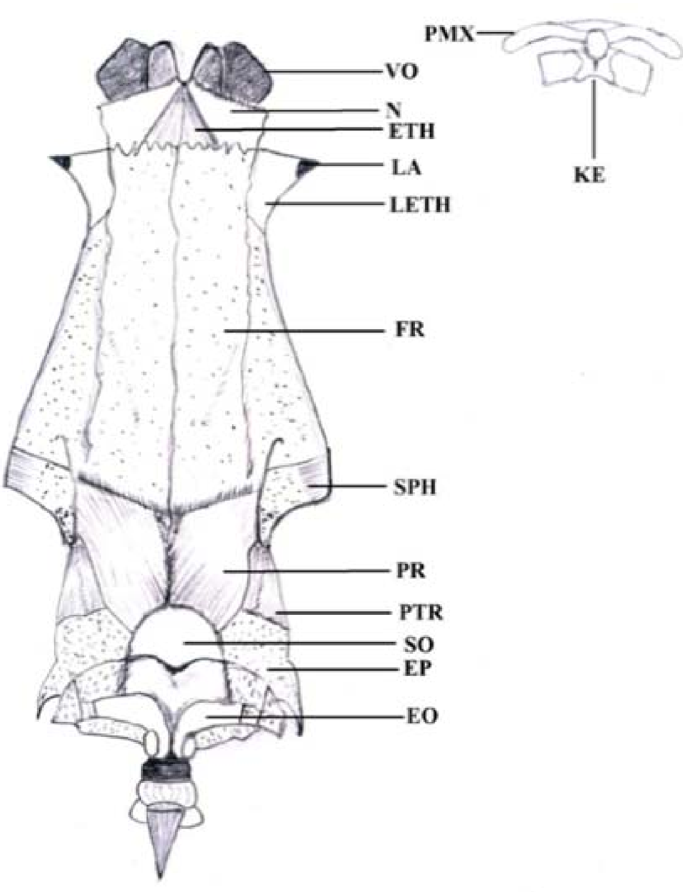

Fig i: Dorsal view of cranium

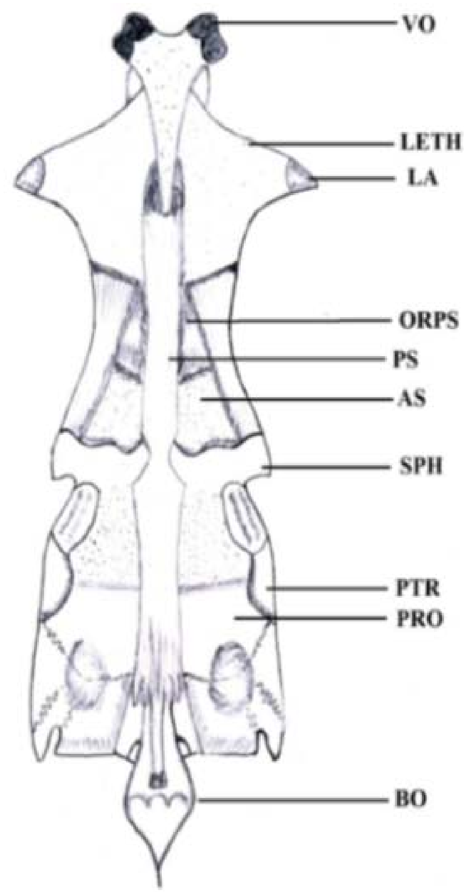

Fig ii: Ventral view of cranium

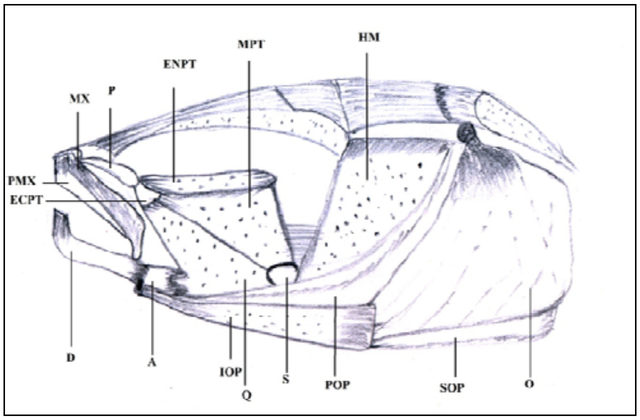

Fig iii: Lateral view of cranium of *S. richardsonii* (Gray)

3.7 Vertebral Column and Positions of Fins

A total of 40 specimens were studied ranging from 124.18+2.5mm to 328.19+2.7mm in total length, 105.51+3.2mm to 272.32+2.9 mm in standard length and 20.45+2.3 g to 292.702+3.1g in weight. These were divided into 6 groups to locate the position of different fins. In the first group the dorsal fin was found to be inserted corresponding to 14th vertebra, anal fin 29th vertebra and ventral fin 13th vertebra. The variation in position of dorsal fin was noticed from second to fifth group where its attachment varies from 14th to 15th vertebra. Further, the position of anal fin also varies from second group onwards where the attachment varies from 29th to 30th vertebra. The position of ventral fin is constant and is along the vertical line of 13th vertebra. The total numbers of 43 vertebrae have been found in all the groups studied (Table 1).

Table1: Vertebral count and positions of different fins in *S. richardsonii* (Gray)

S. No.	Class Range	No. of samples	SL	Vertebral count	Position of dorsal fin	Position of anal fin	Position of ventral fin
1	105-135	5	105.51-34.91	43	14th	29th	13th
2	135-165	6	150.39-58.59	43	14th -15th	29th	13th
3	165-195	7	165.5-190.25	43	14th -15th	29th -30th	13th
4	195-225	12	198.37-24.81	43	14th -15th	29th -30th	13th
5	225-255	7	238.22-43.06	43	14th -15th	29th -30th	13th
6	255-285	3	257.49-272.32	43	14th	30th	13th

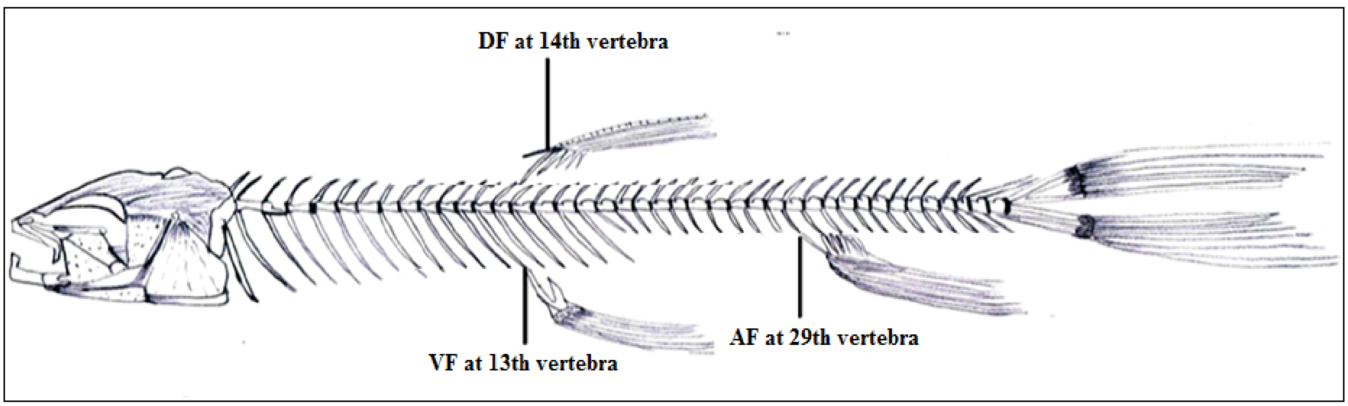

Fig 4. Vertebral Column and Positions of Fins in *S. richardsonii* (Gray)

The osteological characters play a very important role in identification of Schizothoracid fishes at specific as well as generic level. Based on present as well as earlier worker's observation, some important characters noticed in different *Schizothoracids* species, such as in *S. niger* (Heckel) nasal bone is broad but sickle shaped in *S. richardsonii* (Gray). Parasphenoid formed suture with alisphenoid posteriorly to enclose large cranial space in *S. niger* (Heckel) but in *S. richardsonii* (Gray), alisphenoid partially underneath to parasphenoid and encloses small cranial space. The epiotic lamellae are well developed in *S. richardsonii* (Gray) and *Schizothoraichthys labiatus* (McClelland) as compared to other Schizothoracids. Parietal is longer in *Diptychus maculatus* Steindachner and *Schizopygopsis stoliczkae Steindachner*. The length of supraoccipital spine is species specific and longer in *S. richardsonii* (Gray) and *Schizothoriachthys esocinus* (Heckel). Further, it takes part in the formation of foramen magnum but does not take part in *Schizoraichthys micropogon* (Heckel) and *S. labiatus* (McClelland). Kinethmoid is well developed in all the Schizothoracids except *S. richardsonii* (Gray). The rostral process of premaxilla is absent in *Schizopygopsis stoliczkae* and *Diptychus maculatus* Steindachner. The shape of the posterior projections of the dentary is species specific. The opercular process is small and pointed in *S. richardsonii* (Gray) but broader in *Diptychys maculatus* Steindachner, *Ptycobarbus conirostris* Steindachner and *Schizothoraichthys spp.*

4. Acknowledgement

The authors are grateful to the Director, Zoological Survey of India, Kolkata and Officer-in-charge, HARC, ZSI, Solan (H.P.) for encouragement and providing facilities. The Dean, CSK, HPKV, Palampur is greatly acknowledged for the facilities for the work. The reviewer (Anonymous) is also thankful for useful corrections and suggestions.

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