



A NEW SPECIES OF COPEPOD *Caligus polynemi* N. SP. FROM POLYNEMID FISHES OF VISAKHAPATNAM COAST, BAY OF BENGAL, ANDHRA PRADESH, INDIA

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ABSTRACT

Caligids are one of the most frequently occurring copepod parasites of marine and brackish water fishes, which cause a great havoc to the mariculture. There is a huge biodiversity of marine fishes and their parasites in the Bay of Bengal. The present study was aimed to unravel the copepod parasite diversity in marine thread fin fishes of Visakhapatnam coast, Andhra Pradesh (India). A new species of *Caligus* Muller, 1785 was obtained from the gills of 77 *Polydactylus plebeius* (Broussonet) and 55 *Leptomelanosoma indicum* (Shaw) of Visakhapatnam coast, India, fixed in 10% formalin and cleared in a few drops of lactic acid for 12-24 h before identification. The species was characterized by having urn-shaped genital segment, large lunules, indistinctly two-segmented abdomen, converging tines of sternal fork, presence of three rows of teeth on the middle segment of endopod of 2nd leg, three segmented 4th leg and absence of ventral setae on distal segment of 1st leg. Based on above differences in vital diagnostic features and their occurrence in polynemid fishes, they are regarded as new species of *Caligus* and named as *Caligus polynemi* n. sp. Further monitoring surveys may enable sustainable marine fish farming by exploring unknown species from different geographical locations and develop various pest management and conservation strategies against them to safeguard marine life.

Keywords: *Caligus polynemi*, *Leptomelanosoma indicum*, *Polydactylus plebeius*, Visakhapatnam coast

INTRODUCTION

Parasitic copepods are commonly observed among the marine and brackish water fishes, which cause severe pathological lesions and disorders (Özak and Yanar, 2016). There are nearly 30 families of parasitic copepods, of which associates of *Caligidae* Burmeister, 1835 are most widespread and well-known. Amongst the family *Caligidae* that includes nearly 31 genera and 487 species (Francisco *et al.*, 2016), the genus *Caligus* Muller, 1785 was considered to be the most species-rich genus with more than 371 species (Boxshall and El-Rashidy, 2009; Walter and Boxshall, 2020). Majority of them being parasitic on teleosts, only 17 species were accounted from elasmobranchs (Tang and Newbound, 2004; Morgan *et al.*, 2010). Caligids or ‘Sea lice’ are considered to be the utmost notorious parasites ruining the marine aquaculture. Though, these caligids are not harmful to wild fish but can cause serious problems in marine or brackish water cultured fish (Nagasawa, 2004; Johnson *et al.*, 2004; Francisco *et al.*, 2016) and aquarium fishes (Kik *et al.*, 2011).

The impact of caligids as disease-causing mediators, is gaining significance as 54% of copepod infestations in marine cultured fishes are known to be caused by them resulting in mild skin damage, corneal ulceration to severe stress-induced death of the fish (Costello, 2006; Maran *et al.*, 2009, 2012a; Helna *et al.*, 2018). These parasites usually damage epidermal tissues thereby pave the line of attack for bacterial invasion leading to the death of host fish (Lin *et al.*, 1994; Lin, 1996; Ho, 2000; Bergh *et al.*, 2001). A vast amount of work has been contributed on the caligids affecting marine and brackish water fishes all over the world since 19th century (Wilson, 1905, 1908, 1911, 1913, 1928, 1937; Yamaguti, 1936, 1954; Yu, 1933; Ho, 2000; Ho *et al.*, 2000; Yuniar *et al.*, 2007; Maran *et al.*, 2008, 2009, 2012a, 2012b, 2014; Vo *et al.*, 2008; Dojiri and Ho, 2013; Francisco *et al.*, 2016, Helna *et al.*, 2018). Only a meager work on caligids has been conducted from India (Basset-Smith, 1898; Rangnekar, 1956; Pillai, 1961, 1963, 1968, 1974, 1985; Prabha and Pillai, 1983, 1986; Ramesh Kumar *et al.*, 2014; Helna *et al.*, 2018; Nikhila *et al.*, 2019). Polynemid fishes or thread fin fishes serve as very good hosts for a number of parasites. Only meager work has been conducted on the parasites of polynemids from Visakhapatnam coast (Gudivada *et al.*, 2010, 2012). In present study, a new caligid copepod collected from polynemid fishes of Visakhapatnam coast is has been described.

MATERIALS AND METHODS

Study site

Two marine thread fin fishes of family Polynemidae, *Polydactylus plebeius* (Broussonet) and *Leptomelanosoma indicum* (Shaw) were collected from boat seine and trawlers of fishing harbour, fish landing centers and fish markets in and around Visakhapatnam coast (17.67°N and 83.32°E), Bay of Bengal for a period of two years (Fig. 1). The fishes were dead at the time of the collection. The ethics and consent concerning the use of fish for this research were deemed unnecessary according to the Animal ethics committee (Control and Supervision of Experiments on Animals - CPCSEA) as the fish was dead at the time of collection, edible and was easily available to carry out research. Meanwhile the number of fishes used for this research (77 *Polydactylus plebeius*, Broussonet and 55 *Leptomelanosoma indicum*, Shaw) was regulated by the Institute (Andhra University).



Fig. 1: Map showing the study area-Visakhapatnam Coast, Andhra Pradesh, India

Sample collection and identification of the parasite

Morphometric and meristic information of the hosts were recorded on specially prepared data sheets, along with their spectrum of metazoan parasites. Before dissecting the fish, its length, weight and sex were noted. Various external surfaces i.e., fins, body surface, buccal cavity and gills were screened for ectoparasites. Caligid copepods were generally found attached to the gill cover. Gills were cautiously removed and placed in a saline solution, then teased and contents were washed and observed under a stereo zoom microscope. Copepod parasites were collected and fixed in 10% formalin. The parasites were identified by keeping them in cavity blocks with a few drops of lactic acid for 12-24 h for clearing. Parasites were observed, identified and captured in microphotographs taken with Nikon microscope at 10, 30 and 40X magnifications and scale was given accordingly. Figures were drawn with the assistance of Reiss camera lucida using 4, 10, 15 and 40X magnifications. All measurements

were expressed in millimeters, unless otherwise mentioned. Holotypes and paratypes of the copepods were preserved and stored in Museum of Zoology Department, Andhra University (ZDAU), Visakhapatnam, Andhra Pradesh (India).

RESULTS AND DISCUSSION

Caligus polynemi n. sp. (Figs. 2-17)

Name of the host: *Polydactylus plebeius* Broussonet 1782; *Leptomelanosoma indicum* Shaw, 1804

Habitat: Gill filaments

No. of the hosts examined: 77 (*P. plebeius*); 55 (*L. indicum*)

No. of hosts infected: 15 (*P. plebeius*); 12 (*L. indicum*)

No. of specimens: 36 (22 female and 14 male specimens)

Material examined: *Holotype*. Female: India: Andhra Pradesh, Visakhapatnam coast, Andhra University campus, Coll. Mani. G, 15. viii. 2007 (ZDAU) and Male: India: Andhra Pradesh, Visakhapatnam coast, Andhra University Campus, Coll. Mani. G, 15. viii. 2007 (ZDAU). *Paratype*. 20 females and 12 males, India: Andhra Pradesh, Visakhapatnam coast, Andhra University campus.

Etymology: The species name is named after the family name of the host.

Female

(Based on 10 specimens - total length - 1.604-1.75 mm) [Fig. 2-12 & Fig. 16]: Cephalothorax large and broad with projecting frontal plates measured 0.631-0.684 x 0.66-0.71 mm. Lunules large and posterior median lobe larger than the lateral lobes and overlaps 4th thoracic segment. Posterior sinuses open. Fourth thoracic segment had four pair of legs and measured 0.13-0.16 x 0.16-0.18 mm. Genital segment urn-shaped with a short, anterior neck; postero-lateral lobes round; measured 0.53-0.57 x 0.5-0.52 mm. Abdomen indistinctly two segmented, basal longer than distal segment. Basal segment measured 0.18-0.21 mm and distal segment 0.11-0.13 mm. Caudal rami squarish with three long and three short

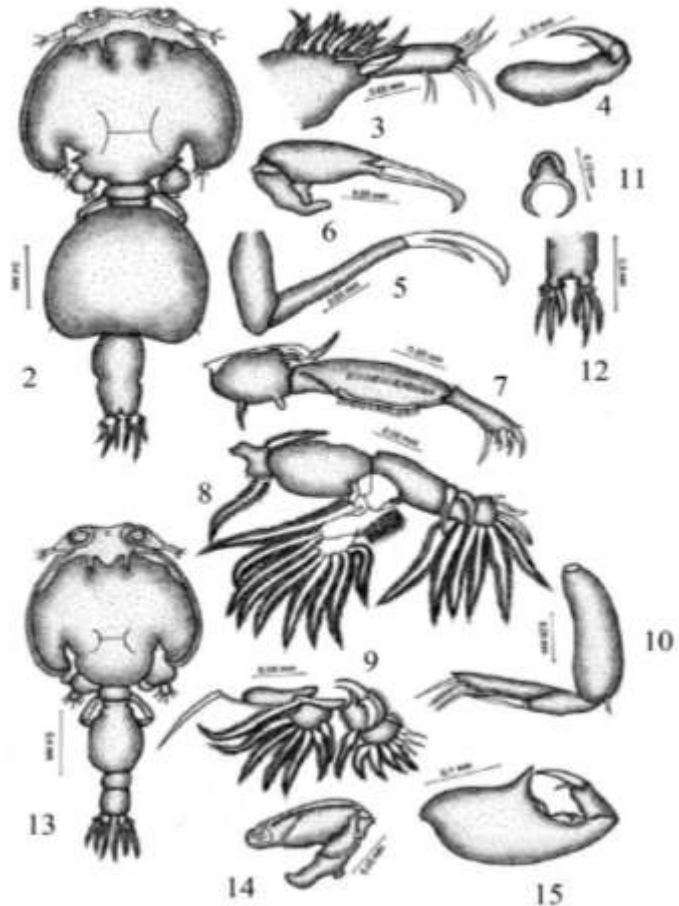


Fig. 2-15: *Caligus polynemi* n. sp.

Fig. 2: Line diagram of *Caligus polynemi* n. sp. (female), 45X

Fig. 3: First antenna, 150X

Fig. 4: Second antenna, 150X

Fig. 5: Maxilla, 150X

Fig. 6: Maxilliped, 150X

Fig. 7: Leg-I, 150X

Fig. 8: Leg-II, 150X

Fig. 9: Leg-III, 150X

Fig. 10: Leg-IV, 150X

Fig. 11: Sternal fork, 150X

Fig. 12: Caudal rami, 60X

Fig. 13: Line diagram of *Caligus polynemi* n. sp. (male), 45X

Fig. 14: Second antenna, 150X

Fig. 15: Maxilliped (Male), 240X

plumose setae and inner margin hairy; measured 0.026-0.05 mm. First antenna: Two segmented, basal segment large with 18 short scattered plumose setae; distal segment long and cylindrical with nine apical naked setae. Second antenna: Two segmented, basal small with a conical basal process and distal long and curved to a claw. Maxilla: Two segmented, basal long and narrow, distal long and slender with a pair of unequal claws, upper claw long and flanged but lower claw short and pectinated was observed. Maxilliped: Two segmented, basal stout and distal curved to a claw with a basal spine. Sternal fork: Base triangular, narrow, tines pointed and converging. Leg I: Basipod with a short plumose seta in the

inner proximal margin, and one long plumose seta on the outer distal corner. Vestigial endopod finger like. Basal segment of exopod long, broad and with hairy inner margin, and one small naked spine on the outer distal corner. Distal segment with three claws and a spine like long and naked seta. Claws decreasing in length, 1st claw ventrally barbed and 2nd & 3rd claws with accessory processes. Ventral setae absent. Leg II: First claw of exopod long, pectinated and projecting beyond the segment. Second claw was pectinated, longer and bent inwards. The claw of 3rd segment was short and straight. Third segment with two setae, 1st seta winged and 2nd seta semi-pinnate. Six long plumose setae present terminally. Endopod three segmented, basal with a row of four spines on the outer margin, and one long plumose seta on the inner margin. Middle segment with three rows of sharp bent teeth on outer margin and two plumose setae on the inner margin. Distal segment naked with six long plumose setae terminally. Leg III: Velum large and hairy. All segments of exopod were with hairy outer margins. Tip of the basal claw touches the 2nd segment, claw externally flanged. Basal segment is with one spine and one plumose seta and distal segment with three spines and four plumose setae. Endopod segment with seven long plumose setae. Leg IV: Three segmented, basal long and broad, middle short, distal longer than the middle. Middle and distal segments with five claws, 4th segment pectinated, while fifth long and naked. Basal crest present on first four claws, absent on last claw. Leg V: Vestigial, lodging on posterior lobes of genital segment.

Male

(Based on 10 specimens; Total length - 1.29-1.32 mm) [Fig. 13-15 & 17]: Cephalothorax large, posterior median lobe larger than the lateral lobes. Lunules large. Posterior sinuses open. Genital segment flask shaped, narrow, having an anterior neck. It measured 0.38-0.40 x 0.29-0.32 mm. Abdomen two segmented, basal shorter measured 0.15-0.16 mm and distal longer measured 0.09-0.10 mm. Caudal rami squarish, with three long and three short plumose setae and hairy inner margin. Second antenna: Two segmented, large basal with a long basal process; distal short and modified to three claws and one seta. Maxilliped: Two segmented, basal segment large with a median, conical basal process; distal segment curved to a claw and with a median short spine.

Discussion

Parasitic copepods especially Caligids are the most notorious metazoan ectoparasites which stand as great barrier to the sustainable marine finfish aquaculture across the globe and cause economic loss of nearly 100 million US dollars annually (Johnson *et al.*, 2004; Monir *et al.*, 2015; Shinn *et al.*, 2015). They are the 2nd highest ever-mentioned parasitic copepods reported so far (Ganeshmurthy *et al.*, 2014). The genus *Caligus* was first proposed by Müller (1785) with *Caligus curtus* as its type-taxon.



Fig. 16: Microphotograph of *Caligus polynemi* n. sp. (Female), 40X



Fig. 17: Microphotograph of *Caligus polynemi* n. sp. (Male), 40X

Table 1: Comparison of *caligus polynemi* n. sp. with the other related species of *Caligus*

Characters	<i>C. annularis</i>	<i>C. diaphanous</i>	<i>C. epinepheli</i>	<i>C. laticaudus</i>	<i>C. phipsoni</i>	<i>C. polynemi</i> n. sp.
Host	<i>Hapalogenys mucronatus</i> <i>Otolithus maculatus</i>	<i>Authisthes puta</i> , <i>Lates calcarifer solea vulgaris</i> <i>Trigla</i> sp.	<i>Epinepheles septem-fasciatus</i> <i>E. akkara</i> <i>E. merra</i> <i>Chorinemus tola</i>	<i>P. heptadactylus</i> <i>Pagrosomusmajor</i> <i>Acanthopagurus olivaceus</i>	<i>P. plebeius</i> <i>P. tetradactylum</i> <i>Cybiumguttatum</i>	<i>Leptomelano-soma indicum</i> <i>P. plebeius</i>
Genital segment	Triangular	Inverted U- shaped	Large, pear shaped, without anterior neck	Large, swollen, hemisphere	Slightly longer than broadening backwards	Urn shaped
Abdomen	One segmentd	Two segmented	Distinctly two segmented	Distinctly two segmented	One segmented	Indistinctly two segmented
Sternal fork	With narrow diverging apical incurved limbs	Base long, limbs epically blunt and diverging	Base long, diverging limbs	Limbs as long as base, broad and flattened, apical round, slightly diverging	Large narrow base, limbs short, slightly diverged	Base oval, limbs longer than base, pointed, converging
Second antenna	Two median setae on distal segment	Distal with 2 median spines	With 2 median spines	Distal curved to a claw	Distal curved to a claw	Distal without median setae
Maxilla	Both claws flanged	2 unequal claws upper claw – 4 rows of spines. Lower 2 rows of spines	2 unequal claws upper claw – 4 rows of spines. Lower 2 rows of spines	2 unequal flanged claws	2 unequal claws, 1 claw - wavy margins 2 claw – pectinated	2 unequal claws, upper claw flanged, lower pectinated
Leg I Exopod – Basal	Hairy inner margin and a naked spine on outer distal corner	Hairy inner margin and a naked spine	Hairy inner margin and a naked spine	Spiny inner margin and naked spine on outer distal corner	Hairy inner margin and a naked spine on outer distal corner	Hairy inner margin and a naked spine on outer distal corner
Distal	Minute spine present	3 claws, 2 nd & 3 rd with small accessory spine	Without lower setae, spine like seta longer than claws, first claw bifid	Clinging claws	Normally developed claws, 2 nd & 3 rd with accessory process	Without minute spine
Vestigial endopod	With a spine	With a spine	Without spinule	With a spine	With a spine	Without spinule
Ventral setae	Absent	Absent	Absent	Present	Present	Absent
Leg II Exopod claws	First two claws long, with narrow serrate flange	First two claws long, slightly bent, externally winged	First two claws stout, with serrate flange	Large & barbed	First two claws large, with broad serrate flange	First claw very long, projecting beyond the segment & pectinated
Leg III	Basal claw not flanged	Basal claw externally winged	Basal claw moderately curved	Basal claw stout, with a membranous flanged	Claw curving inwards and externally flanged	Basal claw flanged
Leg IV	5 th claw pectinated, basal crest present on all claws	5 prominent winged claws, each claw with long patches of denticles near its base	Claws winged, basal crest absent for 5 th claw, pectinated on ventral side	Claws long and winged, spiny crest absent for last claw	Claws with moderately broad wings	5 th claw naked. Basal crest absent on last claw

It is the most species-rich genus including more than 371 species (Boxshall and El-Rashidy, 2009, Walter and Boxshall, 2020). They are common parasites to both marine and brackish water fishes. There are only two species of *Caligus* reported, *C. phipsoni* Bassett-Smith, 1898 and *C. laticaudus*,

Shiino, 1960 from polynemidae fishes (Pillai, 1985). The parasites were characterized by the presence of large lunules, urn-shaped genital segment, indistinctly two-segmented abdomen, converging tines of sternal fork, absence of ventral setae on distal segment of first leg, presence of three rows of teeth on the middle segment of endopod of second leg and presence of three segmented fourth leg (Pillai, 1985). The parasites differ from *C. phipsoni* in having indistinctly two segmented abdomen, absence of median seta on distal segment of antenna, converging tines of sternal fork, absence of ventral setae on distal segment of leg-I, vestigial endopod without a spine, absence of basal crest on fifth claw of leg-IV and in many other characters. The present parasites differ from *C. laticaudus* in having indistinctly two segmented-abdomen, converging limbs of sternal fork, absence of ventral setae on distal segment of leg-I and in many other characters. There are a few species that possess two segmented abdomen and absence of ventral setae like *C. diaphanus* and *C. epinepheli* (Pillai, 1985). Though these parasites resemble with the present parasites in not having a ventral seta on distal segment of leg-I, they differ from these parasites in many other characters like diverging tines of sterna fork, distinctly two segmented abdomen, winged claws in IV leg, etc. (Pillai, 1985). The parasite resembled *C. annularis* in absence of ventral setae on distal segment of leg-I but differed in the nature of appendages. Differentiating characters of present species of *Caligus* with other closely related species of *Caligus* are given in Table 1. In view of these differences, the present species is considered and reported as a new species and named as *Caligus polynemi*. This study affirms that the parasitic community structure of threadfins from Visakhapatnam coast, Bay of Bengal, Andhra Pradesh, India has many new species of parasitic copepods which need to be disclosed. This study shall immensely be useful to provide a database on host-parasite relationship in the Bay of Bengal.

Conclusion: The fishes of Visakhapatnam coast, Bay of Bengal has wide range of undisclosed parasitic biodiversity which is yet to be unveiled. The identification of a new species of caligid copepod, *C. polynemi* from marine threadfin fishes paves a pathway to conduct many more parasitological surveys to unveil the parasite diversity of this geographical location.

Competing interest and author's contribution: The authors declare that they have no competing interest related to the work. The first author MG was involved in the collection of fish samples and parasites, literature collection; while the second author APV helped in writing and drafting the manuscript. All authors have read and approved the manuscript.

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