Available online at: www.mbai.org.in doi:10.6024/jmbai.2025.67.1.2562-06

Taxonomic revision of six species of the genus *Thryssa* from Indian waters: A step towards diversity assessment

Ashly Gopinath[1,2]*, E. M. Abdussamad[2], N. S. Jeena[2] and Elizabeth Tomy[1,2]
[1]Cochin University of Science and Technology, Kalamassery, Kochi- 682 022, Kerala, India.
[2]ICAR-Central Marine Fisheries Research Institute, Kochi- 682 018, Kerala, India.

*Correspondence e-mail: ashlyavish9402@gmail.com
ORCiD: http://orcid.org/0000-0002-7589-8068

Received: 28 Feb 2025 Revised: 21 Apr 2025
Accepted: 22 Apr 2025 Published: 10 Jun 2025

Original Article

Abstract

Anchovies belonging to the genus *Thryssa* (family:Engraulidae) are common pelagic fishes that constitute a significant fishery resource along the Indian coast. A total of 314 specimens belonging to six species of the genus *Thryssa* were collected to generate data for exact field identification and species-specific molecular signatures. The specimens were identified by morphometric and meristic characters, along with COI-based DNA barcodes. This integrative study also aimed to reconstruct a phylogeny to clarify the evolutionary relationships among the species. The morpho-meristic results indicated that maxilla length, number of belly scutes, number of lower gill-rakers, and anal fin ray count were the main characteristics for identifying *Thryssa* species. The nucleotide content of each species was examined, and the genetic distance was calculated. The interspecific genetic distance (K2P) ranged from 13% to 22%, with the highest distance observed between *T. baelama* and *T. cuvierii* (22%) and the lowest between *T. mystax* and *T. polybranchialis* (13%). The average genetic distance within the genus was 19%. The topology of the phylogenetic tree showed that all the species in this study formed separate clades with a common ancestor. This work provides the first COI-barcode of the least investigated *T. polybranchialis* from the Indian coast, and establishes the relationship between *T. mystax* and *T. polybranchialis*. Further morphological and molecular studies are recommended to elucidate the relationship between all extant species within this genus.

Keywords: *Anchovy, barcode, COI, Engraulidae, India, phylogenetic tree*

Introduction

Anchovies are an important small-sized marine food fish of the world. They have a maximum of standard length 40 cm (Froese and Pauly, 2025), and their fishery is high volume, valuable, and priced. Anchovies belong to the family Engraulidae of the order Clupeiformes. They are widely distributed along the Indian coastline and form a major contribution to the finfish fishery along with Indian mackerel and Bombay duck (CMFRI, 2023). In total, 42.9% of pelagic fish catch, 3.72% were composed of Anchovies. However, major contributions are due to *Stolephorus*, *Engraulis, Thryssa, Setipinna*, and *Coilia* genera. There are 17 valid genera in anchovies, including *Thryssa,* that are distributed entirely in the sea habitats of the temperate and tropical regions (Eschmeyer *et al.,* 2025).

Thryssa is a senior synonym for *Thrissina* (Jordan and Seale, 1925). Kottelat (2013) revised the generic name of *Thryssa* to *Thrissina*. Grande and Nelson (1985) classified anchovy into two subfamilies, Coilinae and Engraulinae, and the genus *Thryssa* falling under the latter. *Thryssa* is found throughout the Indo-Pacific region and forms commercially important resources in India, especially on the southwest and southeast coasts. They are silvery-coloured, highly abundant, marine, and carnivorous fishes that form large schools in near-shore habitats at depths of less than 50 meters. *Thryssa* contributed 1.3% of the total marine landings in India (CMFRI, 2023). Gill nets and mini purse seines are the major gear employed for their fishery. There are 38 species reported worldwide (Hata *et al.,* 2025), of which 16 are reported in India. They have a medium-compressed or strongly compressed body with 21-32 keeled scutes on the abdomen that are present from before the pectoral fin base to the anus. The length of their maxilla can vary from short to very long, with 25-45 branched anal fin rays. They possess 6-7 pelvic fin rays, a pre-dorsal scute, and small

jaw teeth that are not canine-like. Their upper pectoral fin rays do not extend as a filament (Whitehead *et al.,* 1988).

The identification and classification of fish are mainly based on morphometric and meristic characteristics (Triantafyllidis *et al.,* 2011). Recently, many authors have reported the taxonomic analysis of various *Thryssa* species (Hata and Motomura, 2019; Hata and Nakae, 2019; Hata, 2020, 2022; Hata and Koeda, 2020; Hata *et al.,* 2021, 2022; Hata *et al.*, 2023a, 2023b; Hata and Lavoue, 2024). In general, species identification of Anchovies is very difficult because of their small size, soft body, presence of ambiguous characters, and rapid spoilage (Whitehead *et al.,* 1988; Khan *et al.,* 2010).

Recent studies have revealed new insights into the genus *Thryssa*. Hata and Motomura (2019) identified specimens of *T. cultella* from the Bay of Bengal and *T. serena* from the northwestern Indian Ocean. A new species, *T. supra,* with a redescription of *T. whiteheadi,* was identified by Hata *et al.* (2021). Hata *et al.* (2022) described another new species, *T. katana,* from the Western Pacific Ocean. Hata *et al.* (2023) also described a new species, *T. aurora,* from the Andaman Sea and conducted a reassessment of the taxonomic status of *T. cuvierii* and *T. malabarica*. Their research established that *T. malabarica* is the senior synonym of *T. hamiltonii*. Additionally, they concluded that the species formerly recognised as *T. malabarica* should correctly be referred to as *T. cuvierii*.

DNA barcoding is an effective method for identifying species using short standardised DNA sequences and helps determine the phylogenetic relationship within the species (Hebert *et al.,* 2004). Numerous studies have shown that the mitochondrial cytochrome c oxidase subunit I (COI) gene's roughly 650 bp-long sequence is more suited for DNA barcoding than other genes (Lakra *et al.,* 2011; Ma *et al.,* 2015; Zhang *et al.,* 2016). The DNA barcoding of three species of anchovies (*Stolephorus*, *Encrasicholina*, and *Thryssa*) was reported by Lakra (2011). Lavoue *et al.* (2017) described the molecular systematics of the anchovy in the Northwest Pacific. Many authors have hinted at the phylogenetic analysis of various anchovy species (Bloom and Lovejoy, 2012; Chairi and Rebordinos, 2014; Afrand *et al.,* 2017; Lavoue *et al.,* 2010; Lavoue *et al.,* 2017). Recently, Mahrus *et al.* (2022) observed the phylogenetic relationship of Anchovies using COI genetic markers.

Integrative taxonomic studies of the genus *Thryssa* in Indian waters are limited. The present study aims to provide valuable information about the classical taxonomy, molecular identification, phylogenetic relationship, and genetic divergence of six species of *Thryssa* from Indian waters. The current work combines morphology, morphometry, meristic, and genetic traits of six species from different locations in India. Six species of genus *Thryssa, viz. T. setirostris*, *T. cuvierii*, *T. mystax*, *T. baelama*, T. *polybranchialis*, and *T. serena* have been analysed in this study.

Material and methods

Taxon sampling and morphological identification

A total of 314 specimens of the six species of *Thryssa* were collected from four localities in Indian waters from January 2022- July 2023 (Fig. 1). These fish were caught by gillnets and mini purse seines at a depth of 15-20 meters. The collected sample was ice-packed on the site and brought to the laboratory. Morphological characters were observed using a vernier calliper with 0.1 cm accuracy, and the total weight (TW) of each fish was taken using an electronic weighing balance with 0.01g accuracy. Meristic characters were observed and counted. Morphometric and meristic characters were measured using Hubbs and Lagler (2004). Most important taxonomic characters, like gill raker count, were analysed using a stereo zoom microscope. Species identification was carried out by using Hata and Motomura (2019); Hata *et al.,* 2023; Hata and Lavoue (2024); Whitehead *et al.* (1988).

Table 1. Name and geographic location of fish samples collected

Sl. No.	Scientific name	Common name	Geographic Location	Code (Lat. and Long.)	Period of collection
1.	*T. baelama*	Baelama *Thryssa*	Andaman and Nicobar Islands	Jungalighat (11°39'33.21"N, 92°43'41.9088" E)	January 2022
2.	*T. cuvierii*	Malabar *Thryssa*	Karnataka	Mangalore (12°51'28.728" N, 74°49'57.2736" E) Malpe (13°20'50.19" N, 74°42'4.842" E).	February 2022
3.	*T. setirostris*	Low jaw *Thryssa*	Tamil Nadu	Tuticorin (08°47' 36.96" N, 78°9'37.7676" E)	August 2022
4.	*T. polybranchialis*	hump head *Thryssa*	Kerala	Alappuzha (9°29'53.04" N, 76°20'19.85" E)	September 2022
5.	*T. mystax*	Middle-jawed *Thryssa*	Kerala	Kalamukku (09°59'24" N, 76°4'564" E) Vizhinjam (08°22'52.59" N, 76°59'4.25" E)	June 2023
6.	*T. serena*	Orange mouth Anchovy	Tamil Nadu	Kanyakumari (8°5' 5.649" N, 77°32'30.46" E)	July 2023

Fig. 1. Map showing the distribution of the sampling sites of the genus *Thryssa* along Indian waters (Source: Metalab)

Approximately 100 mg of white muscle tissue from twelve samples was extracted from each specimen and preserved in 95% ethanol for genetic analysis. The names and geographic locations of the collected samples are presented in Table 1. The GenBank and accession number, collection location, and type locality of each species were listed in Table 2.

DNA extraction and PCR conditioning

The genomic DNA of each species was extracted from the muscle tissue using a standard phenol-chloroform extraction method (Sambrook and Russell, 2006). Amplification of partial sequences of mitochondrial cytochrome oxidase subunit (COI) sequence was done by using the primer set (Ward *et al.,* 2005) LCO1490/ HCO2198 under standard conditions. The PCR products were purified using the Qiagen PCR purification kit. The purified PCR product was used as the template for sequencing PCR with the same primer. The sequencing was done at Eurofins Scientific, Bangalore, India. The chromatograms were visually with the aid of ABI sequence editor 3.3 (Applied Biosystems, Waltham, MA).

DNA sequencing and data analysis

Sequencing was carried out with the forward and reverse primers to confirm the nucleotide bases. The raw sequences were edited

Table 2. List of sequences used for analysis

Sl. No.	Scientific name	GenBank Accession number	Collection Location	Type locality of the species
1	*T. setirostris*	MH380700	Sri Lanka	Tanna Island, Southwestern Pacific
2	*T. setirostris*	MH380701	Sri Lanka	Tanna Island, Southwestern Pacific
3	*T. setirostris*	*PP038216	Current study	Tuticorin, Tamil Nadu
4	*T. setirostris*	*PP038217	Current study	Tuticorin, Tamil Nadu
5	*T. cuvierii*	MH380708	Sri Lanka	Tamil Nadu, India
6	*T. cuvierii*	MH380709	Sri Lanka	Tamil Nadu, India
7	*T. cuvierii*	*PP038238	Current study	Mangalore, Karnataka
8	*T. cuvierii*	*PP038239	Current study	Mangalore, Karnataka
9	*T. mystax*	MW498834	Malaysia	Calicut, India
10	*T. mystax*	MW498835	Malaysia	Calicut, India
11	*T. mystax*	*PP038274	Current study	Kalamukku, Kerala
12	*T. mystax*	*PP038275	Current study	Kalamukku, Kerala
13	*T. baelama*	GU674112	Indonesia	Sudan
14	*T. baelama*	HQ564499	Indonesia	Sudan
15	*T. baelama*	*PP038031	Current study	Jungalighat, Andaman and Nicobar Islands
16	*T. baelama*	*PP038032	Current study	Jungalighat, Andaman and Nicobar Islands
17	*T. polybranchialis*	ON166076	India	Mumbai, India
18	*T. polybranchialis*	MH380703	Sri Lanka	Mumbai, India
19	*T. polybranchialis*	MK962527	India	Mumbai, India
20	*T. polybranchialis*	*PP053492	Current study	Alappuzha, Kerala
21	*T. polybranchialis*	*PP053493	Current study	Alappuzha, Kerala
22	*T. serena*	MH380705	Dubai	Pakistan
23	*T. serena*	MH380704	Dubai	Pakistan
24	*T. serena*	*PP038226	Current study	Kanyakumari, Tamil Nadu
25	*T. serena*	*PP038227	Current study	Kanyakumari, Tamil Nadu

*indicates sequences generated in the present study

manually, and the forward and reverse sequences of the genus were combined using BioEdit (Hall, 1999) sequence alignment editor version 7.0.5.2 (M. J. Research Inc., Waltham, MA). The alignments were manually checked and corrected by using the BioEdit assembly program; aligned sequences had a trimmed length of 597 bp. All sequences generated from the study have been deposited in NCBI GenBank (Table 2). The analysis of GC, AT content, and sequence divergence was carried out based on the Kimura 2 model in MEGA Version 11 (Tamura *et al.,* 2021).

The phylogenetic tree was reconstructed by using MEGA Version 11 (Tamura *et al.,* 2021). Fifteen sequences collected from NCBI GenBank and 12 sequences identified from this study were used for the analysis (Table 2). Closely related outgroups like Buccaneer anchovy, *Encrasicholina punctifer* (GenBank accession number: HQ564397), and Hardenberg's anchovy, *Stolephorus indicus* (GenBank accession number: FJ347956), were included in the sequence analysis. The phylogenetic tree was constructed using the Maximum Likelihood (ML) method.

Results

Our results for the morphological identification of six *Thryssa* species are represented in (Figs. 2-7 and Table 3). We successfully identified species within this genus, including *T. setirostris*, *T. cuvierii*, *T. mystax*, *T. baelama*, *T. polybranchialis* and *T. serena*.

Morphometric characteristics

Thryssa spp. are characterised by medium-sized compressed or strongly compressed bodies. They have a spine-like small scute present in front of the dorsal fin ray. The belly is sharply keeled with pre-and post-pelvic scutes from the isthmus to the anus. Maxilla may be short, moderate, long, or very long. The anal fin is long usually with 26-45 fin rays. Tip of the snout in the middle of the eye centre or above the level of the upper rim of the eye. The serrae on the gill rakers are clumped or even.

Species identification key

The major distinguishing characteristics of all the *Thryssa* species are as follows (adapted from Whitehead *et al.* (1988); Hata *et al.* (2023), and observations made during the present studies)

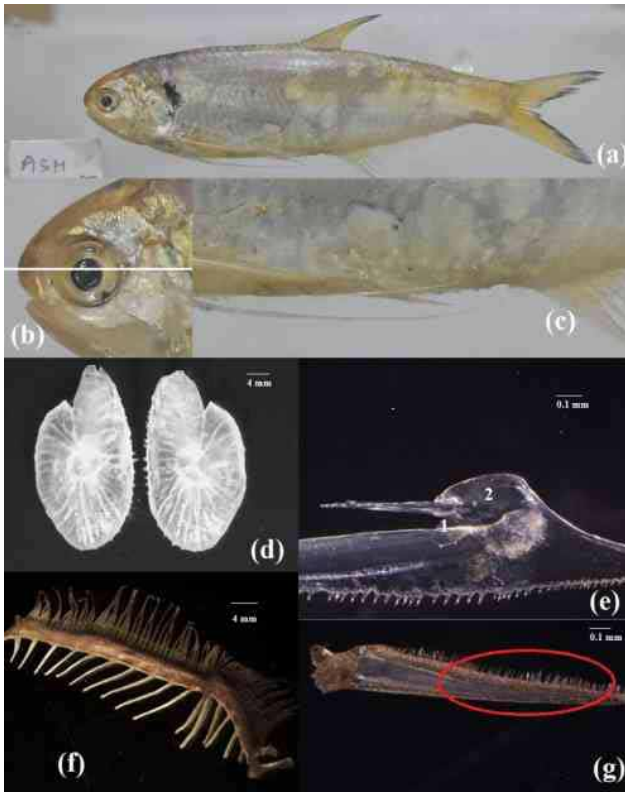

Fig. 2. Morphological characteristics of *T. setirostris* collected from Tuticorin, Tamil Nadu. a) represent *T. setirostris.* b) snout tip at about level of eye center. c) maxilla very long, reaching at the pelvic fin d) otolith e) two supra-maxillae on upper jaw (first supra-maxilla oval in shape). f) first gill arch g) serrae on the gill rakers not clumped

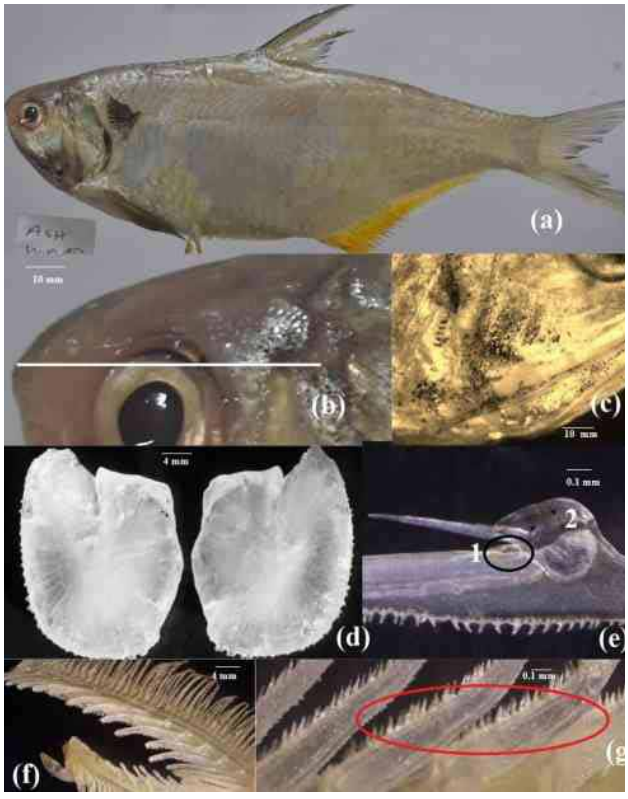

Fig. 3. Morphological characteristics of *T. cuvierii* collected from Karnataka a) represent *T. cuvierii* b) tip of snout at about level of upper rim of eye c) maxilla moderate, projecting a little beyond edge of gill cover d) otolith e) two supra-maxilla on upper jaw (first supra maxilla small, oval) f) first gill arch g) serrae on the gill rakers not clumped

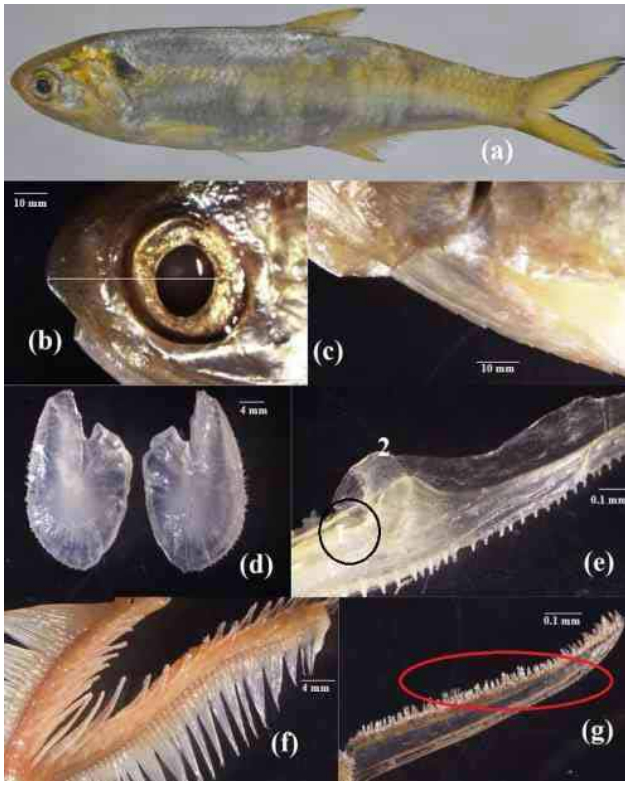

Fig. 4. Morphological characteristics of *T. mystax* collected from Kerala. a) represent *T. mystax*. b) snout tip at the level of eye centre c) maxilla long reaching at the end of pectoral fin rays. d) otolith e) two supra-maxilla on upper jaw (first supra maxilla oval, minute) f) first gill arch g) serrae on the gill rakers not clumped

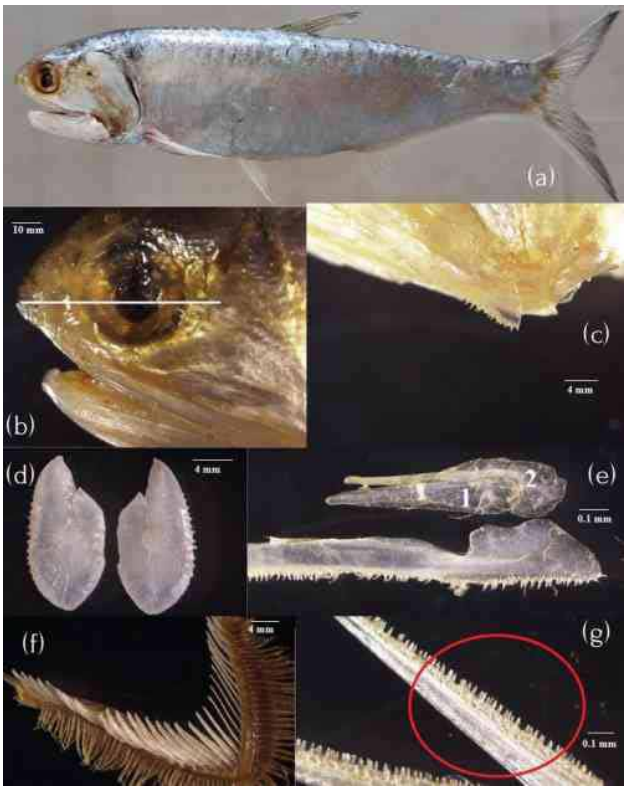

Fig. 5. Morphological characteristics of *T. baelama* collected from Jungalighat, Andaman and Nicobar Islands a) represent *T. baelama* b) snout tip at the level of eye centre c) maxilla short reaching to just or beyond front border of pre-operculum. d) otolith e) two supra-maxilla on upper jaw (first supra maxilla oval and tip is pointed). f) first gill arch g) serrae on the gill rakers not clumped

1a. Maxilla very long, reaching at least to base of pectoral fin ray; dark blotch present posterior to upper part of gill opening 2
1b. Maxilla shorter, usually not extending posterior to gill cover; dark blotch behind gill opening present or absent 3

2a. Lower gill rakers 10-12; high coronoid process on lower jaw ..... *T. setirostris* (Broussonet, 1782).
2b. Lower gill rakers 14-16; coronoid process in lower jaw not rising steeply; anal fin rays 29-37; 1TGR and 2TGR ≥ 25; pre-dorsal length ≤ 53.3% of SL; second supra-maxilla length ≤ 6.3% of SL ..... *T. mystax* (Bloch & Schneider, 1801).

3a. Tip of snout above the level of eye centre 4
3b. Tip of snout at the middle of eye centre or lower 5

4a. Body depth 33.8-38.8% of SL; melanophores scattered on cheek, gill cover, and maxilla; gill arches pink-orange; inside of gill cover yellow-gold; inner part of anal fin deep yellow with milky white margin ..... *T. cuvierii* (Swainson, 1839).
4b. Distinct hump at nape; lower gill rakers on first-gill arch 25-27 ..... *T. polybranchialis* (Wongratana, 1983).

5a. Gill rakers on first lower gill arch 19-23; anal fin rays 34-36; pelvic fin short (7.8-8.6% of SL); body depth 26.6-29.4% of SL; 10 transverse scale; longitudinal scale 38-41 ..... *T. serena* (Hata and Motomura, 2019).
5b. Total gill raker count in first-gill arch 40 or less; snout shorter than 4.8% of SL; 12-18 keeled scutes on abdomen ..... *T. baelama* (Fabricius, 1775).

In this study, species were genetically characterised using the COI regions, where no deletions or insertions were observed. The ATGC content of each species was calculated in Table 4. A comparison of the ATGC content revealed that the AT content is higher than the GC content. The average nucleotide composition was 25.6%, 28.2%, and 19.3%. 26.9% of A, T, G, and C, respectively. The pairwise genetic distance is given in Table 5. The interspecific genetic distance ranged from 13% to 22%. The average was found to be 19%. The highest genetic distance was observed between *T. baelama* and *T. cuvierii* (22%). The lowest (13%) was between *T. polybranchialis* and *T. mystax*. The phylogenetic tree was reconstructed using the best-fit maximum likelihood (ML) method (Fig. 8). The tree topology

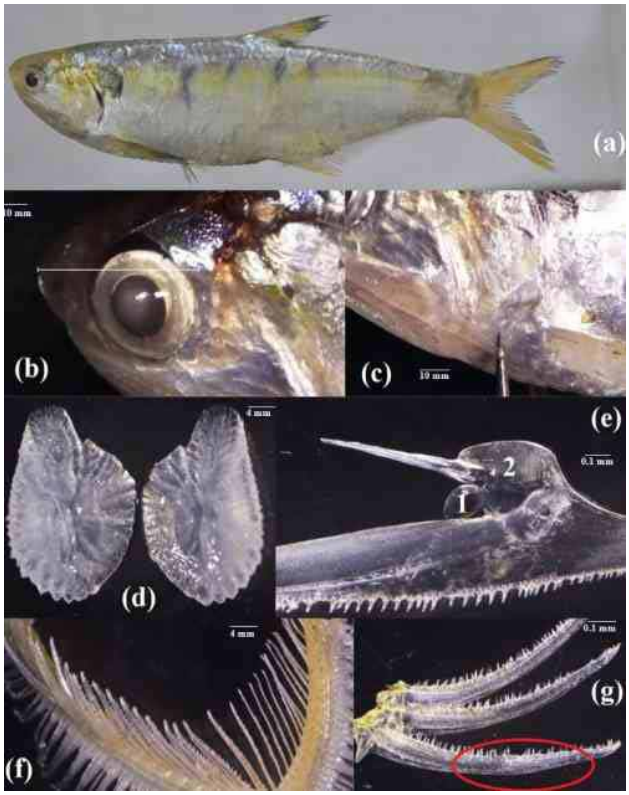

Fig. 6. Morphological characteristics of *T. polybranchialis* collected from Alappuzha, Kerala. a) represent *T. polybranchialis* b) tip of the snout above upper rim of the eye. c) maxilla short reaching to just of the gill cover d) otolith e) two supra-maxillae on upper jaw (first supra maxilla oval, minute) f) first gill arch g) serrae on the gill rakers not clumped

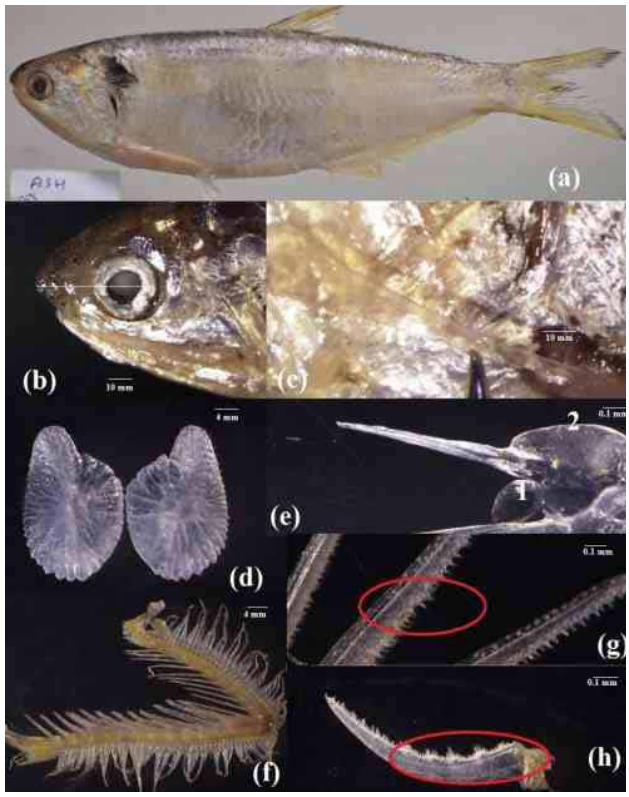

Fig. 7. Morphological characteristics of *T. serena* collected from Kanyakumari, Tamil Nadu. a) represent *T. serena.* b) snout tip at about level of eye centre. c) maxilla moderate, reaching beyond base of first pectoral fin ray d) otolith e) two supra-maxillae on upper jaw (first supra maxilla oval in shape). f) first gill arch g) serrae on the gill rakers not clumped in juveniles. h) the gill rakers serrae in distinct clumps in larger fishes

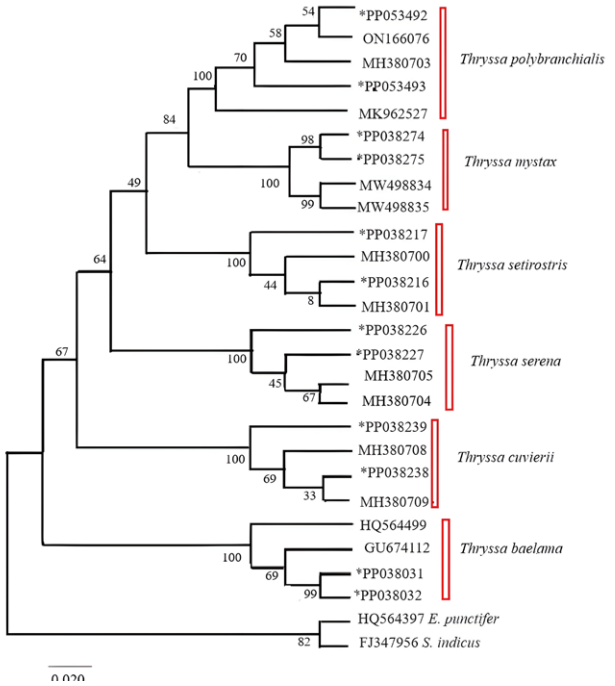

Fig. 8. Maximum-likelihood (ML) phylogenetic tree of the genus *Thryssa* based on MEGA 11. Number of nodes indicates the bootstrap value. HQ564397 (*E. punctifer*) and FJ347956 (*S. indicus*) from GenBank are included as out-group species

indicates that each species observed in this study sprang from a common ancestor to create distinct clades.

Discussion

Morphological characteristics

The present study successfully identified the characteristics of six species within the genus *Thryssa, viz. T. setirostris*, *T. cuvierii*, *T. mystax*, *T. polybranchialis*, *T. baelama*, and *T. serena* in Indian waters. The latter five species are widely distributed along the southern coast of India, and *T. baelama* was observed in the Andaman and Nicobar Islands (Rajan *et al.,* 2013). In our work, the fish were initially identified as the genus *Thryssa* based on their physical traits. The taxonomic characteristics of each species were compared with earlier studies (Al-Faisal, 2012; Ma *et al.,* 2015; Afrand *et al.,* 2020; Hata and Motomura, 2019). These previous studies focused on the taxonomic classification of *Thryssa* species, emphasizing characteristics such as the length of the maxilla, number of lower gill-rakers, number of scutes (both pre-and post-pelvic), and anal fin ray number.

Table 3. The main morphological characters to identify the six species of the genus *Thryssa*

Species	Specimen number	Gill-rakers on the first lower gill-arch	Total abdominal scutes	Scale rows in longitudinal series	Transverse scales	Anal fin rays
T. setirostris	60	11-12	24-26	40-42	10-12	32-34
T cuvierii	50	16-20	24-25	37-40	10-12	35-37
T. mystax	100	15-18	26-30	41-43	11	30-35
T. baelama	4	20-23	14-16	33-34	8-9	25-30
T. polybranchialis	50	25-27	24-28	41-43	11	38-43
T. serena	50	21-23	26-29	39-40	10-11	35-36

Table 4. Average ATGC percentage in the COI region (597 bp) of various species in the genus *Thryssa*

Species name	T(U)	C	A	G	Total
T. setirostris	27.3	27.9	25.7	19.1	597
T. cuvierii	27.5	27.8	24.3	20.4	597
T. mystax	28.2	26.6	27	18.2	597
T. baelama	28.6	27.8	24.6	18.9	597
T. polybranchialis	28.9	25.6	25.7	19.8	597
T. serena	28.6	25.8	26	19.6	597
Avg.	28.2	26.9	25.6	19.3	597

Table 5. Average genetic distance (%) based on Kimura 2-parameter for the COI gene in the genus *Thryssa*

Species name	*T. setirostris*	*T. mystax*	*T. cuvierii*	*T. serena*	*T. polybranchialis*	*T. baelama*
T. setirostris	-	-	-	-	-	-
T. mystax	16.1	-	-	-	-	-
T. cuvierii	18.4	21.0	-	-	-	-
T. serena	16.9	16.8	19.2	-	-	-
T. polybranchialis	15.8	13.1	19.6	15.2	-	-
T. baelama	21.0	20.6	21.6	18.8	20.6	-

In our study, we observed that in *T. setirostris*, *T. mystax*, *T. serena,* and *T. baelama*, the tip of the snout was found to be at the level of the eye centre, while in *T. cuvierii* and *T. polybranchialis,* it was found to be at the upper rim of the eye. Every species is distinguished by having a prominent supra-maxilla in both the first and second positions. It is noted that the first is tiny, round, and positioned under the second.

Khamees *et al.* (2018) stated the comparative measurements of fin rays and five gillrakers of five *Thryssa* species, *viz. T. whiteheadi, T. vitrirostris, T. baelama, T. hamiltonii,* and *T. setirostris* from the north-western Arabian Gulf. In our study, the anal fin ray count of *T. setirostris* was found to range from 32-39, whereas in their work, it was reported as 34-37. Upon comparing our findings with that of the two species (*T. baelama*, and *T. setirostris*), it was found that the specimens from our study have anal and pectoral fin ray counts that fall within the same range. Iwatsucki (2013) reported that the meristic characteristics of fish may vary in different habitats.

Hata and Motomura (2019) observed that *T. serena* has long, slender gill rakers, and the serrae on the gill raker were clumped and ranged from 20-24. Hata *et al.* (2023) examined the original description of *T. malabarica* and *T. cuvierii* and revealed that *T. malabarica* was a senior synonym of *T. hamiltonii* (Gray, 1835). We compared the characteristics of *T. cuvierii* and found that the anal fin ray count fell between 35-39 and the lower gill raker count ranged from 16-20, which is in agreement with Hata *et al.* (2023).

The present study includes the most important taxonomic characters of six species of the genus *Thryssa*. The data presented (Table 3) aligned with the findings of Hata's studies, indicating that *T. mystax* and *T. serena* have morphological similarities that make them difficult to differentiate without accurate taxonomic information. In our results, we found that the maxilla length of *T. mystax* was identical to *T. serena*, both have long maxilla that reaches the base of the pectoral fin. Serrae on the gill rakers are clumped in adult fish of *T. serena*, while in *T. mystax*, they are not clumped.

The lower gill raker of *T. baelama* differs from those of *T. setirostris* and *T. mystax*. *Thryssa mystax* can be distinguished from *T. polybranchialis* by its elongated maxilla.

In *T. polybranchialis*, the maxilla extends to the level of the gill opening, and in *T. mystax*, the maxilla extends to the pectoral fin base. The tip of the snout is in the middle of the eye in *T. mystax*, whereas it is above the upper rim of the eye in *T. polybranchialis*. Gill rakers count was a useful trait for distinguishing between these species. *T. mystax* showed a count of 15-18 gill rakers on the first lower gill arch, whereas *T. polybranchialis* has 25-27 gill rakers. Other morpho-meristic characteristics such as anal fin ray number, transverse scales, and scute count do not differentiate these two species. After examining their morphology, we noted there are huge phenotypic uncertainties in the samples, along with observable variations in morphometric features.

Molecular analysis

The identification of the genus *Thryssa* is extremely challenging due to the overlapping meristic counts. The limitations of morphologically based fish identification systems have been addressed by the application of genetic approaches. The phylogenetic construction of *Thryssa* was reconstructed. The tree topology indicates that individuals of the same species formed distinct clusters. This is the first attempt that reveals the relationship between the *T. mystax* and *T. polybranchialis* from Indian waters. The morphological characteristics of *T. mystax* were identical to those characteristics of *T. serena,* and from this study, we observed that the species *T. mystax* and *T. serena* diverge at the species level.

In the present study, six *Thryssa* species were characterized using barcodes, and the average interspecific genetic distance of each species was calculated. According to Hebert *et al.* (2003), the minimal genetic distance between species is 2%. The genetic distance values in this study lie well above the accepted species threshold value.

Zhang *et al.* (2016) examined the morphological description and phylogenetic relationship of six species from coastal waters in China, which includes three species included in our study. They observed a genetic divergence of 16% between *T. setirostris* and *T. vitrirostris*. They constructed the phylogenetic tree, and the tree topology indicated that *T. setirostris* was the first species derived from the genus. According to their results, *T. vitrirostris* and *T. mystax* showed the closest relationship with each other. They found that the base composition of A and T was higher than that of G and C, which is concordant with our results. The separate genetic status of *T. vitrirostris* from *T. mystax* also corresponded with their investigation.

We reconstructed a phylogenetic tree that includes *T. polybranchialis*, a species commonly found in Indian waters. Our work indicated that *T. mystax* has the closest connection with *T. polybranchialis* and *T. setirostris,* and the tree topology showed that *T. polybranchialis* and *T. mystax* clustered together with a common ancestor. The barcodes in the present study showed that *T. polybranchialis* is quite different from *T. mystax*. Ma *et al.* (2015) recorded the utility of the COI sequence as DNA barcoding for the identification of the genus *Thryssa* from the Zhejiang Sea in China. They found an average of 29.2% T, 26.2% C, 26.2% A, and 18.1% G in their sequences. The genetic distance ranged from 0.003 to 016, with an average of 0.137. The tree topology indicated that four species formed monophyletic clusters. The phylogenetic relationship of the genus *Thryssa* in their study indicated a closer relationship between *T. vitrirostris* and *T. mystax,* with a very low genetic distance of 0.003. However, their morphological analysis revealed that these two might be one species. We observed that our results differed from their conclusions. The life habits of various fish species are related to convergent evolution, which may be the reason for this variable outcome (Ma *et al.,* 2010; Kruck *et al.,* 2013)

Afrand *et al.* (2020) combined the taxonomic traits of Engraulidae in the Persian Gulf and the Oman Sea. They successfully discriminated the DNA barcode of five species of *Thryssa* using the mitochondrial COI gene. The tree topology showed that *T. hamiltonii* and *T. vitrirostris* were clustered together. In our work, the phylogram shows that *T. baelama* was the first species to diverge, and T. *polybranchialis* and *T. mystax* formed a group. The results from the DNA barcoding and identification based on morphological characters in our study agree with Afrand *et al.* (2020). This study reveals an integrative taxonomic identification of the six species of the genus *Thryssa,* and a further detailed revision of other *Thryssa* species is recommended.

Conclusion

We successfully examined the morphology and the DNA barcode of six *Thryssa* species. Our study marks a comprehensive phenotypic and genotypic evaluation of *T. mystax*, *T. polybranchialis,* and *T. baelama* from Indian waters. This work registers the first COI-barcode of the least investigated *T. polybranchialis* from Indian waters. The current study will be useful as baseline information for evolutionary biologists for future studies.

Acknowledgements

The authors would like to thank the Director, Central Marine Fisheries Research Institute and the Dean, Cochin University of Science and Technology, Kochi, for the support and facilities provided. They acknowledge the University Grants Commission for financial support, and the fishers for providing samples for the study.

Author contributions

Conceptualisation: AG, EMA; Methodology: AG, EMA, ET; Data collection: AG; Data analysis: AG, EMA, JNS, ET; Writing original draft: AG; Writing Review and Editing: AG, JNS; Supervision: EMA

Data availability

The data are available and can be requested from the corresponding author. The data supporting this study are publicly available at the repository [CMFRI, Marine Biodiversity Museum, https://www.cmfri.org.in/museum]

Conflict of interest

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Funding

This research was supported by the University Grants Commission (UGC) of India through the Junior Research Fellowship granted to Ashly Gopinath under grant number 986/2019.

Publisher's note

The views and claims presented in this article are solely those of the authors and do not necessarily reflect the positions of the publisher, editors, or reviewers. The publisher does not endorse or guarantee any claims made by the authors or those citing this article.

References

Afrand, M., I. Sourinejad, S. A. Hahzadeh Fazeli and A. Akbarzadeh. 2017. Identification of Engraulids (*Encrasicholina punctifer* and *E. pseudoheteroloba*) using the COI gene as a DNA barcoding marker, *J. Aquat. Ecol.*, 7 (3): 39-47.

Afrand, M., I. Sourinejad, S. A. S. Fazeli, A. Akbarzadeh, L. P. Yeganeh, M. Sadeghi and R. Azarbaijani. 2020. Morphological identification and molecular validation of anchovies (Engraulidae) in the Persian Gulf and Oman Sea, *Zootaxa*, 4742 (2): 375-391.

Al-Faisal, A. J. 2012. Taxonomic study of three species of genus *Thryssa* fishes from Iraqi marine water, *J. King Abdulaziz Univ. Mar. Sci.*, 23 (1): 147-163.

Bloom, D. D. and N. Lovejoy. 2012. Molecular phylogenetics reveals a pattern of biome conservatism in New World anchovies (family Engraulidae), *J. Evol. Biol.*, 25(4): 701-715.

Chairi, H. and L. Rebordino. 2014. A rapid method for differentiating four species of the Engraulidae (Anchovy) family, *J. Agric. Food Chem.*, 62 (13): 2803-2808.

CMFRI. 2023. CMFRI Annual Report 2022, Technical Report, ICAR-Central Marine Fisheries Research Institute, Kochi. p. 21-22.

Eschmeyer, W. N., R. Fricke and R. Van der Laan. 2025. Catalogue of fishes: genera, species, references. California Academy of Sciences. https://www.calacademy.org/scientists/projects/eschmeyers-catalog-of-fishes.

Froese, R. and D. Pauly. 2025. FishBase. https://www.fishbase.org

Grande, L. and G. Nelson. 1985. Interrelationships of fossil and recent anchovies (Teleostei: Engrauloidea) and description of a new species from the Miocene of Cyprus, *Am. Mus. Novit.*, 2826: 1-16.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symp. Ser.*, 41: 95-98.

Hata, H. and H. Motomura. 2019. Two new species of *Thrissina* (Clupeiformes: Engraulidae) from the northern Indian Ocean and redescription of *Thrissina vitrirostris* (Gilchrist and Thompson 1908), *Ichthyol. Res.*, 67: 155-166.

Hata, H. and M. Nakae. 2019. First Japanese record of the engraulid *Thrissina chefuensis* (Teleostei: Clupeiformes), from Yamaguchi prefecture, *Species Divers.*, 24: 290. https://doi.org/10.12782/specdiv.24.287.

Hata, H. 2020. Redescription of the specimen of *Thrissina dussumieri* (Teleostei: Clupeiformes: Engraulidae) collected from the Ogasawara Islands, *Species Divers.*, 25: 123-127.

Hata, H. and K. Koeda. 2020. *Thrissina encrasicholoides* (Actinopterygii: Clupeiformes: Engraulidae): First record from Taiwan and northernmost record of the species, *Acta Ichthyol. Pisc.*, 50: 107-111.

Hata, H. H., P. N. Psomadakis, H. B. Osmany and H. Motomura. 2021. A new species of *Thrissina* from Pakistan (Arabian Sea), with redescription of *Thrissina whiteheadi* (Wongratana 1983) (Clupeiformes: Engraulidae), *Ichthyol. Res.*, 68: 486-495.

Hata, H., S. Lavoué and H. Motomura. 2022. *Thrissina katana* sp. nov., a new *Thryssa* from the western Pacific Ocean, and redescription of *Thrissina hamiltonii* (Gray, 1835) (Teleostei: Clupeiformes: Engraulidae), *Mar. Biodivers.*, 52 (1): 11.

Hata, H. 2022. A new species of *Thrissina* from the northwestern Pacific Ocean, with redescription of *Thrissina adelae* (Rutter 1897) (Clupeiformes: Engraulidae), *Ichthyol. Res.*, 70: 419-430.

Hata, H., I. Mandagi and A. Masengi. 2023. Resurrection of nominal species previously regarded as junior synonyms of *Thrissina baelama* (Fabricius, 1775) and their re-descriptions (Teleostei: Clupeiformes: Engraulidae), *Raff. Bull. Zool.*, 71: 279-302.

Hata, H., S. Lavoué, S. Chungthanawong and H. Motomura. 2023. A new species of *Thrissina* from the Andaman Sea and re-assessment of the taxonomic status of *Thrissina cuvierii* (Swainson, 1839) and *Thrissina malabarica* (Bloch, 1795) (Teleostei: Clupeiformes: Engraulidae: Coiliinae), *Ichthyol. Herpetol.*, 111 (4): 549-562.

Hata, H. and S. Lavoué. 2024. Resurrection and redescriptions of nominal species previously regarded as synonyms of *Thrissina mystax* (Bloch and Schneider, 1801) (Teleostei: Clupeiformes: Engraulidae), *J. Fish Biol.*, 104 (5): 1445-1467.

Hata, H., S. Lavoué, P. N. Psomadakis, H. B. Osmany and H. Motomura. 2025. *Thrissina mystica* sp. nov., a new *Thryssa* (Teleostei: Clupeiformes: Engraulidae: Coiliinae) from the northern Indian Ocean, *Ichthyol. Res.*, 17: 1-13.

Hebert, P. D., A. Cywinska, S. L. Ball and J. R. DeWaard. 2003. Biological identifications through DNA barcodes, *Proc. R. Soc. Lond. B Biol. Sci.*, 270 (1512): 313-321.

Hubbs, C. L. and K. F. Lagler. 2024. Fishes of the Great Lakes Region. Revised. University of Michigan Press, 199 pp.

Iwatsuki, Y. 2013. Review of the *Acanthopagrus latus* complex (Perciformes: Sparidae) with descriptions of three new species from the Indo-West Pacific Ocean, *J. Fish Biol.*, 83 (1): 64–95.

Jordan, D. S. and A. Seale. 1925. Analysis of the genera of anchovies or Engraulidae, *Copeia*, (141): 27-32.

Khamees, N. R., T. K. Adday and J. M. Abed. 2018. Occurrence and redescription of *Thryssa setirostris* (Broussonet, 1782) (Clupeiformes, Engraulidae) from Iraqi Marine water, *Bull. Iraq Nat. Hist. Mus.*, 15 (2): 123-130. .

Khan, S. A., P. S. Lyla, B. A. John, C. P. Kumar, S. Murugan and Jalal K. C. A. 2010. DNA barcoding of *Stolephorus indicus*, *Stolephorus commersonnii* and *Terapon jarbua* of Parangipettai coastal waters, *Biotechnol.*, 9: 373-377.

Kottelat, M. 2013. The fishes of the inland waters of Southeast Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries, *Raffles Bull. Zool.*, 27: 1–663.

Kruck, N. C., I. R. Tibbetts, R. D. Ward, J. W. Johnson, W. K. Loh and J. R. Ovenden. 2013. Multi-gene barcoding to discriminate sibling species within a morphologically difficult fish genus (*Sillago*), *Fish. Res.*, 143: 39-46.

Lakra, W. S., M. S. Verma, M. Goswami, K. K. Lal, V. Mohindra, P. Punia, and P. Hebert. 2011. DNA barcoding Indian marine fishes, *Mol. Ecol. Res.*, 11 (1): 60-71.

Lavoue, S., M. Miya and M. Nishida. 2010. Mitochondrial phylogenomics of anchovies (family Engraulidae) and recurrent origins of pronounced miniaturization in the order Clupeiformes, *Mol. Phylogenet. Evol.*, 56 (1): 480-485.

Lavoue, S., J. A. Bertrand, H. Y. Wang, W. J. Chen, H. C. Ho, H. Motomura and M. Miya. 2017. Molecular systematics of the anchovy genus *Encrasicholina* in the Northwest Pacific, *PLoS ONE*, 12 (7): e0181329.

Ma, C., L. Ma, Y. Ni, A. Shen, Y. Zhang, F. Zhang, and Y. Zhao. 2010. Phylogenetic relationship of *Thryssa* inferred from morphological characteristics and mitochondrial 16S rRNA gene sequences, *J. Fish. Sci.* China, 49 (3): 1-203.

Ma, C. Y., H. Y. Ma, Y. Ni, W. Wang and L. B. Ma. 2015. Molecular identification of the genus *Thryssa* based on DNA barcoding, *Genet. Mol. Res.*, 14 (4): 18580–18586.

Mahrus, H., A. G. I. L. Al-Idrus, and L. Zulkifli. 2022. Molecular phylogeny of anchovy (Clupeiformes: Clupeidae) from southern waters of Lombok using mitochondrial DNA CO1 gene sequences, *Biodiversitas J. Biol. Divers.*, 23 (5): 2433-2443.

Rajan, P. T., C. R. Sreeraj and T. I. T. U. S. Immanuel. 2013. Fishes of Andaman and Nicobar Islands: a checklist, *J. Andaman Sci. Assoc.*, 18 (1): 47–87.

Sambrook, J. and D. W. Russell. 2006. Purification of nucleic acids by extraction with phenol: chloroform, *Cold Spring Harb. Protoc.*, 2006 (1): 4455.

Tamura, K., G. Stecher and S. Kumar. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.*, 38(7): 3022-3027.

Triantafyllidis, A., D. Bobori, C. Koliamitra, E. Gbandi, M. Mpanti, O. Petriki and N. Karaiskou. 2011. DNA barcoding analysis of fish species diversity in four north Greek lakes. *Mitochondrial DNA*, 22 (supp1_1): p. 37-42.

Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Lat and P. D. N. Hebert. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360: 1847-1857.

Whitehead, P. J., G. J. Nelson and T. Wongratana. 1988. Clupeoid fishes of the world (Suborder Clupeoidei). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings. Part 2: Engraulididae. In: FAO Species Catalogue, Vol. 7, FAO Fisheries Synopsis No. 125(7/2), Food and Agriculture Organization, Rome. p. 305-579.

Zhang, J., Y. Li, N. Song, L. Lin and T. Gao. 2016. Species identification and phylogenetic relationship of *Thryssa* species in the coastal waters of China. *Biodivers. Sci.*, 24 (8): 888-895.