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Comparative Osteology Study of Ariid Catfishes along the Coast of Gulf of Mannar and Wadge Bank, Tamil Nadu

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ABSTRACT

Background: Taxonomic ambiguity still exists in ariid catfish species identification. Morphological similarities occur in ariid catfishes which lead to misidentification of the species. To overcome this taxonomic ambiguity osteology is one of the effective integrated taxonomic tools for species discrimination. From this study, we provide an osteotaxonomic key for the field identification of ariid catfish species.

Methods: The fish samples were cleaned and washed with the freshwater put into the sample for 1% potassium hydroxide solution (KOH). Then fish samples were allowed for alkali digestion of muscle in the solution for 24-48 hours depending on the condition of the specimen. After the completion of the muscle digestion process, Alizarin S dye was used for staining the fish bones.

Result: The present paper deals with a comparative study of the five Ariidae family species discussed, they are *Arius arius* (Hamilton,1822), *Plicofollis layardi* (Gunther,1866), *Netuma thalassina* (Ruppell, 1837), *Nemapteryx caelata* (Valenciennes, 1840) and *Osteogeneiosus militaris* (Linnaeus, 1758). The osteological portions used for this study like premaxillary, dentary, neurocranium, otolith, vertebral and caudal bone. The aforementioned osteological portions made the important primitive characters that will be used to differentiate the species.

Key words: Ariid, Bones, Catfishes, Osteology, Taxonomic ambiguity, Taxonomic tool.

INTRODUCTION

Osteology is the study of bones, which helps in identifying fishes based on the size and shape of the skeleton structures (Dhanze, 1980). It is an important taxonomic tool for the identification of their systematic positions such as species, generic, family, or at higher levels. It is expected to understand their within and between species category using osteological characteristics for a better empathy of their taxonomic status. Osteology is advanced for the huge contribution to fish taxonomy for the reason that the bone structure arose from the fish quite peculiar which is not influenced by the environment (Shukla and Verma, 1973). In spite of using molecular techniques such as DNA sequencing and barcoding, osteology, plays a vital role in discriminating the fish species. At present most researchers used osteology as one of the important taxonomic tools for identification of the fish. A Group of the species was already identified earlier based on the morphological similarities, in which, some of the species identification are contradictory (Kumar *et al*., 2015). To overcome the taxonomic difficulties in species identification comparative osteology has been described as the best option (James, 1985).

The family Ariidae or fork-tailed catfishes are the most taxonomic problematic Siluriformes group attributable to the maximum resemblance of the morphological characteristics (Ferraris, 2007). There are over 200 species of Ariidae or sea catfishes worldwide. Only these Siluriform groups have such an enormous distribution extended in all regions. Although many revisions have been completed to define and reclassify ariids (Sullivan *et al.,* 2006; Marceniuk and Menezes 2007; Betancur-R 2009; Marceniuk *et al.,* 2012; Aguilera and Marceniuk 2018), species identification based on morphology purely still can be mystifying (Yu and Quilang 2014). To avoid the misidentification of ariid catfish integrative taxonomic tool is the best selection. Osteotaxonomy is an effective method to overcome species misidentification that is solely done by morphological observation of fish bones. The aim of the study is to provide osteological key characteristics to identify the ariid catfish species deprived of mistakes.

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MATERIALS AND METHODS

Sample collection

The present study of Ariid fish samples were collected from various fish landing centers along the Gulf of Mannar and Wadge bank fisheries on the southeast coast of Tamil Nadu. The major sampling sites in the Gulf of Mannar region, are Thoothukudi (latitude 8.7945°N, longitude 78.1584°E)., Mandapam (latitude 9°16′14″N; longitude 79°7′10″E) and Pamban (Lat.13°04.53′N and Long. 80°27.69′E) whereas in the Wadge bank fishery the major sampling sites are Chinnamuttom (latitude 8.094345°N, longitude 77.561445°E), Kolachal (latitude 8.1728°N, 77.2509°E) and Muttom JPR fishing harbour latitude (latitude 8.1246°N, latitude 77.3307°E). These fish samples were contributed mainly by commercial catches and bycatch of trawlers, gill net and hook and line. The collected fish samples were placed in an insulated box with ice packs and transported to the laboratory, where they were photographed. In the laboratory, the collected fish samples were frozen at -20°C.

Sample identification

The collected ariid fish samples were identified by FAO Fish Identification Sheets and several fish taxonomy guidelines were used to identify the collected fish samples (Dhanze and Jayaram 1982; Jayaram and Dhanze 1978a,1978b; Jayaram 1984; Alexandre and Menezes 2007; Kailola 1999; Kumar *et al*., 2015).

Osteology

In the Present osteological study parts of the Ariid fish samples were prepared by the methods of Hollister (1934) and Clothier (1950). The fish samples were cleaned and washed with the freshwater put into the sample for 1% potassium hydroxide solution (KOH). Then fish samples were allowed for alkali digestion of muscle in the solution for 24-48 hours (or more depending on the species and nature of the specimen: whether fresh or preserved). The preserved samples are become too hard, so they took more time for digestion compared with the fresh samples. After completed the digestion using hand gloves and forceps the samples were cleaned and wash with water. After the completion of the muscle digestion process, the samples are stained Using Alizarin S dye. In completion of the staining process the bones were washed with the water and dried in the sunlight. Finally, premaxillary bone, dentary bone, neurocranium, otolith, vertebral and caudal bone used for this study and labelled all the samples kept in a box.

RESULTS AND DISCUSSION

The current study compared the premaxillary, dentary, neurocranium, otolith, vertebral and caudal bone for differentiating five ariid catfishes.

Premaxillary bone of ariid catfishes

It is a paired bone; the tooth is present only in the premaxillary bone so it is also called a toothed bone (Fig 2).

Arius arius

Feather shaped narrow sized bone, the caudal process is narrow with a blunt edge, Pair of premaxillary connected through the U shape bridge.

Osteogeneiosus militaries

The caudal process is short and sharp, the Pair of premaxillary connected through T shaped bridge and also a gap between two sides very less compared to *Arius arius*

Nemapteryx caelata

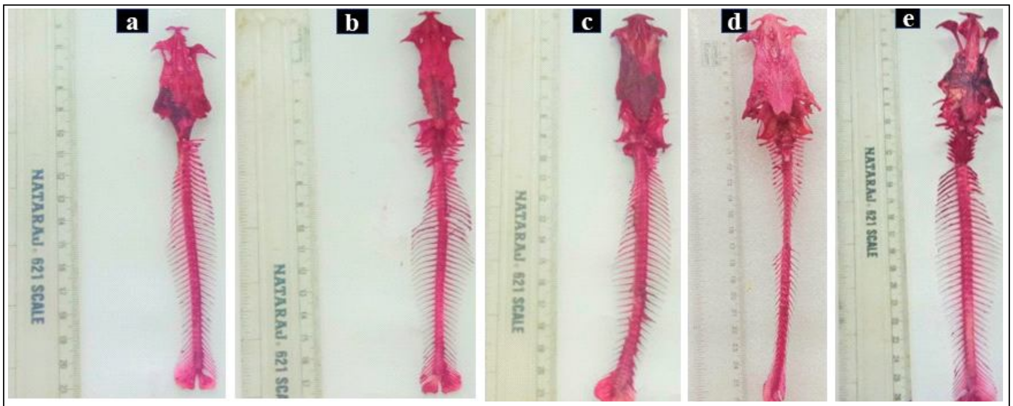
Caudal process is long and sharp, the premaxillary process also extended up to the edge of caudal process, it looks like a small spatula, Pair of premaxillary connected through the large hump like continued step, Ascending and Articular process parallel to each other.

Plicofollis layardi

Fan feather shaped broader sized bone, Premaxillary process is short and broad, the Pair of premaxillary connected through cup or U shape bridge.

Netuma thalassina

Saw shaped, it also a short and sharp but lower side of the caudal process having minute innumerable blades. Pair of



a. *Arius arius, b. Plicofollis layardi,* c. *Netuma thalassina,* d. *Nemapteryx caelata, e. Osteogeneiosus militaris.*

Fig 1: Full skeletal structure of ariid catfishes.

premaxillary connected through the small hump, the premaxillary process connected elevated structure.

Dentary bone of ariid catfishes

A pair of dentary forms the anterior part of the lower jaw (Fig 3).

Arius arius

A pair of dentaries forms the frontal part of the lower jaw. The size of the dentary bone is long and broad. The origin of the coronoid process and ventral process are the same, but the coronoid starts narrow it ends with a broad edge. The mental foramen is absent. meckelian fossa is broad. The length of the sensory canal is large.

Osteogeneiosus militaries

The size of the dentary bone is short and narrow. There are 3 numbers of mental foramen present. The length of the sensory canal is short. The coronoid process starts narrow it ends with a broad edge, but compared to *Arius arius* it is large, it formed the V shape.

Nemapteryx caelata

The size of the dentary bone is very long and broad. The mental foramen is absent. The length of the sensory canal is large.

Netuma thalassina

It is too sharp and narrow. There are 2 numbers of mental foramen present. The sensory canal is not clearly visible. Meckalian fossa short and straight. Mental foramen is absent.

Plicofollis layardi

The size of the dentary bone is short and broad. Only one mental foramen present.

Neurocranium bone of ariid catfishes

The neurocranium comprises four parts constituting the major portion of the skull. Namely, they are the olfactory region, orbital region, Otic region and Occipital region (Fig 1-4) and (Table 1-7).

The vertebral column of the Ariid catfishes

The number of vertebral bones present in the vertebral column is one of the significant osteological key characters to discriminate the catfish species (Fig 1 and 6).

Arius arius

There are forty-two numbers of vertebral bones present in the vertebral column of *Arius arius* among which 1st six ventral side bones are reduced. In the mid portion, six vertebral bones are bifurcated.

Fig 2: Premaxillary bone of ariid catfishes.

Fig 3: Dentary bone of Ariid catfishes.

Plicofollis layardi

There are fifty-two numbers of vertebral bones present in the vertebral column of *Plicofollis layardi* among which the 6th to 10th ventral side bones are reduced. In the mid portion, dorsal side of the vertebral bone elongated, the ventral side was reduced and also the seven bones vertebral bones are bifurcated.

Netuma thalassina

There are forty-five numbers of vertebral bones present in the vertebral column of *Netuma thalassina* among which 1st 5 bone ventral side bones are reduced. Posterior side eight numbers of vertebral bones are equally reduced. Nine numbers of vertebral bones are bifurcated in the mid portion of vertebral column.

Osteogenus military

There are forty-seven numbers of vertebral bones present in the vertebral columun of *Osteogenus militaris* among which only one ventral side bones are reduced. In the mid portion, five or six vertebral bones are bifurcated.

Nemapteryx caelata

There are fifty numbers of vertebral bones present in the vertebral columun of *Nemapteryx cealata.* No reduced bone. In the mid portion, five numbers of vertebral bones are bifurcated.

Caudal bone of the ariid catfishes

The caudal bone comprises of uroneural, hypural, parlendural or parhypural or preural flange, neural spine and hemal spine. Among these uroneural, hypural and parlendural bones were mainly used as the primary character of differentiating the ariid catfishes (Fig 1-6).

Arius arius

The *Arius arius* has three hypural bones, third hypural bones are shorter than the others. Parelandural bones are shortened and narrowed. Uroneural bones are Paired bones, they are connected to the hypural bones, size is large and somewhat broader bone.

Netuma thalassina

The *Netuma thalassina* has three hypural bones, among these first and second bones are broader. Parelandural bones are narrowed. Uroneural are thin and shorter bone.

Nemapteryx caelata

The *Nemapteryx cealata* has three hypural bones, third hypural bones are broader than the others. Parelandural bones are broader compared to others except for *Osteogeneiosus militaris.* Uronerural are thick and larger bones.

Plicofollis layardi

The *Plicofollis layardi* has four hypural bones, third and four hypural bones are larger than the others. Parelandural bones are thin and medium sized. Uroneural are thin and larger bones.

Osteogeneiosus militaris

The *Osteogeneiosus militaris* has three hypural bones, third hypural bones are broader than the others. There is no demarcation between the first and second hypural bones. Parelandural bones are large and broader. Uroneural are large and broader bones except for *Nemapteryx cealata*.

Otolith morphology study of ariid catfishes

Generally, ariid catfishes otoliths are circular, thick and bulbous in shape. All five different catfish species of otolith have similarities because they were from the same family but had enough differences to be distinguished from each other. The shape of the otolith is also used to differentiate catfishes from other groups of fishes. It has been divided into 4 regions they are distal, dorsal, proximal and ventral among these regions distal and ventral portions are used for otolith morphological identification (Fig 6).

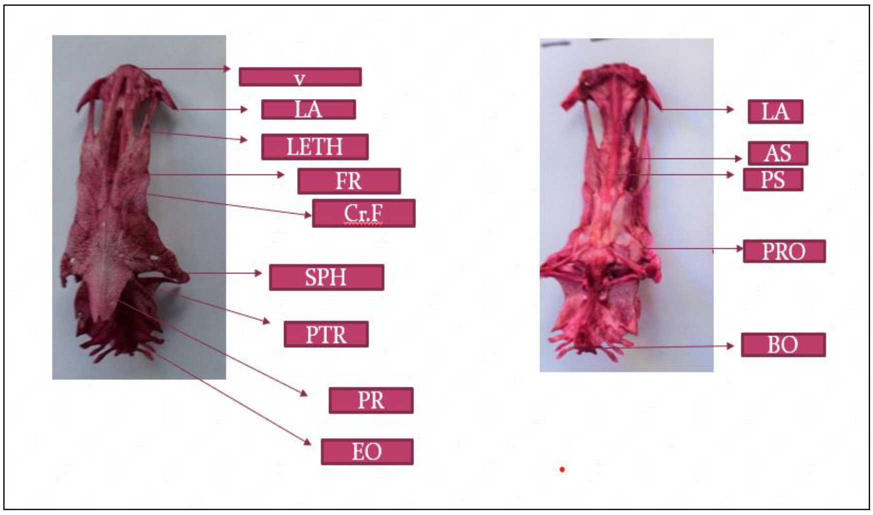


Fig 4: Neurocranium (dorsal and ventral view) of ariid catfish.

Arius arius

The distal portion is long and straight. The ventral portion is V-shaped.

Netuma thalassina

The distal portion is short and straight. The ventral portion is broad and U shaped.

Plicofollis layardi

The distal portion is long and slanting. The ventral portion is broad and circular shaped.

Nemapteryx caelata

The distal portion is larger in size and its end portion is hook-shaped. The ventral is half-moon shaped.

Osteogeneiosus militaris

The distal portion is large in size. The ventral portion is bowl-shaped.

Catfish head skeleton one of the key character used for identification of marine catfishes (Arratia, 2003). Tilak (1965) described the Lateral ethmoid of *A. bilineatus* species are backside bulged which was helpful for eye protection. The head shield of *A. dussumieri* is generally the more rugose, having a broader, more granulated supraoccipital process than does *A. bilineatus* (Tilak, 1965). Kailola (1986) explained the count of free vertebrae in *A. thalassinus* and *A. bilineatus* is an important discriminating feature of the identification of species. Al-Hassan *et al*. (1988) enlightened that there are differences in the spines and neurocranium of Ariid catfish species. They also found that the snout of *A. thalassinus* is significantly sharper than that of the other Gulf catfish which is clearly viewed ventrally. Rodrigues *et al*., 2020 discovered that sequence of thoracic and caudal bone similar in all the order of siluriform, in which the differences were found in only the vertebral bone number in the vertebral column of the catfishes. The vertebral column plays a major role in swimming and propulsion (Laerm 1976; Lindsey 1978; Weihs 1989). Vertebral counts also aid in the identification of closely related taxa such as species of clupeids (Houde *et al*., 1974). The intraspecific variation in vertebral counts must be known when using this character to separate closely related taxa (Berry and Richards 1973). Weitzman (1962) also explained the total number of vertebrae varies according to the fish species which may be lower or higher than certain taxa. Licar-Rodrigues *et al*. (2020) demonstrated descriptions of the osteology of *S. couma*, which assisted to the area of

Fig 5: The vertebral column of ariid catfish.

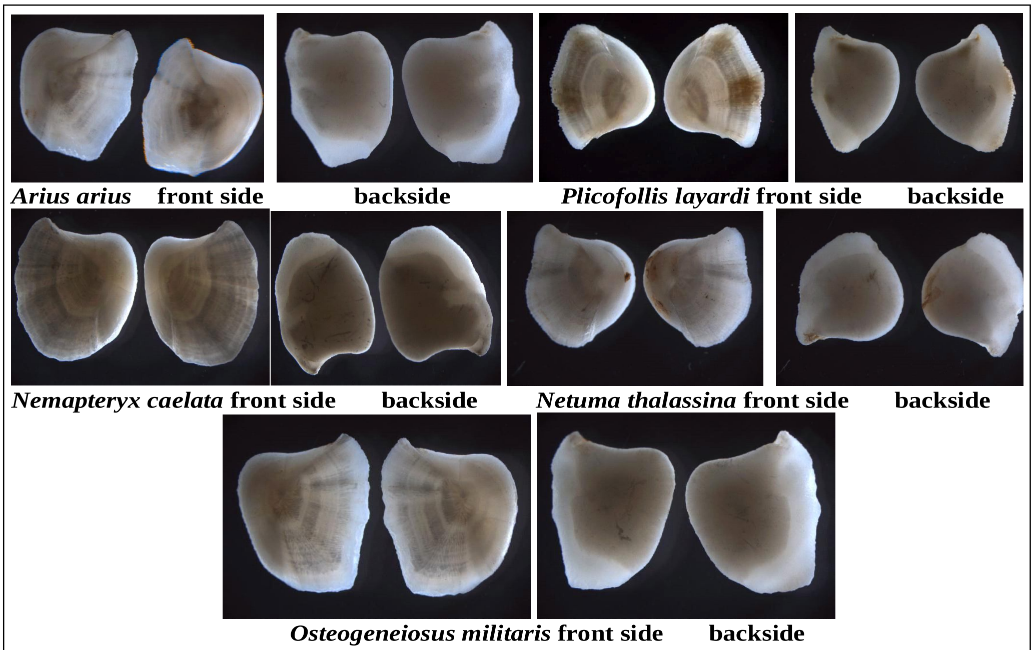


Fig 6: Stereomicroscopic images of otolith of Ariid catfishes front side view and backside view.

Table 1: A comparison of the olfactory region of five ariid catfishes.

Olfactory region	*Arius arius*	*Plicofollis layardi*	*Nemapteryx caelata*	*Netuma thalassina*	*Osteogeneiosus militaries*
Vomer (VO):	Trapezoid shape.	Feather-shaped.	Flat and pointed shape.	Arrow-shaped.	It is a trapezoid-shaped/ T shaped bone.
Nasal (N)	Paired triangle-shaped	Reduced	It is paired cup-shaped	Oval-shaped/ reduced	Inverse right triangle shape
Ethmoid (ETH):	It is a V-shaped.	U-shaped bone.	It is tapered.	Two parallel lines joined shape each other to form rectangular.	Rectangular shape.
Lateral ethmoids (LETH):	The paired bones are attached to the frontals on the ventral side.	A narrow-shaped bone with 2 holes located on each side.	It is short and slanting.	It is short and broad.	Attached to the posterior portion of frontals.

Table 2: A comparison of the orbital region of five ariid catfishes.

Orbital region	*Arius arius*	*Plicofollis layardi*	*Nemapteryx caelata*	*Netuma thalassina*	*Osteogeneiosus militaries*
Lachrymal (LA):	These are paired with flat wing bones, which look like an axe edge.	It is a paired, pointed and arrow-shaped bone.	These are paired with flat wing bones, which look duck nose-shaped.	Leaf-shaped bone.	Honeycomb-shaped in which n are numbers of circular shapes closely connected to each other.
Cranial fontanelle (s): Cr.F	The Cr.F are oval-shaped and the most elongated bone of the neurocranium.	It is a very long bone; the upper part looks triangle-shaped and the lower part U shaped bone.	The Cr.F are oval-shaped Compared to other species width of the frontals are broad.	It is an oval-shaped bone, compared to all other species the length is very short.	The Cr.F are petal-shaped, narrow and sharp edge.
rontals (FR):	Frontal bones are broad with demarcation.	The size is small compared to *Arius arius*.	It originates nearby upper portion of the cranial fontanellae.	Small sized bone.	It originates the nearby end The portion of the Cr.f bone. ength of the bone is too small

Table 3: A comparison of the otic region of five ariid catfishes.

Otic region	*Arius arius*	*Plicofollis layardi*	*Nemapteryx caelata*	*Netuma thalassina*	*Osteogeneiosus militaries*
Sphenotics (SPH):	These are called connecting bones, It looks like a spatula edge arrow-shaped bone.	A pair of V-shaped sphenotic bone.	It looks like an arrow-shaped bone.	It looks like a small spatula-shaped bone.	small arrow or pointed bones.
Parietals (PR):	It is a butterfly-shaped bone.	A pair of wings attached to make W shaped bone.	It is a butterfly-shaped bone.	It is a pair of wing-shaped bones connected by U-shaped sutures.	Wing-shaped flat bones connected to each other middorsal by sutures
Pterotics (PTR)	It is a leaf-shaped bone.	It is a Crescent-shaped bone.	It is a leaf-shaped bone.	Pterotics are the paired bones, it has leaf-shaped structures.	spindle-shaped paired bones.
Epiotics (EP):	These are flower-shaped, 2 petals of the flower make crescent-shaped bone.	Broad Leaf-shaped bone to make a pillar.	Crescent-shaped bone.	It is a heart-shaped paired bone.	These are also flower-shaped, 2 petals of the flower joined to make a garbage disposal plate.

Table 4: A comparison of the occipital region of five ariid catfishes.

Occipital region	*Arius arius*	*Plicofollis layardi*	*Nemapteryx caelata*	*Netuma thalassina*	*Osteogeneiosus militaries*
Exoccipitals (EO):	It is a paired sickle-shaped bone.	Narrow leaf-shaped bone.	It is a paired leaf-shaped bone.	Pillar-shaped bone.	It is small finger-shaped.

Table 5: A comparison of the orbital region (ventral side) of five ariid catfishes.

Orbital region	*Arius arius*	*Plicofollis layardi*	*Nemapteryx caelata*	*Netuma thalassina*	*Osteogeneiosus militaries*
Orbitosphenoids (ORPS):	It is an irregular-shaped narrowed bone, the back bone of the frontal bone.	A pair of fin-shaped bone.	It is an irregular-shaped narrowed bone, the backbone of the frontal bone.	These are irregular bones, located ventral to the frontals and join alisphenoids posteriorly.	There no suture in the OPRS.
Parasphenoid (PS):	The elongated bone of the ventral side of the neurocranium.	It is a broad and elongated bone.	The elongated and wide bone of the ventral side of the neurocranium.	It is a compressed and narrow bone.	It is very thin, needle bone.
Alisphenoid (AS):	It is a posterior portion of an irregular-shaped broader bone.	Reduced.	It is a posterior portion of an irregular-shaped broader bone.	It is a Broad and bulged bone.	It is connected to OPRS without any demarcation, all other species having the dividing bone.

taxonomy in fish. Bemvenuti (2005) explicated that *Odontesthes bonariensis* species having high number of precaudal bone than tail region. Serra and Langeani (2006) highlights the occurrence of hypural in the caudal fin that vary and such variation is detected in the caudal fin of *S. couma*. A high number of hypural bones is often taken as a primitive character (*i.e*., seven in Elops and salmonids); reduction is advanced (*i.e*., four in pleuronectids). The hypural bones may be variously fused, ultimately into a single plate in adults of some species, *e.g*., Coryphaena (Potthoff 1980). When fusion of hypural bones occurs, it may be observed in ontogeny in some fishes (*e.g*., *Thunnus atlanticus*, Potthoff 1975; Coryphaena , Potthoff 1980) but not in others (*e.g*., *Ophichthus gomesi*, Leiby (1979); *Microgadus proximus*, Matarese *et al*. (1981). Uroneurals, paired bones generally occurring dorso laterally to the ultimate vertebra, are often the first bones to ossify in the caudal complex. According to Patterson (1968), they are modified ural neural arches.

Javadzadeh *et al.* (2014) studied the otolith of *A. dussumieri* and *A. thalassinus,* different species from the same family can have similarities in appearance but have enough differences to be distinguished from each other. The otolith of sea catfish *Bagre panamensis* is a large, compressed, thick circle with a well-developed rostrum (Maldonado-Coyac *et al*., 2021). The shapes of an otolith morphology from four species of catfishes are basically having similarities in their presence (Chen *et al*., 2010). The Goat fishes otolith morphology can be used to distinguish species and genera (Echreshavi *et al*., 2021).

However, the present study supports earlier osteological studies and describe a additional key characters for catfish identification. Ariid catfish species identified by using various osteological key characteristics such as premaxillary, dentary, neurocranium, otolith, vertebral and caudal bone. Each bone structure varies with different fish species which will help to contribute to osteology as one of the important taxonomic tools for discriminating the ariid catfishes.

CONCLUSION

The comparative osteology of ariid catfishes was studied, premaxillary, dentary, neurocranium, otolith, vertebral and caudal bone were used to segregate Ariid catfishes. Osteology is one of the integrated taxonomy tools that helped for discriminate the catfishes. This study mainly assists in the accurate identification of the ariid fishes which leads to avoiding misidentification. There are many taxonomic tools available like morphometric, meristic, truss networking and DNA barcoding and sequencing compared to all osteology is a simple, cost-effective, eco-friendly technique favored by all taxonomic researchers. The catfish landings were decreased (CMFRI, 2015) to implement the conservative measures required for accurate field identification this will be further helped for conserved the exploited fishes.

Conflict of interest

All authors decalre that they have no conflict of interest.

Table 6: A comparison of the otic region (ventral side) of five ariid catfishes.

Otic region	*Arius arius*	*Plicofollis layardi*	*Nemapteryx caelata*	*Netuma thalassina*	*Osteogeneiosus militaries*
Prootics (PRO):	These are paired and small size bones.	A pair of javelin shaped bone.	These are paired and large size bones.	It is a butterfly-shaped bone.	Oval shaped paired bone, started the posterior portion of parasphenoid.

Table 7: A comparison of the occipital region (ventral side) of five ariid catfishes.

Occipital region	*Arius arius*	*Plicofollis layardi*	*Nemapteryx caelata*	*Netuma thalassina*	*Osteogeneiosus militaries*
Basioccipital (BO):	It is a small pumpkin-shaped bone.	Small pumpkin-shaped bone.	It is a small round-shaped bone.	It is an apple-shaped bone.	It is a small flower bone, that looks like a flower without tips.

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