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# Ultrastructure of the Sagitta Otolith in Different Body Size Groups of Climbing Perch *Anabas testudineus* (Anabantidae)

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**Abstract**—The ultrastructural characteristics of the sagitta of the climbing perch, *Anabas testudineus* (Bloch, 1792) in different body size groups are described for the first time for the family Anabantidae using scanning electron microscopy. The sagitta is an ovate-shaped structure with a well-developed rostrum and anti-rostrum. The sulcus is S-shaped and has ostio-pseudocaudal type structure. The sulcus lacks collum. The ostium is a spoon-headed structure and is comprised of a triangular ostial colliculum. The excisura major is a prominent V-shaped structure and its width declines along with the growth of the total fish length. The cristae are prominent on both sides of the sulcus. The caudal colliculum is relatively well-developed in the smaller size groups than that of larger groups. The posterior end of otolith is blunt. The Kruskal–Wallis H test revealed that some sagitta features show significant differences in different body size groups of *A. testudineus*. The otolith weight is more closely related to the body length than the otolith length and width. Therefore, otolith weight can be used as an important predictor to evaluate the fish size.

**Keywords:** Anabantidae, climbing perch *Anabas testudineus*, morphometry, otolith, sagitta, ultrastructure

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## INTRODUCTION

The sagitta otoliths of the fishes are larger than the other otoliths (i.e., asterisci and lapilli) except the members of the orders Siluriformes and Cypriniformes (Harvey et al., 2000; Yilmaz et al., 2015; Sanchez and Martinez, 2017; Jawad et al., 2018). The sagittae are developed with various morpho-structural ornamentations (i.e., shape, types of the sulcus, surface sculptures, etc.) which provided some important species-specific characteristics (Smale et al., 1995; Campana and Thorrold, 2001; Jawad et al., 2018; Vilizzi, 2018). It has been reported that the growth of the sagitta constituents is strongly related to the individual body length/weight (Harvey et al., 2000; Jawad et al., 2018) and is also influenced by the ecological factors (Omar and Moselhy, 2016; Omar and Mohamed, 2016; Pracheil et al., 2019). However, the morphological features of sagittae in freshwater Perciformes are more or less described (Nargis, 2010; Ahmed and Latifa, 2012; Bremm and Schulz, 2014; Omar and Moselhy, 2016; Gierl et al., 2018; Pracheil et al., 2019), but detail ultrastructural characteristics of the climbing perch are unknown. Additionally, the Anabantidae family possesses four genera (Rüber et al., 2006), but the information about otolith structures of its members is absent. The present study aim is to investigate detail ultrastructural characteristics of the sagitta in different body size groups of the climbing perch *Anabas testudineus*. A comparison of the sagitta structure with other freshwater Perciformes is also conducted.

## MATERIALS AND METHODS

A total of 125 individuals of *Anabas testudineus* were collected from the local markets at Kolkata, West Bengal, India. The specimens were identified by the Zoological Survey of India (ZSI), Kolkata, West Bengal, India. The specimens were divided into four groups according to total fish length (*TL*): group I (Gr-I), 8–9 cm ($8.57 \pm 0.35$), $n = 30$; group II (Gr-II), 10–11 cm ($10.55 \pm 0.25$), $n = 40$; group III (Gr-III), 12–13 cm ($12.55 \pm 0.35$), $n = 30$; group IV (Gr-IV), 14–15 cm ($14.57 \pm 0.29$), $n = 20$.

The pair of sagittae were removed from the individuals (Ruck, 1976; Jawad et al., 2018) cleaned with water and 70% ethanol and stored dry in individual plastic tubes. Scanning electron microscope (SEM) was used to investigate the ultrastructural characteristics on the medial (inner) surface of the right sagitta. The sagittae to be used for SEM were air dried and

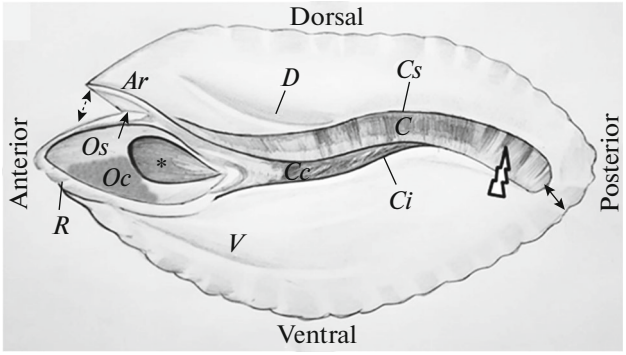


**Fig. 1.** Schematic diagram of the typical right sagitta of *Anabas testudineus* showing various characteristics in its medial surfaces. Here and in Fig. 2: ( ⟶ ) notch; ( ◂⋯▸ ) width of excisura major; ( ⫴ ) growth stripes; (↔) width between posterior caudal end and the postero-ventral margin; (*) bean-shaped impression. (*Ar*) anti-rostrum; (*C*) cauda; (*Cc*) caudal colliculum; (*Ci*) crista inferior; (*Cs*) crista superior; (*D*) dorsal depression; (*Oc*) ostial colliculum; (*Os*) ostium; (*R*) rostrum; (*V*) ventral depression.

mounted on an aluminium stub using double-sided carbon tape (Avigliano et al., 2016). When dry, the sagittae and stubs were gold coated by DWARDS, RV5 coater and were viewed in an EVO18, ZEISS.

According to the terminology of Smale et al. (1995) and Avigliano et al. (2016), a number of sagitta measurements have been documented. These were otolith length (mm), otolith width (mm), otolith weight (g), rostrum length (mm), anti-rostrum length (mm), sulcus length (μm), sulcus width (μm), sulcus depth (μm), ostium length (μm), ostium width (μm), ostial colliculum length (μm), ostial colliculum width (μm), cauda length (μm), cauda width (μm), caudal colliculum length (μm), caudal colliculum width (μm), width of excisura major (μm), width between posterior caudal end and the postero-ventral margin (μm), width between ventral margin to crista inferior (μm), and width between dorsal margin to crista superior (μm). The measurements (mean value ± SD) were taken for the sagittae from the four size groups using image-processing software "ImageJ 1.51t" (Wayne Rasband, NIH, USA). The weights of otolith were taken with a digital weight machine (Mettler Toledo ME204). A normality test was applied to test the distributions of the sagitta features. The Kruskal–Wallis H test was performed using XLSTAT statistical program to determine the significant differences of growth of the studied sagitta features between the four groups of *A. testudineus*.

## RESULTS

### *General Morphology of the Sagitta*

The sagitta of *A. testudineus* is an ovate-shaped, laterally concavo-convex structure. The medial surface is slightly convex. The dorsal and ventral margins are distinct, crenate shaped and sinuate shaped respectively (Figs. 1, 2). The sagittae possess well-developed rostrum and anti-rostrum structure. The medial surface of the otolith is well decorated with various morpho-anatomical features such as sulcus, ostium with ostial colliculum, cauda with caudal colliculum, dorsal depression, ventral depression, and different cristae on both sides of sulcus groove. The sulcus is a well-developed, S-shaped ostio-pseudocaudal type structure, which is comprised of a prominent, spoon-headed ostium and an elongated, curved cauda (Figs. 1, 2). The sulcus lacks collum. The ostium contains well-developed ostial colliculum which is more or less triangular. A bean-shaped impression is observed near the posterior end of the ostium. The cauda possesses an elongated caudal colliculum which is gradually smaller in width toward the posterior end. The dorsal depression of the medial surface of sagittae is more prominent than its ventral depression (Fig. 1).

### *Variations of the Sagitta Constituents in Different Size Groups*

The margin of both sides of the sagitta is gradually prominent in large size fishes (Fig. 2; Table 1). The development of different morpho-structural characteristics of the medial surface of the sagitta varied in different total fish length groups (Figs. 2–4; Tables 1, 2). A small deep notch is observed at the base of the anti-rostrum and it decreases with the increase of the total fish length (Fig. 2). Numerous small, smooth, teeth-shaped surface concretions are found on the surface and their development related to the increment of the total fish length (Fig. 2). These surface concretions are comparatively more distinct in the ventral side than those of the dorsal side (Figs. 2b–2d). The surface concretions in the smaller group (Gr-I) is indistinct, whereas the measurement of each tooth of the surface concretions in the group IV fishes is 0.19 ± 0.05 μm in length and 0.13 ± 0.05 μm in width (Fig. 2d).

The growth of the ostium length (*Osl*), width between crista inferior-ventral margin (*CriV*), ostial colliculum width (*Ocw*) are positively correlated with

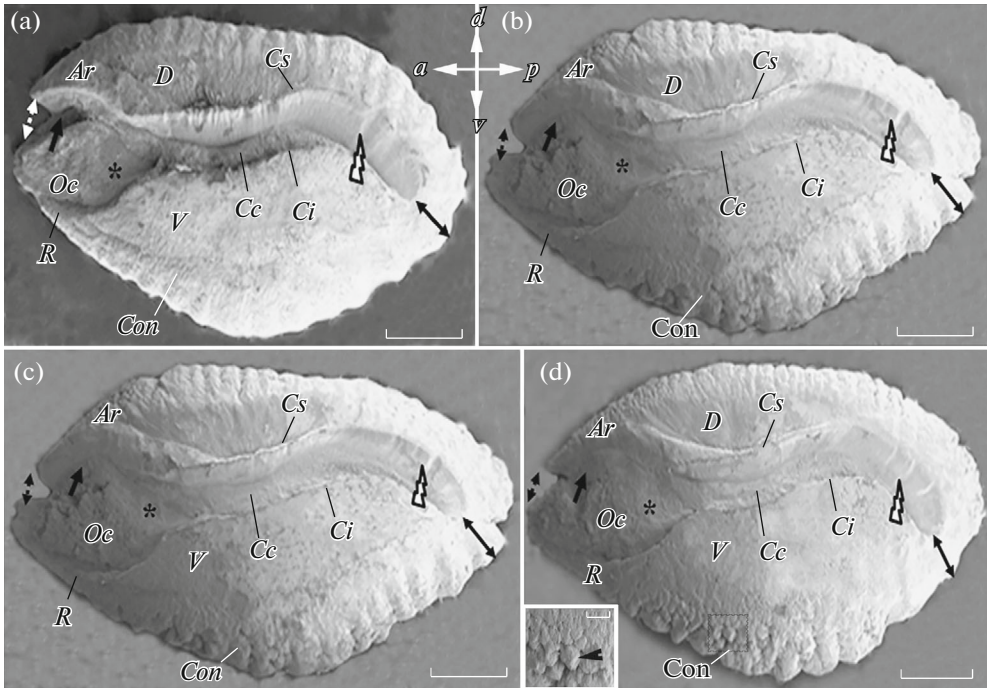


**Fig. 2.** SEM of medial surface of the right sagittae of four groups of *Anabas testudineus*: (a) Gr-I, well-developed spoon-headed ostium and caudal colliculum; (b) Gr-II, deep dorsal depression, pointed spoon-head and anti-rostrum; (c) Gr-III, reduced notch and spoon head, elongated rostrum; (d) Gr-IV, prominent growth stripes on the wall of cauda, deep dorsal depression, enlarged view of a part (black marked area) of the surface concretion is shown inset. (Arrow head) concreted structure, (*) bean-shaped impression (⟶) deep notch; (*A, D, P, V*) anterior, dorsal, posterior, ventral side; (*Con*) concretion. Scale bar: 500 μm for only the figure inset and 1mm for Figs. 2a, 2b, 2c and 2d.

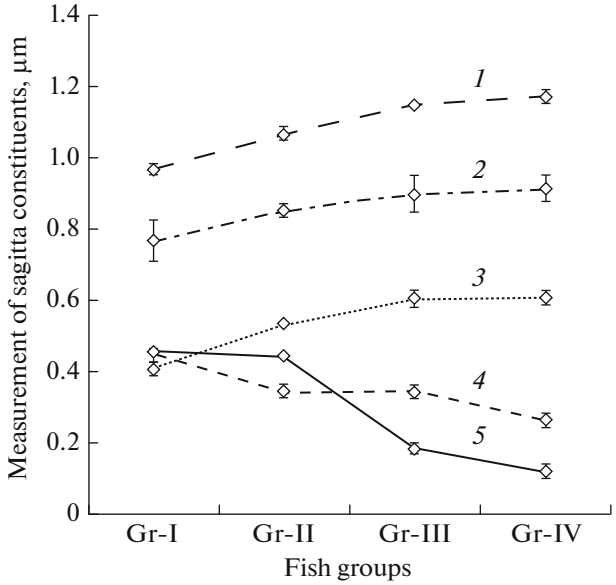


**Fig. 3.** Comparative relationships of some sagitta constituents in four groups of *Anabas testudineus*. Here and in Fig. 4: group I (Gr-I)—8–9 cm *TL*, group II (Gr-II)—10–11 cm *TL*, group III (Gr-III)—12–13 cm *TL*, group IV (Gr-IV)—14–15 cm *TL*. (*1*) ostium length (*Osl*), (*2*) width between ventral margin to crista inferior (*CriV*), (*3*) ostial colliculum width (*Ocw*), (*4*) width between dorsal margin to crista superior (*CrsD*), (*5*) width of excisura major (*ExW*).

**Table 1.** Sagitta characteristics in the four body size groups of *Anabas testudineus*

| Sagitta characteristics | Group | | | |
|---|---|---|---|---|
| | Gr-I | Gr-II | Gr-III | Gr-IV |
| Notch | Deep, clearly visible, promote in formation of wider gap between rostrum and anti-rostrum | Shallow | Reduced | Indistinct |
| Rostrum | Larger > Gr-II | Comparatively smaller | Larger > Gr-I | Largest |
| Anti-rostrum | Moderately developed | Moderately developed | Developed | Well-developed |
| Excisura major | Largest in size | Moderate | Smaller | Relatively very smaller |
| Outer margin | Poorly developed | Ventral margin relatively well developed than dorsal | Developed | Highly developed |
| Ostium | Well developed with prominent spoon-shaped head with a bean-shaped impression | Prominent, slightly degenerated dorsally | Reduced | Indistinct |
| Ostial colliculum | Developed | Well-developed | Developed | Well-developed |
| Cauda | Well-developed, with indistinct marking on the caudal wall near posterior end | Well-developed, with distinct marking on the caudal wall near posterior end | Well-developed, with indistinct marking on the caudal wall near posterior end | Well-developed, with indistinct marking on the caudal wall near posterior end |
| Caudal colliculum | Well developed, curved | Indistinct | Reduced | Absent |
| Width between posterior caudal end and the postero-ventral margin | Comparatively larger | Smaller | Smaller | Smaller |
| Dorsal side | Concave at median region | Slightly concave | Slightly concave | Oval |
| Dorsal depression | Shallow | Deep | Deep | Deep |
| Ventral depression | Developed | Ill-developed | Reduced | Highly reduced |
| Surface concretion | Poorly developed | Well-developed | Well-developed | Well-developed |

Here and in Table 2: group I (Gr-I)—8–9 cm *TL*, group II (Gr-II)—10–11 cm *TL*, group III (Gr-III)—12–13 cm *TL*, group IV (Gr-IV)—14–15 cm *TL*.

the total fish length, while the growth of the width between crista superior-dorsal margin (*CrsD*) and width of excisura major (*ExW*) is negatively correlated with the total fish length (Fig. 3). The caudal colliculum is very indistinct or absent in larger groups (Figs. 2c–2d; Table 2). The sagitta weight is more closely correlated with the total fish length than their length and width (Fig. 4). The normality test shows that most of the studied otolith features are normally distributed and many of them are significantly different in the four size groups (Table 2).

The relative variability of some major sagitta features of *A. testudineus* and some other freshwater Perciformes fishes is illustrated in Table 3. Many sagitta

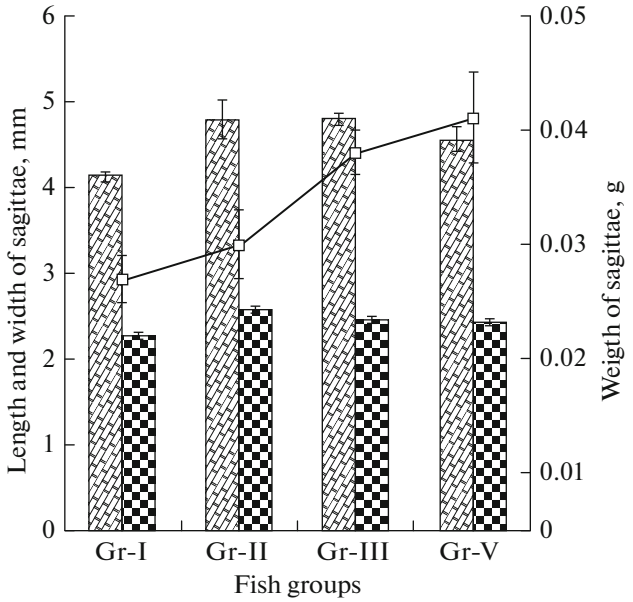


**Fig. 4.** Relationships between sagitta length ( ▨ ), width ( ■ ), weight ( —□— ) and total body length in four groups of *Anabas testudineus.*

features such as general shape, sulcus shape, ostium shape, surface concretions, excisura major, and outer margins in *A. testudineus* substantially differ from those of other freshwater Perciformes fishes.

## DISCUSSION

The ultrastructural characteristics of the medial surface of the sagittae of *A. testudineus* are described for the first time in the Anabantidae fishes. Several sagitta morphological features of *A. testudineus* differ (Table 3) from those of other freshwater Perciformes (Ahmed and Latifa, 2012; Bremm and Schulz, 2014; Omar and Moselhy, 2016; Gierl et al., 2018). The sagitta of *A. testudineus* is an ovate shaped structure which varies among the Perciformes fishes both freshwater (Ahmed and Latifa, 2012; Bremm and Schulz, 2014; Omar and Moselhy, 2016; Gierl et al., 2018) and marine (Jawad, 2007; Kontas and Bostanci, 2015; Jawad et al., 2018). The medial surface of the sagitta of *A. testudineus* comprises a well-developed sulcus as it is reported in other fishes (Dehghani et al., 2016; Jawad et al., 2018; Khedher and Fatnassi, 2018), and this groove may help connecting the medial surface with the sensory macula of the internal ears (Popper and Hoxter, 1981; Popper and Lu, 2000).

The development of the sagitta characteristics of *A. testudineus* is varied between the smaller and larger groups as reported in other fishes (Jawad et al., 2018). It is assumed that the development of sagitta constituents in different body size groups might be associated with deposition of calcium carbonate crystals (Campana and Thorrold, 2001; Kontas and Bostanci, 2015; Vilizzi, 2018). Some researchers stated that the correlation of the total fish length with the otolith length is stronger than that of the total fish length with the otolith weight (Campana, 1990; Lombarte et al., 1991; Lombarte and Lleonart, 1993; Zorica et al., 2010, Skeljo and Ferri, 2012; Kontas and Bostanci, 2015), whereas the Kruskal–Wallis test in the present study revealed that the growth of the sagitta weight in *A. testudineus* more strongly correlate with the growth of total fish length than the sagitta length and width. Since the otolith weight is much easier estimate than the otolith length and width (Gümüs et al., 2007; Gümüs and Kurt, 2009; Bostanci, 2009; Kontas and Bostanci, 2015) it might be used as an important predictor to evaluate the fish size (Yilmaz et al., 2014; Dehghani et al., 2016; Khedher and Fatnassi, 2018).

In the present study, the ostio-pseudocaudal sulcus with heterosulcoid type, heteromorph colliculum of *A. testudineus* is a feature shared by several freshwater and marine Perciformes (Table 3) supporting their taxonomic relatedness (Smale et al., 1995; Ahmed and Latifa, 2012; Bremm and Schulz, 2014; Omar and Moselhy, 2016; Gierl et al., 2018; Jawad, 2007; Schwarzhans, 2014; Jawad et al., 2018). The morphometry of the excisura major, ostium, ostial colliculum, and width between crista inferior-ventral margin show significant differences in the smaller and larger body size group of *A. testudineus* whereas the other studied sagitta features do not show any significant differences between these groups. The development of the spoon-headed ostium in the sagittae and its strong correlations with the total fish length is important for systematics of *A. testudineus*. The growth of the caudal colliculum of the sagitta of *A. testudineus*

**Table 2.** Morphometry (mean value ± SD) of different sagitta constituents in the four body size groups of *Anabas testudineus*

| Sagitta features | Group | | | |
|---|---|---|---|---|
| | Gr-I , $n = 30$ | Gr-II, $n = 40$ | Gr-III, $n = 30$ | Gr-IV, $n = 20$ |
| Otolith length, mm | $4.12 \pm 0.06^{a}$ | $4.78 \pm 0.25^{b}$ | $4.79 \pm 0.05^{b}$ | $4.55 \pm 0.14^{a, b}$ |
| Otolith width, mm | $2.26 \pm 0.04^{a}$ | $2.54 \pm 0.05^{b}$ | $2.45 \pm 0.04^{a, b}$ | $2.42 \pm 0.04^{a, b}$ |
| Otolith weight, g | $0.02 \pm 0.002^{a}$ | $0.03 \pm 0.003^{a, b}$ | $0.03 \pm 0.002^{b, c}$ | $0.04 \pm 0.004^{c}$ |
| Sulcus length, μm | $2.83 \pm 0.05^{a}$ | $3.25 \pm 0.01^{b}$ | $3.19 \pm 0.03^{a, b}$ | $3.22 \pm 0.03^{a, b}$ |
| Sulcus width, μm | $0.29 \pm 0.02^{a}$ | $0.28 \pm 0.01^{b}$ | $0.35 \pm 0.02^{a, b}$ | $0.37 \pm 0.02^{a, b}$ |
| Sulcus depth, μm | $0.18 \pm 0.01^{b, c}$ | $0.11 \pm 0.01^{c}$ | $0.08 \pm 0.01^{a}$ | $0.12 \pm 0.02^{a, b}$ |
| Rostrum length, μm | $0.60 \pm 0.03^{a}$ | $0.56 \pm 0.04^{a}$ | $0.91 \pm 0.08^{a, b}$ | $1.07 \pm 0.04^{b}$ |
| Anti-rostrum length, μm | $0.49 \pm 0.06^{a, b}$ | $0.49 \pm 0.04^{a, b}$ | $0.45 \pm 0.03^{a}$ | $0.68 \pm 0.02^{b}$ |
| Width of excisura major, μm | $0.45 \pm 0.01^{b}$ | $0.44 \pm 0.001^{a}$ | $0.18 \pm 0.015^{a, b}$ | $0.12 \pm 0.02^{a}$ |
| Ostium length, μm | $0.96 \pm 0.01^{a}$ | $1.06 \pm 0.02^{a, b}$ | $1.14 \pm 0.01^{b}$ | $1.17 \pm 0.02^{b}$ |
| Ostium width, μm | $0.68 \pm 0.02^{a}$ | $0.75 \pm 0.02^{a, b}$ | $0.86 \pm 0.03^{b}$ | $0.72 \pm 0.02^{a, b}$ |
| Ostial colliculum length, μm | $0.87 \pm 0.01^{a, b}$ | $1.03 \pm 0.04^{b, c}$ | $0.83 \pm 0.02^{a}$ | $1.12 \pm 0.02^{c}$ |
| Ostial colliculum width, μm | $0.41 \pm 0.02^{a}$ | $0.53 \pm 0.001^{a, b}$ | $0.60 \pm 0.02^{b}$ | $0.61 \pm 0.01^{b}$ |
| Cauda length, μm | $1.88 \pm 0.37^{a}$ | $2.15 \pm 0.01^{b}$ | $1.87 \pm 0.03^{a}$ | $2.06 \pm 0.02^{a, b}$ |
| Cauda width, μm | $0.28 \pm 0.01^{a, b}$ | $0.26 \pm 0.005^{a}$ | $0.29 \pm 0.002^{b, c}$ | $0.31 \pm 0.01^{c}$ |
| Caudal colliculum length, μm | $1.23 \pm 0.05^{a, b}$ | $1.33 \pm 0.04^{b}$ | $0.77 \pm 0.05^{a}$ | * |
| Caudal colliculum width, μm | $0.13 \pm 0.01^{a, b}$ | $0.21 \pm 0.01^{b}$ | $0.06 \pm 0.01^{a}$ | * |
| Width between posterior caudal end and the postero-ventral margin, μm | $0.24 \pm 0.01^{a, b}$ | $0.20 \pm 0.01^{a}$ | $0.20 \pm 0.02^{b}$ | $0.19 \pm 0.02^{a}$ |
| Width between crista superior-dorsal margin, μm | $0.45 \pm 0.01^{b}$ | $0.34 \pm 0.02^{a, b}$ | $0.32 \pm 0.02^{a, b}$ | $0.26 \pm 0.02^{a}$ |
| Width between crista inferior-ventral margin, μm | $0.76 \pm 0.06^{a}$ | $0.85 \pm 0.02^{a, b}$ | $0.89 \pm 0.05^{b}$ | $0.91 \pm 0.04^{b}$ |

* Absence of the structures in the group; the same superscript letters mean the absence of significant differences between the groups.

is significantly reduced with the increment of the total fish length.

Since the most of the sagitta features in *A. testudineus* remarkably differ from those of other freshwater Perciformes they can be useful in taxonomic and trophic studies (Avigliano et al., 2016). The growth of the various morpho-structural features of the sagittae and their relationships to the body size of *A. testudineus* may be convenient for future studies of the otolith of other Anabantidae fishes.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interest.* The authors declare that they have no conflict of interest.

**Table 3.** Relative characteristics of the sagittae of *Anabas testudineus* and some other freshwater Perciformes based on published figures and descriptions

| Sagitta features | *Anabas testudineus* | *Oreochromis niloticus*[1] | *Micropterus salmoides*[2] | *Australoheros facetus*[2] | *Glossogobius giuris*[3] | *Pomatoschistus marmoratus*[4] | *Rhinogobius zhoui*[4] |
|---|---|---|---|---|---|---|---|
| Shape | Ovate | Ovoid | Elliptic | Oval | Oval | Circular | Discoid |
| Rostrum | Broad, pointed | Broad, blunt | Broad, blunt | Broad, blunt | Short, blunt | Indistinct | Indistinct |
| Antirostrum | Broad, pointed | Short, pointed | Broad, pointed | Short, bunt | Broad, blunt | Indistinct | Indistinct |
| Excisura major | Well developed | Well developed | Absent | Absent | Well developed | Absent | Absent |
| Excisura minor | Absent | Poorly developed | Poorly developed | Developed | Absent | Poorly developed | Poorly developed |
| Sulcus shape | S-shaped | Curved, wave like | Straight in 2/3 of the anterior part | Cone-shaped | Almost straight | Shoe-sole shaped | Slender, pointed |
| Sulcus type | Ostio-pseudocaudal | Ostio-pseudocaudal | Ostio-pseudocaudal | Ostio-pseudocaudal | Ostio-caudal | Mesial | Mesial |
| Ostium | Spoon headed with a bean-shaped impression | Narrow | Cap like | Narrow, V-shaped, triangular | V-shaped | Triangular | Poorly developed, pointed anteriorly |
| Cauda | Well-developed, bent posteriorly | Well-developed, bent posteriorly | Well-developed, bent posteriorly | Poorly developed | Indistinct (?) | Well-developed, straight | Poorly developed |
| Dorsal margin | Rounded, crenate | Pointed, serrated | Irregular | Lobed | Smooth | Smooth | Smooth |
| Ventral margin | Oval, sinuate | Pointed, serrated | Irregular | Sinuate | Serrated | Smooth | Smooth |
| Dorsal depression | Deep, wider | Absent | Present | Present | Absent | Deep, wider | Deep, triangular |
| Surface concretion | Concreted Very well | Indistinct | Smooth | Indistinct | Smooth | Smooth | Smooth |

Described by: [1]Omar and Moselhy, 2016; [2]Bremm and Schulz, 2014; [3]Ahmed and Latifa, 2012; [4]Gierl et al., 2018; (?) not adequately described.

*Statement on the welfare of animals.* All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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