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Research Article

A report of the Four-lined tonguesole, *Cynoglossus quadrilineatus* (Bleeker, 1851) and Lachner's tonguesole, *Cynoglossus lachneri* Menon, 1977 from the Andaman and Nicobar Islands, India

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This study reports the occurrence of *Cynoglossus quadrilineatus* (Four-lined tonguesole) and *Cynoglossus lachneri* (Lachner's tonguesole) from the Andaman and Nicobar Islands, India, extending their known distribution range. Notably, *C. lachneri* is recorded from the Andaman Sea for the first time. Three specimens of *C. quadrilineatus* and one specimen of *C. lachneri* were collected from the vicinity of the South Andaman Islands. *Cynoglossus quadrilineatus* is characterised by 12 caudal fin rays and two lateral lines on each side of the body, while *C. lachneri* is identified by 10 caudal fin rays, two lateral lines, and 15 interlinear scales. Species identification was further validated through partial cytochrome coxidase I (COI) gene sequencing. The genetic distance analysis using the Kimura-2-parameter model revealed no overlap among closely related species within the family Cynoglossidae. This study also provides the first COI gene sequence for *C. lachneri*, contributing to the assessment of phylogenetic diversity in future research.

[**Keywords**: Andaman Sea, COI, Cynoglossidae, *Cynoglossus lachneri*, *Cynoglossus quadrilineatus*, Distributional record, Flatfishes]

Introduction

Tongue soles, members of the Cynoglossidae family, are small to medium-sized fish that typically inhabit the ocean floor. Cynoglossidae is a heterogeneous family comprising 166 valid species organised into 3 genera *viz*. *Cynoglossus*, *Paraplagusia* and *Symphurus*. Among these, the genus *Cynoglossus* includes 75 recognised species[1]. These species are commonly encountered in marine habitats from the eastern tropical Atlantic to the Indo-West Pacific waters[2]. The genus *Cynoglossus* is characterised by one to three lateral lines on the eyed side of the body and the absence of fringed lips[3]. The diversity and distribution of the tonguefishes have been investigated from time to time along the Indian coastline[4-9]. To date, the family Cynoglossidae encompasses 27 species identified in Indian waters, and 8 species were reported from the Andaman waters[10–14].

Cynoglossus quadrilineatus (Bleeker 1851), mostly distributed in the coastal areas of the Red Sea and the Indo-West Pacific[1,15]. This species prefers habitats such as mangroves as well as coastal sandy and muddy regions[16]. *Cynoglossus quadrilineatus* is recognised as a passive swimmer and exhibits carnivorous feeding habits[17]. The four-lined tongue soles are distinguished from the closely related species by having 12 caudal fin rays and two lateral lines on both sides of the body[2]. This species has been documented from Kerala, Mumbai, Odisha and Tamil Nadu coastal waters of India[1,9].

Cynoglossus lachneri Menon, 1977, prefers the sandy ocean floor, also known for its relatively large size, often exceeding 40 cm in length[18]. From the Indian coast, this species is recorded from Andhra Pradesh and Maharashtra[10,19].

The partial sequence of the mitochondrial COI (cytochrome c oxidase subunit I) gene has been established as a supportive tool in the identification of fishes for its greater precision[20–22]. The present study aims to report two flatfish species, *Cynoglossus quadrilineatus* and *C. lachneri* from the Andaman and Nicobar Islands with notes on its taxonomy and distribution.

Materials and Methods

Sample collection and preservation

Three specimens of *Cynoglossus quadrilineatus* and a single specimen of *Cynoglossus lachneri* were collected on January 25, 2023 and October 04, 2023, by a gill net operated in the shallow coastal region near Swaraj Dweep Island (11°53'43.15" N; 92°58'7.47" E) (Fig. 1). Meristic characters were recorded, and the measurements were taken to the nearest 0.1 mm by a Vernier calliper. Tissue fragments were collected and stored in ethanol (95 %) for the DNA isolation. Both species have been identified based on the standard identification keys and previous literature with description[2,10,19,23]. The binomial counts and measurements followed the methodology outlined in standard literature[24,25]. Family, genus, and species classifications followed the criteria established by Nelson *et al.*[26], Fricke *et al.*[25]. All 4 specimens were preserved and deposited in the Museum of the Department of Ocean Studies and Marine Biology, Pondicherry University, Port Blair (PUMB 3548 to 3551).

DNA isolation, COI sequence analysis

Genomic DNA was extracted using a commercially available kit (QIAGEN GmbH, Hilden, Germany) from 20 mg of muscle tissue. Partial mitochondrial COI gene was amplified using a set of primers Fish F1 (5' TCAACCAAC CACAAAGACATTGGC AC 3') and Fish R1 (5' TAGACTTCTGGGTGG CCAAAGAATCA 3')[27]. PCR reaction mix was prepared with a total volume of 25 µl by a combination of 9.5 µl ultra-pure nuclease-free water, 12.5 µl of PCR master mix (R GT PCR Emerald Amp master mix), 1 µl of each primer, and 1 µl of isolated

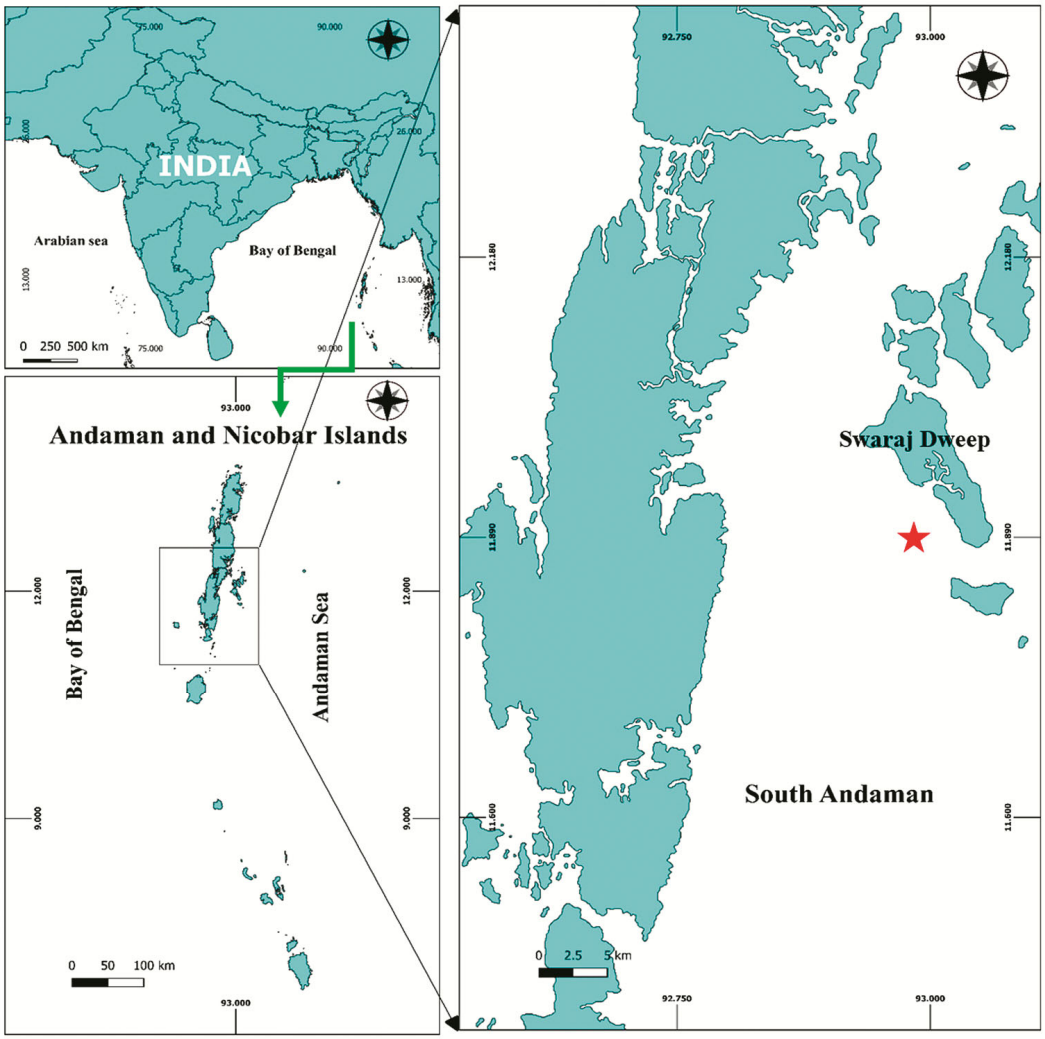

Fig. 1 — Map of the region where the fish were captured, indicated by a red star symbol

fish DNA. The temperature profiles that were used for PCR were initial denaturation at 95 ℃ for 5 min, followed by 30 cycles of 95 ℃ for 1 min, 52 ℃ for 30 sec, and 72 ℃ for 1 min. 72 ℃ for 5 min as a final extension[28]. The resulting PCR products were analysed using agarose gel electrophoresis. The nucleotide sequence of the amplicons was determined using Sanger dideoxy sequencing. 585 and 652 long base pair sequences were generated for *Cynoglossus lachneri* and *C. quadrilineatus*, respectively, after the deletion of ambiguous base pairs. COI sequences of *C. lachneri*, *C. quadrilineatus*, and morphologically similar flatfishes were retrieved from the Genbank database (www.ncbi.nlm.nih.gov/Genbank) for phylogenetic reconstruction. *Atule mate*, belonging to the family Carangidae, was kept as an outgroup to establish a point of comparison in phylogeny. A robust Maximum Likelihood tree was reconstructed using Mega X Version 10.2.6(ref. 29) using the specified substitution model and bootstrap resampling with 1000 replicates. The intra- and interspecific genetic distance analysis was estimated using Kimura-2-Parameter (K2P)[30] model in Mega X version 10.2.6. The obtained sequence of the present study was submitted to GenBank and received accession numbers OR51515, OR415697 (*C. quadrilineatus*) and PP326262 (*C. lachneri*).

Results

This study is the first instance of *Cynoglossus quadrilineatus* and *Cynoglossus lachneri* (Figs. 2, 3) reports belonging to the family Cynoglossidae from the coast of Andaman Nicobar Islands, India. The meristic and morphometric measurements are presented in Tables 1 and 2.

Cynoglossus quadrilineatus (Bleeker 1851)

Systematics

Class: Actinopterygii Klein, 1885
Order: Pleuronectiformes
Family: Cynoglossidae Jordan, 1888
Genus: *Cynoglossus* Hamilton, 1822
Cynoglossus quadrilineatus (Bleeker, 1851) (Fig. 2)

Materials examined

PUMB 3548, 249 mm SL; PUMB 3549, 251 mm SL; PUMB 3550, 281.5 mm SL; Andaman and Nicobar Islands, Swaraj Dweep (11°53'43.15" N, 92°58'7.47" E), 5 m depth; collected by Ameen Ummath, 25 January 2023.

Fig. 2 — *Cynoglossus quadrilineatus* (Bleeker, 1851): a) Eyed side; and b) Blind side

Fig. 3 — *Cynoglossus lachneri* Menon, 1977: a) Eyed side; and b) Blind side

Table 1 — Meristic counts of *Cynoglossus quadrilineatus* and *Cynoglossus lachneri*

Meristics	*Cynoglossus quadrilineatus* (*n = 3)	*Cynoglossus lachneri* (n = 1)
Dorsal fin rays	108–109	117
Anal fin rays	85–86	94
Caudal fin rays	12	10
Pelvic fin rays	4	4
Number of lateral lines eyed side	2	2
Number of lateral lines blind side	2	2
Lateral line scales	88-90	113
Lateral line scales transverse scales	13–15	15

*n = Number of individuals

Diagnosis

Eyes on left side of the body. Caudal fin with 12 rays. Dorsal fin rays 108 – 109; anal fin rays 85 – 86. Lateral line scales 88 – 90. Scales between lateral lines 13 – 15. Body compressed, oval, with broad depth. Head length 23.24 % SL, head depth

Table 2 — Morphometric measurements of *Cynoglossus quadrilineatus* and *C. lachneri* in percentage of Standard Length (% SL) and Head length (% HL)

Morphometrics (mm) in percentage of SL	***Cynoglossus lachneri* (n = 1)**	***Cynoglossus quadrilineatus* (n = 3)**		**Morphometrics (mm) in percentage of HL**	***Cynoglossus lachneri* (n = 1)**	***Cynoglossus quadrilineatus* (n = 3)**	
		Mean	**SD**			**Mean**	**SD**
Head length (HL)	18.2	23.24	0.90	Head depth	103.84	100.21	0.81
Head depth	19.87	23.29	0.84	Body depth	154.8	120.51	0.76
Body depth	24.0	27.57	0.57	Upper snout length	33.43	35.72	0.85
Pre-orbital length upper	6.1	8.46	0.56	Lower snout length	41.9	42.11	0.83
Pre-orbital length lower	7.6	9.79	0.39	Inter-orbital width	7.3	9.06	0.67
Inter-orbital width	1.3	2.10	0.09	Post-orbital length upper	57.7	53.34	0.91
Post-orbital length upper	10.5	12.44	0.82	Post-orbital length lower	55.02	49.69	0.71
Post-orbital length lower	10.0	11.49	0.85	Pre-dorsal length	7.34	4.37	0.33
Pre-dorsal length	1.3	1.01	0.04	Eye diameter upper	8.6	6.37	0.29
Dorsal fin height	4.6	4.91	0.52	Eye diameter lower	9.83	5.68	0.07
Dorsal fin base	96.4	98.28	0.17	Upper jaw	24.19	22.66	0.23
Pre-anal length	18.4	21.68	0.87	Lower Jaw	25.73	22.56	0.67
Anal fin length	5.0	4.61	0.22				
Anal fin base	84.1	77.33	0.71				
Pre-pelvic length ocular side	15.8	19.92	0.87				
Pelvic fin length ocular side	3.9	3.98	0.23				
Pelvic fin base	3.1	2.49	0.12				
Caudal fin height	5.4	7.20	0.23				
Trunk length	84.1	79.01	0.87				
Eye diameter upper	1.6	1.48	0.08				
Eye diameter lower	1.8	1.47	0.15				
Upper jaw	4.4	5.26	0.20				
Lower Jaw	4.7	5.32	0.12				

23.29 % SL, nearly equal. Body depth 27.57 % SL, slightly larger than head length. Upper eye slightly advanced to lower eye; posterior edge of upper eye vertically aligned with the middle of lower eye. Upper eye diameter slightly larger than lower eye diameter; eyes separated by 3 – 4 interorbital scale layers. Two nostrils on the eyed side; anterior nostril tubular, positioned above mid-upper jaw, vertical to anterior margin of upper eye; posterior nostril non-tubular, located in the middle of interorbital space. Mouth angle extends beyond rear margin of lower eye; closer to opercular opening than snout tip. Snout rounded, with short rostral hook. Dorsal fin origin slightly posterior to snout tip. Ocular side with ctenoid scales; blind side with cycloid scales. Two lateral lines on eyed side; two lateral lines on blind side. Lateral lines composed of tubular cycloid scales on both sides. Dorsal lateral line on ocular side ends at seventh dorsal fin ray (counted from posterior). Head on blind side with numerous cirri.

Colouration

The freshly collected specimens display a consistent dark brown hue across their ocular side. Fins are nearly translucent and a yellowish shade on the dorsal and anal fins on the blind side. Head and the body are uniformly white at the blind side. While, the colouration of preserved specimens is similar to that of freshly collected specimens, except the blind side turned paler.

***Cynoglossus lachneri* Menon, 1977**

Systematics

Class: Actinopterygii Klein, 1885
Order: Pleuronectiformes
Family: Cynoglossidae Jordan, 1888
Genus: *Cynoglossus* Hamilton, 1822
Cynoglossus lachneri Menon, 1977 (Fig. 3)

Materials examined

PUMB 3551, 333.1 mm SL; Andaman and Nicobar Islands, Swaraj Dweep (11°53'43.15" N, 92°58'7.47" E), 5 m depth; collected by Ameen Ummath, 04 October 2023.

Diagnosis

Eyes sinistral. Caudal fin with 10 rays. Two lateral lines on the eyed side (dorsolateral and mid-lateral);

15 transverse scales between them. Body elongated and compressed, with greatest depth at anterior one-third of total length (24.0 % SL). Head length (18.2 % SL), slightly less than head depth (19.87 % SL). Upper (migratory) eye slightly advanced over lower eye. Eyes moderately sized, separated by scaly interorbital space. Two nostrils on both sides; anterior nostril tubular on both eyed and blind sides; posterior nostril a simple opening, located anterior to mid-interorbital space. Snout rounded, with short rostral hook barely reaching vertical anterior margin of upper eye. Mouth angle positioned at anterior half of lower eye. Mid-lateral line with 113 scales. Two lateral lines on blind side. Scales ctenoid on ocular side; cycloid on blind side and lateral lines. Dorsal fin with 117 rays; anal fin with 94 rays; pelvic fin with 4 rays.

Colouration

In fresh condition, the brown-coloured ocular side has scattered irregular blotches. A beige appearance along the fins (dorsal and anal) ridge, when observed from the blind side. White colour borders the dorsal and anal fin tips. The ventral side of the body displays a uniform white colouration.

Phylogeny and genetic distance analysis

PCR amplification of the COI gene yielded sequences of 582 bp for *Cynoglossus lachneri* and 652 bp for *Cynoglossus quadrilineatus* after removing ambiguous bases. Sequences were submitted to GenBank. Among these, the COI sequence of *C. lachneri* (PP326262) was the first to be submitted to NCBI. Nucleotide composition analysis of *C. lachneri* (PP326262) revealed a G + C content of 41.18 % (G = 16.09 %, C = 25.09 %) and A + T content of 58.82 % (A = 26.99 %, T = 31.83 %). The sequences obtained for *C. quadrilineatus* (OR51515, OR415697) showed a close match with existing COI sequences of *C. quadrilineatus* in the NCBI database. The G + C content of these sequences was 42.33 % (G = 18.10 %, C = 24.23 %), while A + T content was 57.67 % (A = 27.15 %, T = 30.52 %).

Phylogenetic analysis using the Maximum Likelihood (ML) method (Fig. 4) grouped the obtained sequences into a distinct clade along with other sequences of the same species. This clustering was strongly supported by bootstrap analysis (1000 replications, ML method).

Genetic distance analysis showed that the intraspecific genetic distance within *C. quadrilineatus* was 0.4 % (Table 3). The mean genetic distance between *C. quadrilineatus* and its closely related species was 33.8 %. The difference between the greatest intraspecific (0.004) and the least interspecific (0.165) genetic distance was 0.161. Among the compared species of the same genus, *Cynoglossus macrostomus* showed the lowest interspecific genetic distance (13.2 %) with *C. lachneri* (Table 4).

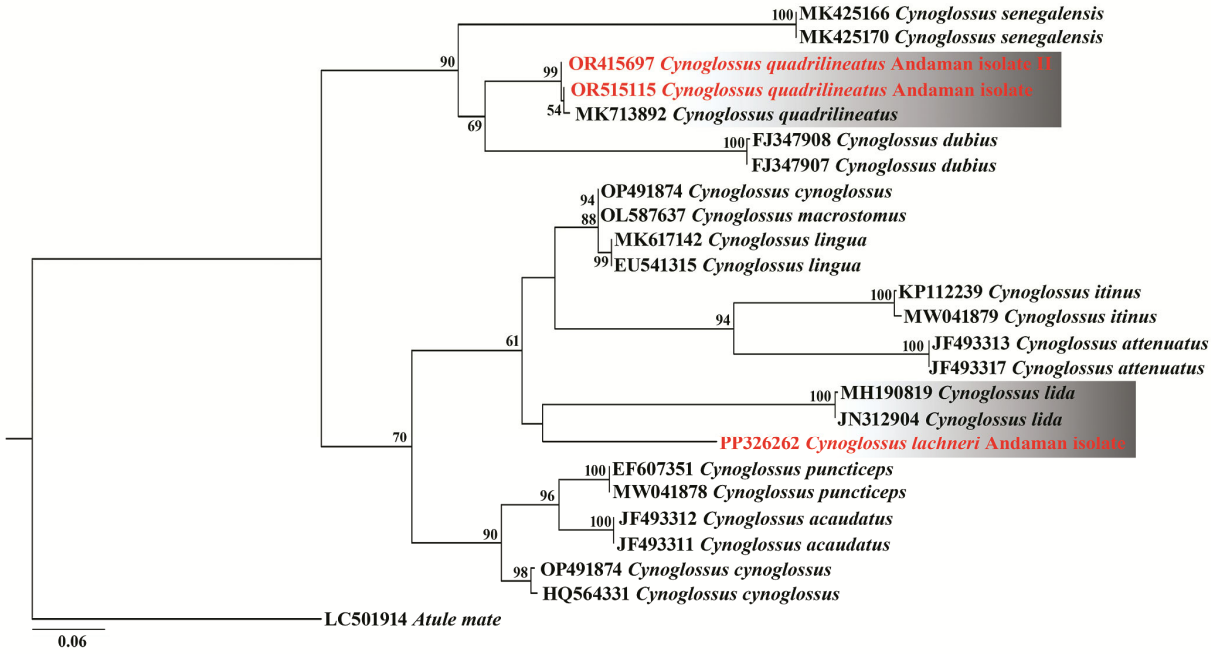

Fig. 4 — Maximum likelihood phylogenetic tree reconstruction based on COI gene. The Andaman isolate of *Cynoglossus quadrilineatus* and *C. lachneri* are presented in red colour in the tree

Table 3 — Genetic distance (interspecific and intraspecific) amongst the Andaman isolate of *Cynoglossus quadrilineatus* with the closely related species of the genus *Cynoglossus*

Sl. No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	OR515115 *Cynoglossus quadrilineatus* Andaman isolate													
2	MK713892 *Cynoglossus quadrilineatus*	0.004												
3	FJ347907 *Cynoglossus dubius*	0.165	0.152											
4	HQ564331 *Cynoglossus cynoglossus*	0.191	0.189	0.261										
5	MK425170 *Cynoglossus senegalensis*	0.191	0.193	0.252	0.218									
6	MW041878 *Cynoglossus puncticeps*	0.200	0.201	0.271	0.086	0.229								
7	JF493311 *Cynoglossus acaudatus*	0.205	0.212	0.279	0.088	0.246	0.066							
8	OL587637 *Cynoglossus macrostomus*	0.209	0.209	0.274	0.140	0.247	0.147	0.151						
9	EU541315 *Cynoglossus lingua*	0.215	0.213	0.276	0.143	0.250	0.145	0.152	0.009					
10	PP326262 *Cynoglossus lachneri* Andaman isolate	0.235	0.238	0.287	0.186	0.266	0.163	0.180	0.132	0.138				
11	KP112239 *Cynoglossus itinus*	0.248	0.246	0.275	0.217	0.232	0.204	0.213	0.170	0.168	0.198			
12	JN312904 *Cynoglossus lida*	0.249	0.253	0.325	0.197	0.222	0.194	0.197	0.160	0.170	0.190	0.240		
13	JF493317 *Cynoglossus attenuatus*	0.259	0.265	0.294	0.215	0.236	0.208	0.234	0.172	0.173	0.219	0.170	0.213	

Table 4 — Interspecific genetic distance analysis between the Andaman isolate of *Cynoglossus lachneri* with the closely related species of the genus *Cynoglossus*

Sl. No.	species	1	2	3	4	5	6	7	8	9	10	11	12
1	PP326262 *Cynoglossus lachneri* Andaman isolate												
2	OL587637*Cynoglossus macrostomus*	0.132											
3	EU541315 *Cynoglossus lingua*	0.138	0.009										
4	EF607351 *Cynoglossus puncticeps*	0.163	0.147	0.145									
5	JF493311 *Cynoglossus acaudatus*	0.180	0.151	0.152	0.065								
6	MN511874*Cynoglossus macrostomus*	0.142	0.000	0.009	0.148	0.150							
7	JN312904 *Cynoglossus lida*	0.190	0.160	0.170	0.191	0.197	0.162						
8	MW041879 *Cynoglossus itinus*	0.196	0.170	0.170	0.211	0.218	0.181	0.243					
9	OR515115 *Cynoglossus quadrilineatus* Andaman isolate	0.235	0.209	0.215	0.197	0.205	0.210	0.249	0.245				
10	FJ347907 *Cynoglossus dubius*	0.287	0.274	0.276	0.271	0.279	0.271	0.325	0.285	0.165			
11	JF493317 *Cynoglossus attenuatus*	0.219	0.172	0.173	0.213	0.234	0.181	0.213	0.165	0.259	0.294		
12	MK425166 *Cynoglossus senegalensis*	0.264	0.247	0.250	0.225	0.246	0.242	0.222	0.237	0.189	0.248	0.238	

Discussion

The morphological characters and range of fin counts of *Cynoglossus quadrilineatus* recorded in this study closely correspond to previous studies[2,31]. This species has been documented along the western and eastern coasts of India, with distribution records from the Mumbai coast[32], Kerala coast[6,9,11], Madras coast[3,12,33], and the Odisha coast[2,34]. However, no previous ichthyological surveys have reported its occurrence from the Andaman and Nicobar Islands[4,13,35]. The present record of the species extends the known distribution range further eastward in the Indian waters. Beyond Indian waters, *C. quadrilineatus* has also been recorded from Thailand, Myanmar, and Indonesia[2] in the north-eastern Indian Ocean. Earlier studies referred to the species under the nomenclature *Cynoglossus bilineatus*. However, Kottelat[36], established that *bilineatus* is not a valid name for any species within *Cynoglossus*, confirming *C. quadrilineatus* as the correct nomenclature for the four-lined tongue sole. The subsequent studies have since adopted *C. quadrilineatus* as a valid name for four-lined tongue sole[31].

The known distribution of *Cynoglossus lachneri* includes Madagascar, Seychelles, Comoros, Mauritius, Eastern Africa, India, the Gulf of Oman, and Pakistan[2,10,18-19,37-41]. The earlier records in the Indian waters were limited to the Mumbai, Kerala and Vishakapattanam coasts. The occurrence of the species was not listed from the Andaman and Nicobar Islands[13], Myanmar coast[42], Southwestern coast of Thailand[43,44], and the Northern Sumatra[45,46] of the Andaman Sea. Hence, the present work reports the first occurrence of *C. lachneri* in the Andaman Sea. The morphometric and meristic observations in the present study largely align with previous descriptions of *C. lachneri*[2,10,19,38], though minor variations were noted in the ocular side colour pattern and interlinear scale numbers. The interlinear scale number recorded in this study was 15, whereas most earlier reports documented a range of 16 to 18[(refs.2,19,37,38)]. Likewise,

the ocular side colouration described by Menon[2] and Heemstra[37] consistently depicted a uniform brown hue for the species. However, the present investigation revealed irregular blotches on the ocular side. Notably, similar minor variations in *C. lachneri* have also been reported in a recent study from the east coast of India[10], suggesting a possible morphological variation within the species.

The maximum likelihood phylogenetic tree constructed in this study formed well-defined clades for each species with strong bootstrap support. The genetic distance between species was more than ten times higher than intraspecific variation, aligning with the threshold proposed by Hebert *et al*.[47]. Additionally, the nucleotide composition of both species closely matched previously reported trends in tongue soles[48]. This study also reports the first COI gene sequence for the *C. lachneri*. Given the established role of the *COI* gene in species identification[49], these findings may provide a valuable molecular resource for analysing phylogenetic diversity among tongue soles.

Conclusion

This study documents the first record of *Cynoglossus lachneri* in the Andaman Sea and the first report of *Cynoglossus quadrilineatus* in the Andaman Islands. Morphological and meristic analyses align with previous descriptions, with minor intraspecific variations noted. Phylogenetic analysis supports species identification, and the study provides the first COI gene sequence for *C. lachneri*. These findings expand the known distribution of both species and enhance the molecular and taxonomic understanding of *Cynoglossus* in Indian waters.

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Conflict of Interest

The Authors declare that they have no known conflicts of financial or personal interest that could have appeared to influence this article. Also, there is no conflict of interest among authors or with other researchers in our institution or with any other institution working in the same topic or region.

Author Contributions

AU: Species identification and manuscript preparation. KK, PVMR, AU, PSFP & SK: Sample collection and field surveys. AU, PSFP & KK: Molecular analysis. SV: Supervision and manuscript All authors have read and approved the final version of the manuscript.

Data Availability

The datasets generated and or analysed during this study are available from the corresponding author on reasonable request

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