

## Morphological discrimination of female specimens of *Pseudodiaptomus annandalei* from Cochin estuary and Corbyn's Cove Creek, India

\*K.S. Santu & S. Bijoy Nandan

Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin 682 016, Kerala, India

\*[E-mail: santucusat@gmail.com]

Received 08 August 2017; revised 08 January 2018

*Pseudodiaptomus annandalei* is a brackish water calanoid copepod first reported and described by Sewell in 1919 from Chilka lake. Preliminary information on morphological differences observed in the examination of female specimens of *P. annandalei* collected from two geographically separated locations namely, Cochin estuary in Kerala and Corbyn's Cove Creek, in Andaman & Nicobar (A&N) Islands are presented in this paper. The specimens collected from both localities showed variations in length of antennules, total body length, spinulation on Pdg segments, ornamentation on posterior corner of metasome, proportional lengths of Pr and Ur, length width ratio of P5, urosome spinulation, and size and shape of caudal setae. These intra-specific differences are described to expand the known morphological range of this species and to provide the first comparative analysis of *P. annandalei* from two locations, Cochin estuary and A&N islands in India.

**[Keywords:** *Pseudodiaptomus annandalei*, copepods, Cochin estuary, Corbyn's Cove Creek, Andaman and Nicobar islands]

### Introduction

*Pseudodiaptomus annandalei* Sewell, 1919 is a subtropical coastal calanoid copepod commonly distributed in Indian Ocean, Gulf of Thailand and China seas. The genus *Pseudodiaptomus* are mainly epi-benthic, show typical diel vertical migration<sup>1-4</sup> and commonly found in highly eutrophic polluted brackish water ecosystems with negligible chlorophyll level<sup>5</sup>. *P. annandalei* was first reported and described by Sewell in 1919 from Chilka lake India and numerous studies are available<sup>6-21</sup>. Previously, *P. annandalei* was mistaken as a separate species, *P. Nostradamus*<sup>22</sup>, *P. dubius*<sup>23</sup>, *P. speciosus* and *Schmackeria speciosa*<sup>24</sup>, however now they are considered as synonymized names for the same because they showed no morphological differences between them<sup>25</sup>. Since, Sewell's<sup>6,7</sup> drawings of *P. annandalei* were less accurate, Reddy & Radhakrishna re described this species with some additional characters<sup>14</sup>. The specimens examined from Cochin estuary and Andaman & Nicobar (A&N) Islands showed some interesting morphological variations.

### Materials and Methods

#### Collection and preservation of the samples

Cochin estuary (9° 40' 10" 12'N- 76° 10' 76"30'E) is a complex shallow estuarine network (250 km<sup>2</sup>) and

forms a major part of the Vembanad backwater system, a Ramsar site on the south-west coast of India, running parallel to the coastline of Kerala. It opens into Arabian Sea through two permanent channel; northern boundary at Azheekode and southern boundary at Thanneermukkam bund (Fig. 1A). The Andaman and Nicobar Islands is an archipelago in the Bay of Bengal, consisting of 306 named islands and 206 rocks and rocky outcrops with outstanding natural beauty and ecological diversity. Mesozooplankton samples were collected using a plankton net with a mesh size of 200 µm from Cochin estuary, Kerala, during the monsoon season (June 2015) and Corbyn's Cove Creek (11°38' N and 92°44' E), A&N Islands, during the late monsoon season (August 2016) (Fig. 1B). A calibrated flow meter (General Oceanics model number-2030®, 2012) was attached to the net and was towed horizontally just below the surface at a fixed speed of approximately 1 knot for 10-15 minutes. The samples were then fixed in 4 % buffered formalin for morphological identification.

#### Morphological examination

All *Pseudodiaptomids* were sorted from mesozooplankton samples; other taxa were counted and identified to species<sup>25-28</sup>. *Pseudodiaptomus*

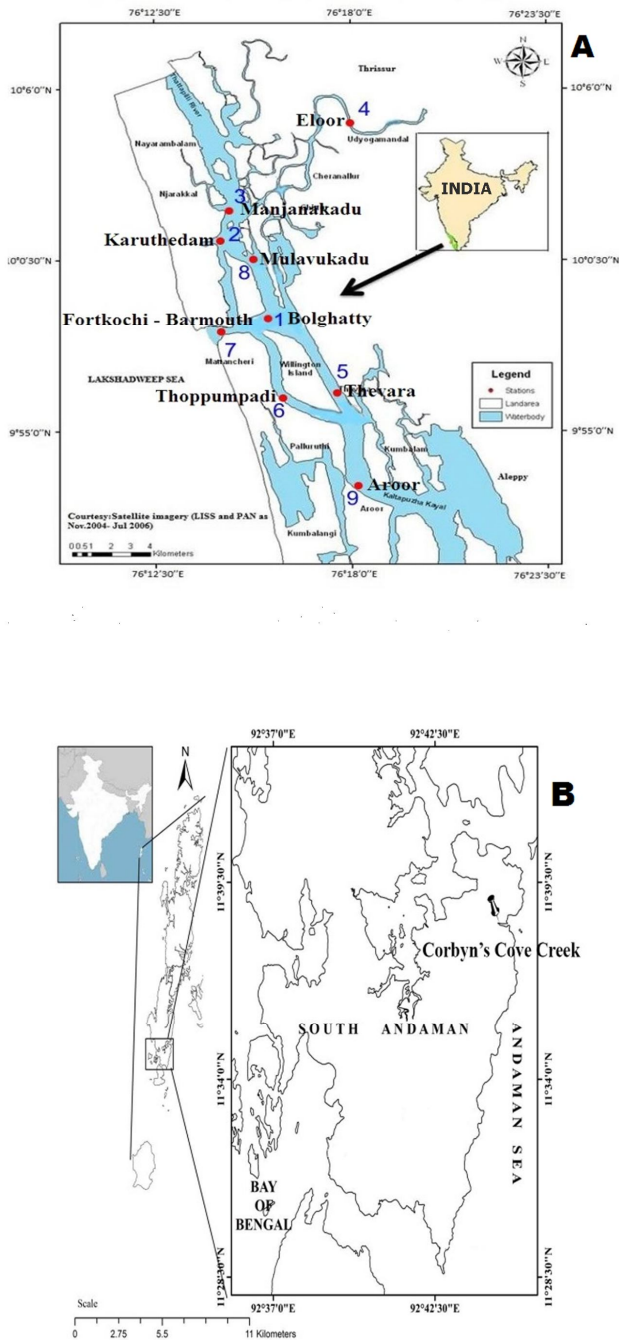


Fig.1 — The study area and sampling stations a) Cochin estuary; b) Corbyn's Cove Creek, Andaman and Nicobar Islands

*annandalei* were sorted out from the original samples and dissected using stereoscopic dissecting microscope with fine needles on a cavity slide. Oral parts and swimming legs were dissected from glycerin-mounted specimens. All the figures have been prepared using a drawing tube attached to an Olympus CX21i bright-field compound microscope.

Measurements were done by using CAT CAM CC300, microscopic camera. The specimens were identified to species level using standard keys<sup>13,11, 29,32</sup>. The specimens were incorporated into the copepod collection of the Department of Marine Biology Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology (catalog entry numbers DMMB/PAANI,11/16 to DMMB/PAANI,15/16 and DMMB/PACE/06/16 to DMMB/PACE /10/16). Total body length and ratio of prosome to urosome (excluding caudal rami setae) were recorded the sum of the mid-dorsal lengths of individual somites measured in lateral view for Cochin estuary specimens and dorsal view for A&N Islands specimens. The descriptive terminology for body and appendages used throughout the paper are: DMMB: Department of Marine Biology Microbiology and Biochemistry; PAANI: *P. annandalei* Andaman and Nicobar; PACE: *P. annandalei* Cochin estuary; prosome: Pdg1-5; pedigers 1-5; Ur1-4: urosomites 1-4; A1: first antenna; P1-5: swimming legs 1-5; CR: caudal rami; B1-B2: basipods 1-2; Re: exopod; Ri: endopod; and Se1-5: setae 1-5 of caudal ramus. Measurements to the nearest micrometer ( $\mu\text{m}$ ) or millimeter (mm) were recorded using a CatCam CC300 (Catalyst Biotech, Panvel, Maharashtra, India) microscope camera.

## Results

Subclass: Copepoda<sup>33</sup>

Order: Calanoida<sup>34</sup>

Family: Pseudodiaptomidae G.O<sup>35</sup>

Genus: *Pseudodiaptomus*<sup>36</sup>

Species: *annandalei*<sup>6</sup>

*Pseudodiaptomus annandalei*<sup>6,13,15,16</sup>

## Description

### Materials Examined

*Pseudodiaptomus annandalei* female specimens:

India, Kerala, Cochin estuary (9° 40' and 10° 12' N and 76° 10' and 76° 30' E), collected on March 2013 (DMMB/PACE/06/16 to DMMB/PACE /10/16). Salinity: 25 ppt temperature: 30 °C.

Total length (Measured in lateral aspects) (n=50)

(Fig. 2.): 1.2 mm; prosome length: 0.78 mm; prosome width: 0.26 mm; Urosome length: 0.31 mm; Urosome width: 0.22 mm. Proportional length of prosome and urosome is 67:33. Prosome is 2.9 times longer as broad. Head and Pdg1 fused as also Pdg4 and Pdg5. Anterior cephalosome in lateral view

sharply rounded; rostrum consists of two filaments processes. The posterior thoracic margin rounded and ornamented with 6-8 dorsal spines; 2<sup>nd</sup> and 3<sup>rd</sup> thoracic segment bears row of small spines dorsally where last thoracic segment bears a double row of small spines laterally.

Urosome (Fig. 3): 4 segments and caudal rami. Ur1 is almost symmetrical devoid of spines on dorso-posterior and large recurved spines on each side. Posterior margin of Ur2-3 armed with row dorsally. CR symmetrical with hirsute inner margins and bears five setae which are short and stout of which the 3<sup>rd</sup> one is much dilated. Inner margin of all the setae are fringed with bristle like hairs. The proportional lengths of urosome and caudal rami are 38:14:16:11:20=100. The length-width ratio of caudal setae is represented in Table 1. Mature females usually bear two egg sacs, each containing 4-6 ova.

Antennule: Folded back reaches to the posterior end of the genital segment. It consistent of 22 segments having the proportional lengths:

5: 4:20: 30:29: 17: 35: 23: 26: 30: 47: 55: 59: 66: 66: 66: 55: 46: 55: 54: 40: 66  
79: 71: 75: 70: 66: 62: 61: 59: 53: 52: 47: 45: 42: 36: 29: 29: 24: 20: 19: 22: 22: 18

All the antennal setae are spine like and devoid of plumose hairs. Of the three setae abided by segment 3, one is distinctly larger than the other two. Segments 6, 7 and 9 bear a minute spine each. Characteristics of antennae, mandible, maxillule, maxilla, maxilliped, P1-P4 are common to the genera and shall not be repeated in the descriptions<sup>38</sup>. Armature formula of swimming legs of both specimens is given in Table 2.

Leg 5 (Fig. 4.): Each consists of a three-jointed ramus. Basis provided with setae on the posterior surface. Exopodite 1 bears a row of spines on its lateral margin. Exopodite 2 is armed at its distolateral with a single marginal serrated spine ornamented with spinules and inner margin provided with one small spine projected outward; there is no trace of lamelliform process as seen in *P. malayalus*, *P. binghami* and *P. tollingeriae*. Exopodite 3 bears a small marginal spine without serration and three end spines, of which the outer is by far the longest and stoutest and is serrated on both margins.

***Pseudodiaptomus annandalei* female:** India, Corbyn's Cove Creek (CC) (11°38' N and 92°44' E) A&N Islands (6° 45' N to 13° 41' N and 92° 12' E and 93° 57' E), collected on August 2016, DMMB/PAANI,11/16 to DMMB/PAANI,15/16. Salinity: 15 ppt; temperature: 25 °C.

Total length (measured from dorsal aspects n=25) (Fig. 5): 1.5 mm; Prosome length: 0.85 mm; prosome width: 0.33 mm; Urosome length: 0.42 mm; Urosome width: 0.27 mm. Proportional length of prosome and urosome is 63:37. Prosome is 2.5 times longer as broad (4.2:1.65). Head and Pdg1 fused as also Pdg4 and Pdg5. Anterior cephalosome forms sharply rounded prominence; pair of rostrum present. The posterior thoracic margin rounded and ornamented with 6-8 coarse curved teeth; 2<sup>nd</sup> and 3<sup>rd</sup> thoracic segments bear double row of small spines dorsally,

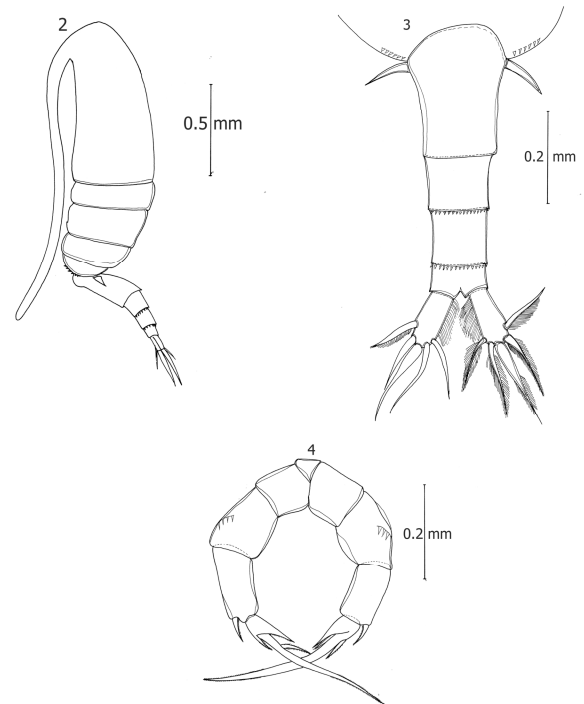


Fig. 2 — *Pseudodiaptomus P. annandalei* habitus (lateral view); Fig.3 — urosome (dorsal view); Fig.4 — P5, dorsal view. Scale bars in mm.

Table 1 — Length-width ratio of caudal setae of *P. annandalei* from Cochin estuary

	Se1	Se2	Se3	Se4	Se5
Length	13	18	30	20	19
Width	17	11	42	15	15

Table 2 — Armature formula of P1-P4 of *P. annandalei* from Cochin estuary and A&N Islands

	Coxa	Basis	Exopodal segment	Endopodal segment
Leg1	0-1	0-0	I-1;0-1;II,I,3	0-1;0-1;1,2,3
Leg2	0-1	0-0	I-1;I-1;II,I,5	0-1;0-2;2,2,4
Leg3	0-1	0-0	I-1;I-1;II,I,5	0-1;0-2;2,2,4
Leg4	0-1	1-0	I-1; I-1; II,1,5	0-1; 0-2; 2,2,3

\*Roman numerals indicating spines, Arabic numerals setae.

whereas last thoracic segment bears a double row of small spines laterally.

Urosome (Fig. 6): Consists of four segments and caudal rami. Ur1 is almost symmetrical; longer than the rest and anteriorly provided with a large recurved spine on either side that project outwardly. Ur1 segment not protruded as seen in *P. binghami malayalus* and above the genital opening, circularly arranged dense patch of needle like spinules across the ventral aspects. The right posterior margin of Ur1 ornamented with double layer of dense slender spinules, which becomes minute in mid dorsal and scattered left side just below the genital opening. Ur2 armed with a row of coarse spines (12 to 14) on the posterior margin dorsally, discontinuous laterally; Ur3 furnished with a complete circle of large spines (about 12-14 spines) around the posterior margin. Ur4 is devoid of spines. CR is symmetrical with five setae and a small sensory setae between the 4<sup>th</sup> and 5<sup>th</sup> caudal setae (length=54.12µm; width=3.31 µm). The 3<sup>rd</sup> setae is normal and much longer and firmer than others and not in spear shaped or dilated as seen in species reported from Cochin estuary. All the setae and inner margin of CR are fringed with bristle like hairs; in addition, the posterior margin of CR forms a wedge like prolongation between 2<sup>nd</sup> and 3<sup>rd</sup> caudal

setae. The proportional lengths of urosome and caudal rami are 45:17:14:8:16=100. The length-width ratio of caudal setae is presented in Table 3. Female specimens observed with pair of egg sacs each containing 6-8 ova.

Antennule: Folded back reaches to the posterior end of the caudal rami. It consistent of 22 segments having the proportional lengths:

38: 41:21: 30:30: 30: 30: 48: 23: 27: 34: 33:61: 61:70: 68: 36: 37: 48: 33: 39: 62  
80: 70:68: 67:61: 66: 61: 36: 35: 32:30: 44:42: 39:35: 28: 26: 26: 24: 17: 17: 16

All the antennal setae seem to be spine like and devoid of plumose hairs. Of the three setae borne by segment 3, one is distinctly larger than the other two. Segments 6, 7 and 9 bear a minute spine each.

Leg 5 (Fig. 7): Same as specimen described in Cochin estuary. Each consists of a three-jointed ramus. Basis provided with setae on the posterior face. Exopodite 1 bears a row of spines on its outer surface. Exopodite 2 armed at its distal external angle with a single marginal serrated spine and there is no trace of lamelliform process. Exopodite 3 bears a small marginal spine and three end spines, of which the outer by far the longest and stoutest and is serrated on both margins. Comparison of length-width ratio of P5 of both specimens is given in Table 4.

Egg sacs and spermatophore: The ovigerous female bears a pair of egg sacs, each containing 5-10 eggs.

**Distribution**

Indian Ocean; Java Coast: Karun River, Perak, Burma; Salt Lake, Calcutta; Chilka Lake; Vizaghapatanam Coast; Madras Coast; Trivandrum Coast; Vizhinjam inshore waters; Quilon Coast; Cochin estuary; Calicut Coast; 'Bandra, Isle de salsette' (Mumbai waters); Krusadai Island; Kuram River, Perak.

Present study covers: A&N Islands and Cochin estuary.

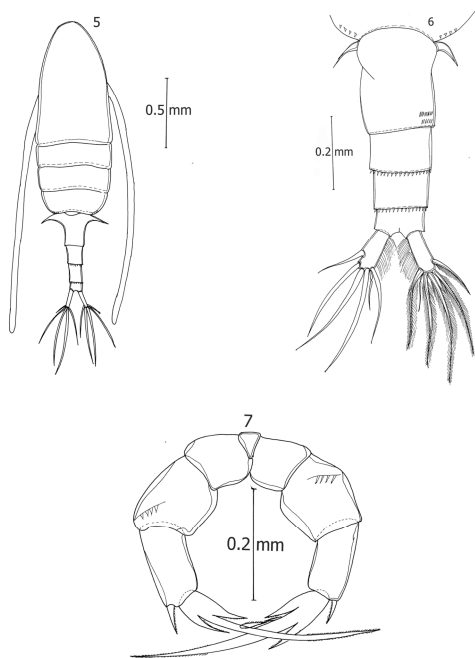


Fig.5 — *Pseudodiaptomus P. annandalei* habitus, dorsal view; Fig.6 — urosome, dorsal view; Fig.7 — P5, dorsal view. Scale bars in mm.

Table 3 — Length width-ratio of caudal setae of *P. annandalei* from A&N Islands

	Se1	Se2	Se3	Se4	Se5
Length	9	16	45	15	15
Width	18	18	25	18	21

Table 4 — Comparison of length -width ratio of P5 of *P. annandalei* from Cochin estuary and A&N Islands

	Cochin estuary		Andaman and Nicobar	
	Length (µm)	Width (µm)	Length (µm)	Width (µm)
Coxa	44.24	43.08	45.86	41.76
Basis	62.15	51.38	56.41	50.69
Exopod1	64.70	29.58	71.08	36.15
Exopod2	31.07	23.65	27.80	23.06

## Remarks

Since, good account on *P. annandalei* is available mainly by the reports of Sewell<sup>6,7</sup>, Brehm<sup>22</sup> and Reddy & Radhakrishna<sup>14</sup>, the geographical variations perceived among the same species collected from Cochin estuary and A&N Islands are presented in this paper. The main characteristic features of the female specimen of *P. annandalei* are: Prominent recurved spine on anterior part of Ur1, vertically ornamented 6-8 large corona of spines on metasome corner, much dilated 3<sup>rd</sup> caudal rami setae etc. When compared with the original descriptions of Sewell<sup>6</sup> from Chilka Lake, females from *P. annandalei* from Cochin estuary showed almost similar body size and shape; however, the specimen collected from A&N Islands showed major morphological variations. The female has typically been separated from other *pseudodiaptomid* species by the shape of caudal setae (much dilated 3<sup>rd</sup> caudal setae), finely ornamented metasome corners and by the prominent recurved spines on Ur1. In Cochin estuary specimen smaller than A&N specimen, antennae reach to the posterior end of the genital segment of the abdomen; posterior margin of Ur1 devoid of any kind of spinulation or spine patches except the large recurved spines on anterior margin. The CR is symmetrical and bears five setae which are short and stout and the 3<sup>rd</sup> seta is much dilated. The specimen collected from A&N Islands is much larger than Cochin estuary specimen. Critical evaluation of the specimen collected from A&N Islands showed several variations: Antennae much longer and extending beyond caudal rami, needle like strong spines just above the urogenital segment and dense spinules patches on right posterior margin of Ur1; double row of ornamentation of spinules on each metasome segments; 3<sup>rd</sup> setae rami setae normal and longer than the rest were observed from A&N Island species.

Table 5 provides a comparison of morphological features of *P. annandalei* from Cochin and A&N Islands.

Table 5 — A comparison of *P. annandalei* from Cochin estuary and A&N Islands

Morphological characters	Chilka Lake	Cochin estuary	A&N Islands
Total length (mm)	1.18	1.18	1.5
Proportional length of prosome: urosome	71:29	67:33	63:37
Length of urosomal somite and CR	40:14:14:9:23	38:14:16:11:20	45:17:14:8:16
Ratio of length CR	-	13:18:30:20:19	9:16:45:15:15
Ratio width of CR	-	17:11:42:15:15	18:18:25:18:21

## Discussion

Extensive studies on *P. annandalei* are available worldwide<sup>6-21</sup>. Even though, reports on *P. annandalei* from A&N Islands were done by Phukum, The detailed morphological discrimination of the same species has not been studied<sup>21</sup>. In this scenario, our study provided the morphological variations of *P. annandalei* from A&N Islands and Cochin estuary. The specimens collected from Cochin estuary accord well with Sewell's descriptions<sup>6</sup>; however, smaller than those from A&N Islands. Variations in body size and form among geographically separated species were reported by Chen & Zhang; Fleminger et al., and Othman & Toda. They studied the extreme morphological variations displayed by *L. rotunda*<sup>37-39</sup>. In a similar study, Radhika et al., redescribed the morphological variations in body size of *C. crassiusculus* and *O. catus* from Lakshadweep Islands<sup>40</sup>. The specimens from A&N Islands clearly have larger and stronger spinules in U3 compared with Cochin estuary specimens. CR setae showed clear intra-specific variation among *P. annandalei* populations. Commonly 3<sup>rd</sup> CR seta of this species was much dilated and spear in shaped; but in A&N Islands it was very thin and normal. Antennule much elongated reaching up to the tip of CR is another discrepancy observed in A&N Island specimens; urogenital segment provided with dense patches of spinules also observed in A&N Island specimens. According to Walter, extensive morphometric variation occurs within the genus and female genital double somite is also in taken care especially regarding the ventral genital flaps and egg sac number<sup>18</sup>. Even though extensive studies which discriminate the calanoid copepod species based on the morphological structures from geographically separated areas are sparse, several studies based on cyclopoid copepods are available. Böttger-Schnack demarcated the variant of *O. bispinosa*, a cyclopoid copepod from northern part of Gulf of Aqaba with an account of morphological variations<sup>42</sup>. Studies stated that intra-specific morphological variations among some copepod have been related to seasonal changes but such variations are related to the formation of species complexes<sup>43&44</sup>. Literature shows that copepod populations which are diverging from the Asian ones and that even among the Asian forms there is some degree of differentiation, particularly between the Indonesia-Thailand and Taiwan-Vietnam groups<sup>45</sup>. In our study, characters which are used to distinguish species within genus show subtle intra-specific

differences among *P. annandalei* and it is clear that these morphological characters are not uniform throughout the species range. This study also offers intra-specific differences to expand the known morphological range of *P. annandalei* species and to provide the first comparative analysis of *P. annandalei* from different locations in India.

### Acknowledgement

The authors are thankful to the University Grants Commission India, for providing financial support and the Head of the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology for providing necessary facilities to carry out the work. The authors thank Dr. Sreelekshmi S (Research scholar, CUSAT) and P. Marimuthu (Research scholar, Pondicherry University, Andaman and Nicobar Islands) for helping in sample collection and support during the study. The authors are also grateful to T. Chad Walter, Smithsonian Institute for his valuable suggestions in preparing the manuscript.

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