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Two new distributional records of *Palaemonella* Dana, 1852 shrimps (Decapoda: Caridea: Palaemonidae) from Lakshadweep Islands, India

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ABSTRACT

The present study reports two new distributional records of palaemonid shrimp, *Palaemonella tenuipes* Dana, 1852, and *P. rotumana* (Borradaile, 1898) from the Lakshadweep region, Arabian Sea. The study provides detailed information on, and insight into, diagnostic characters, distinctiveness, and illustrations of both species. The individuals of *P. tenuipes* were collected from a depth of about 0.5 m in shallow barren pools on rocky flats near the shore, while a specimen of *P. rotumana* was collected from a dead coral patch at a depth of 1–2 m. The intraspecific genetic divergences were estimated for both species using COI and 16S sequence data.

Keywords

Arabian Sea, First record, Genetic divergences, *Palaemonella*, Palaemonid shrimp

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INTRODUCTION

In the Lakshadweep Sea, the corals form a reef system that is home to globally significant populations of green and hawksbill turtles, whale sharks, reef sharks, and many marine invertebrates (Ajith Kumar, 2012). Despite this biodiversity, more knowledge is needed on the diversity of marine invertebrates in the region of the Lakshadweep islands. Continuous biodiversity studies have been carried out in the Lakshadweep intertidal regions since 2018, and have successfully yielded many caridean shrimps such as Lysmata amboinensis (de Man, 1888 [in de Man, 1887-1888]), L. hochi Baeza and Anker, 2008, L. ternatensis de Man, 1902, Palaemonella pottsi (Borradaile, 1915), Periclimenella agattii Bharati, Purushothaman, Akash, Jose, Madhavan, Dhinakaran, Saravanane, Ajith Kumar and Kumar Lal, 2019, Pontoniopsis comanthi Borradaile, 1915, Synalpheus carinatus (de Man, 1888 [in de Man, 1887-1888]), S. comatularum (Haswell, 1882), S. stimpsonii (de Man, 1888 [in de Man, 1887-1888]), Thor hainanensis Xu and Li, 2014, and Urocaridella arabianensis Akash, Purushothaman, Madhavan, Ravi, Hisham, Sudhakar, Kumar and Kuldeep, 2020 (see Bharathi et al., 2019; Madhavan et al., 2019; Akash et al., 2020; Jose et al., 2020; Prakash and Marimuthu, 2020).

Shrimps of the genus Palaemonella Dana, 1852 are commonly distributed in the Indo-West Pacific region and occur in wide bathymetric ranges from intertidal to 544 m depth (Bruce, 1970; 2002a, 2002b; 2006; 2008; 2010; Hayashi, 2001; Li and Bruce, 2006; Marin, 2008; Komai and Yamada, 2015; Okuno, 2017). Currently, the genus consists of 23 valid species worldwide (De Grave and Fransen, 2011; Komai and Yamada, 2015; Okuno, 2017), among which three species are reported from Indian waters: Palaemonella rotumana (Borradaile, 1898) and Palaemonella lata Kemp, 1922 from Andaman and Nicobar Islands (Radhakrishnan et al., 2012; Samuel et al., 2016), and Palaemonella pottsi (Borradaile, 1915) from the Lakshadweep islands (Prakash and Marimuthu, 2020). Similar to other members of the family Palaemonidae Rafinesque, 1815, they have a subcylindrical body, a well-developed rostrum with ventral and dorsal teeth, a fourth thoracic sternite with spiniform median

process, and a simple dactylus on the third to fifth pereiopods. However, the morphological characters of this genus are very close to the genus Cuapetes Clark, 1919, and specifically differ by the presence of a mandibular palp and slender dactylus on the third to fifth pereiopods in Palaemonella (Chace and Bruce, 1993). The species of the genus Palaemonella are mostly free-living, while some species are symbionts such as: Palaemonella aliska Marin, 2008 associated with alpheid shrimps; P. pottsi associated with the crinoids Stephanometra tenuipinna (Hartlaub, 1890) (Bruce, 1970), and Phanogenia gracilis (Hartlaub, 1893) (Prakash and Marimuthu, 2020); and P. rotumana (Borradaile, 1898), which is known as a facultative symbiont of scleractinian corals, but also occurs in dead coral boulders (Kemp, 1922; Bruce, 1970; 1975; Marin, 2008).

During the recent survey conducted off Agatti Island, Lakshadweep, some specimens of *Palaemonella* were collected with *Cuapetes* sp. and *Periclimenella agattii* from the intertidal regions of 0.5–2 m depth. Careful examination of the *Palaemonella* specimens revealed the presence of *Palaemonella tenuipes* Dana, 1852, and *P. rotumana*, representing new distributional records for the Lakshadweep waters. A detailed study on morphology and molecular characters were carried out on these two species.

MATERIAL AND METHODS

The specimens were collected from the intertidal regions of Agatti Island, Lakshadweep, India, by handpicking and snorkeling during March 2020. The specimens were examined under a stereo zoom microscope (Nikon SMZ1270) and identified according to Kemp (1922), Bruce (2002a), and Komai and Yamada (2015). The examined specimens are deposited in the National Fish Museum and Repository of ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow, India. The standard measurement used was carapace length (CL: measured from posterior margin of orbital angle to posterior margin of the carapace). The illustrative line images were drawn using the GNU Image Manipulation Program (Version 2.10.12) and all other images were edited with Adobe Photoshop CS2.

Genetic analyses

To find out the genetic divergences and phylogenetic relationships, the total genomic DNA was isolated from 95 % alcohol preserved tissue samples (pleopods and pereiopods) with a DNeasy Blood and Tissue Kit (Qiagen, UK). Partial sequences of the mitochondrial genes cytochrome c oxidase I (COI) and 16S rRNA (16S) were amplified through polymerase chain reaction with the universal primers: COI, Forward (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and Reverse (5'-GGTCAACAAATCATAAAG ATATTGG-3') (Folmer et al., 1994) and 16S, AR (5'-CGCCTGTTTATCAAAAACAT-3' and BR (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al., 1991), respectively. The 25 µl PCR reaction mixture contained 60 ng of total DNA, 0.5 units of Taq DNA polymerase, 0.3 mM of each primer, 2.0 mM of MgCl₂ 200 mM of dNTPs, and 1X reaction buffer. PCR amplifications were conducted with ProFlex[™] 3 x 32–well thermocycler (Applied Biosystems, USA). The annealing temperature for both the gene regions was 50 °C. The PCR, sequencing process and data analysis were carried out following the methods of Akash et al. (2020).

The sequence quality was screened with an ABI Sequence Scanner v1.0. The sequence editing and aligning process were performed with Clustal W multiple alignment program at Bioedit tool v. 5.0.9 (Thompson *et al.*, 1994). The edited sequences were submitted to the public NCBI database (https:// www.ncbi.nlm.nih.gov/). Available COI and 16S sequences of the genus *Palaemonella* were retrieved from GenBank and BOLD system (Tab. 1). The pairwise genetic divergences were performed in MEGA 10.0.5 (Kumar *et al.*, 2018). Consequently, the phylogenetic trees for both genes were constructed using the Maximum Likelihood (ML) method with 1000 bootstraps in MEGA 10.0.5 (Kumar *et al.*, 2018).

ΤΑΧΟΝΟΜΥ

Family Palaemonidae Rafinesque, 1815

Genus Palaemonella Dana, 1852

Palaemonella tenuipes Dana, 1852 (Figs. 1–3)

S. No.	Species Name	Voucher Number	COI	165
1	Palaemonella rotumana	NBFGR:DBTLD65	MW351713	MW349979
2	Palaemonella rotumana	MTQ 33176	KR088755	_
3	Palaemonella rotumana	MTQ W-33176	-	KU064830
4	Palaemonella rotumana	-	-	JX025197
5	Palaemonella rotumana	-	JN107956	_
6	Palaemonella pottsi	SNU PH PI58	-	MK688425
7	Palaemonella pottsi	SNU PH PI56	-	MK688424
8	Palaemonella pottsi	-	-	JX025198
9	Palaemonella pottsi	JOD_0651	KU496491	-
10	Palaemonella pottsi	JOD_0529	KU496490	-
11	Palaemonella pottsi	JOD_0634	KU496489	-
12	Palaemonella pottsi	JOD_2036	KU496488	-
13	Palaemonella pottsi	RMNH:D 53933	JX185714	-
14	Palaemonella pottsi	RMNH:D 53928	JX185713	-
15	Palaemonella tenuipes	NBFGR:DBTLD81A	MW380740	MW350006
16	Palaemonella tenuipes	NBFGR:DBTLD81B	MW380741	MW350007
17	Palaemonella tenuipes	1CC1Flb8f32	JQ180254	-
18	Palaemonella tenuipes	1CC1Flb8f29b	JQ180252	-
19	Palaemonella disalvoi	-	-	KJ019655
20	Cuapetes americanus	ULLZ13670	-	MK971328
21	Cuapetes americanus	ULLZ13671	MN184203	-

 Toble 1. GenBank accession numbers and voucher details of present study materials.

Palaemonella tenuipes Dana, 1852: 25. — Bruce, 2002a: 288. — Komai and Yamada, 2015: 360.

Palaemonella tridentata Borradaile, 1898: 1007, pl. 64, figs. 8a–c Palaemonella elegans Borradaile, 1915: 210.

Material examined. NBFGR/PALPTEN.01 (1 ovigerous female, CL: 2.5 mm, ID no: NBF-GR:DBTLD20) Arabian Sea, off Agatti Islands, Lakshadweep, India (10°83'4"N 72°18' 25"E); 5 females (CL: 2.5–2.7 mm, ID no: NBFGR:DBTLD81, 81A, NBFGR:DBTLD206, 206A, 206B), Arabian Sea, off Agatti Islands, Lakshadweep, India (10°83'5"N 72°18'33"E), 0.5–1 m depth, temperature 28.1 °C, salinity 35 ppt, March 2020.

Diagnosis. Body glabrous, small; rostrum usually straight, slightly extended beyond distal end of third antennular peduncle, distal end directed slightly upward, dorsally armed with 7 strong teeth including 1 epigastric tooth; epigastric tooth situated on carapace behind orbital angle; rostrum ventrally armed with 2 teeth, situated at level of fourth and fifth dorsal teeth. Carapace smooth, without supraorbital spine; hepatic spine appears directed slightly upward; inferior orbital angle extended like triangle; antennal spine well developed, reaching near to the middle of eyestalk; pterygostomial region rounded. Antennal scaphocerite broad, with distolateral tooth overreaching level of distal margin of lamella (Fig. 2A). Abdominal tergites smooth, third one extending over fourth tergite dorsoposteriorly; posteroventral angle of fourth and fifth pleura extended like triangular lobe, sixth pleura with posteroventral, posterolateral teeth. Telson about 1.5 times as long as sixth abdominal segment length, bearing 2 pairs of dorsolateral spines, and 3 pairs of posterior telson spines (Fig. 2H), lateral spine similar to dorsolateral spines, intermediate spine slender, long, submedian pair extended to middle of intermediate one. Mandible with small 2-segmented palp (Fig. 2B).



Figure 1. Preserved specimen of *Palaemonella tenuipes* Dana, 1852 (ID no: NBFGR: DBTLD206, female, CL 2.5 mm) from Lakshadweep Islands, India.

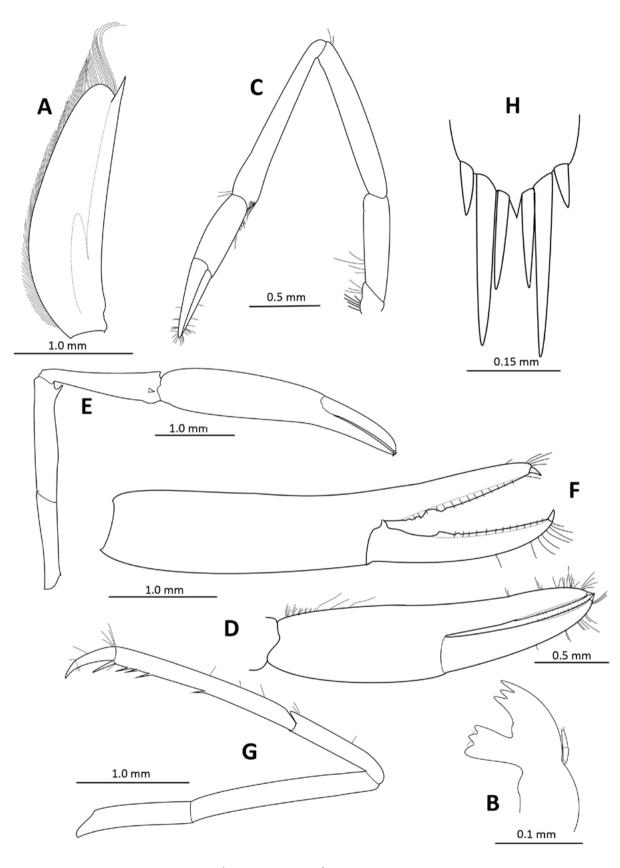


Figure 2. *Palaemonella tenuipes* Dana, 1852 (female, CL 2.5 mm) from Lakshadweep Islands, India. **A**, dorsal view of antennal scaphocerite; **B**, lateral view of the mandible, closer; **C**, lateral view of the first pereiopod; **D**, dorsal view of first pereiopod chela, closer; **E**, lateral view of the second pereiopod; **F**, dorsal view of second pereiopod chela, closer; **G**, lateral view of the third pereiopod; **H**, dorsal view of the posterior part of telson.

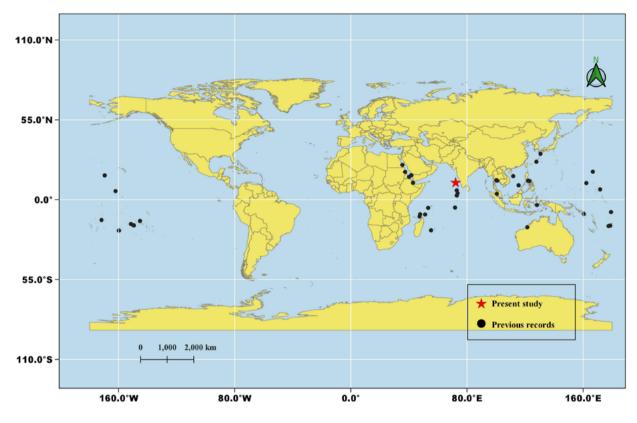


Figure 3. Biogeographical distribution of Palaemonella tenuipes Dana, 1852 worldwide.

Third maxilliped reaching almost to distal end of third antennular peduncle; exopod slightly thick, reaching distal end of antepenultimate segment; penultimate segment about 0.8 times as long as antepenultimate, 1.3 times longer than ultimate segment. First pereiopod thin, overreaching distal end of antennal scaphocerite by length of carpus and chela; merus equal to carpus; carpus about 2.0 times as long as palm, 1.3 times as long as chela (Fig. 2C), few long sensory setae present distoventrally; fingers about 0.95 times as long as palm, inner margin smooth, distally armed with tooth (Fig. 2D). Second pereiopod slightly asymmetrical, extending beyond distal end of scaphocerite by halflength of carpus; merus bearing strong distoventral tooth, about 1.06 times as long as carpus; carpus about 3.5 times as long as distal width, 0.7 times palm length, with sub-terminal spine distally; palm about 3.8 times as long as maximum width, 1.75 times longer than fingers; fingers with two small teeth in proximal region (Figs. 2E, F). Pereiopods III-V slender and extending beyond the distal end of scaphocerite; merus about 1.7-1.8 times as long as carpus; carpus about 0.5 times propodal length; propodus long, with 3-4 spines, and long distal pair

ventrally, about 3.6–4.0 times as long as dactylus; dactylus simple, curved slightly, with few long setae present dorsally and ventrally, about 4.5–5.0 times proximal width (Fig. 2G).

Ecological information. The specimens were found in shallow barren pools on rocky flats near the shore of Agatti Island, Lakshadweep, at the depth of 0.5–1 m.

Coloration in life. Carapace, abdomen, and appendages are transparent. All of the pereiopods and pleopods are transparent. The second pereiopod fingers have a muddy brownish band; each joint with a muddy-brownish band. Eyes are translucent with a dark brownish cornea with a small dark spot dorsally. Matured eggs are muddy greenish.

Geographical distribution. Palaemonella tenuipes widely occurs in shallow water depths and is extensively distributed throughout the Indo-West Pacific region (Fig. 3). Scattered records extend from the Red Sea to the Line Islands, through Madagascar, Seychelle Islands, Maldive Islands, Chagos Islands, Malaya to the Ryukyu Islands, Marshall Islands, Fijian Islands, Society Islands, Wake Island, and Johnson Atoll (Bruce, 2002b). However, this species has not previously been reported from the Lakshadweep region, and Indian waters. Agatti Island is a new record among these regions.

Remarks. The six specimens examined were collected from 0.5-1 m depth. One specimen lost both the second pereiopods, but other specimens were in good condition with mature eggs. Accordingly, we could positively characterize it as P. tenuipes by the presence of a moderately slender dactylus on ambulatory pereiopods about 4.4-5.6 times as long as basal width, the second pereiopod of the merus with a strong distoventral spine, carpus with a subterminal distal spine, and absence of a supraorbital spine. The present Indian materials agreed well with the illustrated keys of Bruce (2002a), and Komai and Yamada (2015). The present report of P. tenuipes from India represents the shallowest depth recorded from the Indian Ocean and it is the first report from Lakshadweep, Indian waters.

Palaemonella tenuipes is morphologically very close to P. rotumana and P. pottsi, which were previously collected from the Lakshadweep Islands. Palaemonella tenuipes differs from P. rotumana (Indian material) by the length of the carpus (more than 6.7 times the distal width, and 2.0 times as long as palm) compared to P. rotumana (carpus is 6.1 times as long as distal width, and 2.2 times palm length). Palaemonella tenuipes is distinguished from P. pottsi by the presence of the transparent body and a muddy brownish band on the fingers of the second pereiopods, the presence of a carpal sub-terminal spine, and scaphocerite length 3.8 times as long as maximum width; P. pottsi has a transparent body with a dark black band on the fingers of second pereiopods, and the scaphocerite is 3.6 times longer than the maximum width.

Palaemonella rotumana (Borradaile, 1898) (Figs. 4, 5)

Periclimenes rotumanus Borradaile, 1898: 383.
Palaemonella vestigialis Kemp, 1922: 123–126, figs. 1, 2, pl. 3.
Palaemonella rotumana — Bruce, 1970: 276–279, pl.

1e-f. — Ďuriš, 2017: 7.

Material examined. NBFGR/PALPROT.01 (1 male, CL: 2.4 mm, ID no: NBFGR:DBTLD65), 1 male (CL: 2.5 mm, ID no: NBFGR:DBTLD65A), Arabian Sea, off Agatti Islands, Lakshadweep, India, (10°49'50"N 72°10'10"E), 1–2 m depth, temperature 28.1 °C, salinity 35 ppt, March 2020.

Diagnosis. Rostrum straight, reaching distal end of third antennular peduncle, distal end pointing slightly upward, dorsally armed with 6 teeth, and 2 teeth ventrally (Fig. 4A). Carapace smooth, glabrous, without supraorbital spine; an epigastric tooth found far behind orbital margin; inferior orbital angle well formed; antennal spine well developed, extended far from distal end of inferior orbital angle (Fig. 4B); hepatic spine small, sharp; pterygostomial region rounded. Eyes well developed, with thick eyestalk, shorter than cornea. Abdominal tergites smooth, rounded dorsally. Mandible with 2-segmented palp. Third maxilliped extending beyond distal end of the rostrum. First pereiopod thin, overreaching distal end of antennal scaphocerite by length of carpus (Fig. 4C). Fifth pereiopods slender, with dactylus slightly curved inferiorly. Telson bearing 2 welldeveloped pairs of movable dorsolateral spines; 3 pairs of posterodistal spines, lateral one similar to dorsolateral spine, intermediate spine slender, about 0.3 times as long as telson length, sub-median pair shorter, barely extended past middle of intermediate spine (Fig. 4D).

Ecological information. The present specimens were collected from dead coral and rocky areas, southwest side of Agatti Island, Lakshadweep, India, at depths of 1–2 m.

Geographical distribution. Palaemonella rotumana is widely distributed throughout the Indo-West Pacific region (Fig. 5): from the Red Sea to Mozambique, eastwards to Hawaiian Islands with wider bathymetric ranges from 10–126 m (Bruce, 1974; 1979; 2002a; 2002b; Ďuriš, 2017). The type locality of this palaemonid shrimp is the coast of Rotuma Island, in the Fijian Islands of the central Pacific region (Bruce, 1970). Presently, the distribution of this species is noted in Agatti Island, Lakshadweep, Arabian Sea, in Indian waters at a depth of 1–2 m.

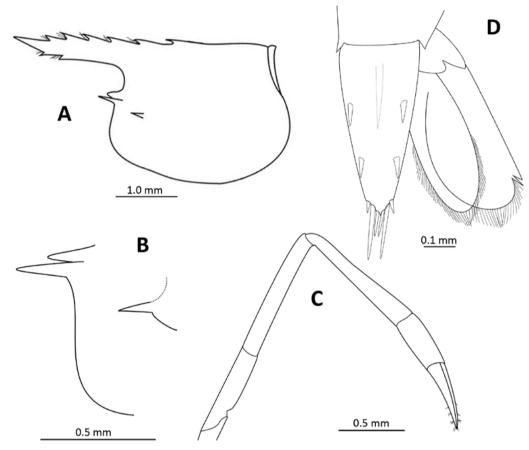


Figure 4. *Palaemonella rotumana* (Borradaile, 1898) (male, CL 2.5 mm) from the Lakshadweep Islands. A, lateral view of carapace; B, closer view of antennal spine; C, lateral view of the first pereiopod; D, dorsal view of telson.

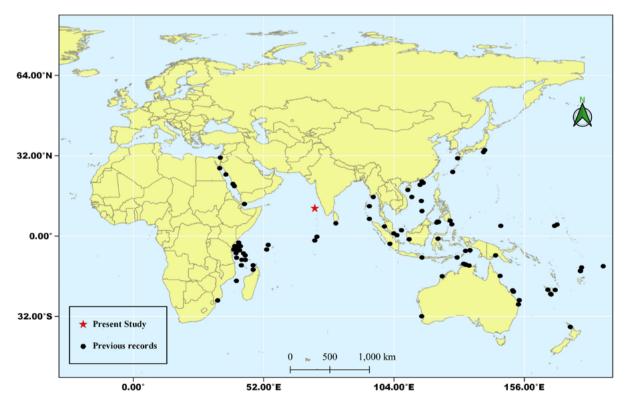


Figure 5. Biogeographical distribution of Palaemonella rotumana (Borradaile, 1898) worldwide.

Remarks. The present material closely agrees with the morphological keys of Bruce (2002a), and Komai and Yamada (2015). However, the second to fourth pereiopods are missing from the present Indian material with only the fifth pereiopod present. Generally, the preserved specimen of P. rotumana closely resembles P. pottsi in the morphology of the carapace, rostrum orientation, abdominal, and telson structures. Recently, Prakash and Marimuthu (2020) have reported P. pottsi from Amini Island of Lakshadweep at a depth of 10 m, associated with crinoids (Phanogenia gracilis). However, both species can be clearly distinguished by the configuration of the dactylus, and propodus of the third to fifth pereiopods (Bruce, 1970; 2002a; De Grave, 2000). In the present material of P. rotumana, the ventral margin of the dactylus of the fifth pereiopods are smoothly concave, and the distal propodal spines are slender, and overreaching the middle of the dactylus length. In P. pottsi, the ventral margin of the dactylus of the third to fifth pereiopods are sinuous and the distal propodal spines are small (Bruce, 2002a; Komai and Yamada, 2015). Palaemonella rotumana are commonly reported from shallow waters to 126 m depth, but the present Indian material was observed at 1-2 m depth in Agatti Island, which is the shallowest recorded depth for this species. Similarly, Li *et al.* (2004) had noted a depth record of 1–2 m at Lingshui County, China.

Genetic analyses

Partial sequences of mitochondrial genes COI and 16S sequences were generated for two individuals of P. tenuipes (COI: MW380740, MW380741, and 16S: MW350006, MW350007) and one individual of P. rotumana (COI: MW351713 and 16S: MW349979), which have subsequently been submitted to GenBank. The pairwise genetic divergences were estimated using the Kimura 2-parameter model with a gamma distribution for both genes against available sequences from Genbank (Tabs. 2, 3). The intraspecific genetic divergence of P. rotumana is 0.08 - 2.7 % for COI and 0.07 % (KU064830; Horká et al., 2016) for 16S data, except for sequences of JX025197, which showed greater genetic divergence (> 5 %) with present Indian material (Kou et al., 2013). It is recommended that these specimens be reexamined in the future (see Tab. 2). For *P. tenuipes*, the intraspecific genetic divergence ranged from 0.0 – 0.08 %. However, the COI sequences MH339412, MH339413 from Saudi Arabia, and JQ180253 from French Polynesia are not incorporated in the present study, as these sequences show greater genetic variation compared to the total divergence.

Table 2. Pairwise genetic analysis conducted using available COI gene sequences in GenBank with the present Indian materials of the genera *Palaemonella* and *Cuapetes*.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	MW351713 P. rotumana*														
2	KR088755 P. rotumana	0.025													
3	JN107956 P. rotumana	0.027	0.008												
4	KU496491 P. pottsi	0.200	0.179	0.179											
5	KU496490 P. pottsi	0.200	0.179	0.179	0.000										
6	KU496489 P. pottsi	0.200	0.179	0.179	0.000	0.000									
7	KU496488 P. pottsi	0.197	0.177	0.176	0.002	0.002	0.002								
8	JX185714 P. pottsi	0.205	0.185	0.185	0.015	0.015	0.015	0.017							
9	JX185713 P. pottsi	0.205	0.185	0.185	0.015	0.015	0.015	0.017	0.000						
10	MW380740 P. tenuipes*	0.202	0.193	0.190	0.202	0.202	0.202	0.205	0.194	0.194					
11	MW380741 P. tenuipes*	0.202	0.193	0.190	0.202	0.202	0.202	0.205	0.194	0.194	0.000				
12	JQ180254 P. tenuipes	0.196	0.187	0.185	0.196	0.196	0.196	0.199	0.189	0.189	0.008	0.008			
13	JQ180252 P. tenuipes	0.196	0.187	0.185	0.196	0.196	0.196	0.199	0.189	0.189	0.008	0.008	0.000		
14	MN184203 C. americanus	0.256	0.239	0.240	0.250	0.250	0.250	0.246	0.261	0.261	0.237	0.237	0.233	0.233	

*Present study

	8	1									
	Species	1	2	3	4	5	6	7	8	9	10
1	MW349979 P. rotumana *										
2	KU064830 P. rotumana	0.007									
3	JX025197 P. rotumana	0.055	0.052								
4	MK688425 P. pottsi	0.114	0.111	0.108							
5	MK688424 P. pottsi	0.114	0.111	0.108	0.000						
6	JX025198 P. pottsi	0.114	0.111	0.108	0.000	0.00					
7	MW350006 P. tenuipes*	0.123	0.120	0.116	0.127	0.127	0.127				
8	MW350007 P. tenuipes*	0.123	0.120	0.116	0.127	0127	0.127	0.00			
9	KJ019655 P. disalvoi	0.111	0.108	0.105	0.127	0.127	0.127	0.116	0.116		
10	MK971328 C. americanus	0.177	0.177	0.192	0.174	0.174	0.174	0.156	0.156	0.187	
-											

Table 3. Pairwise genetic analysis conducted using available 16S gene sequences in GenBank with the present Indian materials of the genera *Palaemonella* and *Cuapetes*.

*Present study

This might indicate a possible case of misidentification. Further, the phylogenetic tree constructed from Maximum Likelihood (ML) analysis with the available data of *P. rotumana*, *P. tenuipes*, and *P. pottsi* for both gene sequences (Fig. 6) clearly demonstrates that *P. rotumana* forms a monophyletic clade, which is more closely related to *P. pottsi* than it is to *P. tenuipes*. The present molecular findings confirm the morphological identification of these species (Bruce 2002a; Komai and Yamada, 2015). The present reports also strengthen the knowledge of the caridean fauna of Lakshadweep waters.

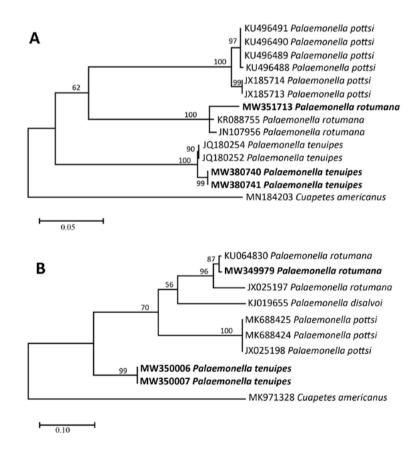


Figure 6. Phylogenetic tree constructed by the Maximum likelihood (ML) analysis Hasegawa-Kishino-Yano model using the available data of COI (A) and 16S (B) genes.

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