

Research Article

Coincident use of fish taxonomy with sagitta and asteriscus otoliths to improve the identification of ponyfishes (Family: Leiognathidae)

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Keywords

Leiognathidae,
Discriminant function analysis,
Otolith morphology,
Species identification,
Northern Arabian Sea,
Taxonomy

Abstract

Despite their small size, ponyfishes play a significant role in fisheries, both as catches and bycatches. However, their diversity remains poorly understood due to their similar appearances and limited knowledge. This study evaluates integrating body morphometrics with quantitative and morphological analysis of both sagittal and asteriscus otolith can provide reproducible diagnostic traits that resolve species-level ambiguities and clarify generic placement. Using discriminant function analysis (DFA) of truss network landmarks and otolith shape parameters, we examined 556 specimens representing 15 species from the northern Arabian Sea, including five new regional records. Furthermore, the statistical analysis of DFA revealed strong discriminatory power among genera, with the first four discriminant functions explaining 62.3%, 11.4%, 8.9%, and 6.1% of the variance (eigenvalues 33.85, 6.20, 4.84, and 3.29, respectively). Moreover, morphometrical study of otoliths showed the genera formed distinct clusters, and otolith characters provided consistent, complementary support: *Aurigequula equula* exhibited the largest sagittal otoliths (mean length 69.29 mm) with pronounced rostra (~15.5 mm) and deep notches (~16.6 mm), whereas members of *Deveximentum* had the smallest otoliths (2.51–4.15 mm) with markedly shorter rostra and shallower notches. Sagittal and asteriscus otoliths provided complementary diagnostic features, with asteriscus morphology offering novel resolution at species level. Collectively, these results indicate that three species from genus *Leiognathus* reassigned to their original generic placements under current nomenclatural standards. These findings demonstrate that otolith-based morphometrics, when combined with traditional taxonomy, can strengthen systematic frameworks, improve reproducibility in species identification, and contribute to ecological and evolutionary studies of Leiognathidae.

Article info

Received: February 2026

Accepted: June 2026

Published: July 2026



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Introduction

Precise and reliable fish species identification is a prerequisite for robust biological, chemical, and physical investigations of fish populations. Misidentification may result in distorted trends in stock assessments that fail to represent true population dynamics. To augment the taxonomic description of fishes' various approaches have been used to minimize ambiguity. The aforementioned challenges such as fish morphology, meristic, length-weight, and molecular analysis adequately resolve species discrimination and determine such ambiguities (Caillon *et al.*, 2018; Jiang *et al.*, 2022). However, within the Leiognathidae family, the genus *Leiognathus* presents considerable taxonomic indistinctness among species that are yet to be streamlined. Despite efforts by Jones (1985), and Chakrabarty *et al.* (2009), species distinctions still remain unclear. Morphology together with the osteological parameters of the *Nuclepoma* genus were used to minimize such ambiguities (Chakrabarty and Spark, 2007; Wu *et al.*, 2010). For this purpose, we add morphometric, landmark, truss network, and otolith (sagitta and asteriscus) shape parameters for the accurate identification of complex species in the Leiognathidae family.

Ponyfishes are vital components of marine ecosystems, occupying crucial positions within the food web (Kashani *et al.*, 2025 a, b). Their distribution and diversity exhibit significant variations across different ecosystems and geographical locations. The inconsistencies within the scientific literature concerning this family highlight

the significant taxonomic ambiguities that currently exist. For example, Sparks and Chakrabarty (2015) reported 80 species, whereas Wu *et al.* (2010), Sharifuzzaman *et al.* (2021), Froese and Pauly (2025), and WoRMS, (2025) documented only 54 species globally. Despite decades of research, only 26 species have gained widespread acceptance among fish biologists (Jones, 1985; Woodland *et al.*, 2001; Kimaru *et al.*, 2003; Chakrabarty *et al.*, 2010a; Sparks and Chakrabarty, 2015; Suzuki and Kimura, 2017). Regional contributors such as (Abraham *et al.*, 2011) recorded 16 species in Indian waters, 12 species in Bangladesh (Sharifuzzaman *et al.*, 2021), 12 species from the Persian Gulf and Oman Sea (Valinassab, 2013), 13 species from Taiwan and Sri Lanka (Chakrabarty *et al.*, 2009, 2010a), and merely 8 species recorded in Pakistan (Qamar *et al.*, 2017).

Sagittal otoliths are widely used for fish discrimination (Quigley *et al.*, 2023; Takahashi *et al.*, 2023) but within the family-level taxonomy, they have not yet been used. Sagitta, the largest pair of fish otoliths found exclusively in bony fishes with a few exceptional cases such as Cypriniformes and Siluriformes (Yilmaz *et al.*, 2015). Asterisci, on the other hand, are a minute pair of otoliths that are difficult to handle during fish dissections but possess useful information for species discrimination. The third pair of otoliths, the lapilli is the smallest and is highly challenging to handle owing to its minuscule size. In comparison to the use of fish otoliths which are extremely species specific, cost-effective, simple, and reliable for species recognition, rather than the

development of molecular markers, which may be costly and time-consuming. Therefore, they are widely used for paleontological studies, accurate identification, and assessment of age, growth, and ecological interactions, as they remain intact and provide crucial insights (Vignon and Morat, 2010; Wiff *et al.*, 2020; D'Iglio *et al.*, 2021; Akbay *et al.*, 2022; Dunlop *et al.*, 2023). Typically, traditional morphology can adequately describe species, but the *Leiognathus* and *Secuter* genera of the family Leiognathidae require integrated data to outline species-specific variability. Therefore, this study tests the hypothesis that combined analysis of sagittal and asteriscus otoliths, alongside body morphometrics, can discriminate ponyfish species more effectively than traditional approaches alone. Specifically, we evaluate whether otolith traits can clarify generic placement of species currently assigned to *Leiognathus*, and whether these traits reveal broader ecological or evolutionary patterns. By applying discriminant function analysis to specimens from the northern Arabian Sea, we aim to strengthen the systematic foundation of Leiognathidae taxonomy and highlight the ecological significance of otolith-based diagnostics.

Materials and methods

Fish sampling and species identification

A total of eight study sites in the northern region of the Arabian Sea were randomly selected for sampling. A major portion samples were collected from commercial fish landing sites, such as Keti Bunder, Korangi, Karachi Fish Harbor, Hawks Bay, Sonmiani, Pasni, Gwadar, and Jiwani along

the coastal belt of Pakistan, whereas some samples were collected from fishery-independent surveys of Hawks Bay, Karachi. The fish were mainly caught as bycatch by medium-sized commercial boats using gill nets with 2.5 cm mesh size and trawl nets (3.8 avg knots). A random sampling protocol was adopted while the fish were immediately transferred to an icebox and transported to the fisheries laboratory at the Centre of Excellence in Marine Biology, University of Karachi for further analysis. A gross taxonomic investigation, comprising measurements of lengths (cm), weights (g), and meristic counts, was conducted on each sample in the laboratory (Table 1). By integrating taxonomic information from field identification guides by Jones (1985), Fisher *et al.*, (1990), Carpenter and Niem (2001), Psomadakis *et al.* (2015), Fishbase (Froese and Pauly, 2025), and World Register of Marine Species (WoRMS) (2025) species were identified.

Otolith extraction procedure

After registering the fish body measurements and weights, the right and left otoliths were carefully extracted from the labyrinth system of the skull which contains three parts of the otoliths: the sagittae, the asterisci and the lapilli. The undamaged otoliths underwent a thorough cleaning process with alcohol and were subsequently dried, labeled, and poured into Eppendorf tubes separately. Later on, the left otoliths were subsequently examined under a Nikon digital binocular microscope (model SMZ1270) to analysis the morphological characteristics and image of each otolith. Due to their smallest

size, the lapillus otoliths could not be preserved and thus were not included in this study. Landmarking and subsequent morphometric calculations for both otolith types (sagitta and asteriscus) were conducted with tpsDig2 (v.2.33). The description and terminology of otoliths

followed by Tuset *et al.* (2008), whereas asteriscus has been meticulously described for the first time, with a focus on its detailed morphology and taxonomic classification (Fig. 1).

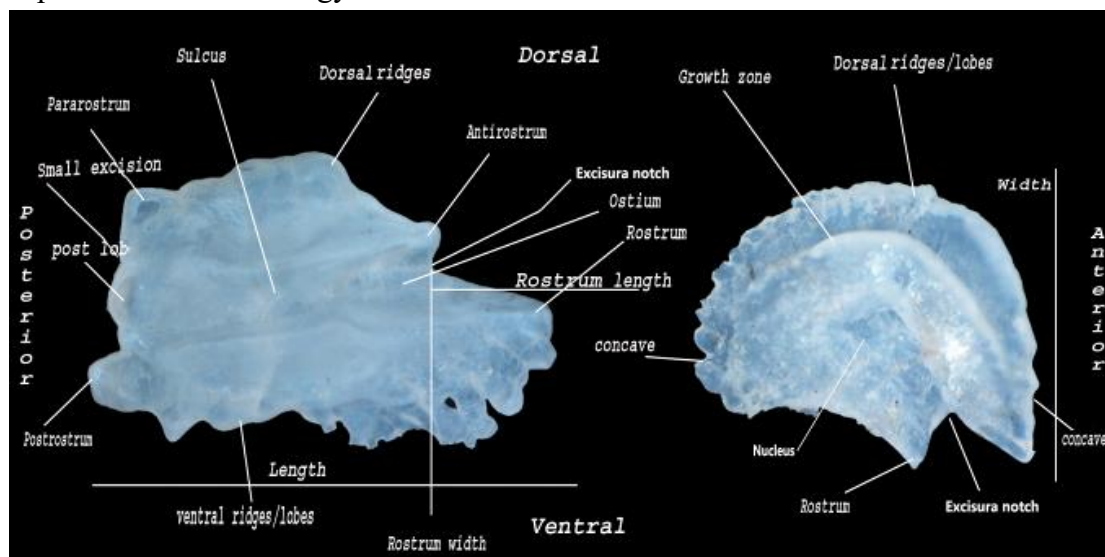


Figure 1: Detailed description of left sagitta and asteriscus otoliths of Ponyfish.

Truss network and landmarks

A truss network comprising 21 anatomical landmarks and 10 interlandmark (truss network) distances were applied to each specimen to capture body shape variation (Fig. 2). Linear distances between the landmarks were measured to 0.1 mm precision using a calibrated divider. The tpsDig 2 v.2.33 software was used for digital landmarking and calculations. The MorphoJ v 1.07a software was used to measure the landmarks and truss distances for accuracy and corroboration.

Statistical analysis

Statistical analyses, including Principal Component Analysis (PCA), and Canonical Discriminant Function Analysis (DFA),

were conducted via SPSS version 27. Prior to analysis, data normality was assessed.

Results

With regard to international nomenclature, this research offers a thorough description of eight genera and fifteen species of family Leiognathidae, including five new regional records. A total of 556 individual specimens, an extensive analysis of fish weights and sizes, and twenty-seven morphometric measurements were carried out (Table 1). The incorporation of cohesive datasets e.g. truss networks, landmarks, and morphometrics resulted in the accurate identification of some complex species and genera. Data normality for length and weight was assessed prior to multivariate analysis (Fig. 3). The

relationships between variables and underlying factors after a rotation technique has been applied. The three axes represent

the principal components that explain the variance in the data (Fig. 4).

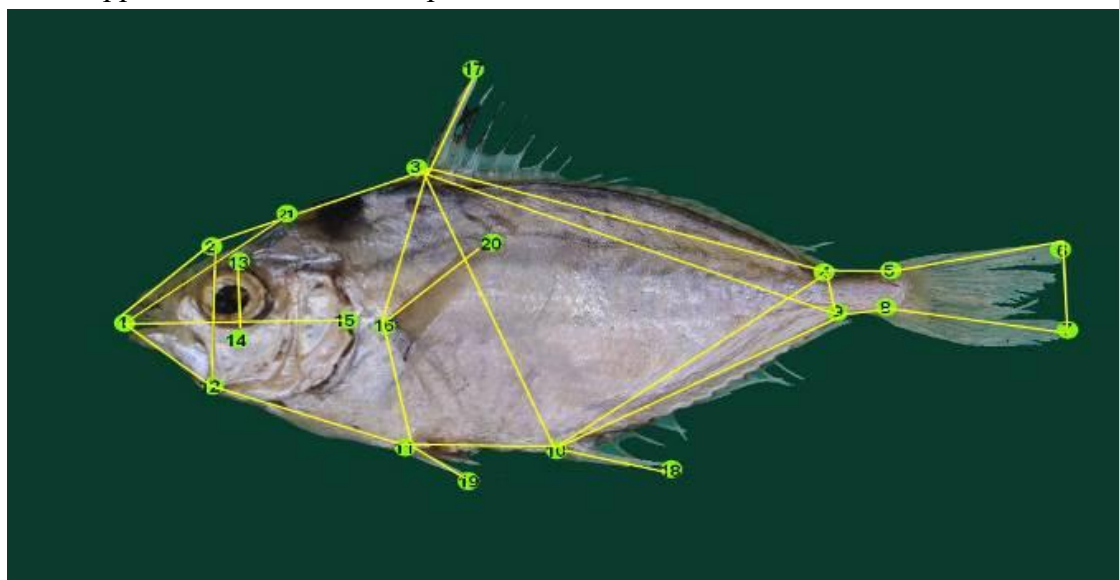


Figure 2: Deployment of landmarks and truss network connections design on the Ponyfishes.

Table 1: The mean value of morphometric measurements of fifteen ponyfishes encountered in this study ($\pm$) stands for the standard deviation.

Species	N	Total length (cm)	Standard length (cm)	Girth (cm)	Total weight (g)
<i>Aurigequula equula</i>	62	15.17 $\pm$ 3.10	12.08 $\pm$ 2.51	15.81 $\pm$ 4.71	69.33 $\pm$ 52.05
<i>Aurigequula fasciata</i>	10	16.40 $\pm$ 0.57	12.85 $\pm$ 0.49	16.05 $\pm$ 0.49	75.30 $\pm$ 6.90
<i>Deveximentum indicum</i>	2	8.90 $\pm$ 0.30	7.45 $\pm$ 0.25	7.45 $\pm$ 0.05	7.295 $\pm$ 0.805
<i>Deveximentum insidator</i>	101	7.81 $\pm$ 0.97	6.47 $\pm$ 0.78	6.4 $\pm$ 1.06	5.60 $\pm$ 2.57
<i>Deveximentum interruptum</i>	1	8.00	6.90	6.20	7.20
<i>Deveximentum ruconius</i>	78	5.04 $\pm$ 0.77	4.13 $\pm$ 0.60	5.09 $\pm$ 0.81	2.02 $\pm$ 0.99
<i>Equulites berbis</i>	4	11.05 $\pm$ 0.07	8.95 $\pm$ 0.07	8.5 $\pm$ 0.00	16.91 $\pm$ 0.26
<i>Equulites leuciscus</i>	14	11.37 $\pm$ 0.76	9.23 $\pm$ 0.57	8.93 $\pm$ 0.31	19.11 $\pm$ 3.41
<i>Equulites lineolatus</i>	65	9.02 $\pm$ 1.58	7.35 $\pm$ 1.28	6.87 $\pm$ 1.42	10.75 $\pm$ 6.23
<i>Gaza minuta</i>	38	10.40 $\pm$ 1.02	8.50 $\pm$ 0.81	8.10 $\pm$ 0.85	16.37 $\pm$ 4.63
<i>Karalla daura</i>	121	11.95 $\pm$ 1.16	9.71 $\pm$ 0.95	10.17 $\pm$ 1.31	26.03 $\pm$ 9.16
<i>Nuchequula blochii</i>	50	6.40 $\pm$ 1.52	5.26 $\pm$ 1.29	5.02 $\pm$ 1.14	4.30 $\pm$ 3.39
<i>Nuchequula flavaxilla</i>	28	9.42 $\pm$ 0.51	7.64 $\pm$ 0.47	8.31 $\pm$ 0.47	12.90 $\pm$ 2.82
<i>Nuchequula gerreoides</i>	53	9.40 $\pm$ 0.49	7.72 $\pm$ 0.41	8.00 $\pm$ 0.43	12.01 $\pm$ 2.18
<i>Photopectoralis bindus</i>	15	8.44 $\pm$ 1.15	6.74 $\pm$ 0.88	7.84 $\pm$ 1.02	7.56 $\pm$ 3.19

By observing the clustering and distribution of points (variables), the variables which are most strongly associated with each component, providing insights into the structure of the data. Understanding the underlying structure of data and the separation between distinct groups is crucial in many analytical endeavors.

Principal Component Analysis (PCA) revealed that the first three components capture the overwhelming majority of variance: PC I=84.97%, PC II=7.65%, and PC III=4.96%, yielding a cumulative explained variance of ~97.58% (Table 2).

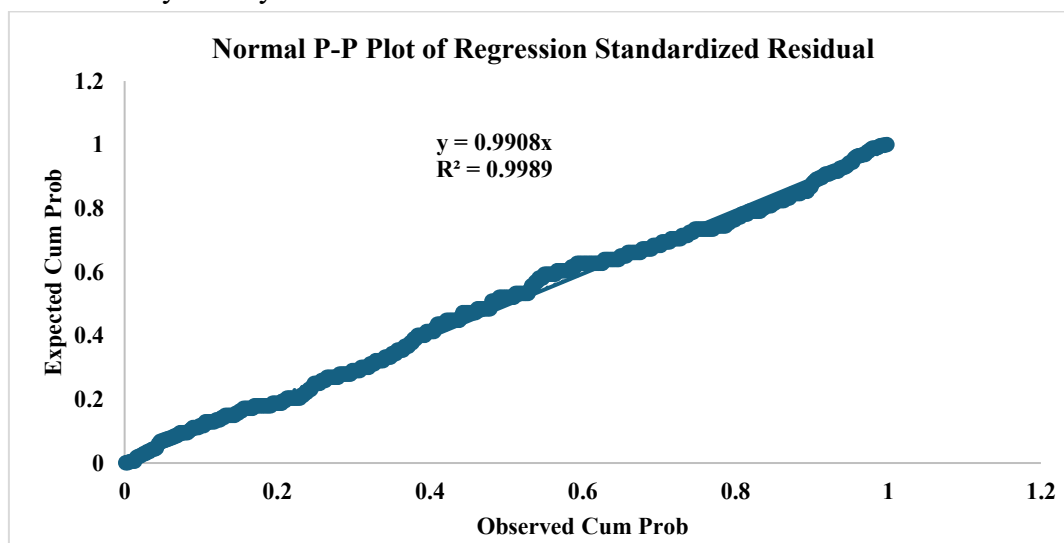


Figure 3: Examining the normality assumption (Shapiro-Wilk test) for length of ponyfishes species.

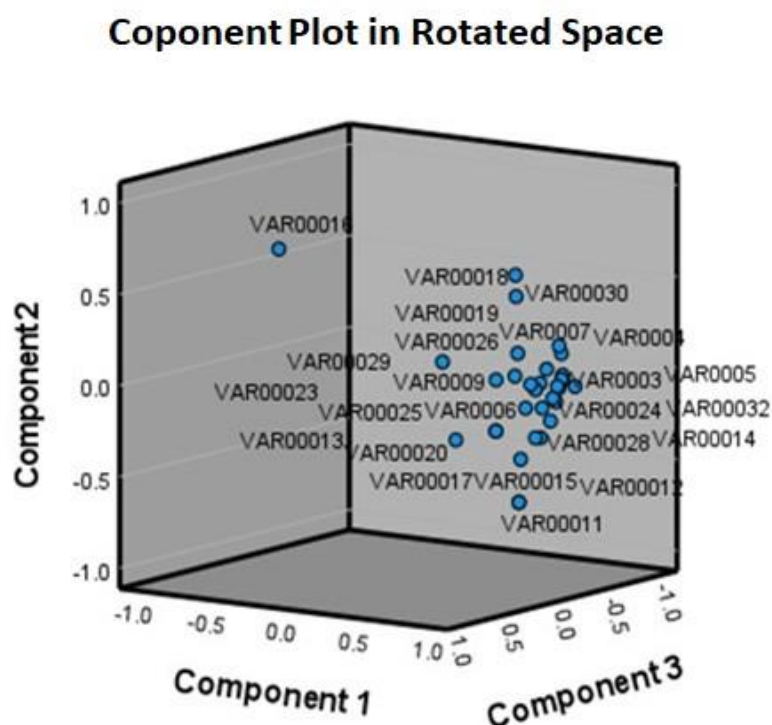


Figure 4: The rotated component loadings of the variables. Each axis represents a principal component (Component 1, Component 2, and Component 3), derived from PCA.

Table 2: Description of principal component analysis (PCA) for the first three components.

Component	Total variance explained ^a				
	Extraction sums of squared loadings			Rotation sums of squared loadings	
	Total	% of Variance	Cumulative %	Total	% of Variance
1	27.191	84.973	84.973	27.166	84.893
2	2.447	7.648	92.621	2.458	7.681
3	1.587	4.961	97.582	1.603	5.008

These results indicate that a small number of orthogonal axes effectively summarize body shape and size variation across the sampled taxa. Canonical Discriminant Function Analysis (DFA) further partitioned

between-group variance, with the first four discriminant functions accounting for 62.3%, 11.4%, 8.9%, and 6.1% of the variance and corresponding eigenvalues of 33.85, 6.20, 4.84, and 3.29 (Table 3).

Table 3: Description of Principal Component Analysis (DFA) for the first fourteen components.

Function	Eigenvalues			
	Eigenvalue	% of Variance	Cumulative %	Canonical correlation
1	33.853 ^a	62.3	62.3	.986
2	6.201 ^a	11.4	73.7	.928
3	4.835 ^a	8.9	82.6	.910
4	3.288 ^a	6.1	88.7	.876

a. First 4 canonical discriminant functions were used in the analysis.

Wilks's lambda tests confirmed that the discriminant functions are statistically significant (Table 4). The DFA identified the strongest discriminating variables as pre-dorsal length, body depth, distance from first dorsal spine to last anal ray, eye

tip to first dorsal fin, and pelvic spine to pectoral fin (breast). Variables contributing less to group separation included length from first dorsal spine to pectoral fin, head length, nuchal crest, and pre-pelvic length.

Table 4: Wilk's lambda test function of the first four components.

Test of Function(s)	Wilks' Lambda			
	Wilks' Lambda	Chi-square	df	Sig.
1 through 14	.000	4363.340	294	.000
2 through 14	.000	3123.996	260	.000
3 through 14	.001	2434.970	228	.000
4 through 14	.005	1819.366	198	.000

Furthermore, Cluster structure in canonical space revealed clear generic separation for several taxa. *Aurigequula equula*, *Equulites berbis*, and *Deveximentum ruconius* formed distinct, well-separated clusters (Fig. 5). *Gaza minuta*, *Karalla daura*, and *Photolateralis bindus* each displayed distinctive generic variation. Species within

Nuchequula (*N. blochii*, *N. flavaxilla*, *N. gerreoides*) and *Equulites* (*E. berbis*, *E. leuciscus*, *E. lineolatus*) showed substantial overlap, indicating close morphological affinity; within *Nuchequula*, *N. flavaxilla* and *N. gerreoides* were particularly similar while *N. blochii* was more dispersed.

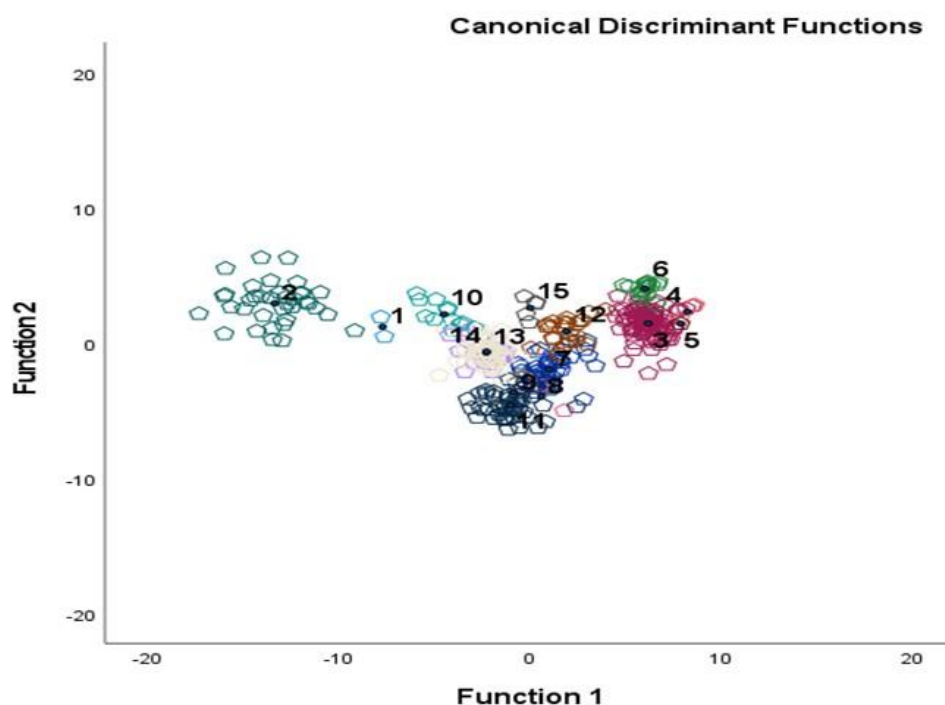


Figure 5: Canonical discriminant function analysis using geomorphometric data of (1) *Aurigequula fasciata*, (2) *Aurigequula equula*, (3) *Deveximentum insidator*, (4) *Deveximentum indicum*, (5) *Deveximentum interruptum*, (6) *Deveximentum ruconius*, (7) *Equulites lineolatus*, (8) *Equulites leuciscus*, (9) *Equulites berbis*, (10) *Gaza minuta*, (11) *Karalla daura*, (12) *Nucchequula blochii*, (13) *Nucchequula flavaxilla*, (14) *Nucchequula gerreoides*, (15) *Photoptoralis bindus*.

Species of *Deveximentum* clustered tightly, reflecting strong within-genus similarity. Furthermore, Otolith morphometrics provided complementary and often decisive diagnostic characters. Sagittal otoliths of *Aurigequula equula* were the largest (mean length 69.29 mm) and exhibited pronounced rostrum lengths (≈ 15.5 mm) and deep notches (≈ 16.6 mm). In contrast, *Deveximentum* species possessed the smallest sagittae (length range 2.51–4.15 mm) with markedly reduced rostra and shallow notches, producing an almost absent “beak.” Additional diagnostic otolith features include the elevated sulcus height in *Nucchequula* (notably *N. flavaxilla* at 6.42 mm) and the exceptionally deep ostium in *Karalla daura* (9.01 mm) (Table 5).

Across taxa, the asteriscus commonly displays mid-length dominance, producing a circular to oval outline (Table 6). Asteriscus morphology contributed novel species-level resolution that complemented sagittal characters. Furthermore, A strong positive correlation between sagittal otolith height and length was observed across most species, indicating consistent allometric growth patterns. Several taxa exhibited very high model fit ($R^2 > 0.90$), for example *A. equula* ($R^2 = 0.95$) and *K. daura* ($R^2 = 0.951$), implying that otolith height is tightly predictable from length and therefore useful for identification from partial material. By contrast, species such as *N. blochii* showed low R^2 values (0.049), reflecting high individual variation (Table 7).

Table 5: Morphometric variation (mean $\pm$ SD) of the left sagittal otoliths in ponyfish species.

Species	Otolith length	Otolith height	Sulcus height	Ostium depth	Rostrum length	Otolith perimeter
<i>A. equula</i>	69.29 $\pm$ 26.4	35.85 $\pm$ 14.85	5.81 $\pm$ 2.14	6.99 $\pm$ 3.33	15.54 $\pm$ 6.84	30.78 $\pm$ 12.71
<i>A. fasciata</i>	58.95 $\pm$ 1.83	26.42 $\pm$ 0.5	4.32 $\pm$ 1.19	5.54 $\pm$ 1.61	8.29 $\pm$ 0.42	25.17 $\pm$ 0.69
<i>D. indicum</i>	57.78 $\pm$ 0.30	33.44 $\pm$ 3.59	5.38 $\pm$ 0.39	6.20 $\pm$ 0.72	16.81 $\pm$ 3.52	19.33 $\pm$ 5.64
<i>D. insidator</i>	54.54 $\pm$ 2.76	34.17 $\pm$ 2.25	5.27 $\pm$ 0.78	6.59 $\pm$ 1.4	15.62 $\pm$ 1.97	22.58 $\pm$ 1.48
<i>D. interruptum</i>	47.85	40.00	5.86	8.25	11.58	20.55
<i>D. ruconius</i>	40.40 $\pm$ 2.01	42.10 $\pm$ 1.08	5.30 $\pm$ 0.56	6.99 $\pm$ 1.57	11.84 $\pm$ 1.79	17.72 $\pm$ 0.78
<i>E. berbis</i>	57.43 $\pm$ 0.32	33.5 $\pm$ 0.39	4.78 $\pm$ 0.54	6.61 $\pm$ 0.60	11.26 $\pm$ 0.51	25.12 $\pm$ 1.37
<i>E. leuciscus</i>	52.74 $\pm$ 3.73	35.11 $\pm$ 3.1	5.32 $\pm$ 0.48	6.99 $\pm$ 1.41	10.00 $\pm$ 1.91	20.80 $\pm$ 1.28
<i>E. lineolatus</i>	60.92 $\pm$ 16.42	39.41 $\pm$ 10.38	5.91 $\pm$ 1.49	7.61 $\pm$ 2.71	13.53 $\pm$ 2.62	25.61 $\pm$ 7.25
<i>G. minuta</i>	52.28 $\pm$ 3.65	37.66 $\pm$ 2.40	5.60 $\pm$ 0.67	7.63 $\pm$ 1.27	9.38 $\pm$ 1.76	22.97 $\pm$ 1.68
<i>K. daura</i>	64.04 $\pm$ 20.22	32.51 $\pm$ 10.97	5.88 $\pm$ 1.81	9.01 $\pm$ 2.94	16.78 $\pm$ 4.75	26.95 $\pm$ 8.08
<i>N. blochii</i>	54.52 $\pm$ 3.33	36.85 $\pm$ 2.45	4.97 $\pm$ 0.52	6.38 $\pm$ 0.88	15.54 $\pm$ 2.59	22.58 $\pm$ 1.28
<i>N. flavaxilla</i>	55.10 $\pm$ 2.22	31.74 $\pm$ 2.72	6.42 $\pm$ 0.75	7.94 $\pm$ 1.47	16.70 $\pm$ 2.94	22.56 $\pm$ 1.35
<i>N. gerreoides</i>	54.59 $\pm$ 2.63	30.74 $\pm$ 2.73	5.85 $\pm$ 0.67	7.28 $\pm$ 1.03	15.36 $\pm$ 2.56	21.69 $\pm$ 1.70
<i>P. bindus</i>	46.41 $\pm$ 3.73	38.01 $\pm$ 2.9	4.82 $\pm$ 0.46	6.22 $\pm$ 0.84	9.89 $\pm$ 0.56	20.78 $\pm$ 1.67

Deveximentum species generally displayed higher R^2 values, suggesting a conserved otolith growth “blueprint” within the genus.

Beyond DFA and morphometric variations, our findings demonstrate that *Equulites berbis*, *Aurigequula. equula*, and *Deveximentum ruconius* possess distinct biological profiles, differing significantly in body depth (Table 1), mouth protrusion (Fig. 6), feeding habits, and otolith shape (Fig. 7).

Consequently, on the basis of generic discrimination, all tribes were pooled independently, and the species were close to each other. However, the species from the genus *Leiognathus* were found to be significantly different, indicating the presence of notable generic variation within the group.

Morphological description of the sagitta and asteriscus otoliths of the Family: Leiognathidae

Aurigequula equula (Forsskal, 1775) (Fig. 7a)

Previous name: *Leiognathus equula* (Forsskal, 1775)

The sagitta is elongated and sole-like in shape. The rostrum and anti-rostrum are short. Excisura is too shallow. The ostium is deep. The sulcus is deep and narrow. The pararostrum and excisura minor are not obvious. All sides have prominent ridges. The asteriscus is oblong. The rostrum is long and the excisura is deep. The ventral profile has ridges. The sagitta and asteriscus of this species are likely to be striped ponyfish, *Aurigequula fasciata*.

Remarks: This species is erroneously added to the genus *Leiognathus* and therefore should be accounted for in the *Aurigequula* genus owing to its discriminating morphological characteristics.

Table 6: Morphometric variation (mean $\pm$ SD) of the left Asteriscus otoliths in ponyfish species.

Species	Hight	Pre mid length	Mid length	Post mid length	Rostrum length	Notch
<i>A. equula</i>	51.21 $\pm$ 17.77	57.57 $\pm$ 21.2	64.02 $\pm$ 23.94	61.54 $\pm$ 22.78	15.15 $\pm$ 5.55	16.62 $\pm$ 5.86
<i>A. fasciata</i>	42.61 $\pm$ 1.82	39.2 $\pm$ 2.1	47.66 $\pm$ 0.36	48.34 $\pm$ 0.94	12.37 $\pm$ 1.03	17.77 $\pm$ 0.78
<i>D. indicum</i>	24.76 $\pm$ 0.71	29.8 $\pm$ 1.82	40.23 $\pm$ 3.3	40.27 $\pm$ 2.78	4.15 $\pm$ 0.33	3.31 $\pm$ 0.69
<i>D. insidator</i>	27.53 $\pm$ 2.86	33.52 $\pm$ 4.52	42.97 $\pm$ 6.98	38.9 $\pm$ 7.21	3.53 $\pm$ 1	4.02 $\pm$ 0.74
<i>D. interruptum</i>	24.55	30.41	48.46	50.51	2.53	2.74
<i>D. ruconius</i>	18.26 $\pm$ 1.93	29.79 $\pm$ 2.45	37.91 $\pm$ 2.98	35.34 $\pm$ 2.99	2.51 $\pm$ 0.89	3.99 $\pm$ 1.22
<i>E. berbis</i>	32.78 $\pm$ 0.35	32 $\pm$ 3.08	41.08 $\pm$ 2.12	42.45 $\pm$ 0.45	6.64 $\pm$ 0.44	10.81 $\pm$ 0.53
<i>E. leuciscus</i>	33.81 $\pm$ 2.3	27.33 $\pm$ 5.26	41.4 $\pm$ 1.75	40.49 $\pm$ 2.14	7.28 $\pm$ 1.84	9.82 $\pm$ 1.48
<i>E. lineolatus</i>	34.73 $\pm$ 10.26	34.33 $\pm$ 10.49	44.84 $\pm$ 13.93	42.99 $\pm$ 15.13	6.83 $\pm$ 2.28	8.37 $\pm$ 2.45
<i>G. minuta</i>	26.55 $\pm$ 1.89	42.06 $\pm$ 3.50	51.94 $\pm$ 3.59	48.86 $\pm$ 3.86	3.29 $\pm$ 0.76	3.63 $\pm$ 1.42
<i>K. daura</i>	41.17 $\pm$ 13.5	45.66 $\pm$ 17.57	59.5 $\pm$ 22.39	54.04 $\pm$ 20.06	5.94 $\pm$ 2.40	7.46 $\pm$ 3.76
<i>N. blochii</i>	27.32 $\pm$ 5.03	33.20 $\pm$ 5.43	38.31 $\pm$ 6.81	36.28 $\pm$ 7.34	6.05 $\pm$ 2.73	6.4 $\pm$ 2.34
<i>N. flavaxilla</i>	30.03 $\pm$ 2.89	34.25 $\pm$ 3.01	42.1 $\pm$ 2.79	38.41 $\pm$ 3.73	5.22 $\pm$ 1.70	6.4 $\pm$ 2.11
<i>N. gerreoides</i>	31.72 $\pm$ 3.12	33.56 $\pm$ 2.76	40.85 $\pm$ 2.18	37.42 $\pm$ 4.95	6.2 $\pm$ 2.09	7.34 $\pm$ 2.28
<i>P. bindus</i>	29.61 $\pm$ 2.89	34.18 $\pm$ 2.68	42.36 $\pm$ 3.5	38.37 $\pm$ 4.43	5.34 $\pm$ 1.05	6.65 $\pm$ 1.67

Table 7: Relationship between the otolith length and the otolith Hight parameter of Ponyfishes.

Species	Slop (b) $\pm$ SE	Intercept (a) $\pm$ SE	Adjusted R2	t	P value
<i>A. equula</i>	1.7 $\pm$ 0.05	4.94 $\pm$ 2.07	0.95	2.39	<0.001
<i>A. fasciata</i>	2.29 $\pm$ 1.05	1.07 $\pm$ 27.78	0.27	0.04	<0.000
<i>D. indicum</i>	0.83 $\pm$ 0.00	55.02 $\pm$ 0.00	1	-	-
<i>D. insidator</i>	0.86 $\pm$ 0.94	25.26 $\pm$ 3.22	0.49	7.85	<0.001
<i>D. interruptum</i>	-	-	-	-	-
<i>D. ruconius</i>	1.48 $\pm$ 0.14	-16.28	0.64	-3.85	$\leq$ 0.001
<i>E. berbis</i>	-0.075 $\pm$ 0.57	59.94 $\pm$ 19.24	-0.487	3.12	<0.001
<i>E. leuciscus</i>	-0.38 $\pm$ 0.33	66.04 $\pm$ 11.63	0.024	5.68	<0.001
<i>E. lineolatus</i>	1.53 $\pm$ 0.05	0.78 $\pm$ 2.21	0.93	0.36	<0.004
<i>G. minuta</i>	1.23 $\pm$ 0.15	5.99 $\pm$ 5.65	0.64	1.063	<0.000
<i>K. daura</i>	1.75 $\pm$ 0.04	6.8 $\pm$ 1.29	0.951	5.28	$\leq$ 0.004
<i>N. blochii</i>	-0.16	67.5 $\pm$ 6.9	0.049	9.78	$\leq$ 0.001
<i>N. flavaxilla</i>	0.33 $\pm$ 0.15	44.45 $\pm$ 4.66	0.136	9.56	<0.001
<i>N. gerreoides</i>	0.25 $\pm$ 0.11	46.9 $\pm$ 3.53	0.052	13.33	<0.001
<i>P. bindus</i>	1.16 $\pm$ 0.15	2.22 $\pm$ 5.74	0.81	0.387	<0.005

Aurigequula fasciata (Lacépède, 1803)
(Fig. 7b)

Sagitta is elongated with numerous arcs on the anterior edges. The rostrum is long, the anti-rostrum is short, the excisura is

shallow, the ostium is highly deep, and the sulcus is customary. The para rostrum is lobed whereas the post rostrum is discernable. The excisura minor is deep and the anterior part is curvature along with the

rostrum and the anti-rostrum. The dorsal edge is humped while the ventral profile is straight with numerous curves, and the posterior end is inclined toward the ventral region. The asteriscus is semicircular with a long rostrum and deep excisura. The nucleus appeared to have minor plumes reflecting a cyclonic area. The anterior part

is bulged and elongated. The dorsal part has several wedges. The posterior region is circular with prominent curvature. Overall, the asteriscus size is relatively larger than that of the other species.

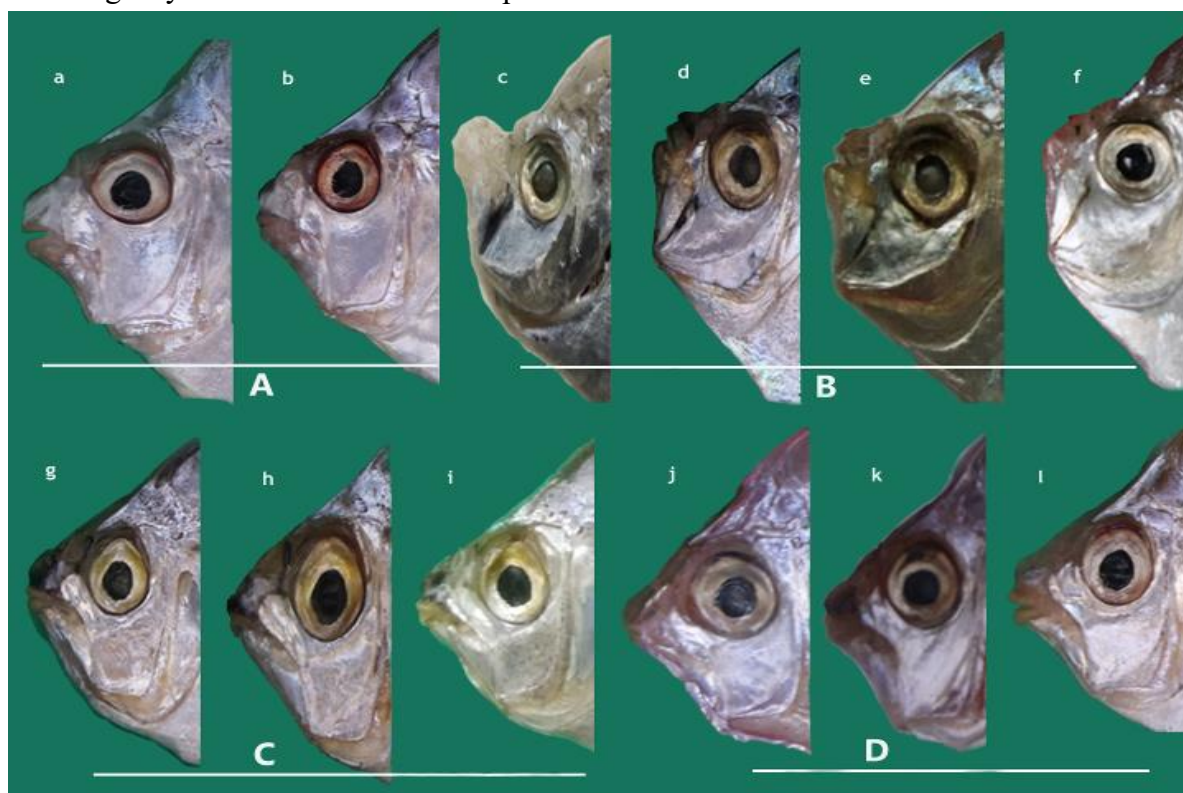


Figure 6: Depicting the morphological diversity of fish heads, with distinct differences in eye placement, and mouth structure. Capital letter denoted for genus A= *Aurigequula*, B= *Deveximentum*, C= *Equulites* and D= *Nuchequula* and small letter represent species a= *A. equula*, b= *A. fasciata*, c= *indicium*, d= *D. insidator*, e= *D. interrptum*, f= *D. ruconius*, g= *E. berbis*, h= *E. leuciscus*, i= *E. lionelatus*, j= *N. blochii*, k= *N. flavaxilla*, l= *N. gerreoids*.

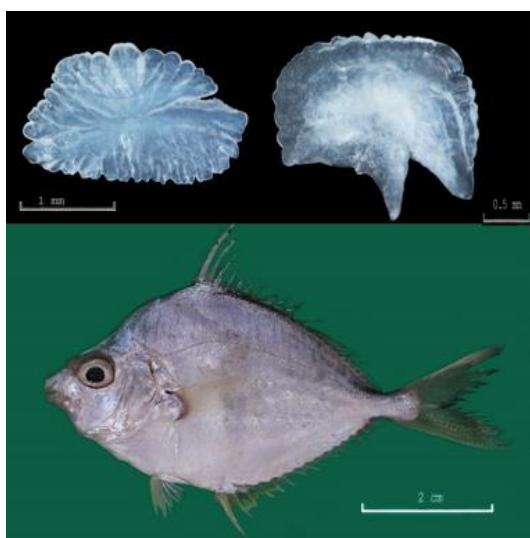
Deveximentum indicium (Monkolprasit 1973) (Fig. 7c)

Sagitta is oval-shaped. The rostrum is long, and the antirostrum is distinctly observable. The ostium and excisura are deep. The dorsal and ventral edges are deeply marked. The sulcus is deep and round at the posterior end. The dorsal end is smooth with hump-like arcs, whereas the ventral end embosses several ridges. The para-

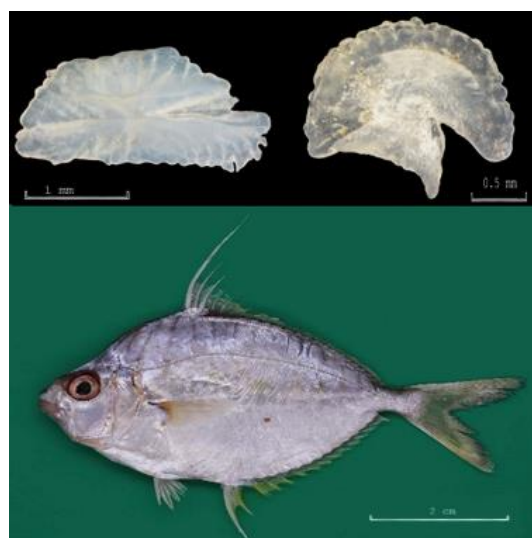
rostrum and excisura minor merged in an oval shape at the posterior end. The post-rostrum is bulk shaped and posteriorly sloppy smooth.

The asteriscus resembles the shape of *D. insidator*, with the modest distinction of dorsal edges that appear to be curved.

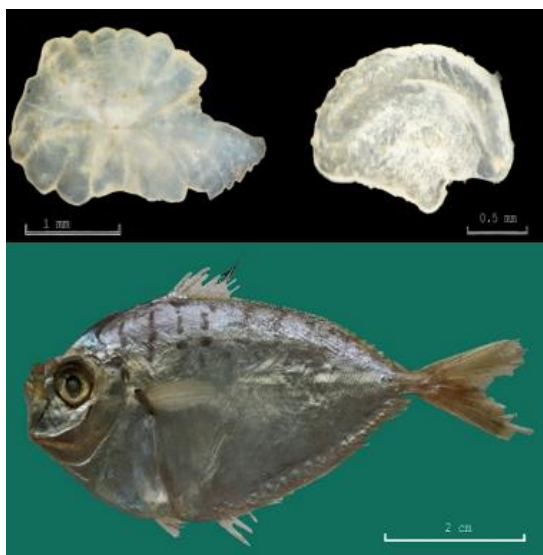
Remarks: This is the first report in the northern Arabian Sea.



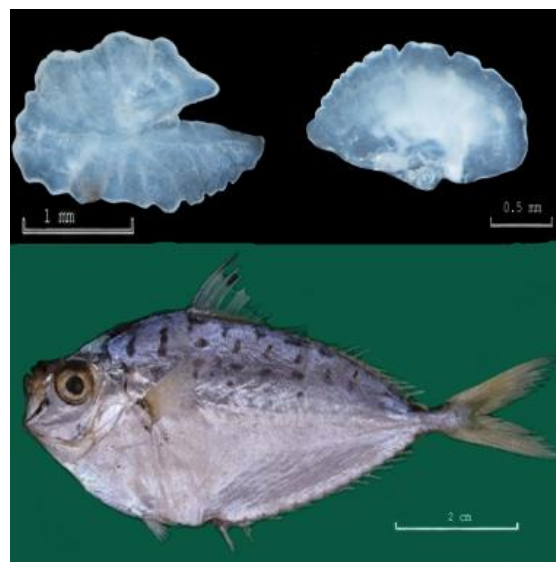
a



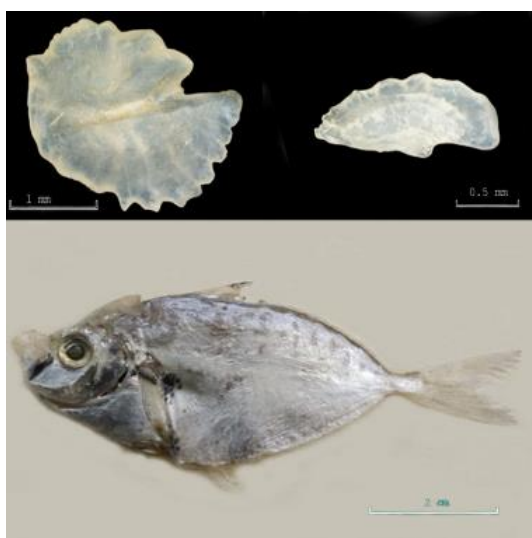
b



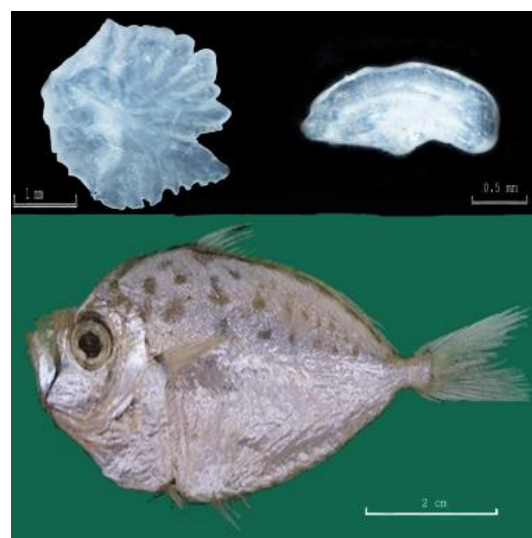
c



d



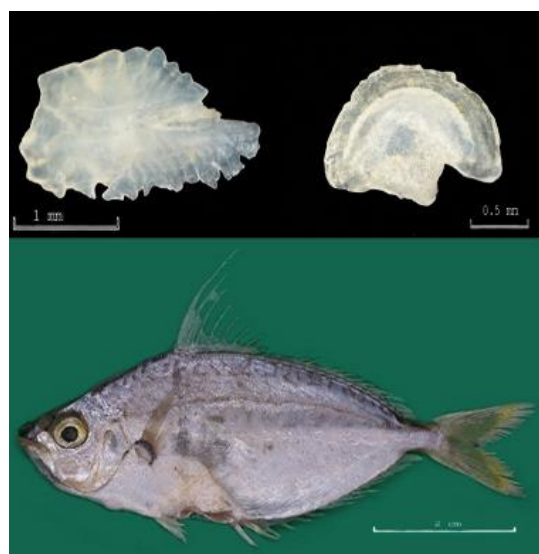
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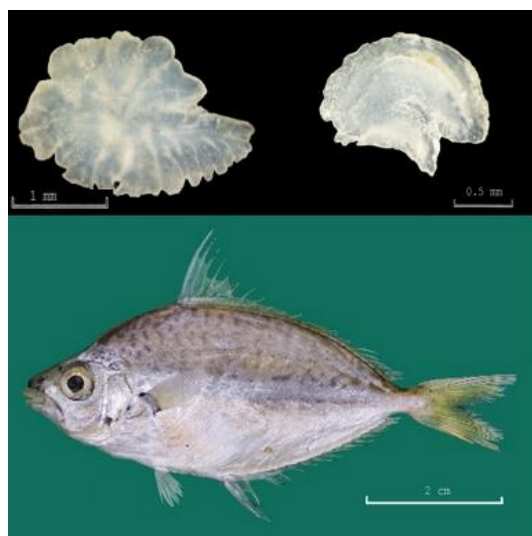
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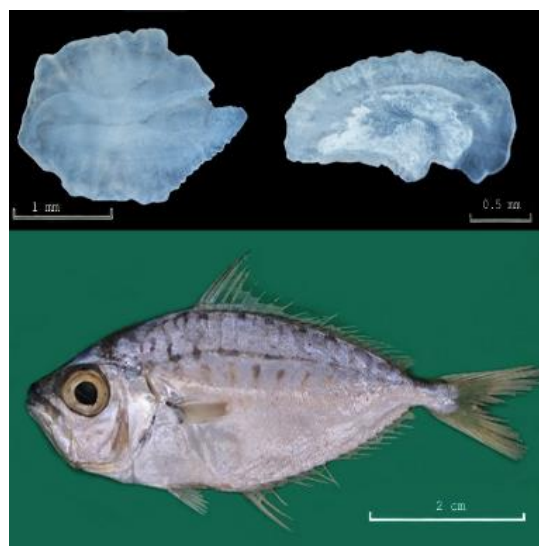
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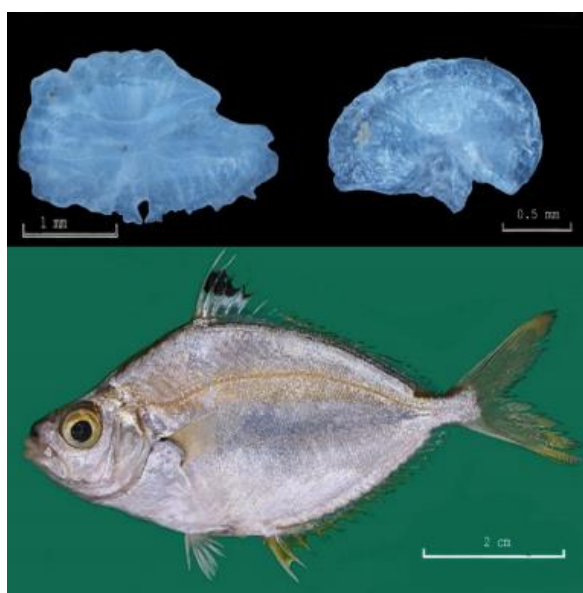
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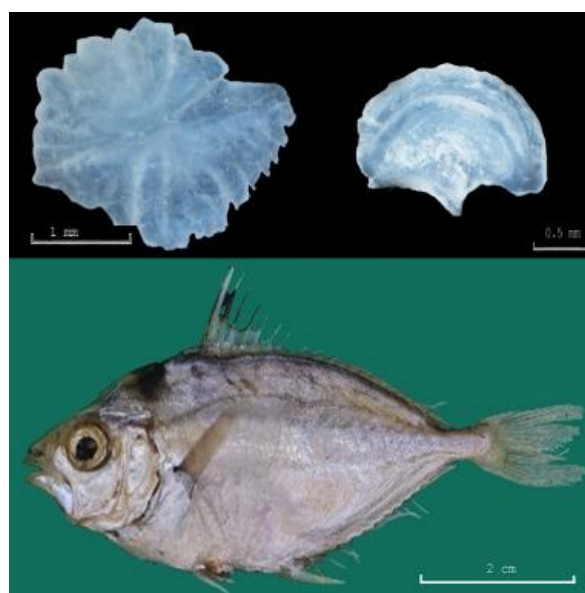
i



j



k



l

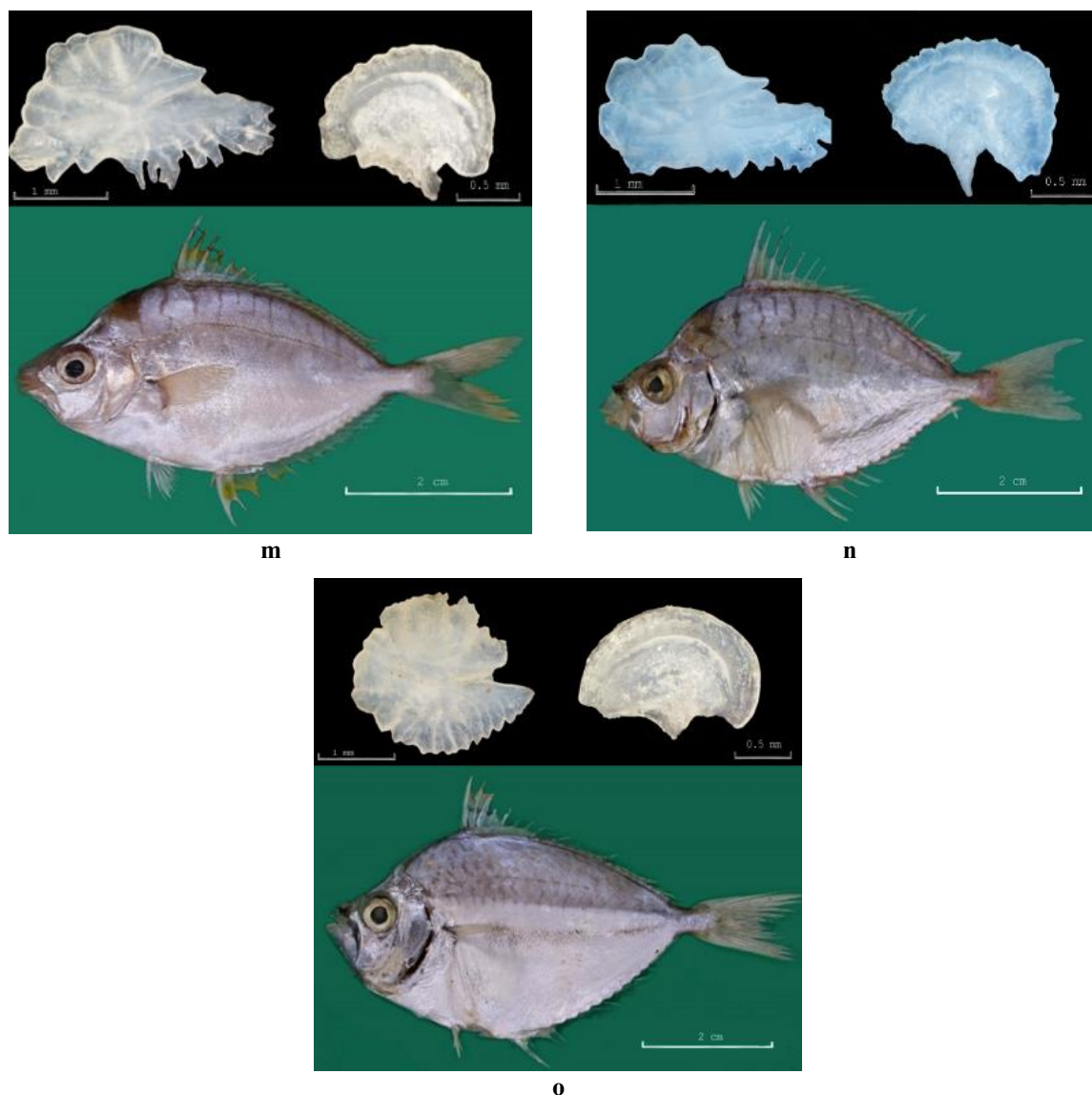


Figure 7: Images of fifteen Ponyfishes species (a-o) including sagitta and asteriscus otoliths.

Deveximentum insidator (Bloch, 1787) (Fig. 7d)

Sagitta is oval and elongated. The ostium is deep, and the sulcus is wider and ends before the posterior. The rostrum is long, along with the anti-rostrum. The excisura is deep. The para rostrum is wedgy by an excisura minor and bulky post rostrum. The posterior end has a thick vertical mark toward the sulcus. The dorsal profile has a smooth curvature whereas the vertical profile has multiple ridges. The posterior ends are curved. The asteriscus is elongated

and unequally shaped. The rostrum is inferior. The excisura is shallow. The nucleus is thin and has cyclonic reflections. The dorsal edges are marked. The anterior part is smooth, and the posterior profile comprises minor ridges.

Deveximentum interruptum (Valenciennes, 1835) (Fig. 7e)

Humped-like sagitta. The rostrum and anti-rostrum are inferior. The ostium is deeply expended. The sulcus is deep slender and posteriorly rounded. The Excisura is short,

however excisura minor and para-rostrum are not prominent. The post-rostrum is knob-like. The ventral end is severely marked while the dorsal end has some ridges, and the posterior end is smooth and curved. The asteriscus has elongated and the rhombus has formed. The rostrum is short. The dorsal profile is rough whereas the posterior end is grumpy.

Deveximentum ruconius (Hamilton, 1822) (Fig. 7f)

Previous name: *Leiognathus ruconius* (Hamilton, 1822)

The sagitta is irregularly shaped, the rostrum is long and the antirostrum is short. The ostium is mild. The sulcus is deep and wide. The para and postrostrum are prominent. Excisura minor is mild. The dorsal-to-ventral profile is elongated. The anterior and posterior profiles are short and thin. The dorsal part has sharp spikes; however, the ventral end is smooth and humped like lobe. The anterior is curvy with a long rostrum and excisura. The posterior profile is bulged and rounded. The sagitta of this species holds exceptional characteristics for the manipulation of phylogenetic traits. The asteriscus is a semicircular structure. The rostrum is prominent, with smooth dorsal and anterior profiles, whereas the posterior profile is concave. In addition, the asteriscus is relatively small when compared with the rest of the species.

Remarks: On the basis of taxonomical characteristics, this species should be accounted for in the genus *Deveximentum*. This species is reported for the first time in the northern Arabian Sea.

Equulites berbis (Valenciennes, 1835) (Fig. 7g)

Previous name: *Leiognathus berbis* (Valenciennes, 1835)

Sagitta is sole-like. The rostrum is long. The excisura is too shallow while the antirostrum is very short. The ostium is deep and broad. The sulcus is exceptionally deep too and ends before the posterior part. The para-rostrum and excisura minor are heavily marked. The dorsal region is humped-like whereas multiple ridges are present ventrally. The posterior part is sloppy and consists of bulge lines that separate at the end. The sulcus is grooved. The asteriscus is kidney-like. The rostrum is distended. The excisura is deep. The nucleus is lightly tarnished. The posterior region is concave and has ridges along with the dorsal edge, while the anterior region is smooth.

Remarks: On the basis of its taxonomic resemblance this species should be accounted for in the genus *Equulites*. This species is reported for the first time in the northern Arabian Sea.

Equulites leuciscus (Gunther, 1860) (Fig. 7h)

Sagitta is elongated compared with *E. lioneolatus*. The anterior owns a concave edge. The rostrum is short and wider. The anti-rostrum is prominent with a thin excisura. The ostium is deep. The sulcus is deep, narrow, and round at the posterior end. The dorsal part consists of the lobule margins while the ventral profile has many wedges. The posterior end is a smooth arc with para rostrum, post rostrum, and excisura minor infusions. The asteriscus is semicircular but wider than that of *E.*

lioneolatus. The rostrum is prominent with a deep excisura. The anterior and posterior ends have smooth and dorsally rough edges.

Equulites lioneolatus (Valenciennes, 1835) (Fig. 7i)

Sagitta is oval-shaped. The rostrum is long with a prominent antirostrum with a concave edge. The excisura is shallow and thin. The ostium is mild. The sulcus is deep and has a marked that extends to the posterior end. The Para rostrum is bulky. The excisura minor is deep and the post rostrum is bulged. The dorsal part possesses bulkheaded projection. The ventral profile has prominent ridges, and the posterior part is bulged. The asteriscus is semicircular in shape with a sharp rostrum. The excisura varies in large specimens (deep) and shallow in small specimens. The dorsal profile is wedgy, and the anterior end has a concave margin. The posterior is smoothly arced.

Remarks: This is the first report in the northern Arabian Sea.

Gaza minuta (Bloch, 1795) (Fig. 7j)

Sagitta is somewhat rectangular. The rostrum is relatively short compared to that of other species within the genus. The ostium is very deep. The sulcus is shallow and wide where the ends meander at the posterior end. The rostrum is gruff and the antirostrum is tiny. The para-rostrum is sloppy whereas the excisura minor is dull. The dorsal part has bulkheaded edges while the ventral part has multiple ridges. The posterior profile is smooth and sloppy. The asteriscus is dome-shaped. The rostrum is short and the excisura is slightly deeper.

The dorsal profile is rough. The posterior part is smooth whereas the anterior part is uneven and comprises minor curvatures.

Karalla daura (Cuvier, 1829) (Fig. 7k)

Sole-like sagitta, the rostrum is wider and longer, and the antirostrum is short. The ostium is mild. The sulcus is deep and the excisura is thin. The para-rostrum and excisura minor are prominent. The dorsal part is humped with severe arcs. The ventral part is sharp and has deep ridges. The posterior end is smooth with a semi-bracket shape. The asteriscus is bean-shaped. The rostrum is long with mild excisura. The dorsal profile has ridges. The interior is smoothly curved, and the posterior region possesses concave edges.

Nuchequula blochii (Valenciennes, 1835) (Fig. 7l)

Elliptical-shaped sagitta, rostrum long, and the antirostrum inferior. The ostium is deep and broader in size. The excisura is mild. The sulcus is deeper and wider. The para-rostrum is predominant while excisura minor is vaguely observed in this species. The dorsal-to-ventral profile is broad and wide. The posterior end is sloped with the pararostrum and postrostrum, however, the ventral end is severely ridged. The asteriscus is bean-like. The rostrum is elongated, and dorsally smooth with the rare appearance of minor arcs. The anterior end is smooth and bulgy, and the posterior end has 2-4 concave edges.

Nuchequula flavaxilla (Kimura and Ikejima, 2008) (Fig. 7m)

Sagitta is oblong, the rostrum is long and mordant, and the antirostrum is short along with the excisura. The ostium is deep with

a broader sulcus. The para and post rostrum are pointed, and the excisura minor is deep. The dorsal part is smooth and humped-like in shape. The anterior is elongated, the ventral end has severe sharp ridges, and the posterior end has a slopy diminished profile. The asteriscus is oblong. The rostrum is long and the excisura is deep. The anterior edge is articulated, whereas the dorsal edges are curved.

Remarks: This is the first report in the northern Arabian Sea.

Nuchequula gerreoids (Bleeker, 1851) (Fig. 7n)

Sagitta is a triangular structure that is elongated at the anterior end and wider at the posterior end. The rostrum is long and the antirostrum is inferior. The ostium is deep, and the excisura is mild. The sulcus is deeper and wider. The para-rostrum and post rostrum are pointed at the posterior end. The excisura minor is deep. The dorsal profile has smooth ridges. The ventral end has sharp or pointed spikes below the rostrum. The posterior part is wing-like with a pointed para and post rostrum. The asteriscus is short at the anterior and wider at the posterior. The rostrum is sharp and elongated. Excisura is very deep. The nucleus is bluish. The anterior and posterior sides are concave. The dorsal and ventral ends are severely ridged.

Photopectoralis bindus (Valenciennes, 1835) (Fig. 7o)

Sagitta is oblong. The rostrum and antirostrum are long with deep excisura. The ostium is deep. The sulcus is deep and narrow and ends up before the posterior part. The para and post rostrum are distinct.

The dorsal edges are bulk-headed margins. The anterior profile is elongated, the ventral profile has multiple small wedges, and the posterior profile is bulged. The asteriscus is depressed with a long rostrum. The excisura is mild, and the outer edges lack dentition.

Discussion

This study demonstrates that integrating sagittal and asteriscus otolith morphology with morphometric analyses provides a reproducible framework for resolving taxonomic ambiguities in Leiognathidae. By applying discriminant function analysis (DFA) to a large dataset of specimens, we show that otolith traits can reliably distinguish species and genera, even in cases where external morphology alone has proven insufficient. Importantly, the inclusion of asteriscus otoliths often overlooked due to their small size; revealed diagnostic features that complement sagittal otoliths and strengthen species-level resolution.

The reclassification proposals for *Aurigequula equula*, *Equulites berbis*, and *Deveximentum ruconius* are supported by distinct otolith morphologies and DFA clustering. While these results align with previous revisions, they also underscore the need for integrative approaches that combine morphological, molecular, and type-based comparisons. Rather than presenting otolith evidence as definitive, we emphasize its role as a robust line of support that can guide future systematic revisions.

Beyond taxonomic clarification, these findings have broader ichthyological implications. Otolith morphology reflects

not only phylogenetic relationships but also ecological adaptations, such as feeding strategies and habitat preferences (Assis *et al.*, 2020). The consistent growth patterns observed in certain genera (e.g., *Aurigequula* and *Karalla*) suggest evolutionary constraints on otolith development, while the high variability in others (e.g., *Nuchequula*) may indicate ecological plasticity. Such insights highlight the potential of otolith-based diagnostics to inform studies of niche differentiation, trophic ecology, and biogeographic distribution.

Ecologically, the addition of five new regional records expands the known diversity of ponyfishes in the northern Arabian Sea, underscoring the importance of comprehensive surveys in biodiversity hotspots. These records contribute to baseline data essential for fisheries management, conservation planning, and ecosystem monitoring. Given the ecological role of ponyfishes as mid-trophic species, accurate identification is critical for understanding food web dynamics and sustaining coastal fisheries (Kashani *et al.*, 2025a, b).

The family Leiognathidae has undergone significant taxonomic revision since, Jones (1985) initially placed all species in the genus *Leiognathus*, subsequent research has established multiple genera. However, some species remain ambiguously classified within *Leiognathus* genus. In recent years, multiple changes have been made, for instance, *D. ruconius* was previously placed in the genus *Leiognathus* and now become a member of the *Secutor* genus (Chakrabarty *et al.*, 2009; 2010 a; Abraham

et al., 2011). Furthermore, *A. equula*, *E. berbis*, and *D. ruconius* were unintentionally included in the genus *Leiognathus* without rational justification. Despite multiple efforts by different authors (Chakrabarty, Spark, Kimura, Suzuki, Woodland, and Jones) to rationalize the identification of the Leiognathidae family, they have yet to be aligned. Therefore, a plausible description of the family is provided in this study. By demonstrating different performances in identifying members of the family Leiognathidae, we have shown the importance of an integrated approach that fully represents the variation in taxonomy and otolith morphology.

Canonical DFA has emerged as a highly promising method for delineating taxonomic divergence in ponyfishes (Chakrabarty *et al.*, 2009). It has been effectively employed to differentiate species such as *Equulites leuciscus* and *Equulites absconditus* (Chakrabarty *et al.*, 2010 b). More recently, Kashani and Panhwar (2023) applied multivariate DFA to characterize population variability in the goldstripe ponyfish *Karalla daura*, revealing significant adaptations to environmental stress despite phenotypic alterations. In consequence, DFA is a useful technique to discriminate population variability and group. The DFA efficiently decreased the species-specific variability. According to DFA data, *L. equula* is more identical to the genus *Aurigequula*: and *L. ruconius* to the genus *Deveximentum*, and *L. berbis* to the genus *Equulites*; therefore, they should be in appropriate taxonomic arrangements. The species belonging to the genus *Leiognathus* are proposed to be

reclassified under their original genus, considering their inherent generic traits. Despite the fact, *D. ruconius* and *D. insidator* exhibit greater morphological similarity since DFA, whereas *A. equula* displays markedly different morphological features, distinguishing it from both other genera. The species from the genus *Nuchequula* and *Equulites* overlap with each other showing closer phylogenetic relationship, but all species from the genus *Leiognathus* are dispersed. PCA and DFA were performed exclusively on intact specimens, with individuals exhibiting damaged or broken fins omitted from the dataset. The key discriminators were pre-dorsal fin length, body depth, the 1st dorsal spine to last anal ray, the eye tip to the 1st dorsal spine, and pelvic spine to pectoral fin (breast), whereas the distances from the 1st dorsal spine to the pectoral fin, head length, nuchal crest, and pre-pelvic length were found to be less distant according to DFA. Furthermore, DFA validated significant taxonomic differentiation among species, as evidenced by overall morphometric variability. While some parameters exhibited overlap, suggesting phenotypic similarities, the observed morphometric variations effectively distinguish taxonomic groups, aligning with previous findings (Gupta *et al.*, 2018). These variations, potentially driven by evolutionary processes (MacLeod and Forey, 2002), contribute valuable insights to phylogenetic studies. In addition to DFA and morphometric variations, our study demonstrates that three species from the genus *Leiognathus* (*berbis*, *equula*, and *ruconius*) exhibit distinct biological traits, these include differences in otolith shape,

feeding habits, mouth protrusion, and body depth.

The otoliths have traditionally provided insights into paleodiversity, they are now widely used to study various biological traits and trophic interactions in fish (Echreshavi *et al.*, 2021). In this study, the statistically otolith morphology were observed between the genera *Aurigequula* and *Deveximentum*. Although they belong to the same family, their 'beak' structures are polar opposites. *Aurigequula* features a robust, elongated rostrum and a deep notch of nearly equal size (~15.1 and ~16.6, respectively), resulting in a sharply defined anterior profile. In contrast, the anterior features of *Deveximentum* are almost invisible, with both the rostrum and notch typically measuring under 4.0. Furthermore, while *Aurigequula* has a greater overall sagittal length and asteriscus height, *Deveximentum* otoliths have a compressed height-to-length ratio, appearing significantly 'taller' relative to their short horizontal span.

Typically, sagittal otoliths are used for species identification (Vu *et al.*, 2022; Ghanbarzadeh *et al.*, 2023). For the first time, the second otolith is being utilized to distinguish species at the taxonomic level. Due to its tiny size, the asteriscus has rarely been used in ichthyological studies but it holds unique characteristics and thus appears to be highly helpful in terms of robust taxonomic studies. Recent studies increasingly utilize all three pairs of otoliths (sagitta, asteriscus and lapillus) for species differentiation (Gallardo-Cabello *et al.*, 2017; Espino-Barr *et al.*, 2018; Panhwar and Kashani, 2024). This approach proves particularly effective because of otoliths,

notably remain intact over time, making them highly reliable for discriminating between species or population variations (Baker, 2006; Thuy *et al.* 2015; Ozpicak *et al.*, 2018). Otolith shape variation is influenced by a complex interplay of factors, as evidenced by numerous studies. These include developmental influences during ontogeny (Vignon, 2012), adaptations related to feeding habits and habitat characteristics (Lord *et al.*, 2012; Qamar *et al.*, 2019), length and weight factors (Khanali *et al.*, 2021) and broader patterns of phylogeographic distribution and phenotypic traits (Tuset *et al.*, 2008).

Additionally, it is generally observed that among the three pairs of otoliths in bony fishes, the sagitta is typically the largest. However, in some species, such as sea catfish, the lapillus is larger than the other two otoliths. In the case of ponyfishes, Thuy *et al.* (2015) reported that the otoliths, sagitta is the largest, the asteriscus is the smallest, and the lapilli are mussel shell shaped. Our study revealed lapilli to be the smallest otolith, contradicting this previous observation. Moreover, lapilli recovered from only a few individuals despite examination of ample specimens. To address these issues, our study precisely resolved the size of the three otolith pairs, including accurate identification of the left and right sagittae with their proximal surfaces.

The body shape, and morphological otolith shapes are somewhat harmonious with each other, for instance, the deeply compressed and rounded sagitta found in *D. ruconius* resemble *D. interrptum*, whereas the asteriscus is elongated with a concave posterior profile, which is a key taxonomic

characteristic. Similarly, the otoliths of *A. equula* and *A. fasciata* are similar in terms of the sulcus, rostrum, and dorsal view. The keel-like projections on the ventral sides distinguish the *Nuchequula* genus, whereas the asteriscus exhibits variation in the dorsal edges and rostrum structure. The genus *Deveximentum* can be characterized by the shape of the rostrum, antirostrum, and posterior region. The *Equulites* are oval-convex with prominent sulcus grooves, whereas *Aurigequula* reflects a sole-like structure with numerous irregular structures and its asteriscus possesses prominent excisura and rostrum shape. In this scenario, ponyfishes with deep body shapes such as *D. interrptum*, *D. insidator*, *D. indicum*, *D. ruconuis*, and *P. bindus* have oval-shaped sagitta and elongated asteriscus. While elongated body-shaped species possess elongated sagitta and semicircular asteriscus. In general, the shapes of sagitta in ponyfishes are categorized as rounded (orange ponyfish), oval (ruconuis), elongated (equula), common (minuta), and common with keel-like projection (decorus).

In conclusion, this study demonstrated the efficacy of a multifaceted approach, including morphology, meristic traits, landmark data, and otolith shape analysis, in accurately differentiating species within the Leiognathidae family. All analyses yielded satisfactory statistical results, highlighting the unique discriminatory power of otolith shape. The research provides a robust framework for species identification, overcoming the challenges posed by morphological similarities. Our findings underscore the importance of a cohesive database, current knowledge of

fish taxonomy, and accurate species delineation.

Conclusion

This study demonstrates that integrating sagittal and asteriscus otolith morphology with morphometric analyses provides a robust, reproducible framework for resolving taxonomic ambiguities within the ponyfish family (Leiognathidae). By applying discriminant function analysis, this study successfully identified key diagnostic otolith traits that consistently distinguish species and genera. Notably, incorporating the frequently overlooked asteriscus otoliths revealed novel, species-level markers that significantly strengthen taxonomic resolution. Beyond systematics study, the observed morphological variations highlight key ecological and evolutionary insights: consistent growth patterns in certain genera suggest evolutionary constraints, whereas variability in others indicates ecological plasticity. Ultimately, these findings extend the utility of otoliths into broader studies of niche differentiation, trophic ecology, and biogeographic distribution, offering a streamlined identification system that unravels the complex taxonomy of Leiognathidae to safeguard their ecological and economic value.

Acknowledgments

The Higher Education Commission is highly acknowledged for funding under NRPU-17461 to SKP.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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