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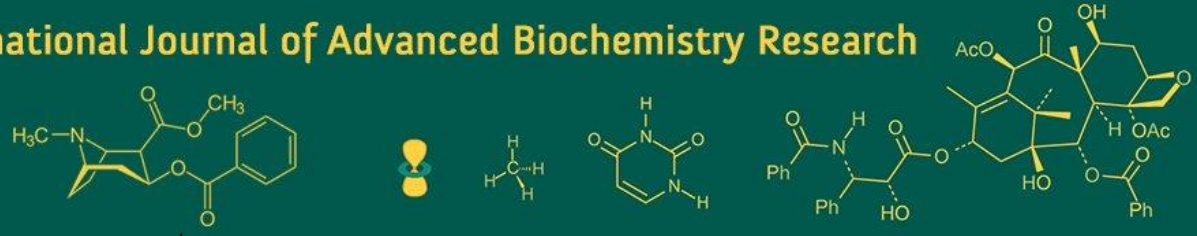

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Resolving the taxonomic ambiguities in the distribution of *Acropoma splendens* (Lloyd, 1909) from Arabian Sea of Indian EEZ: An integrative taxonomic approach

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Abstract
Seventy-three specimens of Acropomatids measuring 40.7-90.8 mm standard length were collected from commercial multi-day fishing trawlers at 25-50 m depth off the Mumbai coast, Arabian Sea, Northwest coast of India. The morphological and morphometric diagnostic characters of the collected specimens matches with *Acropoma splendens* (Lloyd, 1909) with the key features *viz*., the presence of a vertical line on the cheek, four rows of scales between the first dorsal fin and lateral line; gill raker count 19 – 22 numbers; pectoral fin rays 16 numbers; anus situated closer to the pelvic fin than to the anal fin; short and U-shaped luminous gland around the anus with a length of 12 – 16.9 % of standard length. Further, through cytochrome c oxidase subunit I (COI) gene sequencing analysis validated and confirmed the identity of the specimens as *Acropoma splendens* (Lloyd 1909). The comparison of COI gene sequences of *A. splendens* in the present study with the COI gene sequences of its interspecies of the *Acropoma genus* revealed that *Acropoma splendens* had the highest intraspecies distance (5.8) with *A. japonicum* and the least intraspecies distance (1.839) with *A. hanedai* and *A. argentistigma* was 3.0. Thus, integrative taxonomic unraveling the taxonomic ambiguity of the species and confirmed the presence of *A. splendens* for the first time from the Arabian Sea of Indian Exclusive Economic Zone (EEZ).

Keywords: Fish DNA barcoding, Acropomatidae, *Acropoma lacrima*, Glowbelly, Lanternbelly, Indian Ocean

Introduction
Laternbelly of the family Acropomatidae, representing the genus *Acropoma* Temminck and Schlegel 1843, has 12 valid species (Okamoto *et al*., 2021) [27] with global distribution in the Indo-Pacific region from Vanuatu to South Africa (Okamoto and Golani 2017; Okamoto *et al*. 2019; Okamoto *et al*., 2021) [25, 28, 27]. The species includes *A. japonicum* Günther, 1859 Glowbelly, *A. splendens* (Lloyd 1909) Indian lanternbelly, *A. hanedai* Matsubara 1953, *A. lecorneti* Fourmanoir, 1988 Lecornet's lanternbelly, *A. argentistigma* Okamoto and Ida 2002, *A. boholensis* Yamanoue & Matsuura, 2002 Big-headed lanternbelly, *A. profundum* Okamoto, 2014 Solomon's lanternbelly, *A. neglectum* Okamoto & Golani, 2017 Red Sea lanternbelly, *A. leobergi* Prokofiev, 2018 Tropical lanternbelly, *A. arafurense* Okamoto, Williams, Carpenter, Santos & Kimura, 2019 Arafura lanternbelly, and *A. musorstom* Okamoto, Randall & Motomura, 2021 South Pacific lanternbelly. The unique characteristics of the genus *Acropoma* includes luminous organs and an isolated dorsal fin spine between the 1st and 2nd dorsal-fins (Haneda 1950; Matsubara 1953; Haneda and Johnson 1962; Brüss and Ben-Tuvia 1983; Okamoto and Ida 2002; Yamanoue and Matsuura 2002; Yamanoue and Toda 2008; Okamoto 2014; Ghedotti *et al*. 2018) [12, 21, 11, 2, 26, 42, 43, 29, 9]. The location, shape, and length of the luminous gland, shape of the proximal radial of the 1st anal-fin spine pterygiophore, gill raker count, number of scales between the lateral line and 1st dorsal-fin

base, presence/absence of cycloid scales, and vertical line on the cheek are the most important diagnostic characters for species differentiation in the genus *Acropoma* (Katayama 1959; Yamanoue and Matsuura 2002; Yamanoue and Toda 2008; Okamoto & Golani, 2017; Okamoto *et al*., 2021) [15, 42, 43, 25, 27].

A. splendens was first discovered in the Gulf of Oman in 1909 as *Synagrops splendens* by Llyod. In the Arabian Sea, this species has been misreported as *Acropoma japonicum* by many authors (Fowler 1927; Norman 1939; Kotthaus 1974; Heemstra 1984; Balachandran and Nizar 1990; Carpenter *et al*. 1997; Manilo and Bogorodsky 2003; Javadzadeh *et al*. 2012; Psomadakis *et al*. 2015) [7, 24, 17, 13, 1, 3, 20, 14, 31] until 2016, when Okamoto and Golani described this as a new species as *Acropoma lacrima* in 2017 based on paratypes from the northwest coast of India, but later confirmed it as a junior synonym of *A. splendens* (Okamoto *et al*. 2021; Prokofiev 2019) [27, 30].

In the Indian EEZ, *A. splendens* were misidentified as *A. japonicum* by Balachandran and Nizar in the year 1990 [1] from the specimen caught in the Arabian Sea at the depth 140 m by operating bottom trawl net in the Fishery Oceanographic Research Vessel (FORV), Sagar Sampada (Location: Latitude 11°29'N Longitude 74°30'E). Another misidentified report of *A. splendens* by Fowler (1927) [7] from Bombay (currently Mumbai) waters of the Arabian Sea was *A. japonicum*. Another misidentified report of *A. splendens* as *A. argentistigma* from the Bay of Bengal region of the Indian EEZ, based on specimens collected from the Mohana, Digha, Fish markets on the West Bengal coast without confirming the gill raker count, pectoral fin ray count, and dark vertical line mark on the cheek in the preserved specimen (Yennawar *et al*., 2012) [44].

The aim of this study was to resolve the taxonomic ambiguity of this species, A. *splendens*, and to validate the presence of *A. splendens* in the Indian EEZ through an integrative taxonomic approach. This study also confirmed species identification by comparing the sequences of closely related species of the *Acropoma genus* available globally.

Materials and Methods

Sample collection information

Acropoma samples were collected from trawl bycatch landings at the New Ferry Wharf fish landing centre caught off the Mumbai coast, Arabian Sea, Northwest coast of India at a depth range of 25–50 m (Figure 1). The coloration and pigmentation of the fresh specimens were recorded and photographed at the landing centre. A total of 73 specimens of *A. splendens* were brought to the laboratory of the Mumbai Regional Station of ICAR-CMFRI, Mumbai, for further analysis. Of the 73 specimens analysed, 34 specimens were collected in January 2018, 4 specimens were collected in January 2021, and the remaining 35 specimens were collected in January 2023 from the Multiday trawl fish landings at the New Ferry Wharf fish landing centre.

The present study collected 73 *A. splendens* specimens from the heaps of trash fish landings at the New Ferry Wharf fish landing centre, Mumbai, as a component of the trash fish commodity. These trawlers regularly catch *A. splendens* as bycatch, targeting species such as ribbon fish, bombay duck, gold-spotted grenadier anchovy, sciaenids, and marine ariids from September to January annually. Typically, these trawlers bring their bycatch from the last four hauls of the trip as trash fish heaps during the peak fishing season, which runs from September to November. During the lean season, trash fish landings dominated the total catch composition compared with the targeted fish catch by these trawlers, usually from December to March every year. The landing centre price of these trash fish ranged from 15 to 28 INR per kilogram. Fishmeal plants use the trash fish landings as raw materials. The trawl fishery landings in the New Ferry wharf fish landing centre on the Mumbai coast discard 42.19 % of their bycatch at sea, which amounts to 33.25 % of the total catch annually, including juveniles of commercially important fishes that have huge economic potential in domestic markets (Ramkumar *et al*., 2019; Sugumar *et al*., 2015, 2016) [35, 37, 36]. Trash fishes constitute the constituents of the bycatch in the multiday trawl fishery of the Mumbai coast as low-value bycatch (LVB) commodities.

Sample analysis

Morphometric measurements and meristics counts were performed using the methods described by Okamoto (2014) [29]. All morphometric measurements were performed using a digital caliper to the nearest 0.1 mm and were expressed as proportions of standard length (% of SL) (Table 1). For molecular identification and characterization of the specimens, muscle tissue from four fish samples collected in January 2021 was aseptically removed and stored at -20°C in absolute alcohol. The specimens were stored at the Mumbai Regional Station of the ICAR-CMFRI, Mumbai. Gastro Somatic Index (GaSI) and Gonado Somatic Index (GSI) for *A. splendens* were estimated using methods described by De Silva and Anderson (1995) [4]. The maturity stages of *A. splendens* were determined using methods described by Nikolsky (1963) [23]. A single spine between the first and second dorsal fins was counted as the dorsal fin spine according to Okamoto and Ida (2002) [26]. The rudimentary gill raker count was combined with the gill raker count. Species identification was performed using the key diagnostic characteristics of *A. splendens* as reported by Okamoto and Golani (2017) [25], Okamoto *et al*. (2021) [27], and Lloyd (1909) [18]. SL represents the short form of the standard length.

Genomic DNA isolation, amplification and sequencing

DNA was extracted from the muscle tissue of *A. splendens* collected in January 2021 using the CTAB method (Mishra, 2019) [22]. The purity and concentration of the DNA were determined using a spectrophotometer (NanoDrop Thermo Fisher Scientific, USA). Utilizing the cytochrome c oxidase subunit I (COI) gene universal primers (Table 2), a 655 bp region from the 5' end of the gene was amplified. PCR amplification was performed using a C1000 thermal cycler (Bio-Rad Laboratories). The optimized PCR cycles consisted of an initial denaturation at 94°C for 3 min, followed by 29 cycles of 30sec denaturation at 94°C, 30sec annealing at 54°C, 1 min extension at 72°C, and a final extension at 72°C for 7 min. The amplified PCR products were purified using the GeneJet PCR Clean Up Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. The PCR-amplified products were visualized on a 1.5 % agarose gel and viewed using a Gel Documentation System (Bio-Rad Laboratories, USA). After purification, the samples were sent to Macrogen (Seoul, Korea) for sequencing.

Sequence analysis

The sequences obtained were aligned using ClustalW (Thompson *et al*., 2002) [38] in MEGA7 (MEGA7: Molecular Evolutionary Genetics Analysis version 7.0) for larger datasets (Sudhir *et al*., 2015) [34]. The aligned sequences were then passed through (National Center for Biotechnology Information) ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) to obtain the coding sequences. The coding sequences were subsequently searched using the BLAST nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the gene. Pairwise distances were calculated based on the p-distance using MEGA7. Based on the pairwise distances calculated using the COI sequences, a neighbor-joining (NJ) tree with a bootstrap value of 1000 (Kimura 1980) [16] was constructed. The sequences were then submitted to GenBank, and accession numbers were obtained, the details of which are given in Table 3.

Results and Discussions

Systematic Position:

Order: Acropomatiformes

Family: Acropomatidae

Species: *Acropoma splendens* (Lloyd, 1909).

English name: Indian lanternbelly

Fresh and alcohol-preserved specimens of *Acropoma splendens* collected in this study are shown in Figures 2 and 3a, respectively.

Identification character diagnosed

Body laterally compressed with greatest depth in the anterior part of the first dorsal fin. The head length is nearly half of the standard length, and its proportion to SL is 33.9-51.5 % SL. The head and eyes are larger in size. The eye orbit rim is slightly elevated above the upper contour of the head. A large mouth opening with the maxilla extending below the middle of the eye. Palatines and vomers with villiform teeth. Conical teeth observed in the upper and lower jaws. The symphysis of the lower jaw did not protrude. Scales are large and mostly ctenoid, and some ctenoid scales have less or meager serrations at the end. Caudal peduncle covered with ctenoid scales. Few cycloid scales were observed in the lower middle part of the body. Four rows of scales descended from the base of the first dorsal fin to the lateral line (Figure 4). Two dorsal fins were also observed. There are eight spines on the dorsal fin, and the last spine lacking a connection with any of the fin membranes. Anal-fin with three spines and seven rays were noticed. The anus was positioned closer to the pelvic fin than the anal fin. Caudal-tail is forked. Gill rakers are slender, with rudimentary buds at the ends of both the upper and lower limbs. The total gill raker count is 19 -22 in numbers. The luminous gland was short and U-shaped around the anus (Figure 5a). The luminous gland is U-shaped, and its length is proportional to the standard length (SL) ranges from 12-16.9 % SL. The standard length (SL) of the 73 *A. splendens* specimens range from 40.7 mm – 90.8 mm. The preserved specimen showed a vertical line on the cheek. The pectoral fin ray counts were 16. A concavity was observed on the anterior surface of the proximal radial anal fin pterygiophore. The comparative morphometric scale and meristic counts of the present study and earlier records are presented in Table 1. The morphometric ranges of the present study represent wider variation due to the large sample size, representing an SL from 40.7 mm to 90.8 mm.

Distribution

The distribution of *A. splendens* has been previously reported in the Persian Gulf (Kotthanus 1974; Carpenter *et al*. 1997, Javadzadeh *et al*. 2012; Eagderi *et al*. 2019) [17, 3, 14, 5], Gulf of Oman (Llyod 1909; Norman 1939) [18, 24], Gulf of Aden (Heemstra 1984) [13], Mumbai coast of the Arabian Sea (Okamoto and Golani 2017; present study) [25], Arabian Sea (Balachandran and Nizar 1990; Carpenter *et al*. 1997; Manilo and Bogorodsky 2003; Psomadakis *et al*., 2015; Okamoto and Golani 2017) [1, 3, 20, 31, 25], Bay of Bengal (Yennawar *et al*., 2012) [44], Myanmar (Psomadakis *et al*., 2019) [32], and Andaman Sea off southwestern Thailand (Okamoto *et al*., 2021) [27].

Coloration

The fresh specimen was pinkish in color. The operculum, ventral part, and mid-portion of the body are silvery-white. All the fins were slightly pinkish. The ventral side of the body, as well as the pelvic and anal fins, displayed melanophores. The alcohol-preserved specimen displays a dark black vertical line on the cheek (Figure 3b). The luminous gland was yellow-colored in fresh condition (Figure 5b).

Synonym of *A. splendens*

Lloyd in 1909 originally described *A. splendens* as *Synagrops splendens* from the Gulf of Oman as a holotype. In 2017, Okamoto and Golani described a specimen from Bombay (currently Mumbai) coast of the Arabian Sea as a new species, *Acropoma lacrima* sp. nov., previously misidentified as *A. japonicum* in other studies (Fowler 1927; Kotthaus 1974; Balachandran and Nizar 1990) [7, 17, 1]. Similarly, Yennawar *et al*. (2012) [44] misidentified *A. splendens* in the Bay of Bengal as *A.* argentistigma and it was re-identified by Okamoto and Golani (2017) [25] as *A. splendens*, based on morphometric measurements, meristic counts, and photo information obtained from Yennawar. Prokofiev (2019) [30] argued that *A. lacrima* Okamoto and Golani (2017) are synonymous with *A. splendens* (Llyod, 1909). Okamoto *et al*., 2021 also mentioned that the *Acropoma* species recorded from the Arabian Sea and Bay of Bengal in Indian waters is *A. splendens*, while reporting its distribution off southwestern Thailand for the first time. Further, Makoto Okamoto, in personal communication with the first author of this present study, stated that *Acropoma lacrima* is a junior synonym of *A. splendens*. Similarly, the fishbase website mentioned that *Synagrops splendens* Lloyd, 1909 is a senior synonym of *A. splendens* and *Acropoma lacrima* Okamoto & Golani, 2017 is a junior synonym of *Acropoma splendens* (Lloyd, 1909), and the accepted name is *Acropoma splendens* (Lloyd, 1909). Hence, the GenBank accession number of the four specimens of *A. lacrima* in the present study was read as *A. splendens* COI sequences for future comparison globally.

Comparison

Among Acropoma species, a vertical line on the cheek was observed in *A. splendens* and *A. hanedai*. However, *A. splendens* was easily distinguished from *A. hanedai* by its U-shaped luminous gland (vs. elongated) and anus nearer to pelvic-fin origin (vs. anus halfway between pelvic-fin and anal-fin origin) (Okamoto & Golani 2017 [25]; Okamoto *et al*., 2021 [27]; in the present study, the same observation was observed for *A. splendens* in the case of the luminous

gland). The U-shaped Luminous gland has similar characteristics to those of *A. splendens* and *A. argentistigma*, but these species can be easily differentiated with a gill rakers count is 19-24 in *A. splendens* vs 16-18 in *A. argentistigma* and pectoral-fin rays 15 in *A. argentistigma* vs. 16 – 17 in *A. splendens* (Okamoto & Golani 2017; Okamoto *et al*., 2021) [25, 27]. The present study also recorded 19-22 numbers of gill rakers and 16 pectoral fin rays consistent with earlier reports on *A. splendens*. *A. japonicum* has a U-shaped luminous gland and more than 20 gill rakers, such as in *A. splendens*, but lacks cycloid scales on the lateral side of the body vs. weakly cycloid and ctenoid scales in *A. splendens* (Okamoto & Golani 2017; Okamoto *et al*., 2021) [25, 27]. In the present study, cycloid scales were also observed on the mid-lateral side of the body, and weak ctenoid scales were observed throughout the body. Present study presenting the wider range of SL of *A. splendens* (SL = 40.7 – 90.8 mm) than the earlier records which is mainly due to the larger sample size (n=73) vs. Okamoto *et al*. 2021 [27] (n=5) vs. Okamoto and Golani 2017 [25] (n=6) vs. Yennawar *et al*. 2012 [44] (n=30). Therefore, the present study has opened up an avenue worldwide to understand new variations in the morphometric measurements of *A. splendens*. The present study recorded the smallest SL (40.7 mm) for *A. splendens*, whereas Yennawar *et al*. (2012) [44] recorded the highest SL (110 mm) for *A. splendens*. To date, the known SL range for *A. splendens* is 40.7 – 110 mm. Luminous gland length in this study ranged from 12 – 16.9 % SL (vs. 15.6-17.6 % SL in Okamoto *et al*. 2021 [27]; 15 – 16 % in Okamoto and Golani 2017) [25]. The wider variation in the present study is mainly due to the inclusion of smaller-sized *A. splendens* specimens, which were not covered in previous studies. Counts of pectoral-fin rays (15 nos.) and gill rakers (17-18 nos.) of *A. splendens* reported by Yennawar *et al*. (2012) [44] (Table 1) is not matching with accepted diagnostic characters of *A. splendens* but Okamoto and Golani 2017 [25] mentioned that with confirmation of vertical line on the cheek as well as 16 numbers of pectoral-fin rays through personal communication with Yennawar results in the re-identification of *A. argentistigma* of Bay of Bengal as *A. splendens*. Except for these two meristic counts, all other counts as well as morphometric measurements of Yennawar *et al*. (2012) [44] matched with identification characteristics of *A. splendens* of the present study.

Biological observations

The Gastro somatic index (GaSI) helps in determining the feeding conditions of fish. In present study it ranged between 0.61 to 5.89 with an average of 2.47. The major food item was *Acetes* spp., followed by fish bones, scales, and digested matter. Gut content analysis showed that *A. splendens* is a carnivorous species. The gonadosomatic index (GSI) was used to determine the stage of ovarian maturation and the degree of ripeness. It was observed between 0.69 to 3.14 with a mean of 1.82 in the present study. Of the observed females, 46.66 % were mature, 36.66 % were mature or spent, and 16.66 % were immature. This shows that *A. splendens* is a continuous batch spawner that may result in the release of a cohort every month owing to the availability of its food year-round. The overall sex ratio (male:female) for the 73 specimens was 1:7.5, and the dominance of female specimens was observed.

COI Gene sequencing and analyses of *Acropoma lacrima*

Sequence alignment of the four *Acropoma lacrima* individuals yielded a nucleotide base pair ranging from 629 to 640 base pairs and their nucleotide composition is mentioned in Table 3. The Maximum Composite Likelihood (MCL) estimate for nucleotide substitution (Table 4) shows the probability of nucleotide substitution (r) from one base (row) to another base (column). All values are expressed in a tabulated form, where entries within a row should be compared. The rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. The overall transition/transversion bias is 0.437.

Genetic distance

A comparison of the four *Acropoma lacrima* individuals (Al1, Al2, Al3, and Al4) based on COI gene sequences was performed to determine the distances within the same species, and the details are listed in Table 5. The lowest genetic distance of 0.000 was found between Al4 and Al3, whereas the maximum genetic distance of 2.000 was found between Al3 and Al2. The pairwise distances among the different species in *the Acropoma genus* for which nucleotide data were available in the NCBI database were determined using the p-distance model (Table 6). *Acropoma lacrima* had the highest intraspecies distance (5.8) with *Acropoma japonicum* and the least intraspecies distance (1.839) with *Acropoma hanedai*.

Evolutionary relationships using phylogenetic tree

With the help of phylogenetic analysis, it has become easier to identify the most accurate evolutionary relationships among organisms and the distances between nucleotide sequences. In this study, a phylogenetic tree was generated for *Acropoma lacrima* individuals (Figure 6) using the neighbor-joining (NJ) method (Saitou and Nei 1987) [33] in MEGA 7. For the NJ tree, evolutionary distances were computed using the Kimura 2-parameter method and expressed in units of number of base substitutions per site. In addition, a similar NJ method-based phylogenetic tree (Figure 7) using Kimura 2-parameter was constructed for intraspecies individuals. For this purpose, *Acropoma japonicum*, *Acropoma hanedai*, and *Acropoma argentistigma* were used as outgroups to understand the evolutionary relationships among different species of the Genus *Acropoma*. The outgroup sequences were obtained from NCBI and their accession numbers are listed in Table 6.

This study is the first to provide COI gene sequences of *Acropoma lacrima* and *A. splendens*. The COI gene has been used as a DNA barcode because of its high interspecies variation, low intraspecies variation, and universality of priming sites for large taxonomic groups (Folmer *et al*., 1994) [6]. The mutation rate in the COI gene is fast enough to differentiate closely related species and conspecifics owing to conserved sequences (Ha *et al*., 2018) [10]. COI offers a high level of diversity that can be used to understand the relationships between closely related species or estimate phylogeographic groupings within species (Luo *et al*., 2011; Wares *et al*., 2001; Trontelj *et al*., 2005) [19, 41, 39].

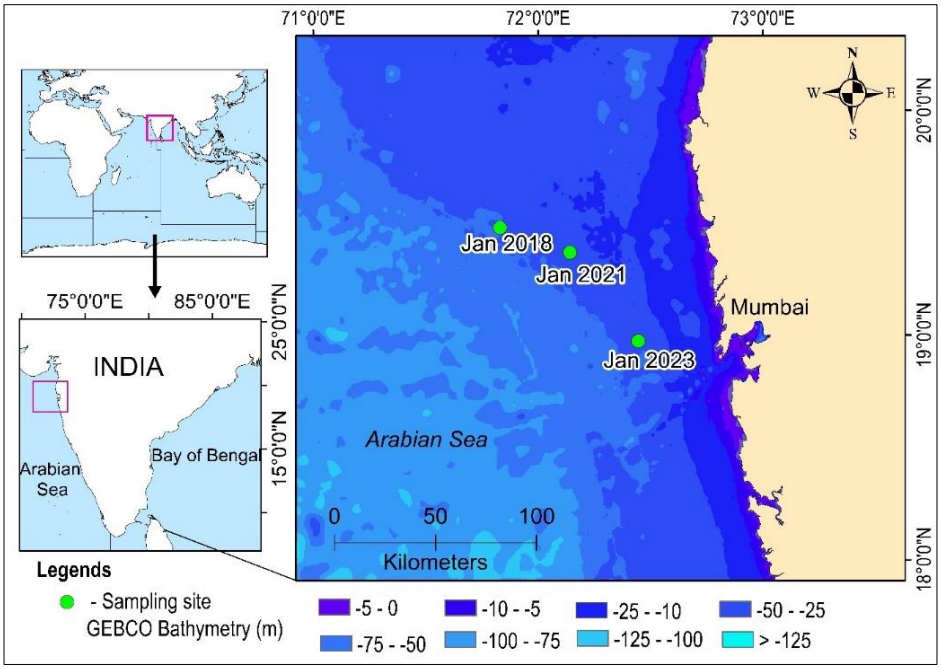

Fig 1: Sampling location (green dots) of Indian lanternbelly, *A. splendens*, caught off the Mumbai coast, Arabian Sea, Northwest coast of India

Fig 2: A fresh specimen of *A. splendens*

Fig 3: a) Alcohol preserved specimen of *A. splendens*; Arrows shows the vertical line on the cheek of *A. splendens* in the figure 3a and 3b

Fig 4: Four rows of scale between the first dorsal fin base (black arrow) and lateral line (white arrow) of *A. splendens*

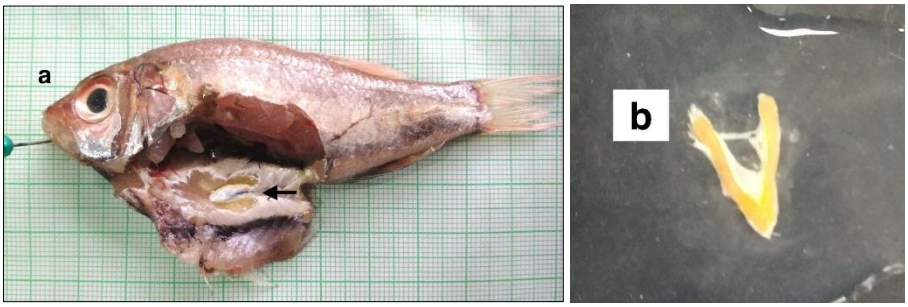

Fig 5: a) Arrow shows the Short U-shaped luminous-gland surrounding the anus of *A. splendens*; b) Luminous gland extracted from a fresh specimen of *A. splendens*

Fig 6: Neighbour-joining tree of COI gene sequences of *Acropoma lacrima* individuals constructed using Kimura 2-parameter with bootstrap value 100 and scale bar: 0.0010 substitution per site

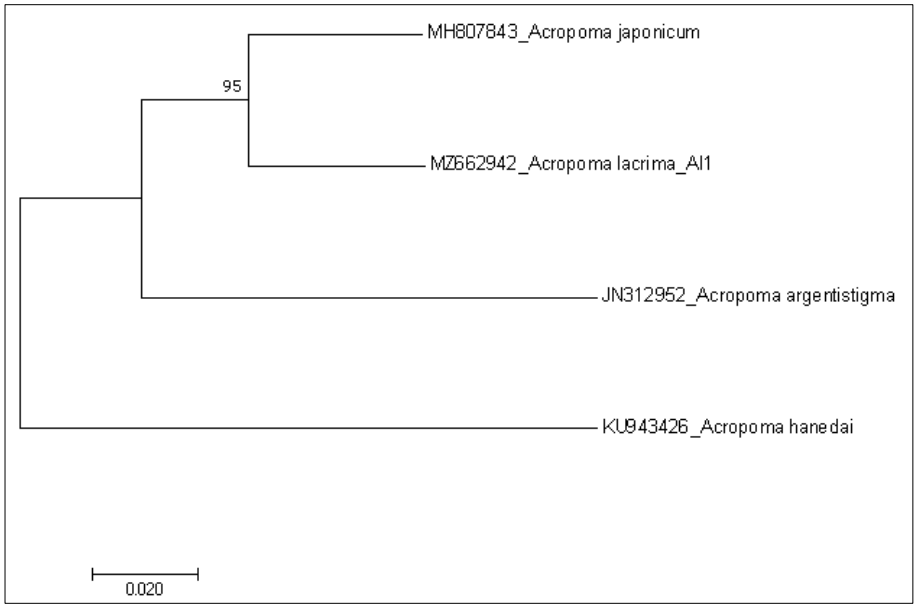

Figure 7: Neighbour-joining tree of COI gene sequences of *Acropoma lacrima* with related outgroup species constructed using Kimura 2-parameter with bootstrap value 100 and scale bar: 0.020 substitution per site.

Table 1: Counts and measurements of *Acropoma splendens*

	Acropoma splendens			
	Present study	**Okamoto *et al.* 2021**	**Okamoto and Golani 2017**	**Yennawar *et al.* 2012**
	***n*=73**	***n*=5**	***n*=6**	***n=30***
	Arabian Sea (Mumbai Coast)	Andaman Sea	Arabian Sea	Bay of Bengal (West Bengal Coast)
Standard Length (mm)	40.7-90.8	81.0-88.6	64.1-77.9	44-110
Counts				
Dorsal-fin rays	VII-I-I, 10	VII-I-I, 10	VII-I-I, 10	VII-I-I, 10
Anal-fin rays	III, 7	III, 7	III, 7	III, 7
Pectoral-fin rays	16	16	16-17	15*
Pored lateral-line scales	-	ca. 45	44-ca. 46	-
Scales above lateral line	4	4	4	-
Scales below lateral line	9	Missing scales	9	-
Gill rakers	19-22	5-6 + 14-15 = 22	6-7 + 14-18 = 20-24	5+12-13=17-18*
Vertebrae	-	10 + 15	10 + 15	-
Measurements (% SL)				
Head Length	33.9-51.5	42.0-44.2	43.1-46.8	39.1-41.1
Head Width	10.1-19.9	13.8-15.5	15.7-18.0	-
Head Height	22.9-34.2	20.2-21.9	22.5-24.0	-
Body depth	27.4-38.6	29.5-32.2	31.8-34.8	28.5-32.9
Body Width	8.2-16.8	11.0-14.3	14.1-17.0	-
Caudal Peduncle Depth	11.8-21.7	11.2-12.2	11.3-12.6	10.4-12.2
Caudal Peduncle Length	17.4-29.1	22.5-25.8	22.1-24.9	22.9-24.8
Orbital Diameter	9.7-15.8	10.6-10.9	11.9-12.7	9.4-10.7
Inter-Orbital Width	6.3-14.1	7.1-7.9	7.3-12.7	6.8-8.2
Post-Orbital Length	12.4-26.3	19.9-22.6	23.0-23.5	-
Upper Jaw Length	12.5-19.7	15.2-16.5	16.2-17.0	-
Lower Jaw Length	15.5-24.4	17.7-19.4	19.3-20.2	-
Pre-First Dorsal Fin Length	38.6-49.9	40.1-41.3	41.2-44.3	40.0-41.9
Pre-Second Dorsal Fin Length	60.9-80.1	61.5-64.2	64.3-66.2	-
Pre-Pectoral Fin Length	35.4-46.8	35.2-37.6	36.7-39.9	-
Pre-Pelvic Fin Length	34.0-50.1	35.8-38.5	38.5-40.7	-
Pre-Anus Length	44.3-58.3	45.8-49.1	46.9-49.8	46.3-47.8
Pre-Anal Fin Length	65.2-88.3	67.3-70.9	67.4-71.0	-
1st Spine Length on 1st Dorsal Fin	1.7-12.8	7.8-8.7	8.3-10.0	-
2nd Spine Length on 1st Dorsal Fin	8.9-22.1	15.7-17.2	14.2-16.9	-
3rd Spine Length on 1st Dorsal Fin	12.1-25.1	16.7	17.0-18.9	-
2nd Dorsal Fin Spine Length	5.7-9.2	7.0-7.6	7.7-9.2	-
1st Anal Fin Spine Length	0.8-2.9	1.5-1.8	1.7-2.7	-
2nd Anal Fin Spine Length	2.9-6.0	3.3-4.8	4.1-5.2	-
3rd Anal Fin Spine Length	6.9-12.9	9.3-10.4	9.9-10.6	-
Pelvic Fin Spine Length	9.7-15.8	10.9-12.6	11.1-13.6	-

1st Dorsal Fin Base Length	13.5-23.5	14.4-15.6	14.9-16.2	17.1-18.7
2nd Dorsal Fin Base Length	11.3-18.8	15.4-16.1	14.9-17.0	13.3-16.3
Anal Fin Base Length	8.6-14.9	11.4-12.1	11.2-13.0	-
Pectoral Fin Length	20.0-36.9	24.7-26.7	24.6-27.3	22.3-25.7
Pelvic Fin Length	13.1-20.9	16.7-18.1	16.8-19.1	14.8-17.6
Luminous gland length	12.0-16.9	15.6-17.6	15.0-16.0	-
*These meristic counts are not a diagnostic character of *Acropoma splendens* (Llyod, 1909)				

Table 2: Primers used for the amplification of COI of *Acropoma lacrima*

Gene	Primer	Primer Sequence	Reference
COI	Fish F2	5' TCGACTAATCATAAAGATATCGGCAC 3'	Ward *et al.* (2005) [40]
	Fish R2	5' ACTTCAGGGTGACCGAAGAATCAGAA 3'	

Table 3: Nucleotide composition of *Acropoma lacrima* for COI gene

GenBank Accession Number	Species	Percentage base composition				GC content (%)	Total length
		A	T	G	C		
MZ662942	*Acropoma lacrima* (Al1)	22.8	28.9	18.1	30.2	48.3	640
MZ662943	*Acropoma lacrima* (Al2)	22.9	28.5	18.3	30.4	48.7	629
MZ662944	*Acropoma lacrima* (Al3)	22.8	28.2	18.5	30.5	49.0	632
MZ951046	*Acropoma lacrima* (Al4)	23.1	28.2	18.2	30.4	48.6	631

Table 4: Maximum Composite Likelihood estimate of the pattern of nucleotide substitution

Base Pairs	COI			
	A	T	C	G
A	-	9.93	10.65	6.94
T	8.01	-	7.46	6.39
C	8.01	6.95	-	6.39
G	8.7	9.93	10.65	-

Table 5: Pairwise distance (p-distance) among *Acropoma lacrima* individuals based on COI gene

Accession Number	Individuals	1	2	3	4
MZ662942	*Acropoma lacrima* (Al1)				
MZ662943	*Acropoma lacrima* (Al2)	0.500			
MZ662944	*Acropoma lacrima* (Al3)	1.000	2.000		
MZ951046	*Acropoma lacrima* (Al4)	0.333	0.400	0.000	

Table 6: Pairwise distances (p-distance) among intraspecies of *Acropoma* based on COI gene

Accession Number	Species	1	2	3	4
JN312952	*Acropoma argentistigma*				
MH807843	*Acropoma japonicum*	2.400			
KU943426	*Acropoma hanedai*	2.846	1.750		
MZ662942	*Acropoma lacrima* (Al1)	3.000	5.800	1.839	

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Conclusion
The present study provides a holistic picture of the morphometric variation of *A. splendens* collected from the Arabian Sea off the Mumbai coast. The current study has also provided the sex ratio, GaSI, GSI, and food preference of *A. splendens* for the first time in Indian waters. This study also confirmed that *A. splendens* is distinct from its closely related species through comparative sequencing analyses of the COI gene of *Acropoma lacrima* (junior synonym of *A. splendens*) with *A. japonicum*, *A. argentistigma*, and *A. hanedai* obtained from the NCBI nucleotide database. Hence, the *Acropoma* species on the Mumbai coast of the Arabian Sea was validated as *Acropoma splendens* (Llyod, 1909). Thus, integrative taxonomic resolving the taxonomic ambiguity of the species and confirmed the presence of *A. splendens* for the first time from the Arabian Sea of Indian EEZ.

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