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Quantification of morphological variability among species of family Cynoglossidae from Indian waters

S K Sharma[a,b], S S Mishra[c] & A K Jaiswar*[,b]

[a]Central Inland Fisheries Research Institute, Barrackpore, Kolkata – 700 120, India
[b]Central Institute of Fisheries Education (Deemed University), Mumbai – 400 061, India
[c]Marine Fish Section, Zoological Survey of India, 27, JLN Road, Kolkata – 700 016, India
*[Email: akjaiswar@cife.edu.in]

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The morphometric and meristic studies of flatfishes belonging to the family Cynoglossidae collected from various parts of the Indian coast were studied. Fourteen species of Cynoglossids belonging to two genera, *Cynoglossus* and *Paraplagusia*, were obtained from commercial landings of fishing trawlers in coastal waters from August 2013 to May 2014. A distance matrix was created using the Euclidian method to show their differences based on their characters. Based on these characters, hierarchical cluster analysis (Ward's Minimum-Variance Method) was used to establish a relationship among genera and species of the family Cynoglossidae. A field-level identification key for the species of the family Cynoglossidae in Indian waters is also proposed.

[Keywords: Cladogram, Cynoglossidae, *Cynoglossus*, Indian waters, Morphological characters, *Paraplagusia*]

Introduction

Cynoglossid species (Order Pleuronectiformes) are primarily marine fishes, commonly known as tongue soles. Most tongue soles are marine inhabitants, but the survivability tolerance of several species extends to freshwater levels. Thus, most tongue soles are recorded from the coastal marine waters, though their distributional range extends from estuarine waters to the deep sea. Fishes of the family Cynoglossidae are characterized by a left-sided eyes, lower jaw not prominent, strongly curved snout and toothed jaw on the blind side, hidden pre-opercular margin, fused gill membranes with free branchiostegal rays, and the asymmetrical position of nasal organ on the eyed side, *i.e*., one is in between the two eyes and the second one on the upper lip of the mouth[1].

Even after many investigations on flatfish taxonomy, a considerable amount of ambiguity still exists in identifying these fishes, especially from the South-East Asia-Pacific region, including India[2]. Hence, the present work attempted to distinguish different species of the family Cynoglossidae found along the Indian coast based on morphological characters. The findings can be utilized for correct identification of the species under the family Cynoglossidae.

Materials and Methods

A total of 341 specimens belonging to 14 species of the family Cynoglossidae were collected from different landing centres along the Indian coasts (Fig. 1), *viz*., Veraval, Mumbai, Ratnagiri, Goa, Mandapam, Digha and Kolkata, mainly from the commercial landings of bottom trawlers during August 2013 to May 2014.

A total of 12 morphometric characters (Standard length - SL; Total length - TL; Body depth at gill opening - BD_1; maximum body depth - BD_2; Distance from the snout tip to the vertical line at BD_2 - LB; Eye diameter - ED, Inter-orbital - IOL; Snout length (preorbital length) - SN; Head length - HL; Snout tip to mouth distance (preoral length) - SM; Distance between angle of mouth to gill opening (post-oral head length) - MG and Jaw length - MD) were measured (Fig. 2) and from that 14 morphometric ratios, were calculated, *viz*., head in total length (TL/HL), head in standard length (SL/HL), body $depth_1$ in total length (TL/BD_1), body $depth_1$ in standard length (SL/BD_1), body $depth_2$ in total length (TL/BD_1), body $depth_2$ in standard length (SL/BD_2), body $depth_2$ in head length (LB/HL), standard length in total length (TL/SL), mouth gill distance in head length (HL/SM), mouth distance in head length (HL/MD), snout in head length (HL/SN), eye diameter in snout length (SN/ED), eye diameter in head length (HL/ED) and inter-orbital distance (ED/IOL). BD_1 is the body depth measured at the operculum, and BD_2 is the maximum body depth. All measurements and counts were taken from the eyed

side of the specimens. Pearson's coefficients of variation (percentage levels of the standard deviations in the mean values of the proportions) have been used for studying the variability between the species of this family.

Six meristic characters were measured following two protocols[3,4]. The characters considered herein were numbers of dorsal fin rays, anal fin rays, caudal-fin rays, pectoral fin rays, rows of scales between above and mid-lateral line on the eyed side, and numbers of scales on the mid-lateral line (from the cephalic junction to last lateral line scales).

Software PAST was used to estimate the descriptive statistics of morphometric and meristic traits. Simple linear correlations between various morphometric characteristics were also estimated employing PAST Software. All the morphometric data were transformed in the PAST software using the (x-mean)/stdev command to remove the size-dependent variation using an allometric approach[5].

Species were identified employing the following key, modified from earlier works of the region[1,5-8] and western Pacific[9,10].

Field level identification key to the genera of the family Cynoglossidae from India

1a. Lips with a row of fringed papillae .... *Paraplagusia*

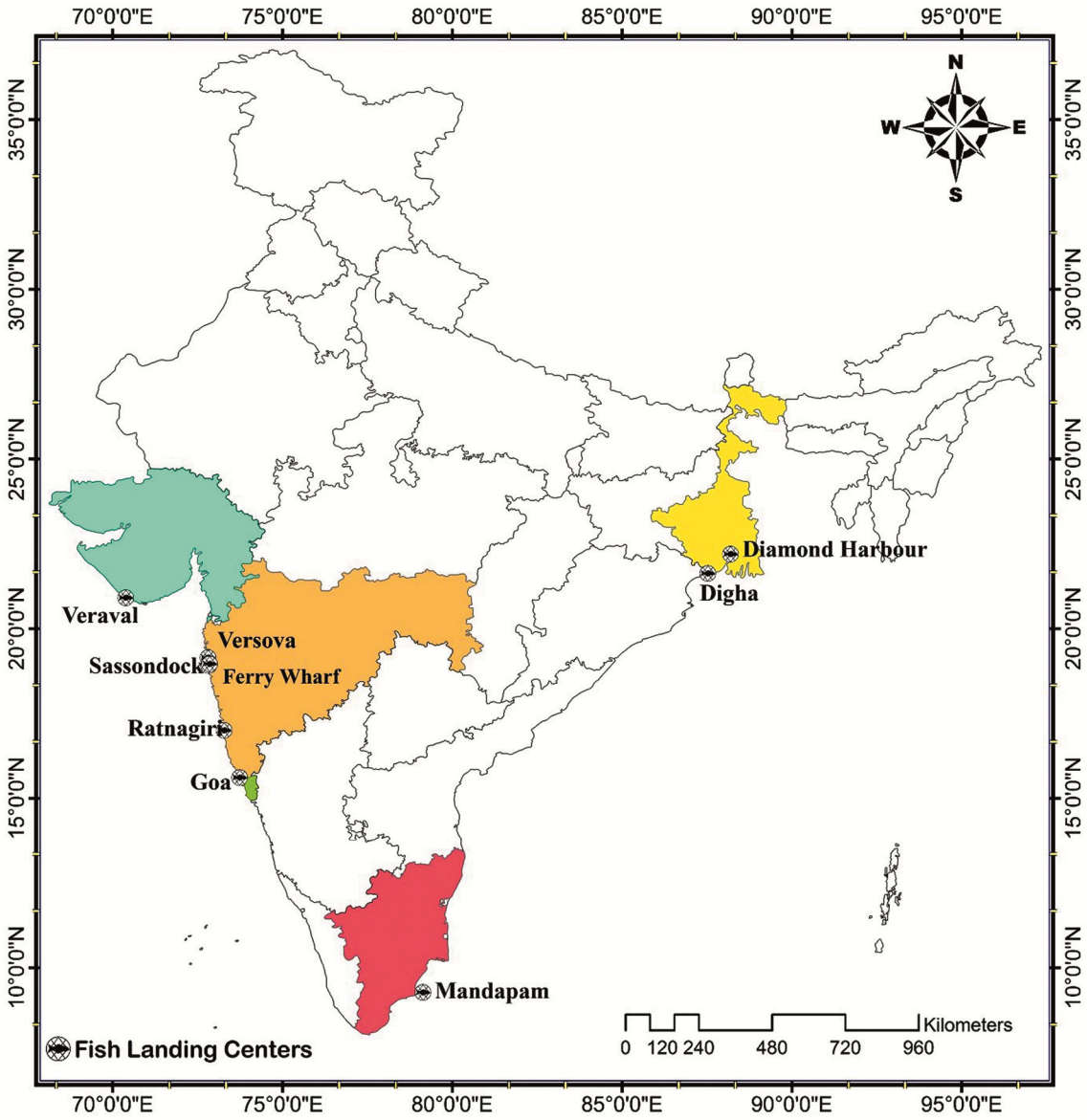

Fig. 1 — Sampling sites along the Indian coast

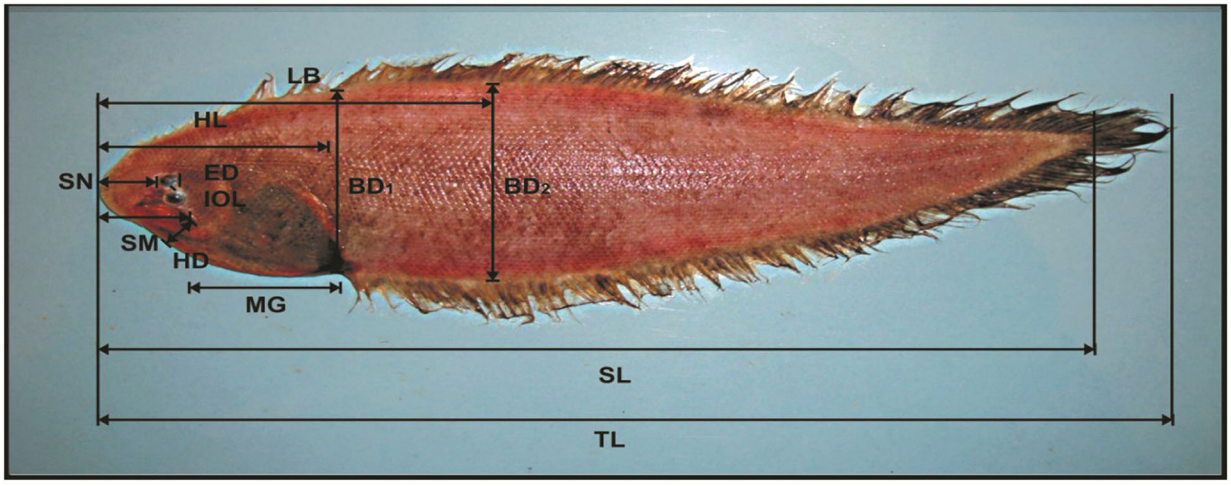

Fig. 2 — Morphometric characters of typical Cynoglossid fish

1b. Lips smooth, without papillae 2

2a. At least two lateral lines on the ocular side .... *Cynoglossus*

2b. No lateral line on ocular side .... *Symphurus*

Key to the species of the Paraplagusia of India

1a. Rostral hook long, reaching to a level far behind lower eye; anal-fin rays 81 - 88; dorsal-fin rays more than 105; interlinear scales 17-20 rows; ocular side of body brown with pale ocelli of various sizes ……..…….…….. *P. bilineata*

1b. Rostral hook shorter, reaching to the level of posterior margin of lower eye; anal-fin rays 66-80; dorsal-fin rays less than 105; interlinear scales 15-16 rows; ocular side of body uniformly pale brown without ocelli ……. *P. bleekeri* (= *P. blochii*)

Key to the species of the Cynoglossus of India

1a. Lateral line present on blind side ……..…... 2

1b. Lateral line absent on blind side ……..…... 6

2a. One lateral line on blind side …..... *C. dubius*

2b. Two lateral lines on blind side …… 3

3a. Lateral line scales on ocular side 88-96; scales between lateral lines 13-16 ..... ……..… *C. quadrilineatus* (= *C. bilineatus*)

3b.Lateral line scales on ocular side 100-120; scales between lateral line 16-20 ….……..….. 4

4a.Three lateral lines on the eyed side (third line incomplete) ……..……..... *C. quinquelineatus*

4b.Two lateral lines on eyed side …….. 5

5a. Scales between lateral line 16-18; D 113-121; A 92-98; vertebrae 55-58 ……..……..… *C. lachneri*

5b. Scales between lateral line 18-20; D 109-113; A 90-92; vertebrae 53-55 ……..….. *C. dispar*

6a. Scales cycloid in the blind side 7

6b. Scales ctenoid on both blind and eyed side ……..……..…… 11

7a. Three lateral lines on the eyed side; scales between lateral line 15-20 ……..…..…..….. 8

7b. Two lateral lines on the eyed side; scales between lateral line 6-12 ...……. 9

8a. Snout shorter, about 30-37% (mean 33%) of head length ……..……...…..…….*C. carpenteri*

8b. Snout longer, about 37-48% (mean 43%) of head length ……..…..……. *C. acutirostris*

9a. Mid-lateral line scales 90-101; scales between lateral line 11-12 ……...……..….. *C. lingua*

9b. Mid-lateral line scales 43-70; scales between lateral line 6-9 …… 10

10a. Scales between lateral line mostly 8 or 9; eyes smaller, 15-17 in the head; lower eye begins at the vertical line from the middle of upper eye ……..…..... *C. arel*

10b. Scales between lateral line mostly 6 or 7; eyes larger, 8-12 in the head; lower eye begins at vertical from the posterior edge of upper eye ……... ……..…….. *C. macrolepidotus*

11a. Eyes small, pedunculate ..……. *C. monopus*

11b. Eyes normal, not pedunculate ……..... 12

12a. One nostril on the eyed side; scales weakly ctenoid on blind side ……. *C. praecisus*

12b. Two nostrils on the eyed side; scales on blind side strongly ctenoid ...…….…..…….…….. 13

13a. Angle of mouth distinctly nearer to the gill-opening than to tip of snout ..... *C. lida*

13b. The angle of mouth nearer to the tip of snout than to gill-opening ….....……..……. 14

14a. Mouth cleft extending far back beyond posterior margin of lower eye …...… *C. macrostomus*

14b. Cleft of mouth extending at most to the posterior border of lower eye ……..….....….. 15

15a. Eyes contiguous; interlinear scales 11 or less …….....…….....….. *C. kopsii*

15b. Eyes distinctly separated, interlinear scales 12 or more …….....……..…….....… 16

16a. Scales between lateral line mostly 15 or 19 ……..…….....…….....….. *C. puncticeps*

16b. Scales between lateral line mostly 11 or 14 ……..…….. 17

17a. Three lateral lines on the eyed side; anal-fin rays 88-90 ……..……... *C. versicolor*

17b. Two lateral lines on the eyed side; anal-fin rays 72-83 ……..……... 18

18a. Anal fin rays 72-78; mid-lateral line scales 77-90; vertebrae 44-47 ……..……… *C. cynoglosuus*

18b. Anal fin rays 76-83; mid-lateral line scales 70-78; vertebrae 47-50 ……..……..... *C. semifasciatus*

Two species, *C. praecisus* and *C. versicolor* are considered valid[11] and recorded from the Indian coast[12]. The record of *C. itinus* in Indian waters may be a misidentification of *C. praecisus* specimens, which were stated to have a single nostril on the ocular side[11] and *C. itinus* is most probably unlikely to occur in Indian Ocean[13,14]. Although *C. quinquelineatus* was described from only one specimen from Madras (Chennai)[15], two more specimens were recorded from the Mumbai coast under this study. Thus, it is treated here as a valid species and can be distinguished from others, as depicted in the above key.

Results and Discussion

All 12 compared morphometric traits showed a significant difference between the species studied (Table 1, affirming the importance of morphometry in species differentiation. Dorsal fin rays, anal fin rays, number of scales on the mid-lateral line, and number of lateral lines on the eyed and non-eyed side are among the significant morphometric variables used in the earlier studies for differentiating species of this family[1,7,10,15,16].

Descriptive statistics of characters of the recorded species indicate that ED and IOL are the major characters for distinguishing species[17]. Some species were larger, like *Cynoglossus dubius*, *C. quadrilineatus*, *C. arel*, *C. macrolepidotus* and *C. quinquilineatus* with a mean SL of 322.79, 288.79, 223.6, 184.94 and 273.16 mm, respectively and some species were medium in size, *P. bilineata* and *C. lachneri* having mean SL of 181.47 and 202.30 mm, respectively. In comparison, smaller species like *C. punticeps*, *C. macrostomus* and *C. cynoglossus* had mean SL of 145.56, 128.70, and 105.62 mm, respectively. The results are as per earlier findings[1,16].

In the present study, two body depths were used for the first time for this family, BD_1, and BD_2 to describe the shape of the fishes (Fig. 2). The difference between BD_1 and BD_2 in *C. arel* and *C. macrolepidotus* were 6.06 and 3.67, respectively, showing that *C. arel* has a more bulging body than *C. macrolepidotus* (Fig. 3). The higher differences were also found in *C. dubius*, *C. macrostomus*, *C. cynoglossus* and *C. lachneri*, *i.e*., 17.34, 11.09, 8.24 and 7.66, respectively, indicating that these species have a more in-depth body after the operculum (Fig. 3). Some fishes have a decreasing trend of body depth from BD_1 to BD_2 like, *C. quinquelineatus*; having a difference of 1.41 means it bulges near the operculum, but it reduces after that (Fig. 3).

The SN in *C. lachneri*, *C. cynoglossus* and *C. punticeps* were smaller compared to the species of comparable measurements. This shows that eyes are closer to the snout in these species, as observed in Menon's monograph[1]. SM compared to SN of *C. cynoglossus* was much less, which shows that its hook length is small compared to other recorded species (Fig. 3), as described by earlier workers[1]. Among all the morphometric traits, *C. dubius* possesses the longest and deepest fish among all the studied species (Fig. 3). The highest variation in ED was also seen in *C. dubius.*

In the current study, 14 morphometric ratios were investigated. The ratio of HL in TL showed that it was not a very suitable character to differentiate the species as it ranged from 3.16 in *C. bilineatus* to 5.86 in *C. punticeps*. Although it gave some idea of the overall figure of the species, the proportion was higher in small-sized species like *C. punticeps*, *C. cynoglossus* and *C. macrostomus*, which shows that these species are more or less round-bodied, not elongated (Fig. 3).

The proportion of BD_1 in TL showed that *C. cynoglossus* (3.07) and *P. bilineata* (3.88) have a bulging body, as the proportion was relatively lower. The total length proportion of BD_1 and BD_2 revealed that *C. macrostomus* and *P. bilineata* have a deeper

Table 1 — Descriptive analysis and ratios of selected morphometric traits

Species	*C. arel* (n = 72)	*C. macrolepidotus* (n=37)	*C. macrostomus* (n = 35)	*C. lachneri* (n = 34)	*C. bilineatus* (n = 33)	*C. punecticeps* (n = 4)	*C. dubius* (n = 34)	*C. quiquilineatus* (n = 2)	*C. cynoglossus* (n = 5)	*P. bilineatus* (n =12)
SL	223.61	184.94	128.70	202.30	283.79	145.56	322.79	273.16	105.62	181.47
TL	243.43	204.20	142.84	216.96	293.18	154.69	322.79	245.51	113.52	193.83
BD1	46.57	39.24	31.71	51.01	67.38	34.01	78.83	69.12	28.72	49.93
BD2	52.63	42.91	42.80	58.67	73.44	38	96.17	67.71	36.96	57.01
LB	59.09	50.39	38.48	55.77	68.58	33.35	101.11	71.50	56.35	52.58
ED	03.47	03.11	02.41	03.40	04.76	02.37	04.77	04.31	02.5	03.16
IOL	02.69	01.91	00.89	03.08	04.91	02.69	07.23	4.87	01.76	03.31
SN	20.62	17.72	08.04	11.91	23.25	08.55	35.28	24.18	07.6	19.05
HL	52.29	43.25	30.78	39.90	61.23	27.03	30.89	63.84	20.87	46.33
SM	26.31	23.32	14.50	16.20	31.84	12.53	42.2	33.77	6.61	24.57
MG	30.69	26.36	25.33	29.09	35.81	19.03	41.59	33.46	13.13	15.99
MD	10.28	08.73	08.59	08.83	14.56	05.95	17.29	14.76	06.60	08.42
Morphometric ratios										
TL/HL	4.7	4.74	4.65	5.44	4.82	5.73	4.25	4.63	5.43	4.18
SL/HL	4.299	4.314	4.19	5.07	4.64	5.38	4.00	4.27	5.06	3.92
TL/BD1	5.24	5.20	4.51	4.26	4.35	4.54	4.39	4.77	3.95	3.89
TL/BD2	4.64	4.75	3.34	3.70	3.99	4.07	3.598	4.37	3.07	3.40
SL/BD1	4.80	4.72	4.07	3.97	4.23	4.28	4.13	4.41	3.67	3.64
SL/BD2	4.25	4.31	3.01	3.45	3.87	3.83	3.39	4.04	2.857	3.19
LB/HL	1.56	1.16	1.22	1.40	1.11	1.23	1.201	1.24	2.70	1.14
TL/SL	1.09	1.10	1.109	1.07	1.03	1.07	1.06	1.08	1.074	1.06
HL/SM	1.99	1.87	2.15	2.47	1.93	2.16	1.91	1.88	3.15	1.89
HL/MD	5.13	4.92	3.59	4.53	4.21	4.54	4.703	4.36	3.35	5.53
HL/SN	2.54	2.44	3.82	3.36	2.64	3.159	2.28	2.64	2.72	2.43
SN/ED	5.97	5.73	3.4	3.54	4.93	3.59	7.68	5.63	3.06	6.057
HL/ED	15.13	14.01	12.80	11.86	12.98	11.37	17. 52	14.92	8.34	14.69
ED/ IOL	1.40	1.88	2.95	1.13	0.98	0.88	0.62	0.88	1.418	0.89

SL–Standard length, TL-Total length, BD_1 - Body depth at gill opening, BD_2 - maximum body depth, LB-Distance from the snout tip to the vertical line at BD_2. ED–Eye diameter, IOL-Inter-orbital, SN-Snout length (preorbital length), HL-Head length, SM-Snout tip to mouth distance (preoral length), MG-Distance between the angle of mouth to gill opening (post-oral head length) and MD-Jaw length

body after the operculum (Fig. 3). *C. cynoglossus* had the highest body depth, suggesting a more or less circular shape of the species (Fig. 3).

The IOL compared to ED was found to be less in some fishes, *C. arel, C. macrolepidotus, C. macrostomus, C. lachneri, C. cynoglossus* while, in others fishes, *C. bilineatus, C. punticeps, C. dubius, C. quinquelineatus* and *P. bilineata* (Fig. 3), it was similar to the earlier reports[1,15].

The proportion of SL in TL had higher values, which revealed that *C. lachneri*, *C. dubius* and *C. bilineatus* have longer caudal fins. The proportion of MG in HL revealed that species like *C. cynoglossus*, *C. macrostomus* and *C. lachneri* were deeply hooked fishes (Fig. 3). The proportion of snouts in HL explained that *C. macrostomus* and *C. lachneri* have longer snouts. The proportion of ED in HL and ED in the snout revealed that *C. dubius* and *C. arel* have larger eyes than other members of recorded fishes. The proportion of IOL distance to ED showed that *C. macrostomus* has the most substantial IOL distances compared to other above-studied fishes (Fig. 3).

In the case of *C. macrostomus*, as the fish increases in SL, its SM, MG and MD decrease (Table 2). For correlation between the BD_1 and BD_2 with the SL, *C. arel* has the highest, and *C. macrostomus* has the lowest. The correlation between the LB of *C. macrostomus* with its SL was significantly low. ED has the highest correlation with SL in the case of

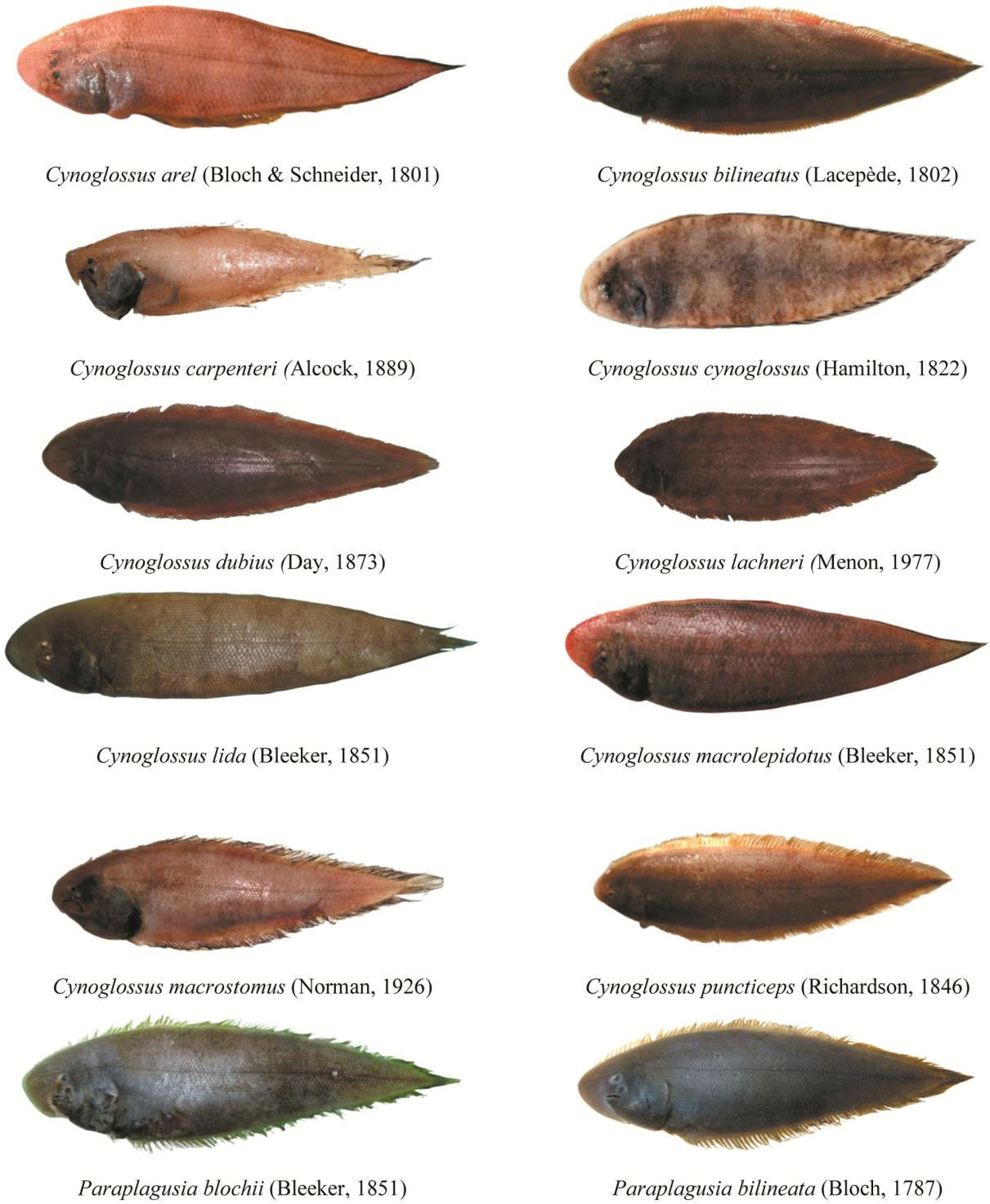

Fig. 3 — Photographs of Cynoglossid species reported from India

P. bilineata. IOL is negatively correlated with ED in the case of *P. bilineata* (Fig. 3).

The SL with other morphometric traits shows that two similar species have a difference in the correlation of IOL distance with the SL, *i.e.*, lower in *C. macrolepidotus* (0.49) and higher in *C. arel* (0.91), similar to the description[1]. *C. macrostomus* showed a negative correlation for the snout-to-mouth and mouth-to-gill distance (Table 2). Since the size of fish is relatively small, but as the TL of fish increases

Table 2 — Correlation between standard length (S.L.) and other selected morphometric traits

Species	TL	BD_1	BD_2	LB	ED	IOL	SN	HL	SM	MG	MD
C. arel	0.96	0.90	0.92	0.93	0.80	0.81	0.88	0.93	0.77	0.83	0.92
C. macrostomus	0.63	0.20	0.26	0.05	0.63	0.75	0.31	0.67	-0.71	-0.85	-0.76
C. macrolepidotus	0.98	0.94	0.95	0.93	0.77	0.49	0.90	0.94	0.90	0.90	0.83
C. bilineatus	0.65	0.85	0.88	0.86	0.83	0.79	0.76	0.86	0.42	0.86	0.92
C. dubius	0.98	0.98	0.97	0.97	0.88	0.79	0.94	0.97	0.95	0.96	0.91
C. lachneri	0.93	0.93	0.92	0.76	0.67	0.84	0.25	0.76	0.09	0.66	0.87
P.bilineata	0.99	0.64	0.59	0.76	0.92	-0.51	0.81	0.94	0.89	0.84	0.67

TL - Total length, BD_1 - Body depth at gill opening, BD_2 - maximum body depth, LB - Distance from the snout tip to the vertical line at BD_2. ED –Eye diameter, IOL - Inter-orbital, SN - Snout length (preorbital length), HL - Head length, SM - Snout tip to mouth distance (preoral length), MG - Distance between the angle of mouth to gill opening (post-oral head length) and MD - Jaw length

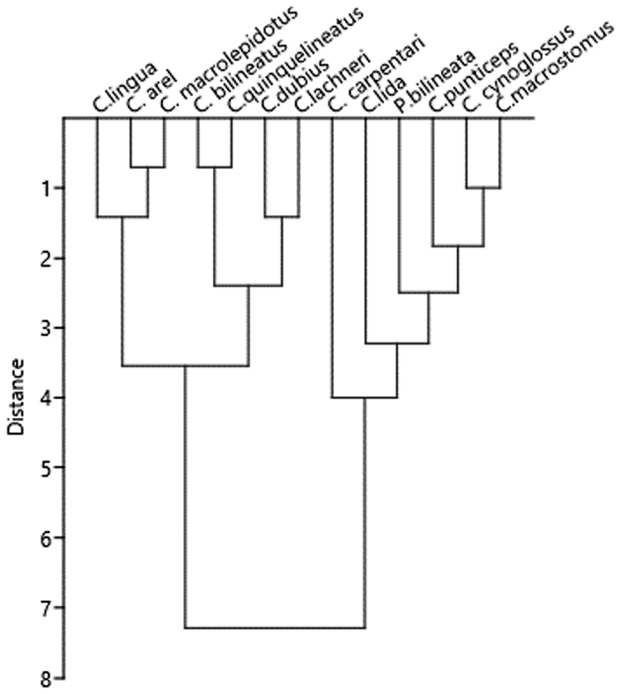

Fig. 4 — Cladogram showing relationship among genera and species of Cynoglossidae based on morphological and qualitative characters

faster, these two characteristics do not correlate positively; that species shows allometric growth. In *P. bilineata*, with the increase in the total size of fish, the ED grows very fast, and in the same way, the IOL distance decreases (Fig. 3).

Relationships within cynoglossids

The character analysis from morphometric, meristic, qualitative, and hard parts (scales, otoliths and radiographs) has yielded 22 characters based on a shared character of 14 species of 2 genera within the Cynoglossidae (Fig. 4). These characters, attributes and points distribution for selected traits for cluster analysis (Table 3), and the Character matrix of the

Table 3 — Characters, attributes and points distribution for the selected traits for cluster analysis

Characters	Attributes	Points
No. of lateral line on the eyed side (NLE)	2	0
	3	1
No. of lateral line on blind side (NLB)	1-2	0
	0	1
Dorsal fin rays	>110	0
	<110	1
Anal fin rays	>85	0
	<85	1
Caudal fin rays	12	0
	<12	1
Scale type on the ocular side	Cycloid	0
	Ctenoid	1
Scale type on the blindside	Ctenoid	0
	Cycloid	1
Number of vertebrae	>50	0
	>=50	1
Number of scales on mid-lateral line on ocular side	>95 <=95	0 1
Eye size	Large small	0 1
Inter-orbital length	Wide	0
	Narrow	1
Snout shape	Rounded	0
	Pointed	1
Size of adult fish	Large	0
	Small	1
Inter-linear scale	> 17 < = 17	0 1
Eyes contiguity	Yes No	0 1
Corner of mouth	Near to	0
	branchial	1
	Near to snout	
Number of nostrils on the ocular side	Single double	0 1
Fringed lip	Lip fringed	0
	Lip not fringed	1
Noticeable nostril on the blindside	Present Absent	0 1
Body shape	Uniform	0
	Rounded	1
Caudal shape	Non-tapering	0
	Tapering	1
Lip with tentacles	Present Absent	01

family Cynoglossidae with the value of their binary character. From the cladogram of the relationship, it is observed that *C. cynoglossus* and *C. macrostomus* have a close resemblance as both the species have a smaller size and prominent hooked snout; *Cynoglossus lingua* and *C. arel* have similar size, scale pattern, and body depth; *C. quadrilineatus* and *C. quinquelineatus* have similar scale pattern, similar size, same scale rows between above and mid-lateral lines and *P. bleekeri* and *P. bilineata* have fringed lips and similar scale pattern.

Conclusion

In this study, the cladogram was subsequently divided into small monophyletic units, providing information on synapomorphies and autapomorphies. The particular single cladogram for the family Cynoglossidae bifurcates into two main branches A and B. Branch "A" is divided into three close groups, first containing *C. dubius* and *C. lachneri*; *C. arel* and *C. macrolepidotus* and *C. bilineatus* and *C. quiquelineatus*, whereas, branch "B" presents two closely related groups namely *C. cynoglossus* and *C. macrostomus*; and *P. bilineata* and *P. blochii* apart from three separate species in the same branch that are *C. puncticeps*, *C. lida* and *C. carpenteri.*

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Conflict of Interest

There is no conflict of interest among the authors.

Ethical Statement

All of the experimental procedures involving flatfishes were conducted in an ethically responsible manner.

Author Contributions

All the authors have contributed to the manuscript's preparation. The first author collected data, and other authors helped in the manuscript's processing, analysis, and writing.

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