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43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: Mauve Stinger *Pelagica noctiluca* by Swaathi Na. Medium used is soft pastels and gelly roll.



The giant clam commensal shrimp *Anchistus miersi* (de Man, 1888) (Decapoda: Palaemonoidae) new to Lakshadweep Sea, India

Manu Madhavan¹ , Purushothaman Paramasivam² , S. Akash³ , T.T. Ajith Kumar⁴
& Kuldeep Kumar Lal⁵

^{1–5} ICAR – National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow, Uttar Pradesh 226002, India.

^{1,4} Kerala University of Fisheries and Ocean Studies, Kochi, Kerala 682506, India.

¹manumadhavan060@gmail.com, ²purushothgene@gmail.com, ³akash03jack@gmail.com

⁴ttajith87@gmail.com (corresponding author), ⁵kuldeepklal@gmail.com

This article is dedicated to the late Dr. P. Purushothaman, the second author.

Abstract: The genus *Anchistus* Borradaile, 1898 is a colourful shrimp from the commensal group. In this study, we are reporting a new occurrence of Giant Clam commensal shrimp *Anchistus miersi* (de Man, 1888 [in de Man, 1887–1888]) caught from Agatti Island, Lakshadweep Sea during February 2020. The present specimens (one each matured male and female) were collected from the mantle cavity of the Giant Clam, *Tridacna maxima* (Roding, 1798) in the coral lagoon at a depth of 4 m. *Anchistus miersi* is morphologically very similar to *Anchistus demani*, which is easily distinguished by the presence of a strong antennal spine and conspicuous accessory spinules in the third–fifth pereopods and presence of small blue spots all over the body. The molecular analysis confirms that, the morphological identification of the present Indian specimens displays an intraspecific genetic divergence of 0.3–1.2%. Besides, the remarkable taxonomic features and colour patterns, distributional ranges are also attested with the report of the species from the present area.

Keywords: Agatti Island, distributional range, giant clam association, Lakshadweep, morphology.

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Competing interests: The authors declare no competing interests.

Author details: Manu Madhavan is working as PhD, research scholar at the ICAR-National Bureau of Fish Genetic Resources. Purushothaman Paramasivam is a researcher at the ICAR-National Bureau of Fish Genetic Resources. S. Akash is working as PhD, research scholar at the ICAR-National Bureau of Fish Genetic Resources. T.T. Ajith Kumar is working as principal scientist at the ICAR-National Bureau of Fish Genetic Resources. He known for his contribution to marine ornamental aquaculture for conservation and livelihood. Kuldeep Kumar Lal was the former director of the ICAR-National Bureau of Fish Genetic Resources and presently the director of the ICAR - Central Institute Brackishwater Aquaculture, have vast experience in conservation genetics.

Author contributions: MM—animal collection and morphological identification of the specimen. PP—species confirmation by morphological and molecular analysis and preparation of the manuscript. SK—animal collection and morphological identification of the specimen. TTAK—manuscript revision. KKL—conceptualization.

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INTRODUCTION

The shrimp fauna of Indian waters was studied majorly in the zones of western and eastern coasts. The insight knowledge on the caridean shrimps from Lakshadweep waters is still very narrow with the current status. Recently, a few surveys have exposed new distributional coral-associated carideans (Baby et al. 2016; Bharathi et al. 2019; Madhavan et al. 2019; Akash et al. 2020; Prakash & Marimuthu 2020).

In general, some of the palaemonid shrimps are endo-commensal species, enticing and fascinating in their colour patterns. These shrimps inhabit mantle cavities and gastric regions of host organisms such as sponges, bivalves, and tunicates (Kemp 1922; Johnson & Liang 1966; Bruce 1977; De Grave 1999). The genus *Anchistus* Borradaile, 1898 is one such taxa and has been symbolized as a commensal group, usually associated with bivalves (mostly in the subfamily Tridacninae) (Bruce 1972, 2000; Jayachandran 2001). In the symbiotic relationship, the giant clam gain benefits from the shrimps with cleaning service, which helps to maintain the health of its tissues. Simultaneously, the shrimps get benefits from the giant clams like safety from predator, stable environment, as well as access to food. This symbiotic relationship was dynamic and can change over time. For example, a cleaner shrimp may become a parasite, if it begins feeding on the giant clam's tissues rather than just its parasites.

Typically, this genus covers seven species which are distributed in the Indo-Pacific regions at shallow water depths (Jayachandran 2001; De Grave & Fransen 2011). Four species are reported from the Indian waters notably in Andaman and Nicobar Islands; *Anchistus custos* (Forskål, 1775), *A. demani* Kemp, 1922, *A. miersi* (de Man, 1888 [in de Man, 1887–1888]), and *Anchistus pectinis* Kemp, 1925 (Samuel et al. 2016). The morphological features of *Anchistus* are distinguished from other groups of palaemonid shrimps by the presence of their movable spine in the lateral border of uropods, rostrum downwards, laterally compressed and extended to the distal end of eyes. Also, the distolateral spine of antennal scaphocerite is not overreaching the distal margin and has a convex structure in the inner margin of the dactylus of 3rd pereopods.

During the recent survey undertaken at Lakshadweep waters, a pair of *Anchistus* specimens were collected at shallow depth lagoon regions of Agatti Island. These specimens were carefully examined and have been identified as *A. miersi*, which is a new distributional record to the Lakshadweep Sea. The authors also

provided taxonomic notes on morphological and molecular features, habitat, and coloration of this species in a unique manner.

MATERIALS AND METHODS

Sampling

A pair of *A. miersi* specimens were caught out from the mantle cavity of the Giant clam, *T. maxima* (Roding, 1798) from the lagoon area of Agatti island at the depth of 4 m (10.8533N & 72.1872E; Image 1). The specimens were picked up using a hand net during snorkeling in the reef region of the lagoon. The specimens were transferred alive to the Germplasm Resource Centre of the ICAR - National Bureau of Fish Genetic Resources (NBFGR) located at Agatti, Lakshadweep, India for further analysis. After transportation, the colouration of the species was captured with a camera (Canon G1X), followed by maintaining the specimens in live condition. However, mortality was noticed on the second day, it might be happened due to the absence of host organisms. Further, the dead specimens were preserved in 95% of ethanol for detailed morphological examination.

Morphology

The preserved specimens were taken to the Peninsular and Marine Fish Genetic Resources (PMFGR) Centre of the ICAR-NBFGR, Kochi, India for further studies. Careful examination with a stereo zoom microscope (0.5–8X) with Nikon SMZ1270 digital camera has been carried out. A compound microscope, Leica ICC50 was used for observation of dactylus and mouth structures. The illustrative images for morphological characteristics were drawn using the GNU Image Manipulation Program (Version 2.10.12) and edited with Adobe Photoshop CS2. The morphological identification and diagnosis were carried out by following the literature of Holthuis (1952), Bruce (1973), and Jayachandran (2001). The examined material was deposited in the National Fish Museum and Repository of the ICAR-NBFGR, Lucknow, India. The measurement of carapace length (CL) was taken from the posterior orbit angle to the posterior margin of the cephalothorax with Vernier caliper (0.1 mm accuracy). The measure of CL is considered as a standard length for both individuals.

DNA barcoding

The partial sequences of barcoding gene mitochondrial cytochrome c oxidase I (COI) data were generated for this species adopting Akash et al. (2020).

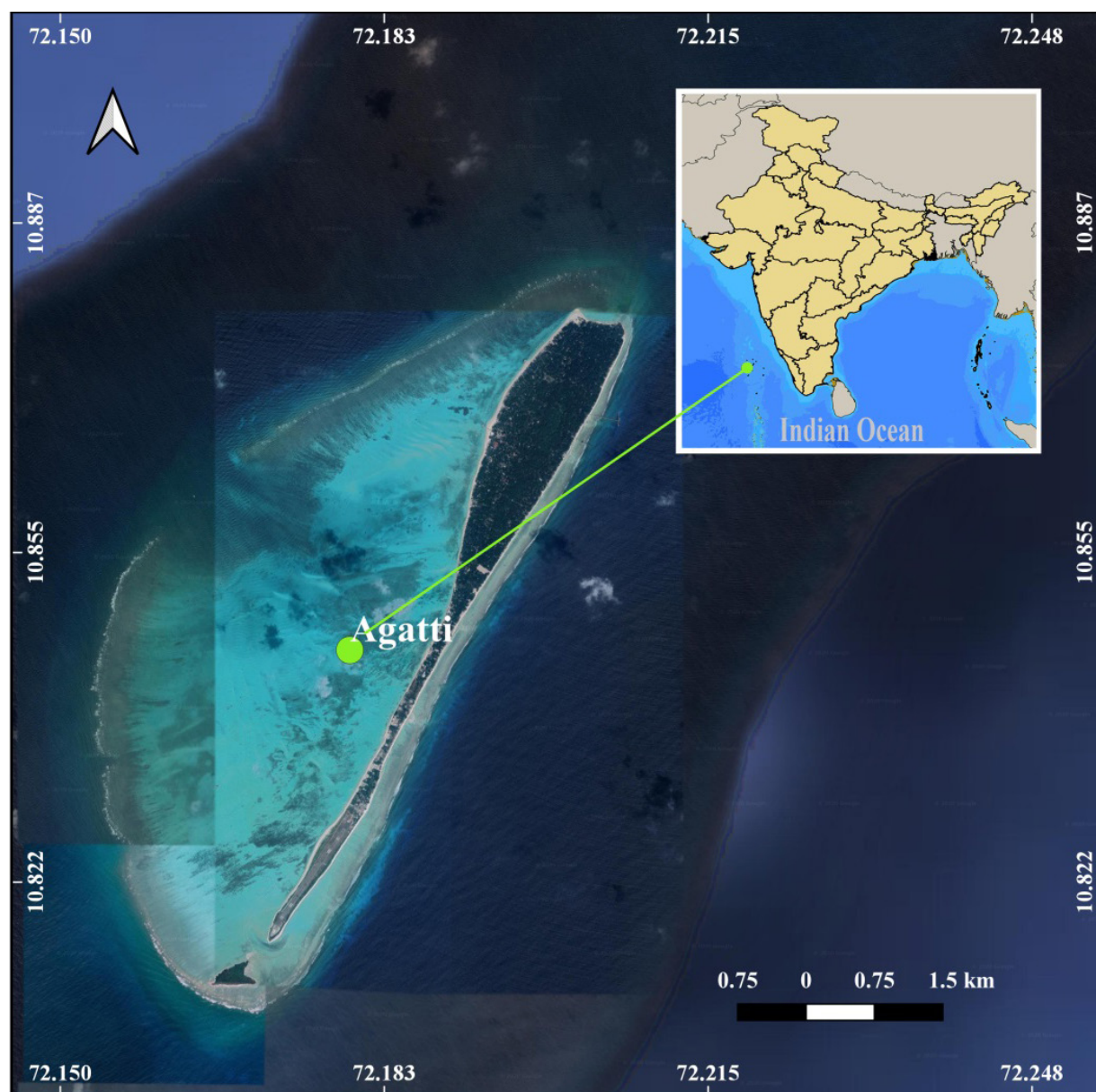


Image 1. Collection site of *Anchistus miersi* (de Man, 1888 [in de Man, 1887–1888]) from a Giant clam, *Tridacna maxima* at Agatti island, Lakshadweep, India.

BioEdit software v. 5.0.9 (Thompson et al. 1994) was used to align and correct the sequence data. The corrected sequences were blasted in Blastn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) of NCBI to find out similarity ranges. 12 COI sequences of *Anchistus* species were retrieved from NCBI (<https://www.ncbi.nlm.nih.gov/>) and were used in this analysis. MEGA X software was used to estimate the pairwise genetic distances and reconstruct the Maximum Likelihood (ML) tree (Kumar et al. 2018) with the implementation of 1,000 replications.

Taxonomic Status

Order Decapoda Latreille, 1802

Infraorder Caridea Dana, 1852

Superfamily Palaemonoidea Rafinesque, 1815

Family Palaemonidae Rafinesque, 1815

Genus *Anchistus* Borradaile, 1898

Species *Anchistus miersi* (de Man, 1888 [in de Man, 1887–1888]) (Images. 2, 3 & 4)

Harpilius miersi De Man, 1888 : 274, Plate 17, figs 6–10 [type locality : Elpninstone Island. Mergui Archipelago. Burma].

A. miersi. – Holthuis. 1952 : 110–111, Fig 45. – Bruce, 1973 : 136, Fig. 1c–e. – Monod, 1976 : 24–26, figs 29–36. De Grave, 1999: 132–133.

Material examined

NBFG/PALAMIE.01, female (CL: 3.0 mm, ID no: DBTLD224) and 1 male (CL 2.5 mm, ID no: DBTLD186), coral reef lagoon at Agatti Island, Lakshadweep, Arabian Sea, Indian Ocean (10.8533N & 72.1872E), 4 m depth, associated with *T. maxima*, temperature 28.2°C, Salinity 35 ppt, February 2020.

Diagnosis

Carapace (Image 2) glabrous and more or less dorsally convex with a strong antennal tooth. Rostrum Fig 1A) short and directed downwards, slightly reaching the distal end of second antennular peduncle; terminal end rounded and bearing 2–3 teeth on the upper border, ventral of the terminal end rounded with few plumose setae. Abdomen tergites dorsally rounded and somewhat compressed. The posteroventral angle of the 4th & 5th somite rounded, 6th somite bearing a strong tooth in postero-ventrally. The nature of the telson smooth and thin, about 1.6 times as long as 6th abdominal somites, and bears two pairs of dorsolateral spines; terminal end rounded and bears with small 4 pairs of posterior-distal spines (Figure 1B). Uropod rounded posteriorly and bearing with a movable tooth in the lateral side, slightly exceeded in the distal end of telson. Basal antennular peduncle (Figure 1C) with anterolateral tooth, second and third segments short. Stylocerite acute distally and exceeding to middle of the first antennular segment; both flagella almost equal, upper antennular flagellum fused with 13 segments and free ramus with 11 segmented, which has few hairy setae in distally. The antennal scale (Figure 1D) with a strong anterolateral spine, and distinctly exceeded distal end with few long plumose setae. Third maxilliped with well-developed exopod; the antepenultimate segment about 1.5 times as long as the penultimate segment with few lateral setae, ultimate segment short and fringed with numerous setae. The first pereopod slender, merus slightly longer than carpus. The ventral side of the carpus with few long setae and fingers with nine groups of setae present dorsally. Second pereopods (Image 2) symmetrical, carpus short, and triangle-shaped with palm stout, dactylus curved hook like structure at anteriorly and proximally with strong tooth and few small teeth; female pereopods somewhat similar in length, dactylus about 0.5 lengths of palm, whereas in male, major dactylus is about 0.5 in the length of the palm and minor dactylus is 0.64 length of the palm.



Image 2. Picture representation of *Anchistus miersi* (de Man, 1888 [in de Man, 1887–1888]) collected from Agatti island, Lakshadweep, India (Ethanol preserved). © Purushothaman Paramasivam.

Pereopods III–VI (Figure 1E–J) similar in size; propodus have few long setae at the distal end, their dactylus short and hook like structure with a microscopic tooth in flexor margin. The third and fourth pereopods stout and similar in structures, merus about 2.3 times as long as carpus; propodus subequal to merus, about 7.3 lengths of dactylus. Fifth pereopods slightly compressed, merus about 1.9 times as long as carpus, propodus about 1.1 times as long as merus, 2.1 times as long as carpus, and 8.5 times of dactylus.

Colouration in life

The body and appendages are commonly translucent with scattered small blue spots (Image 3). Rostrum with few blue dots, antennal scale, and antennular peduncle with blue spots, but flagellum is transparent. The second pereopods are translucent with lined blue dots dorsally. The third–fifth pereopods are transparent without any marks. Eyes are translucent with small dark blue dots in eyestalk, cornea with dark black with translucent. The ovary is greenish.

Habitat and Distribution

The present species was caught from the mantle cavity of the Giant clam, *T. maxima* (Röding, 1798) (Image 4) in the lagoon region of Agatti island (10.8533N & 72.1872E), Lakshadweep at the depth of 4 m. *A. miersi* is widely distributed in Indo-Pacific regions, ranging from the Red Sea, eastern Africa to the Gambier Archipelago through Maldives and Chagos Islands, Seychelles; Zanzibar, Kenya, Tanganyika, Madagascar, Andaman Islands, and the Philippines. In common, this species is associated with the *Tridacna* clam (subfamily Tridacninae). However,

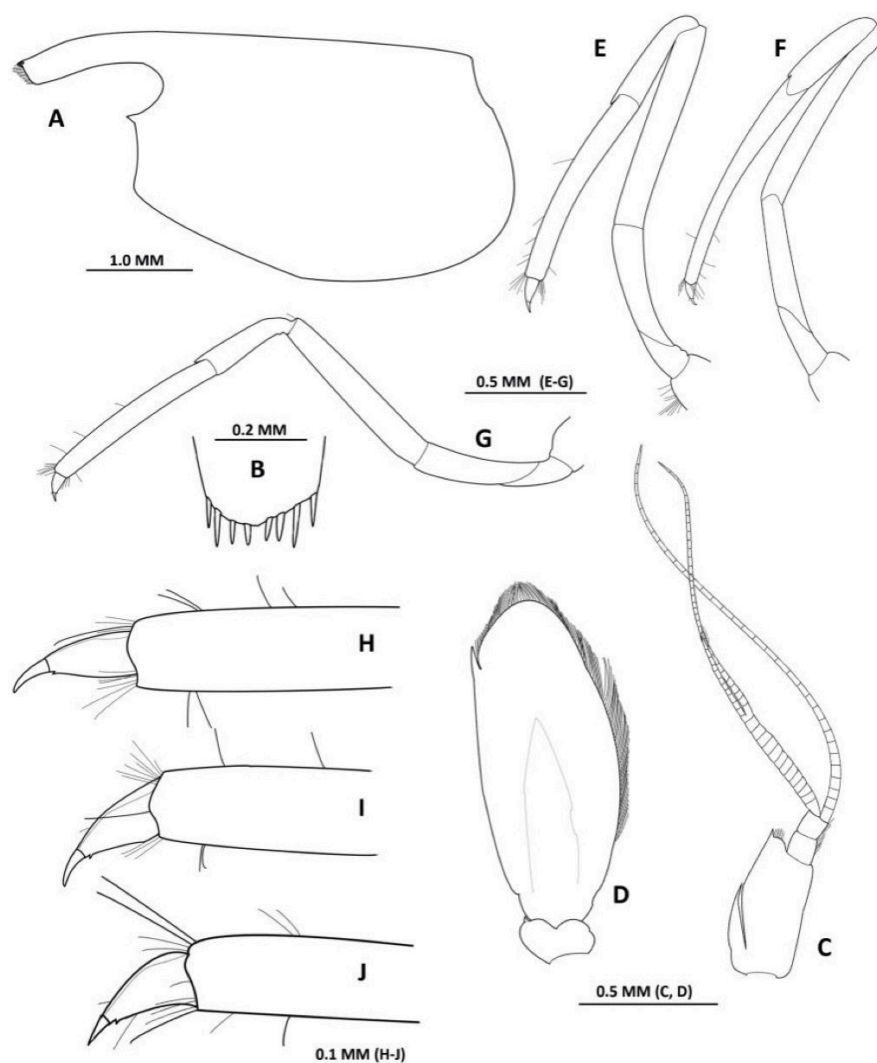


Figure 1. *Anchistus miersi* (de Man, 1888 [in de Man, 1887–1888]) from Agatti Island, Lakshadweep, India: A—Lateral view of carapace | B—Posterior end of the telson (Closer view) | C—Dorsal view of antennular peduncle | D—Dorsal view of antennal scale | E—Lateral view of the third pereopod | F—Lateral view of forth pereopod | G—Lateral view of the fifth pereopod | H–J—Closer view of dactylus of Last three pereopods.

it also occurred within bivalve of the genera *Hippopus*, *Pinna*, *Magnavacula*, and *Meleagrina* (Bruce 1978; Chace & Bruce 1993; De grave 1999; Neo et al. 2014).

Remarks

The present Indian specimens were agreed well with previous descriptions of De Man (1888) and Kemp (1922) with their key characteristics of Jayachandran (2001) with carapace and architecture of rostrum, presence of antennal spines, dactylus dentation of second pereopods, and its colour patterns. The general appearance of the Indian materials is similar with earlier finding, but a female individual is bigger than the male. Also, some remarkable differences were noticed in the present organisms, upper antennular flagellum fused

with 13 segments and free ramus segmented with 11 articles (where de Man mentioned few segments fused in basally), the antepenultimate segment of third maxilliped is broader much than the penultimate segment. Dactylus of ambulatory pereopods with hook-like distal end with a microscopic tooth in flexor margin, fifth pereopod slightly compressed and longer than third and fourth pereopods. However, minute spinules or granules were not able to observe on the anterior border on the dactylus, where Fujino (1975) described the minute granules with the help of scanning electron microscopy.

The present individuals of *A. miersi* had an appealing colour pattern in their overall body with transparent and small dark blue spots, which also agree well with the previous descriptions of Bruce (1976) and Neo et

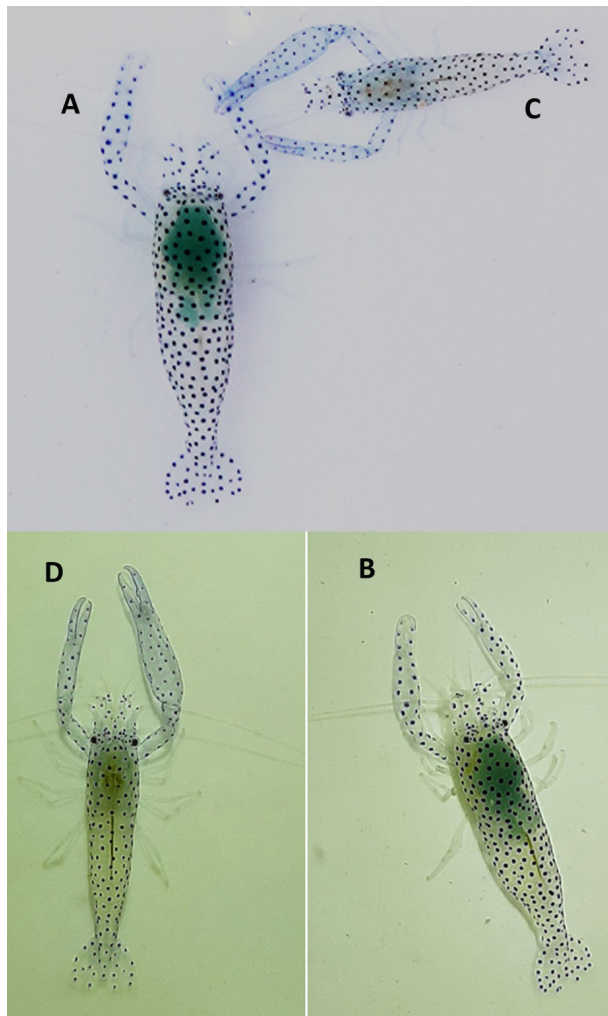


Image 3. The live colouration of *Anchistus miersi* (de Man, 1888 [in de Man, 1887–1888]) from Agatti Island, Lakshadweep, India: A–B—Female | C–D—Male. © Purushothaman Paramasivam.

al. (2014). However, he has noticed two different colour spots (red and blue) over the body and appendages. Commonly, *Anchistus* shrimps live as pairs in the host animals, especially bivalves (Bruce 1975; Fujino 1975). This colour variation is caused due to sexual dimorphism and based on their dwelling habitats (Bruce 1976). De Grave (1999) reported red dots and transparent colour in both the animals obtained from the Hansa Bay associated with *Magnavacula penguin* at the depth of 19 m. Neo et al. (2015) also noticed red dots in the male organism, wherein female blue dots were noticed, which were associated with a fluted giant clam, *Tridacna squamosa* from Singapore waters. In the present study, both the sex had dark blue spots in the body, which were associated with Giant clam, *T. maxima* from Agatti Island, Lakshadweep. In morphological and colour patterns wise, *A. miersi* is closely related to *A. demani*.



Image 4. A Giant Clam *Tridacna maxima* at Agatti island, Lakshadweep, India, where *Anchistus miersi* was collected. © Purushothaman Paramasivam.

However, *A. miersi* differed by the presence of a strong antennal spine (vs absent in *A. demani*), very minute and conspicuous accessory spinules in the third-fifth pereopods (vs inconspicuous and blunt in *A. demani*). The colour patterns also differed between these two species, small blue spots over the body for *A. miersi* and comparatively bigger in size of blue spots for *A. demani* individuals (Kemp 1922; Jayachandran 2001).

DNA Barcoding

The COI DNA barcoding has been commonly applied for revealing cryptic species complex, taxonomic ambiguities, delineating species boundaries of crustaceans (Hebert et al. 2003; Schwentner et al. 2013; Chan et al. 2017). The present study generated two COI sequences for Indian materials of *A. miersi* with greater than 650 bp (Accession No: MW897781 & MW897782). The NCBI has only 12 COI sequences of the *Anchistus* species, which have been retrieved and used for the present analysis where, two sequences are from *A. australis*, four from *A. miersi*, and another four from *A. custoides*. It should also be noted that the sequences of *A. demani* (KP759379 & KC706757) are not used for the present analysis due to the close homogeneity with the sequences of *A. miersi*. It reveals that misidentified sequences are there in NCBI for *A. demani* (KP759379 from Madagascar and KC706757 from French Polynesia), which have >99.5% sequences similarity with the present Indian materials of *A. miersi*. Overall, the present study reveals that the intraspecific genetic divergence for *A. miersi* is 0.3–1.2 % and the highest interspecific divergences were showed between these three species which ranged from 9.7–23.9% (Table 1). On the other hand, a sequence of *A. custoides* (MH287043) showed the greatest intraspecific divergences with the Malaysian materials (Fransen & Reijnen 2012) which has to be restudied with integrative

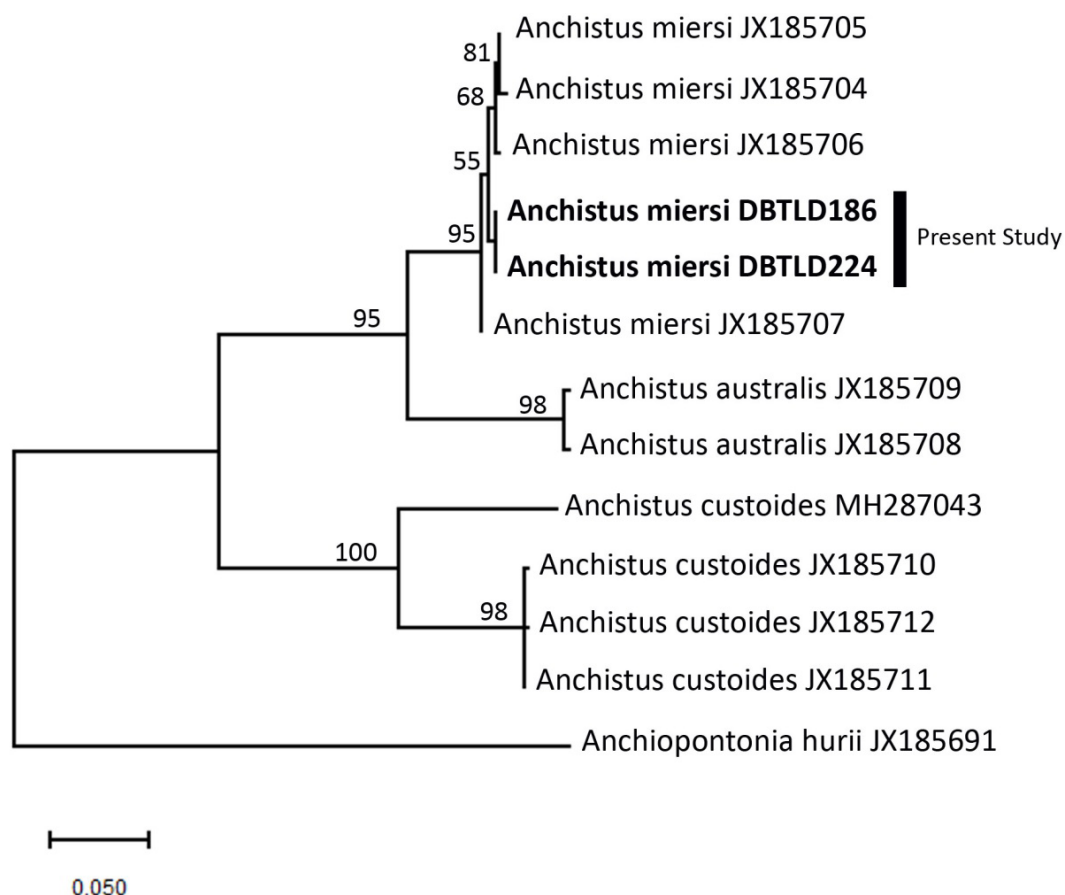


Image 2. Picture representation of *Anchistus miersi* (de Man, 1888 [in de Man, 1887–1888]) collected from Agatti island, Lakshadweep, India (Ethanol preserved).

Table 1. Pairwise genetic distances for *Anchistus* species using COI gene sequence data.

	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	<i>Anchistus miersi</i> JX185707												
2	<i>Anchistus miersi</i> JX185706	0.008											
3	<i>Anchistus miersi</i> JX185705	0.008	0.003										
4	<i>Anchistus miersi</i> JX185704	0.012	0.007	0.003									
5	<i>Anchistus miersi</i> DBTLD224	0.007	0.008	0.008	0.012								
6	<i>Anchistus miersi</i> DBTLD186	0.007	0.008	0.008	0.012	0.000							
7	<i>Anchistus custoides</i> JX185712	0.221	0.227	0.230	0.237	0.221	0.221						
8	<i>Anchistus custoides</i> JX185711	0.217	0.224	0.227	0.233	0.217	0.217	0.002					
9	<i>Anchistus custoides</i> JX185710	0.221	0.227	0.230	0.237	0.221	0.221	0.003	0.002				
10	<i>Anchistus australis</i> JX185709	0.097	0.097	0.101	0.106	0.101	0.101	0.239	0.239	0.242			
11	<i>Anchistus australis</i> JX185708	0.099	0.099	0.104	0.108	0.104	0.104	0.229	0.229	0.232	0.005		
12	<i>Anchistus custoides</i> MH287043	0.201	0.196	0.199	0.204	0.196	0.196	0.125	0.122	0.120	0.227	0.224	

approaches in the future. Additionally, the phylogenetic tree constructed with the Maximum Likelihood analysis for available sequences among the *Anchistus* species is represented in Figure 2. The *A. miersi* sequences from

India formed a clade with the other sequences of *A. miersi* which were retrieved from NCBI and it confirms the morphological identification of the present Indian materials.

Overall, the present study reports new occurrence of small commensal shrimp associated with Giant clam, *T. maxima* (Roding 1798) in the lagoon regions of Agatti Island, Lakshadweep at the depth of 4 m, which distribution extended Andaman Sea–Lakshadweep Sea towards. Additionally, taxonomic, habitat details are represented discussed in detail. The present report is also strengthening the caridean fauna of the Lakshadweep waters.

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ravi@threatenedtaxa.org

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