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Ultrastructural variations of the sagittae in two *Anabantiformes* fishes: *Anabas testudineus* (Bloch, 1792) and *Trichogaster fasciata* (Bloch & Schneider, 1801)

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The sagitta otolith is well-decorated with various species-specific peculiarities, used to support the relatedness in taxonomy and other aspects among fishes. Present study deals with the morphostructural variations of the sagitta in the *Anabas testudineus* and *Trichogaster fasciata* by light and scanning electron microscopy. As these two fishes are belonging to order *Anabantiformes* but their sagitta morphologies are much more varies. The sagitta of the *A. testudineus* is oval with complete dorsal and ventral marginal boundary, while the dorsal boundary is incomplete in the *T. fasciata*. The sulcus of the *A. testudineus* is ostio-pseudocaudal with spoon-headed ostium, whereas the sulcus in *T. fasciata* is ostio-caudal with a simply tubular ostium. The ostium and cauda are demarcated with a prominent constriction in the *A. testudineus*, while this demarcation is maintained by a virgule-like development in the *T.fasciata*. Presence of the orthorostrum patch, and hexangular stony pillars along the marginal sculpture in the *T. fasciata* is a species-specific peculiarity. A phylogenetic tree is made based on the sagitta features between these two fish species and five other *Anabantiformes* fishes and stated that the *A. testudineus* is characteristically more related to *Channa* sp than that of the *T. fasciata.*

Keywords: *Anabantiformes*, Climbing-Perch, Labyrinth-Perch, Sagitta otolith, Ultrastructure

Otoliths are aragonite structures of the protein and calcium carbonate and are located in the inner ear cavity of all teleost fish, perform as a balance organ and support in hearing. The otolith is used as an important tool in the field of origin, species identification, systematics, stock analysis, pollution exposure, relatedness between different trophic levels in a habitat, biogeographical history and palaeontological analysis of the fish fauna.The teleost fishes possess three pairs of otoliths; the sagittae, lapilli, and asterisci.

Sagitta is largest otolith in most teleost fishes and it is commonly used to study the growth, age, intra- and inter-specific variations among the otolith morphology in fishes[1,2]. The sagitta includes an outer (or lateral) and an inner (or medial) surface, which is well-ornamented with numerous morphostructural features most of which have inscribed of the species-specific features[3-5]. There is very inadequate information regarding the detail morphostructural description of the sagitta in the Order *Anabantiformes* fishes which includes a large number of family in different habitats. Although, Bardhan *et al*.[6] described the ultrastructural details of the sagitta in *Anabas testudineus* (Anabantidae: *Anabantiformes*) and a developmental relationship between the various sagitta constituents and the body size. Additionally, Bano and Sirajuddin[7] stated a light microscopic outline of the sagitta morphometrics of the *Trichogaster lalia.* Beside these two studies, no other study on the sagitta morphology among the Order *Anabantiformes* is still yet done. On the other hand, the ultrastructural characteristics of the sagitta and their variations among the *Anabantiformes* fishes are not so far described. Therefore, the present study was accomplished to analyse the morphostructural peculiarities present in the sagitta otolith in two fresh water *Anabantiformes* fishes; *Anabas testudineus* (Bloch, 1792) and *Trichogaster fasciata* (Bloch & Schneider, 1801). A dendrogram is constructed here

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Suppl. Data available on respective page of NOPR

based on the available morphostructural characteristics of the sagitta in fresh water *Anabantiformes* fishes to establish how the fishes are related to each other in respect of their shared and derived sagitta features.

Materials and Methods

Live *Anabas testudineus* (Bloch, 1792) (TL, 12.52 ± 0.35 cm; N = 25) and *Trichogaster fasciata* (Bloch & Schneider, 1801) (TL, 6.5 ± 0.29 cm; N = 30) were collected from the ponds at the Negua village (21.80805N, 87.51114E), Purba Medinipur, West Bengal, India and adjacent areas. The fishes were kept in two separate plastic jars filled with pond water and some fish feeds and those jars are transported to the laboratory. The specimens were identified by the Zoological Survey of India (ZSI), Kolkata. The pair of sagittae were dissected out from the fishes (N = 10 of each species) and washing with water [6] and stored air-dried in individual plastic tubes. Only right side sagittae were used in the studies. For light microscopic studies the right sagittae were placed on a clean glass slide, facing the inner (medial) surface upward and viewed with Leica DMC4500. For scanning electron microscopy, the sagittae were processed following a standard method[6] and viewed the inner surface with EVO18, Zeiss.

According to the terminologies of Smale *et al*.[8], Schwarzhans[9], Bardhan *et al*.[6], and Roy and Bardhan[5], a number of sagitta morphostructural characteristics of *A. testudineus* and *T. fasciata* have been described in the present study. The measurements (mean value ± SD in mm) of various sagitta features were taken using image processing software "ImageJ 1.5t" (Wayne Rasband, NIH, USA).

To investigate the relatedness of sagitta structures in the *Anabantiformes* fishes, a dendrogram was constructed between the *A. testudineus, T. fasciata* and available other *Anabantiformes* fishes in the fresh water with the available sagitta morphostructural character-based maximum parsimony method[10-11].

Results

Morphologies of the inner surface of a typical sagitta

The inner surface of a typical sagitta is ornamented with various morphostructural constituents such as the sulcus, rostrum, anti-rostrum, post-rostrum, para-rostrum, excisura, dorsal depression, ventral depression, cristae, various marginal sculptures, scallops along the margins, and various surface sculpture of the dorsal and ventral part of the inner surface. The sulcus includes the ostium and the cauda. However, the development of various morpho-anatomical characteristics of the sagitta constituents are depended on the taxon of the fishes (Figs 1A-E, 2A-D, 3A & B and 4A & B).

The sagitta in the *Anabas testudineus*

The sagitta (5.9 ± 0.01 × 2.9 ± 0.02 mm) of *A. testudineus* is an ovate-shaped structure that is dorsally slightly concave and ventrally convex. The shape of the dorsal margin is rounded and crenate shaped, whereas the ventral margin is oval and sinuate (Figs. 1A & B and 4A). The scallops of the ventral margin are relatively broader and prominent than those of the dorsal margin (Fig. 1B). The sulcus is 'S'-shaped, hetero-sulcoid, medial, and ostio-pseudo caudal type structure (Figs 1A & B and 3A). The collum is absent in the sulcus, but the ostium and the cauda are demarcated with a prominent constriction at the posterior end of the ostium (Figs 1A-C and 3A). The ostium (1.84 ± 0.02 × 1.27 ± 0.01 mm) is a spoon-headed structure and is comprised of a shallow depression (0.51 ± 0.01 × 0.44 ± 0.01 mm) near the posterior end (Fig. 1A-C). Some prominent stony elevations are observed on the floor of the ostium (Fig. 1C). The cauda (3.65 ± 0.02 × 0.43 ± 0.08 mm) is tubular and ventrally curved. The colliculum is well-developed and frequently found in the ostium and cauda portion. The growth stripes are frequently found on the wall of the sulcus groove. The dorsal and ventral cristae are anteriorly more distinct that those of the posterior end. The rostrum, anti-rostrum, post-rostrum, and para-rostrum are well-developed. The excisura major is distinct between the pointed rostrum and anti-rostrum (Fig. 1A-C, and Table 1). The excisura minor is narrow but prominent between the post-rostrum and para-rostrum. The dorsal depression is irregular and wider dorsally, while the ventral depression is shallow (Fig. 1A & B). The surface sculpture of the inner surface includes some uneven, tetrangular stony pillars and most of which possess roughly rectangular head surface (Fig. 1D & E). It is observed that these stony pillars are relatively prominent and higher in number in the ventral part than those of the dorsal part.

The sagitta in the *Trichogaster fasciata*

The sagitta (0.28 ± 0.01 × 0.2 ± 0.01 mm) of the *T. fasciata* is a mostly oval-shaped structure. The dorsal margin is partially lobate and ventral margin is crenate-shaped but the anterior part of which is entire (Figs 2A & B and 4B). The dorsa l margin is relatively smaller

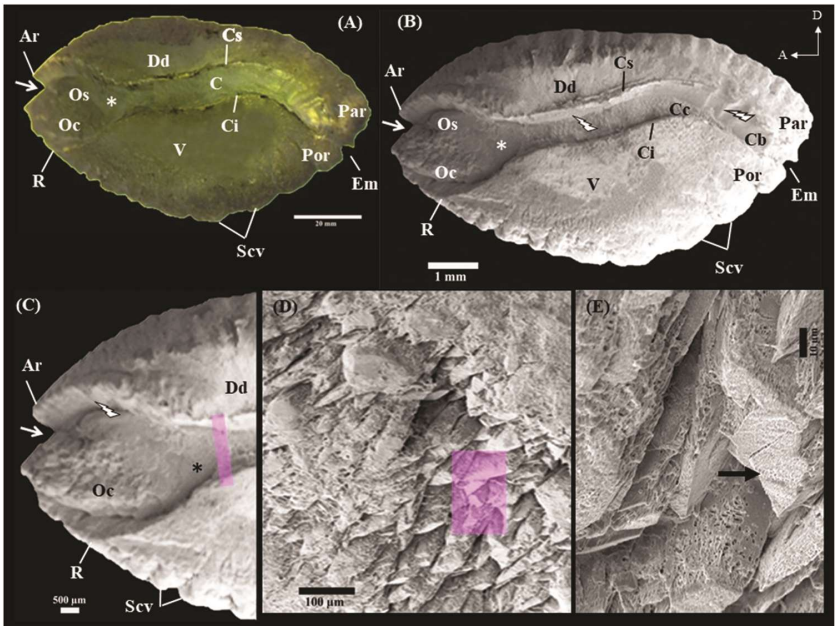

Fig. 1 — The inner surface of the surface of the sagitta (right side) of *Anabas testudineus*, (A) Light photomicrograph; (B) Scanning electron photomicrographs of whole sagitta; (C) Enlarged view of the ostium; the colour area is the demarcation between the ostium and cauda. (D) Enlarged view of the colour portion of the surface sculpture in Fig. C, note the rectangular head surface of the tetrangular stony pillars; and (E) Magnified view of the marked area of the Fig. D; thick arrow, rectangular head of the stony pillar. A, anterior end; Ar, anti-rostrum; C, cauda; Ci, crista inferior; Cs, crista superior; D, dorsal end; Dd, dorsal depression; Em, excisura minor; Os, ostium; Oc, ostial colliculum; Par, para-rostrum; Por, post-rostrum; R, rostrum; Scv, scallop in ventral; V, ventral side; white arrow, excisura major; zig-zag arrow, growth stripes; asterisk, shallow depression

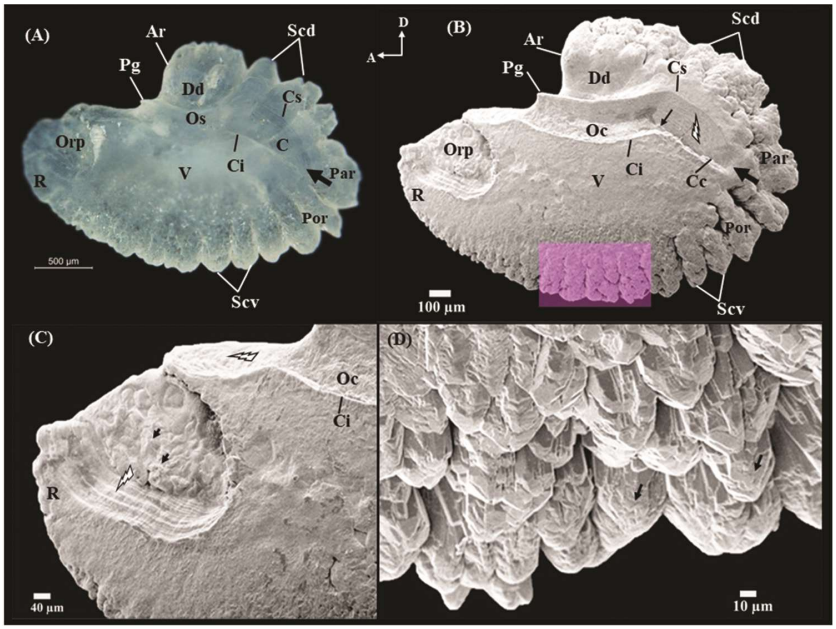

Fig. 2 — The inner surface of the surface of the sagitta (right side) of *Trichogaster fasciata*, (A) Light photomicrograph; (B) Scanning electron photomicrographs of whole sagitta; (C) Enlarged view of the ortho-rostrum patch; and (D) Enlarged view of the colour portion of the surface sculpture in Fig. B, note the blunt headed (arrow heads) hexangular stony pillars. A, anterior side; Ar, anti-rostrum; C, cauda; Cc, caudal colliculum; Ci, crista inferior; Cs, crista superior; D, dorsal side; Dd, dorsal depression; Oc, ostial colliculum; Os, ostium; Par, para-rostrum; Pg, peg-like projection of cristae superior; Por, post-rostrum; R, rostrum; Scd, scallop in dorsal margin; Scv, scallops in ventral margin; V, ventral side; Zig-zag arrow, growth stripes; Black arrow, virgule-like mark in the sulcus groove; Thick arrow, caudal fork; Black arrow head, floor-sculptures in the ortho-rostrum patch

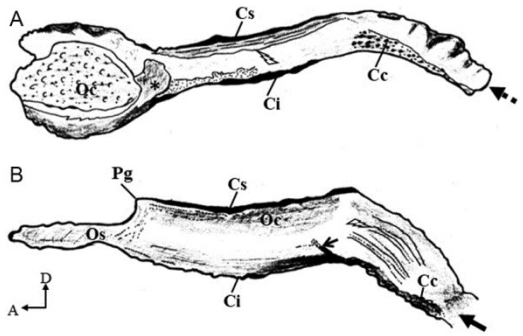

Fig. 3 —Diagrammatic comparisons of the sulcus characteristics of the sagitta between *Trichogaster fasciata* and *Anabas testudineus.* A, anterior side; C: cauda; Cc: caudal colliculum; Ci: cristae inferior; Cs: cristae superior; D, dorsal side; Oc: ostial colliculum; Os: ostium; Pg: peg-like structure of cristae superior; Black arrow head,virgule-like mark; Thick arrow, caudal fork; Zig-zag arrow, growth stripes; Asterisk, shallow depression; bold arrow head, caudal bulb.

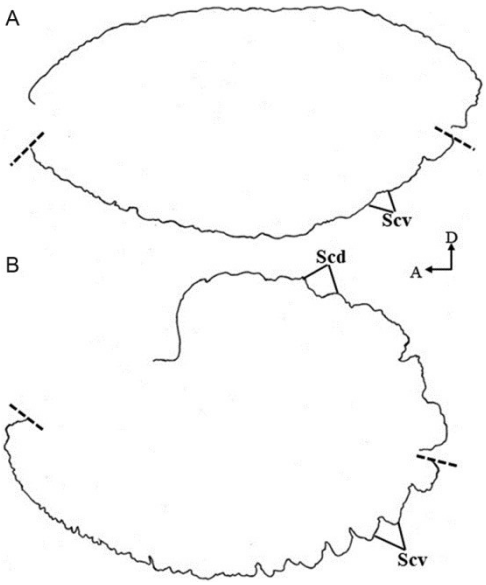

Fig. 4 —Schematic variations of the dorsal and ventral side of margins of the sagitta of *Trichogaster fasciata* and *Anabas testudineus.* A, anterior side; D, dorsal side; Scd, scallop in dorsal margin; Scv, scallops in ventral margin

in length than that of the ventral margin (Figs. 2A & B). The scallops in the dorsal and ventral margins are prominent, but their numbers in the ventral margin are relatively higher than that of the dorsal margin (Figs. 2A-D and 4B). The sulcus is slightly 'J'-shaped, supra-medial, heterosulcoid, and ostio-caudal type structure (Figs. 2A & B and 3B). The collum is absent in the sulcus but the ostium and the cauda are demarcated by a distinct virgule-like mark at the anterior end of the cauda (Figs. 2B and 3B). Both the dorsal and ventral cristae are well-developed. The ostium (0.13 ± 0.01 × 0.03 ± 0.01 mm) is tubular and it opens into the excisura major. The cauda (0.07 ± 0.01 × 0.03 ± 0.01 mm) is also tubular, slightly curve and it opens into the caudal fork. The rostrum is well-developed, while anti-rostrum is poorly developed (Figs. 2A-C and Table 1). The dorsal depression is well-developed and oval shaped. The ventral depression is shallow. A circular-patch is found beneath the rostrum, is described as the ortho-rostrum patch in the present study and it is comprised of some irregular elevations on its floor (Figs. 2A-C). Some growth strips are prominently observed on the wall of the ortho-rostrum patch, and the sulcus groove. The surface sculpture of the dorsal and ventral part of the inner surface contains numerous hexangular, blunt headed, stony pillars that are relatively higher in number in the ventral part than those of the dorsal part (Figs. 2A, B, D and Table 1).

Variations of the sagittae in the two fishes

The morphostructural features of the sagitta in the *A. testudineus* and *T. fasciata* are varies significantly in respect of their otolith shape, size, and the surface sculpture (Figs. 1A-E, 2A-D, and Tables 1-2). The sulcus in the *A. testudineus* contains a spoon-headed ostium which is separated from the cauda by a prominent constriction (Figs. 1A-C and 3A). On the other hand, the sulcus of the *T. fasciata* is comprised of a prominent virgule-like development which performed as a demarcation between the ostium and cauda (Figs. 2A & B, 3B). It is found that the sculpture along with the margin as well as the dorsal and ventral part of the sagittae inner surface is significantly varies between the fish species (Figs. 1B-E, 2B-D, and Table 1). Additionally, the morphometric measurements of the different sagitta constituents between two fishes are showing their species-specific developmental variations (Figs. 1A-E, 2A-D, and Table 2).

The morphological variations of the major sagitta constituents in *A. testudineus, T. fasciata* and four others freshwater *Anabantiformes* fishes (*Channa* spp) are documented here (Table 1). A phylogenetic tree on the sagitta morphostructural characteristics between seven *Anabantiformes* fishes, *i.e.*, *A. testudineus, T. fasciata, T. lalia, C, limbata, C, lucius, C, micropeltes, C, striata* and a Perciformes fish (*i.e.*, *Perca fluviatilis*) is drawn here (Fig. 5 and Table 3). It is found that the *A. testudineus* (Anabantidae, *Anabantiformes*) is closely related to some extend to the *Channa* sp (Channidae, *Anabantiformes*) than that of the *T. fasciata* (Osphronemidae, *Anabantiformes*) (Fig. 5, Table 3, and Suppl. Table 1).

Table 1 — Relative characteristics of the sagitta morpho-anatomical features between *Anabas testudineus* and *Trichogaster fasciata* and five other *Anabantiformes* fishes (*Trichogaster lalia, Channa limbata, Channa lucius, Channa micropeltes,* and *Channa striata*) based on the photographs and descriptions. [1]Banu and Sirajuddin, 2020; [2]Krainara and Wunnitikul, 2004

Sagitta features	*A. testudineus*	*T. fasciata*	*T. lalia* [1]	*C. limabata*[2]	*C. lucius*[2]	*C. micropeltes*[2]	*C. striata*[2]
Thickness of sagitta	Thick	Moderately thick	Moderately thick	Thick	Thick	Thick	Thick
Demarcation between the ostium and cauda	A constriction at posterior end of the ostium	Virgule-like structure at the junction of ostium and cauda development	Virgule-like structure at the junction of ostium and cauda	Constriction at posterior end of the ostium	Constriction at posterior end of the ostium	Constriction at posterior end of the ostium	Constriction at posterior end of the ostium
Shape of sagitta	Ovate, nearly flat medial surface, slightly depressed	Sub-ovate, convex medial surface and concave outer surface	Sub-ovate, convex medial surface and concave outer surface	Ovate	Ovate	Elliptical	Oblong
Sulcus location	Supra-medial	Supra-medial	Supra-medial	Median	Median	Median	Median
Shape of sulcus	S-shaped	J-shaped	J-shaped	Semi "S"-shaped	Semi "S"-shaped	"S"-shaped	"J"-shaped
Dorsal margin	Rounded, crenate	Partially lobate	Smooth	Sinuate	Crenate	Dentate	Crenate
Ventral margin	Oval, sinuate	Crenate but some stretch of the anterior part is entire	Smooth	Lobed	Sinuate	Serrate	Crenate
Rostrum	Well-developed, straight, pointed	Well-developed, bent, pointed	Well-developed, bent, pointed	Well-developed, pointed	Well-developed, pointed	Well-developed, pointed	Well-developed, pointed
Anti-rostrum	Well-developed, pointed	Ill-developed, blunt	Ill-developed, blunt	Short, pointed	Short, pointed	Short, pointed	Short, pointed
Post-rostrum	Ovate margin with 2 wider short scallops	Crenate margin with 2-3 elongated scallops	Smooth margin with 2 short scallops	Lobed margin with 2 short scallops	Sinuate margin with 2 scallops	Serrate margin with 2-3 short scallops	Smooth margin lacks scallops
Para-rostrum	Rounded margin with 4-5 very short scallops	Lobate margin with 2-3 short scallops	Smooth margin with 1-2 short scallops	Sinuate margin with few short scallops	Crenate margin with many short scallops	Dented with narrow scallops	Crenate with few very scallops
Exisura major	Deep, "V"-shaped	Deep, "W"-shaped	Shallow, "W"-shaped	Wider with shallow notch	Wide & shallow	Narrow, shallow notch	Narrow, notched acute angle
Exisura minor	Small, "U"-shaped	Absent	Absent	Absent	Absent	Absent	Absent
Ostium	Spoon-headed	Tubular, anterior half is half-tubular	Tubular, anterior half is half-tubular	Oval, depressed	Rhomboidal	Tear-drop	Oblong
Cauda	Tubular with caudal bulb	Tubular with caudal fork	Tubular with caudal fork	Tubular, leaned down at posterior, caudal bulb present	tubular, leaned down at posterior, caudal bulb present	Curved at posterior end, caudal bulb present	Bent posteriorly, caudal bulb present
Cristae superior	Developed	Well-developed	Well-developed	Developed	Developed	Developed	Developed
Cristae inferior	Developed	Well-developed	Well-developed	Developed	Developed	Developed	Developed
Collum	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Colliculum							
in ostium	Well-developed	Well-developed	Developed	?	?	?	?
in cauda	Well-developed	Well-developed	Developed	?	?	?	?

Table 2 —Morphometry (mean ± SD in mm) of various sagitta features of *Anabas testudineus* and *Trichogaster fasciata**, absent of the features

Sagitta features	*A. testudineus*	*T. fasciata*
Sagitta length	5.92 ± 0.01	0.29 ± 0.001
Sagitta width	2.9 ± 0.02	0.2 ± 0.001
Sulcus length	5.5 ± 0.03	0.2 ± 0.001
Sulcus width	0.5 ± 0.003	0.03 ± 0.001
Ostium length	1.84 ± 0.02	0.13 ± 0.001
Ostium width	1.27 ± 0.001	0.03 ± 0.0001
Cauda length	3.65 ± 0.02	0.07 ± 0.001
Cauda width	0.43 ± 0.02	0.03 ± 0.001
Rostrum-postrostrum length	4.99 ± 0.09	0.28 ± 0.001
Antirostrum-pararostrum length	5.8 ± 0.03	0.19 ± 0.001
Rostrum-pararostrum length	5.53 ± 0.01	0.29 ± 0.001
Antirostrum-postrostrum length	5.45 ± 0.02	0.2 ± 0.001
Orthorostrum patch diameter	*	0.05 ± 0.001

Discussion

The analysis of the morphostructural features of the sagitta otolith has the capability to assess the taxonomic peculiarities in the fish species. It is well-known that the morphological descriptions of the sagitta with only light microscopy are not sufficient to describe the species-specific otolith characteristics in detail of a fish species[7,12]. The morphostructural variations of the sagitta inner surface in the *Anabas testudineus* and *Trichogaster fasciata* are described by light microscope and scanning electron microscope in the present study. There are much more significant structural peculiarities of the sagitta of these two fishes as they are belonging to the same taxon (Order *Anabantiformes*).

The sagitta of the *A. testudineus* possess a complete dorsal and ventral margin whereas in the *T. fasciata*

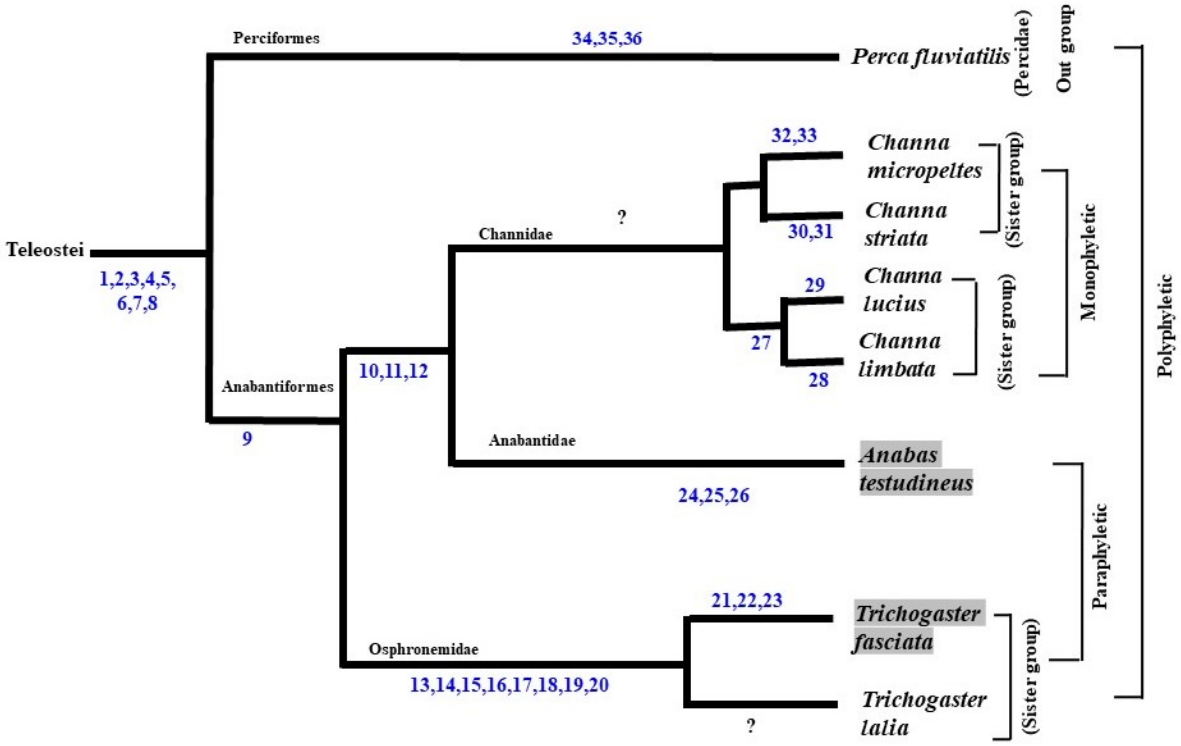

Fig. 5 — A schematic dendrogram on the sagitta morphostructural features in the *Anabas testudineus*, *Trichogaster fasciata* and 4 other *Anabantiformes* fishes and a perciformes fish. The tree showing the relatedness among the species along with the sagitta features. *Channa lucius* and *Channa limbata*; *Channa micropeltes* and *Channa striata* are two separate sister groups in the Channidae family due to some species-specific derived sagitta features. The fishes in the Anabantidae and the Channidae families are shared some sagitta features (10-12) and they have a common ancestor and support a monophyletic relationship. The *A. testudineus* and two *Trichogaster* species possess a paraphyletic relationship by their derived sagitta features from the recent common ancestor. As *T. fasciata* and *T. lalia* belongs to the Osphronemidae family and both are maintained a monophyletic relationship but *T. fasciata* is strongly separated from the *T. lalia* by three autapomorphic sagitta features (21-23). Highlighted species are the studied species in the present work. It is found that some of the sagitta features are significantly related to some extend in the fishes of the Anabantidae and Channidae families and the Percidae family. The showing that all these fish families have a common ancestor and Channidae and Anabantidae are somehow taxonomically closely related to the Perciformes fishes by some specific plesiomorphic characteristics. The blue coloured numbers indicate the serial number of the sagitta features in the Table 3

Table 3 — This table shows the presence (1) or absence (0) of the sagitta characteristics of some *Anabantiformes* fishes (*Anabas testudineus, Trichogaster fasciata, Trichogaster lalia, Channa limbata, Channa lucius, Channa micropeltes,* and *Channa striata*) and a perciformes fish (*Perca fluviatilis*), based on the description and published photographs.
[1]Banu and Sirajuddin, 2020; [2]Krainara and Wunnitikul, 2004; [3]Yilmaz *et al*., 2014

Sl. No.	Sagitta features	*A. testudineus*	*T. fasciata*	*T. lalia*[1]	*C. limbata*[2]	*C. lucius*[2]	*C. micropeltes*[2]	*C. striata*[2]	*P. fluviatilis*[3]
1	Fresh water habitat	1	1	1	1	1	1	1	1
2	Dorsal depression	1	1	1	1	1	1	1	1
3	Ostio-pseudocaudal sulcus	1	0	0	1	1	1	1	1
4	Median sulcus	1	0	0	1	1	1	1	1
5	Constriction at the junction of the ostium and cauda	1	0	0	1	1	1	1	1
6	Ovate-shaped sagitta	1	1	1	1	1	0	0	0
7	Thick sagitta	1	0	0	1	1	1	1	1
8	Well-developed anti-rostrum	1	0	0	1	1	1	1	1
9	Curved cauda	1	1	1	1	1	1	1	0
10	Caudal bulb	1	0	0	1	1	1	1	1
11	"S"-shaped sulcus	1	0	0	0	0	1	0	0
12	Moderately-developed marginal scallops	1	0	0	1	1	1	1	1
13	Supra-median sulcus	0	1	1	0	0	0	0	0
14	"J"-shaped sulcus	0	1	1	0	0	0	1	0
15	Caudal fork	0	1	1	0	0	0	0	0
16	Ostio-caudal sulcus	0	1	1	0	0	0	0	0
17	Orthorostrum patch	0	1	1	0	0	0	0	0
18	Tubular ostium	0	1	1	0	0	0	0	0
19	Moderately thick sagitta	0	1	1	0	0	0	0	0
20	Ill-developed anti-rostrum	0	1	1	0	0	0	0	0
21	Virgule-like mark between the ostium and cauda	0	1	0	0	0	0	0	0
22	Blunt headed, hexangular stony pillars in surface sculpture	0	1	?	?	?	?	?	0

(*Contd*.)

Table 3 — This table shows the presence (1) or absence (0) of the sagitta characteristics of some *Anabantiformes* fishes (*Anabas testudineus, Trichogaster fasciata, Trichogaster lalia, Channa limbata, Channa lucius, Channa micropeltes,* and *Channa striata*) and a perciformes fish (*Perca fluviatilis*), based on the description and published photographs. [1]Banu and Sirajuddin, 2020; [2]Krainara and Wunnitikul, 2004; [3]Yilmaz *et al*., 2014 (*Contd.*)

Sl. No.	Sagitta features	*A. testudineus*	*T. fasciata*	*T. lalia*[1]	*C. limbata*[2]	*C. lucius*[2]	*C. micropeltes*[2]	*C. striata*[2]	*P. fluviatilis*[3]
23	Well-developed marginal scallops	0	1	0	0	0	0	0	0
24	Rectangular headed, tetrangular stony pillars in surface sculpture	1	0	?	?	?	?	?	0
25	Ventral depression	1	0	?	0	0	0	0	0
26	Spoon-headed ostium	1	0	0	0	0	0	0	0
27	Semi-"S"-shaped sulcus	0	0	0	1	1	0	0	0
28	Oval ostium	0	0	0	1	0	0	0	0
29	Rhomboidal ostium	0	0	0	0	1	0	0	0
30	Oblong ostium	0	0	0	0	0	0	1	0
31	Oblong sagitta	0	0	0	0	0	0	1	1
32	Elliptical sagitta	0	0	0	0	0	1	0	0
33	Tear drop-like ostium	0	0	0	0	0	1	0	0
34	Flower vase-shaped ostium	0	0	0	0	0	0	0	1
35	'Y'-shaped sulcus	0	0	0	0	0	0	0	1
36	Straight cauda	0	0	0	0	0	0	0	1

the dorsal margin is incomplete. The scallops morphologies along with the sagitta margins in the *T. fasciata* are much more related to the *T. lalia* to some extent than those of the *A. testudineus* (Anabantidae, *Anabantiformes*). The sulcus, surface and marginal sculptures of the *A. testudineus* are varies with those of the *T. fasciata,* characteristically much more species-specific like other fish species 13-15. The sagitta of the *A. testudineus* is thick with well-developed, pointed rostrum and antirostrum which are relatively alike to those in the *Channa* species (family Channidae) but they are under developed in the *Trichogaster* species (Table 1). It is assumed that according to the sagitta features the *A. testudineus* is more related to the members of *Channidae* family than that of the *Osphronemidae* family[16]. Additionally, the sulcus of the *Channa* spp and *A. testudineus* is an ostio-pseudocaudal type while it is ostio-caudal in the *Trichogaster* spp. The spoon-headed ostium in the *A. testudineus* and the orthorostrum patch in the *T. fasciata* are the important species-specific peculiarities and this feature supports to identify the fish species.

The *Anabas testudineus*[6,17], *Trichogaster fasciata*[18-20], and *Channa* spp (*C. gachua, C. lucius, C. micropeltes, and C. striata*)[16] are described earlier as Perciformes fishes in the freshwater, but according to the recent taxonomic reconstructions all these

fishes are belong to the Order *Anabantiformes*. On the other hand, the species name of the *Trichogaster lalia* (Banu and Sirajuddin, 2020) and *Channa limabata*[16] are changed to *Trichogaster lalius*[21] and *Channa gachua*[21], respectively, (Table 1). The phylogenetic tree on the relative sagitta features between the different families of the order *Anabantiformes* and a percidae fish (*Perca fluviatilis*)[22] of the perciformes order is stated that the family Percidae is very closely related to the family Anabantidae, and Channidae, than that of the Osphronemidae family (Fig. 5 and Table S1). It is assumed that all these fish families have a common ancestor and Channidae and Anabantidae are somehow taxonomically closely related to the Perciformes fishes by some specific plesiomorphic characteristics. It is also assumed that the structural morphologies of the sagitta may be supportive in taxonomic reconstruct among the fish groups.

The inner (or medial) surface of the sagitta is relatively well-decorated with various structural ingredients[1,5,6] than the outer (or lateral) surface[12]. The present study advocated that the morphostructural features of the inner surface of the sagitta have some important inter- and intra-specific relatedness and some species-specific apomorphic and autapomorphic features which helps to make a trophic as well as taxonomic relationship between the fish species. The detail sagitta features with their structural constitutions may help to predict many aspects of the fishes such as habitats, pray-predator relationship, systematics, fish stock analysis, etc. indirectly. The morphometric characteristics of the sagitta in the *A. testudineus* and *T. fasciata* possess a species-specific relationship and it is corroborated to the other fish species[23].

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Conflict of interest

All authors declare no conflict of interest.

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