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Sicyopterus garra Hora, 1925, a valid species of sicydiine goby from the Andaman Islands, India

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Abstract

Sicyopterus garra Hora, 1925 from the insular streams of South Andaman Islands was synonymized with *Sicyopterus microcephalus* described from Java, South East Asia and has retained this taxonomic status since then. Recent collections of *Sicyopterus* from the type locality of *S. garra* and the examination of syntypes of this species revealed significant morphological and genetic differences from *S. microcephalus* and the other *Sicyopterus* species with papillae on upper lip. *S. garra* is thus a valid species and not a synonym of *S. microcephalus*. *S. garra* differs from *S. microcephalus* in having fewer lateral scales 53–59 *vs.* 57–68, fewer zigzag series (12–14 *vs.* 13–16), a longer caudal peduncle length (16–21 *vs.* 13–17), and by having a high percentage of divergence in COI gene (5.5%–5.8%).

KEYWORDS
COI gene, freshwater goby, insular, sicydiine, *Sicyopterus microcephalus*, South East Asia

1 | INTRODUCTION

The sicydiine goby *Sicyopterus garra* was described in 1925 by Sundar Lal Hora based on eight specimens collected by Nelson Annandale from two different locations in the South Andaman Islands: two specimens from a small stream near Birchgunge and the other six specimens from a streamlet originating from a reservoir at the base of the Mount Harriet National Park (Annandale & Hora, 1925). This species, with papillae on upper lip and no lateral clefts, has long been considered to be a synonym of *Sicyopterus microcephalus* (Bleeker, 1855) (type locality: Java, Indonesia). Koumans (1940) received and examined, through Baini Prashad and S. L. Hora, the type specimens of *S. garra* from Birchgunge (now Brichgunj), South Andaman, Port Blair, and synonymized it with *S. microcephalus*, concluding that the distribution of the latter was in Andamans, Java, Celebes and Timor (Koumans, 1940). Since then, authors have used this name for the *Sicyopterus* found in the Andamans (Kottelat, 2013; Fricke *et al.*, 2022). Nonetheless, the distribution of *S. microcephalus* in the Andaman Islands and the synonymy of *S. garra* was questioned without comment by Keith *et al.* (2015), although the species has never been studied in detail, although collected by many workers (see Talwar, 1990; Rao *et al.*, 2000; Palavai & Davidar, 2009; Devi, 2010; Rajan & Sreeraj, 2013). Recent surveys of Andaman streams have been carried out, and several *Sicyopterus* specimens with papillae on the upper lip (and no lateral clefts) have been collected. Examination of several syntypes at ZSI, Kolkata, and of these freshly collected specimens reveals significant morphological and genetic differences between *S. garra* and *S. microcephalus* necessitating the clarification of the status of the Andaman *Sicyopterus*.

The purpose of this article is to clarify this status using genetic and morphometric approaches, using both the new material collected and two syntypes.

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2 | MATERIALS AND METHODS

2.1 | Study site and specimen collection

Specimens were collected from Brichgunj (11° 35′ 44.5″ N 92° 43′ 37.7″ E) and Mannarghat (11° 45′ 18.1″ N 92° 42′ 47.5″ E), the base

of Mt. Harriet National Park, South Andaman Island, preserved in 10% formalin and transferred to 70% ethanol for long-term storage and registered in the collections of the ICAR–Central Island Agricultural Research Institute (CIARI), Port Blair, India and in the freshwater fish collections of the Zoological Survey of India (ZSI/FF), Kolkata, India. Comparative specimens were examined from AMS (Australian Museum, Sydney); MNHN: Muséum national d'Histoire naturelle, Paris; RMNH: Rijksmuseum van Natuurlijke Historie, Leiden; SMF (Senckenberg Museum, Frankfurt am Main, Germany); MZB (Museum Zoologicum Bogoriense, Bogor, Indonesia) and WAM (Western Australian Museum, Perth, Australia).

2.2 | Morphomeristics

Measurements were taken point-to-point using digital callipers, to the nearest 0.1 mm. Methods follow Keith *et al.* (2011). All counts and measurements were taken from the right side. Teeth were counted to the right of the symphysis. Abbreviations for cephalic sensory pores follow Akihito (1986). Abbreviations used in the descriptive account follow Keith *et al.* (2004). Two recent specimens of *S. garra*, CIARI/FF-90 were used for osteology following Potthoff (1984).

2.3 | Genetic analysis

Right side pectoral fins were harvested from six fresh specimens, Brichgunj ($n = 3$) (ZSI/FF-9385), Mannarghat ($n = 3$) (ZSI/FF-9386) and preserved in absolute ethanol. Total DNA was extracted using Macherey & Nagel NucleoSpin Tissue kits following the manufacturer's instructions on an Eppendorf EpMotion 5075. The DNA was amplified for the barcoding fragment of the COI gene (630 bp) with the specific fish primers TelF1 and TelR1 (Dettaï *et al.*, 2011). DNA amplification was performed by PCR in a final 20 µl volume containing 5% DMSO, 1 µl of BSA, 0.8 µl of dNTP 6.6 µM, 0.15 µl of Qiagen Taq DNA polymerase, using 2 µl of the buffer provided by the manufacturer, and 0.4 µl of each of the two primers at 10 pM; 1.2 µl of DNA extract was added. After denaturation for 2 min at 94°C, the PCR was run for 55 cycles of (25 s, 94°C; 25 s, 52°C; 55 s, 72°C) on a Bio-Rad C1000 Touch Thermal Cycler. Successful PCRs were selected on ethidium-bromide stained agarose gels. Sanger sequencing was performed in both directions by a commercial company (Medauxin, Bangalore, India) using the same primers.

Sequences generated as part of the study are deposited in GenBank under accession numbers ON007176-78 ($n = 3$) for the specimens collected from the base of Mt. Harriet, and ON007179–81 ($n = 3$) for the specimens collected from Brichgunj. Data processing and sequence assembly were done in Geneious 11.1.3 (http://www.geneious.com) (Kearse *et al.*, 2012). COI sequences were aligned with Muscle Alignment (Edgar, 2004). The sequence of another gobiid species, *Sicyopus zosterophorum*, was included as out-group, two sequences of *Sicyopterus pugnans*, a species with papillae on upper lip and two lateral clefts, and a sequence of *Sicyopterus lagocephalus*, a species without papillae on upper lip, were added. Sequences of other *Sicyopterus* species were used from the work of Lord *et al.* (2019). A simple PhyML tree was performed on the alignment using SeeView v3.1 by incorporating sequences of *S. garra* from Brichgunj (ON007176) and Mt. Harriet (ON007179), and other *Sicyopterus* sequences (see Appendix 1 for the list of NCBI GenBank sequences). The percentage of divergence between sequences was calculated with the Geneious 11.1.3 software.

FIGURE 1 One of the original syntypes of *Sicyopterus garra* collected by Nelson Annandale from Brichgunj, South Andaman Islands, now lectotype, ZSI F 10831/ 1, 22.8 mm SL

Ethics statement. Fish specimens used in the study are not listed as threatened or endangered by the IUCN Red List or CITES.

3 | RESULTS

3.1 | *S. garra*, Hora *in* Annandale & Hora 1925

S. garra Hora, in Annandale & Hora 1925.

S. garra Hora: Herre (1939: 348).

S. microcephalus (non Bleeker 1855): Koumans (1940:17).

S. garra Hora (Herre 1941: 347).

S. microcephalus (non Bleeker 1855): Talwar (1990); Rao *et al.* (2000: 310); Palavai and Davidar (2009: 12); Rema Devi (2010: 332); Rajan and Sreeraj (2013: 37); Kottelat (2013: 428).

Material examined: S. *garra*, Hora type material from the Andaman Islands; ZSI F ZSI F10831/1, 22.8 mm SL; additional topotypic

FIGURE 2 *Sicyopterus garra* in life. (a) Male, ZSI/FF-9385, 37.5 mm SL, and (b) female, ZSI/FF-9385, 39.9 mm SL, from Brichgunj, South Andaman Islands. (c) Male, ZSI/FF-9386, 54.3 mm SL, and (d) female, ZSI/FF-9386, 50.0 mm SL, from Mannarghat, base of Mt. Harriet, National Park, South Andaman

FIGURE 3 *Sicyopterus garra*, colouration in preservative. (a) Female, ZSI/FF-9385, 39.9 mm SL; (b) male, ZSI/FF-9386, 54.3 mm SL; (c) female, ZSI/FF-9386, 50.0 mm SL. (note: blue colour on the cheeks is due to stain for clarifying sensory pores)

material: ZSI/FF-9385, 35.4–39.9 mm SL (7 ex.), Brichgunj, South Andaman; ZSI/FF-9386, 50.0–54.3 mm SL (2 ex.), Mannarghat water fall, base of Mt. Harriet National Park, South Andaman; CIARI/FF-90, 48.3 & 50.8 mm SL (both used for osteology), same data as above.

Diagnosis: The species has an upper lip with 25–26 papillae and without clefts anteriorly or midlaterally, second dorsal fin with 1 spine and 10 segmented rays, 53–59 lateral scales, 12–14 scales in zigzag series and a long caudal peduncle.

Description: For general shape and appearance see Figures 1–3. Scale counts are given in Table 1, morphometrics in Table 2. Body subcylindrical, snout obtuse. Dorsal fins VI-I, 10; in males, second, third and fourth rays of I dorsal fin longest, usually reaching half the length of second dorsal fin when adpressed. In female, first dorsal fin spine short and not reaching second dorsal fin ray. Anal fin I, 10, origin directly opposite second dorsal fin, reaching procurrent rays of caudal fin in males and not reaching in females. Caudal fin with 14 branched rays, its posterior margin rounded in males and slightly truncated in females. Pelvic disk with one spine and five branched rays on each side, fifth rays joined together over their entire length, a thick frenum between spines; disc adherent to belly between all five rays. Pectoral fins 18–19, posterior margin rounded. LS 53–59, ctenoid scales on flanks and caudal peduncle. TRB 14–17. TRF 18–22. PD 18–23. ZZ 12–13. Belly entirely covered with cycloid scales, extending from anus, almost to pelvic base. Nape with cycloid scales. Upper jaw with a single row of flexible tricuspid teeth, lateral cusps slightly pointed, medial cusps rounded longer than lateral cusps (Figure 4). Dentary with a single straight row of conical teeth, 8 on each side; anterior and posterior teeth usually caniniform; horizontal teeth correspond in position with upper jaw teeth. Upper lip with 25–26 small papillae. Lower lip mostly absent, rudimentary elements present as expanded and folded tissue posterior to lower jaw teeth. Cephalic sensory pore system A, B, C, D, F, H, L, (M), (N) and (O). Pore D single with all others paired. Oculoscapular canal uninterrupted, posterior to eye. Cutaneous sensory papillae developed on head (Figure 5). Males with an elongate urogenital papilla with distal tip rounded. Females with bulbous bilobed urogenital papilla (Figure 6).

Coloration: In live specimens, sexual dimorphism is well developed. Males (Figure 2a,c): Body olive-brown, with six brown saddle-like bands on the sides, distinct on the upper half. Belly pale white. Cheek bluish-grey with orange irregular markings on upper half of the opercle. Lower jaw pale blue, upper jaw olive-green. A black streak below the eye. Pelvic fin pale white. Pectoral fin pale brown, base bluish with orange irregular markings, its anterior margin bordered with pale chrome yellow. Anal fin pale blue. First dorsal fin brown, its first three unbranched rays black; second dorsal fin brown with small curved markings on the fin membrane, resembling reticulated pattern.

TABLE 1 Scale counts in *Sicyopterus garra* and related species

	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	53	54	55	56	57	58	59	60	61	62	63
Zig-zag series																											
SS	1	2	2	1																							
SM		4	13	7	6	1																					
SG	2	8	1																								
Transverse backward series																											
SS			1	2	2	1																					
SM			3	2	8	8	8	2																			
SG			1	–	9	1																					
Transverse forward series																											
SS			1	–	1	1	1	1	1																		
SM							3	2	4	2	2	4	2	4	3												
SG							2	–	5	3	1																
Pre-dorsal midline scales																											
SS					1	–	1	1	1	1	1																
SM								2	7	6	3	3	2	3	1	1											
SG							1	6	–	3	–	1															
Lateral scales																											
SS																		1	1	2	–	1	1				
SM																					1	3	7	10	1	5	1
SG																	2	3	2	2	1	–	1				

Abbreviations: SG: *Sicyopterus garra*; SM: *Sicyopterus microcephalus*; SS: *Sicyopterus stiphodonoides*.

TABLE 2 Morphometric in *Sicyopterus garra* and related species expressed to the nearest whole percentage of standard length

	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	30	31	32	33	34	35	36	37	50	51	52	53	54	55	56	57	58	59	60	61	62
Jaw length																																								
SS		2	3	1																																			
SM	2	3	9	5	10	2																																	
SG		4	6	1																																			
Caudal peduncle depth																																							
SS			1	3	2																																		
SM				2	5	14	7	2																																
SG					3	6	2																																	
Caudal peduncle length																																							
SS							1	1	2	1	1																													
SM					1	6	2	7	6	4																														
SG									1	1	3	4	1	1																									
Head length																																							
SS													1	1	3	1																							
SM													2	8	4	4	5	3																					
SG														1	3	3	1	–	1																					
Pre-dorsal length																																							
SS																					1	–	1	3	1														
SM																				1	–	3	8	7	2	3	4												
SG																				1	–	2	1	4	2	1													
Pre-anal length																																							
SS																													1	1	1	–	–	1	1	1				
SM																												1	1	3	3	–	5	5	2	1	4	2		
SG																																1	3	1	1	1	1	–	1	1

Abbreviations: SG: *Sicyopterus garra*; SM: *Sicyopterus microcephalus*; SS: *Sicyopterus stiphodonoides*.

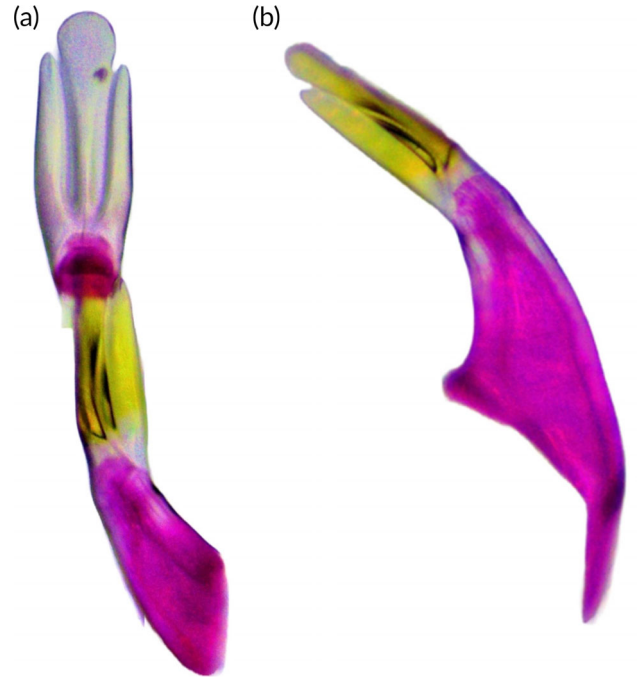

FIGURE 4 Tricuspid teeth of *Sicyopterus garra* from the upper jaw. (a,b) Medial view and lateral view, *S. garra*, CIARI/FF-90

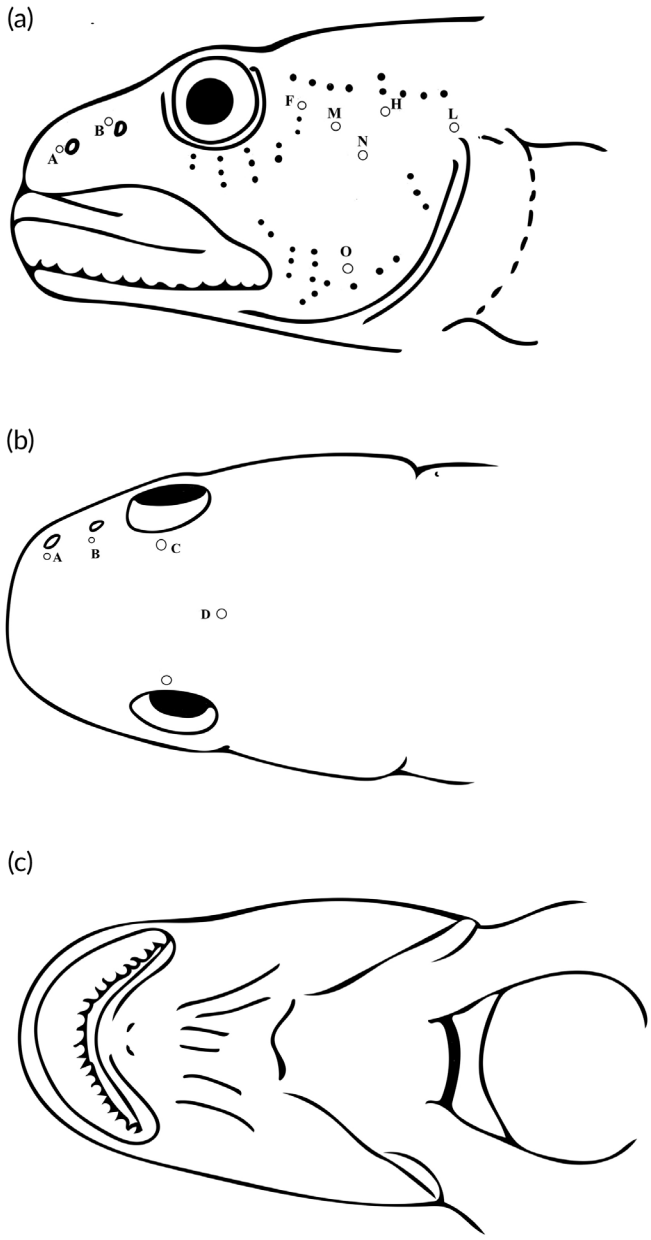

FIGURE 5 Head in *Sicyopterus garra*, ZSI/FF-9386, 54.3 mm SL, showing cephalic sensory pore system. (a) Dorsal view, (b) lateral view, (c) ventral view

Caudal fin pale red, with bluish to reddish interradial membrane and two slightly curved pale maroon blue-edged lines extending from upper and lower caudal peduncle to rear margin of fin. Female (Figure 2b,d): Body pale-brown, with 6–7 beige, saddle-like bands on the sides and dorsum. Two longitudinal brownish black bands on the side, the first one extending midlaterally from snout to caudal-fin base, the second one extending from the head posterior to eye to the upper caudal rays. The two dark bands are interspersed by saddle-like lateral bands. Pectoral fin with upper half black, lower half translucently pale-yellow, margins bordered with pale chrome yellow. Anal fin 3/4th pale-grey, distal margin black. Caudal fin translucent pale grey, with two longitudinal blue lines extending from the caudal peduncle to the distal part bordered by pale red margins distally. First dorsal fin translucently pale grey, the first three unbranched rays black and chrome yellow distally. Second dorsal fin translucent pale yellow. Both first and second dorsal fins with irregular markings on fin membrane similar to males. Pelvic fin pale white. In preservative: Male (Figure 3b): Body greyish, first dorsal, second dorsal and anal fins translucent greyish with black distal margins. Caudal fin greyish, anterior and posterior margin dull white. Pectoral fin greyish, margins dull white. Female (Figure 3a,c): Colouration similar to male, except the body colour in pale grey. The caudal fin superior and inferior margins narrow and dull white.

Distribution: Presently known from the Andaman group of islands (Figure 7).

Ecology: The type locality near Brichgunj, South Andaman, is a very shallow stream of depth of 15 cm. The stream was moderately fast flowing over mixed gravel, cobbles, rocks and sand in open canopy forest (Figure 8a). The type locality at Mannarghat, the base of Mount Harriet, South Andaman, was a slow-flowing mountainous stream with rocky boulders and leaf litter as the substrate (Figure 8b). The species was seen grazing on the red algae *Caloglossa* sp. encrusted on rocks and boulders. Co-occurring species in both habitats include *Redigobius tambujon*, *Giurus* sp., *Microphis insularis*, *Oryzias* sp., *Aplocheilus andamanicus*, *Rasbora* sp. and *Eleotris* sp.

Genetic analysis: A total of 581 bp of the COI gene from 12 *Sicyopterus* individuals was obtained, and percentages of divergence are presented in Table 3. The phylogeny presented two main branches (Figure 9). The first one (A) contains the *Sicyopterus* species without papillae on upper lip according to the results of Lord *et al.*, (2019). The second branch of the tree (B), corresponding to *Sicyopterus* species with papillae on upper lip, is composed of three clades.

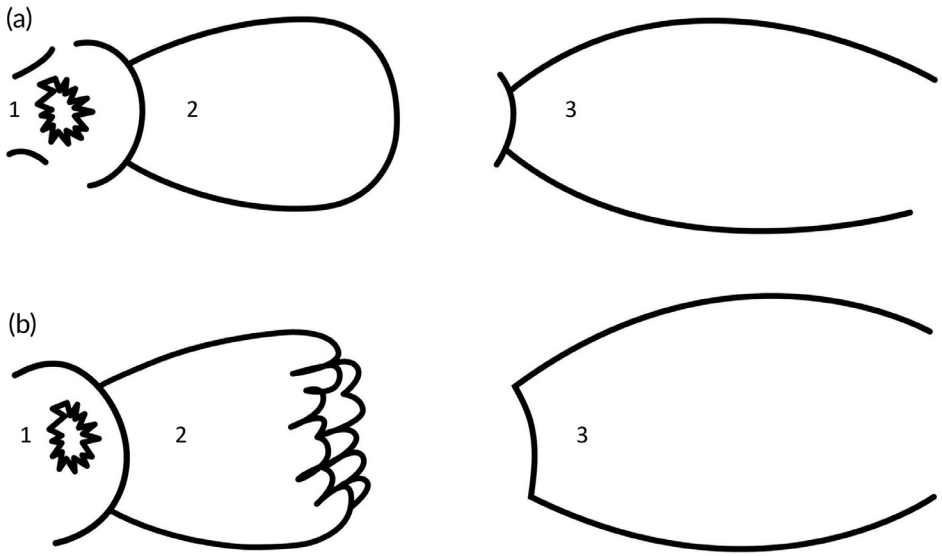

FIGURE 6 Urogenital papilla in *Sicyopterus garra*. 1: anus, 2: urogenital papilla, 3: anal fin. (a) Male, ZSI/FF = 9386, 50.0 mm SL, (b) female, ZSI/FF-9385, 39.9 mm SL

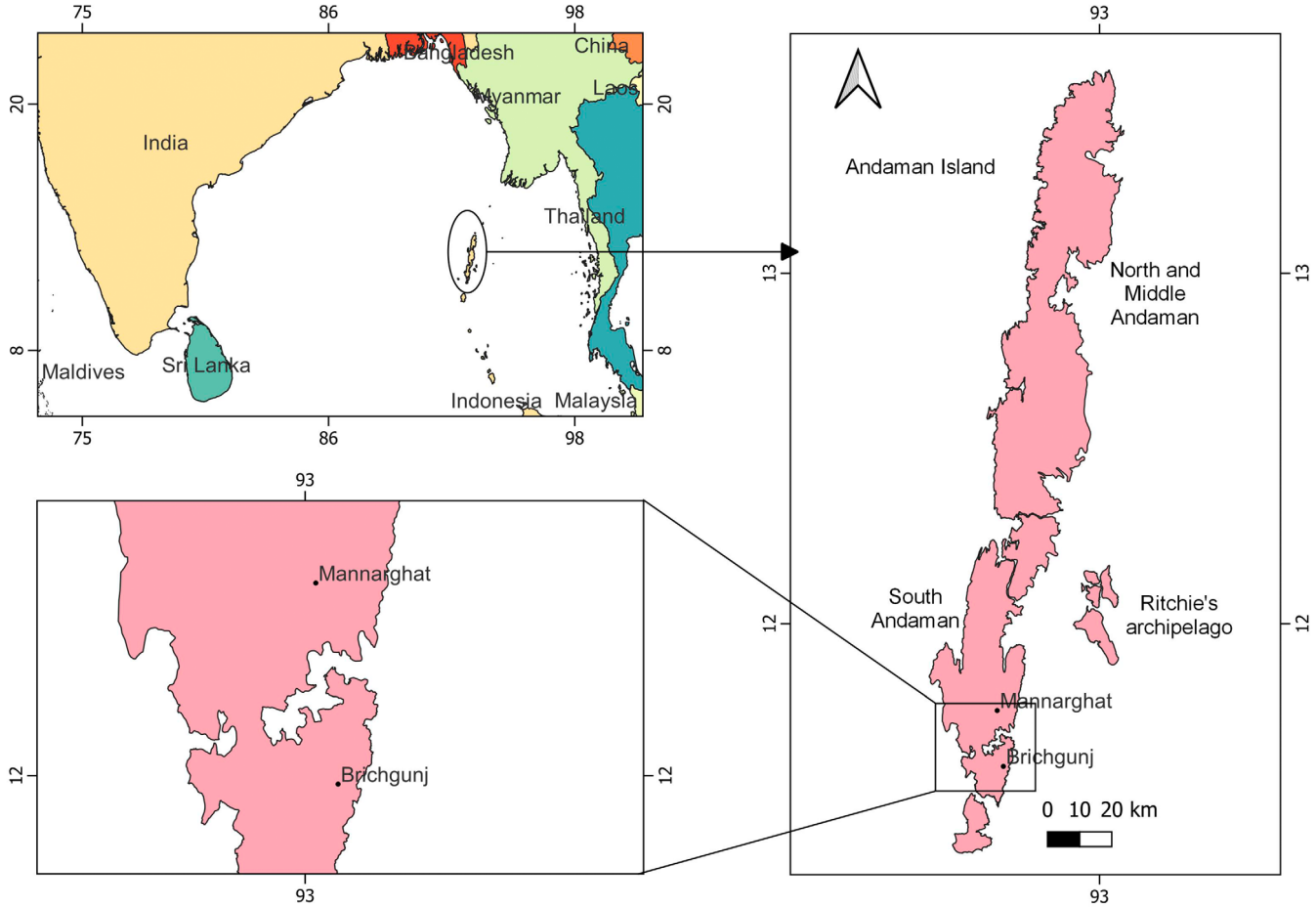

FIGURE 7 Map showing the type locality of *Sicyopterus garra* in the South Andaman Islands

The clade D is composed of specimens of *Sicyopterus stiphonodoides* and *Sicyopterus pugnans*, the clade F is *S. microcephalus* and the clade E is composed of two clades *Sicyopterus griseus* (E1) and *S. garra* (E2).

The tree shows that *S. garra* is a distinct species from all the others with papillae on upper lip and it differs from *S. microcephalus* by 5.51%–5.85% of divergence in the COI gene. It differs from the other *Sicyopterus* with papillae on upper lip by 6.2%–8.43% of

FIGURE 8 Type locality of *Sicyopterus garra* at South Andaman Islands. (a) A stream originating near Brichgunj military station, (b) Mannarghat waterfalls, situated at the base of Mt. Harriet National Park

TABLE 3 Pair-wise distance matrix (percentage of divergence between *Sicyopterus* paired individuals) based on the COI gene sequence

	1	2	3	4	5	6	7	8	9	10	11
Sicyopus zosterophorum	0	12.91	13.25	13.08	13.08	13.43	13.08	12.91	13.25	13.25	13.25
Sicyopterus lagocephalus MK496946	12.91	0	0.69	7.92	7.92	9.64	9.12	8.61	7.92	8.78	8.78
Sicyopterus lagocephalus MK496948	13.25	0.69	0	7.92	7.92	9.81	9.47	8.95	8.26	8.78	8.78
Sicyopterus griseus	13.08	7.92	7.92	0	0	7.75	7.23	6.71	6.54	6.2	6.2
Sicyopterus griseus	13.08	7.92	7.92	0	0	7.75	7.23	6.71	6.54	6.2	6.2
Sicyopterus pugnans MK496973	13.43	9.64	9.81	7.75	7.75	0	0.52	3.96	3.79	8.43	8.43
Sicyopterus pugnans MK496972	13.08	9.12	9.47	7.23	7.23	0.52	0	3.44	3.27	8.09	8.09
Sicyopterus stiphodonoides MK496986	12.91	8.61	8.95	6.71	6.71	3.96	3.44	0	0.86	7.23	7.23
Sicyopterus stiphodonoides MK496988	13.25	7.92	8.26	6.54	6.54	3.79	3.27	0.86	0	7.06	7.06
Sicyopterus garra ON007176 (Mannarghat)	13.25	8.78	8.78	6.2	6.2	8.43	8.09	7.23	7.06	0	0.17
Sicyopterus garra ON007179 (Brichgunj)	13.25	8.78	8.78	6.2	6.2	8.43	8.09	7.23	7.06	0.17	0
Sicyopterus microcephalus MN812976	12.22	7.57	7.92	6.54	6.54	6.88	6.37	6.37	5.68	5.85	5.85
Sicyopterus microcephalus KU693049	11.88	7.57	7.92	6.2	6.2	6.54	6.02	6.02	5.68	5.51	5.51

divergence. Its main characteristics are given below based on type material and recently collected specimens.

4 | DISCUSSION

After examination and measurement of type specimens, the morphological and meristic identification of the recently collected specimens (Tables 1 and 2) show that these last are different from the other known species of *Sicyopterus* with papillae on upper lip and no lateral clefts; their measurements correspond to those of *S. garra* syntypes. The morphomeristic and genetic study confirms the validity of *S. garra*. As the attempt to locate the specimens at ZSI, Kolkata, yielded only two syntypes (17.9 and 22.8 mm SL) from Birchgunge (Brichgunj), according to ICZN (1999: art. 74.1) the authors here designate them as lectotype, to stabilize the name, the larger syntype of

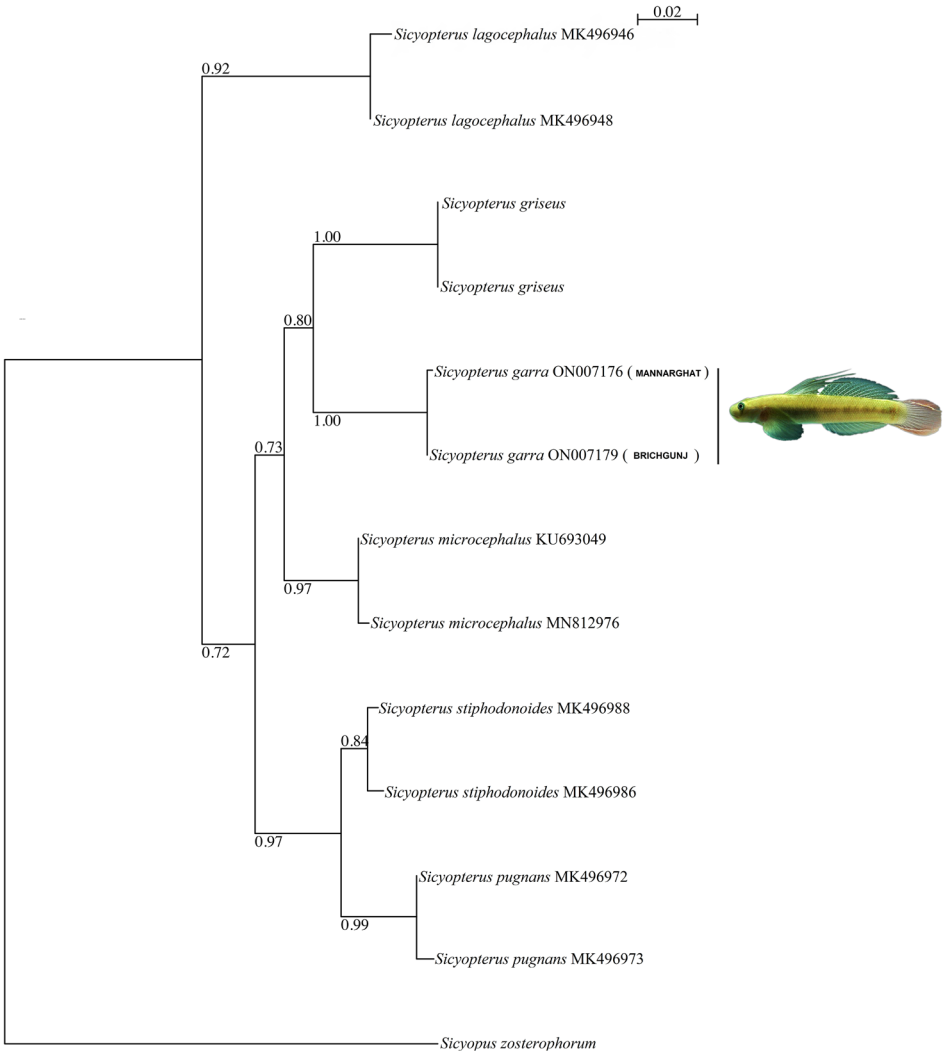

FIGURE 9 Phylogenetic position of *Sicyopterus garra* based on PhyML tree of a COI fragment gene (581 bp). *Sicyopus zosterophorum* is used as the out-group. Sequences of *S. garra* used in the tree includes ON007176 (Mannarghat) and ON007179 (Brichgunj)

S. garra 22.8 mm SL (Figure 4) here labelled with the ZSI number 10831/1 (Figure 1). The remaining specimen (1) of ZSI F 10831/1 becomes a paralectotype. *Sicyopterus garra* differs from *S. microcephalus* by having a high percentage of divergence in COI gene (5.51%–5.85%) (Table 3). In addition to divergence of COI genus mentioned earlier, *S. garra* also differs from *S. microcephalus* by a combination of characters including fewer lateral scales 53–59 *vs*. 57–68, fewer zigzag series (12–14 *vs*. 13–17), and a longer caudal peduncle length (16–21 *vs*. 12–17). It differs from *Sicyopterus stiphodonoides* by having a high percentage of divergence in COI gene (7.06%–7.23%) (Table 3), a longer caudal peduncle depth and length (12–15 *vs*. 10–12; 16–21 *vs*. 14–18) and an upper lip with more papillae (25–26 *vs*. 14–15). It differs from *S. griseus* from mainland India by having a high percentage of divergence in COI gene (6.2%) (Table 3), fewer lateral scales (53–59 *vs*. 69–76), fewer pre-dorsal midline scales (19–25 *vs*. 30–36) and more scales in zigzag series (12–14 *vs*. 9–10). It differs from *S. pugnans* by having a high percentage of divergence in COI gene (8.09%–8.43%) (Table 3) and no lateral clefts on upper lips *vs*. two clefts.

4.1 | Material examined

S. microcephalus (Bleeker, 1855). – Syntypes: RMNH 4768 (2 of 4 and 1 non type), size range 80.2–84.5 mm SL). Tjibiliong, Banten Province, Java, Indonesia. And its two synonyms: *Sicyopterus wichmanni* (Weber, 1894), lectotype: ZMA 111274, 53.7 mm SL; paralectotypes: ZMA 111275, 31.3–48.6 mm SL; ZMA 111276, 36–44.2 mm SL, river near Kupang, Timor Island, southern Malay Archipelago; *Sicyopterus hageni* Popta, 1921, syntypes: SMF 6622–29, 55.1–65.2 mm SL. Sunda Islands, Indonesia.

S. griseus (Day, 1877). – Syntypes: AMS B.8254 (1), 61.0 mm SL, Day coll.

S. stiphodonoides Keith et al. (2012). – Holotype: MZB 20007, 32.7 mm SL, Nantuke Creek, Papua Province, Indonesia, 02° 27.103′ S 140° 29.174′ E, 24 August 2000; G.R. Allen coll. *Paratypes*: WAM P31746.004, female, 34.6 mm SL. Same data as holotype. MNHN 2011–0048, male, 46.3 mm SL, Vila River, Solomon, 21 July 2010, D. Boseto coll. MNHN 2011–0049, 2 males, 1 female, size range 45–57.4 mm SL. Choiseul Island, Tupiri River, Solomon, 8 August 2008, D. Boseto coll.

S. garra, Hora *in* Annandale & Hora 1925. – Syntypes: ZSI F10831/1 (2) Birchgunge; South Andaman Island, Andaman Islands, N. Annandale Coll.

AUTHOR CONTRIBUTIONS

J.P., R.K.S. and K.S. collected, identified, examined the specimens and prepared the manuscript. T.T. performed molecular analysis. P.S. examined the syntypes, from ZSI, Kolkata, and provided essential data. P.K. and J.D.K.M. provided overall guidance and revised the manuscript, and added essential components. All authors read and approved the final version of the manuscript.

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