



***Apocyclops cmfri* sp. nov. (Cyclopoda : Cyclopoida : Cyclopidae), a new copepod species from Arabian Sea off Karwar, Karnataka, India**

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ABSTRACT

A new species of *Apocyclops* Lindberg (Cyclopoda: Cyclopoida: Cyclopidae) is described based on specimens collected from marine waters of Karwar, Karnataka, India. At present, there are 11 valid and accepted species excluding one extinct species (fossil) in this genus which are widely distributed in fresh and brackishwater areas in the tropics and subtropics. Five valid species of *Apocyclops* are reported from the Asian region, including two species from India. The new species is closer to the previously reported Indian species, *A. royi* (Lindberg) and *A. dengizicus dengizicus* (Lepeshkin). *Apocyclops cmfri* sp. nov. differs from these in the size of caudal rami, in the characteristic shape of disto-medial part of coxa and basis of first to fourth pleopods (P1-P4) and in the presence of a single broad spinous expansion with a pointed tip in between exopod and endopod in basis of P1 to P4. Only one terminal spine is present in the second segment of endopod of P1. In P2 to P4, both exopod and endopod terminate in a spine and a seta of almost equal size. Maxillule highly dentate and maxilla basis form a large dentate claw with inner serration. All these characters form distinct identification features of the new species in comparison with the 12 accepted species under the genus. A key to all the species of the genus including the newly described species is proposed here. Molecular identification of the new species was carried out by mitochondrial cytochrome c oxidase 1 (CO1) gene sequencing and the sequence was submitted to NCBI, GenBank. Genetic differentiation and divergence between *A. cmfri* sp. nov. and species belonging to other closely related genera; *Thermocyclops*, *Mesocyclops* and *Eucyclops* were compared using CO1 gene sequences. The new species belonging to *Apocyclops* showed significant divergence from *Apocyclops borneoensis* with K2P value of 10.2% and from species under the genera *Mesocyclops*, *Thermocyclops* and *Eucyclops* with K2P values of 26.6, 27.5 and 34.9% respectively.

Keywords: *Apocyclops cmfri* sp. nov., Cyclopoida, India, Karwar, Marine copepod, Mitochondrial CO1 gene, New species, Taxonomy

Introduction

The copepods of the family Cyclopoidae comprise more than 70 genera and 1200 species (Dussert and Defaye, 2006; Walter and Boxshall, 2016). The genus *Apocyclops* Lindberg, 1942 and related genera of *Metacyclops* complex are generally separated from the rest in having two segmented exopodite and endopodite of first to fourth pleopods (P1-P4) (Karanovic *et al.*, 2011). Fiers (2001) and Karanovic (2004a, b) reported *Metacyclops* as a polyphyletic taxon. Later Karanovic *et al.* (2011) revised *Metacyclops* complex based on cladistics analysis. There are only five valid reports of these two genera from India *viz.*, *M. margaritae*

(Lindberg, 1938), *M. communis* (Lindberg, 1938), *A. dengizicus elamicus* (Lindberg, 1940), *A. dengizicus dengizicus* (Lepeshkin, 1900) and *A. royi* (Lindberg, 1940) (Lindberg, 1961). Monchenko (1975) and Dussart and Defaye (1985) considered the species, *M. margaritae* as a synonym of *M. gracilis*. But Kolaczynsky (2015), Lee and Chang (2015) and Pesce (2016) considered this species as a distinct taxon. The genus *Apocyclops* Lindberg, 1942, previously included as a subgenus of *Cyclops* and even Lindberg (1940) described the two Indian species (*A. dengizicus* and *A. royi*) in the genus *Metacyclops*. At present, there are 11 valid and accepted species in this genus, widely distributed in coastal lagoons,

bays, estuaries, pools, caves and lakes in desert areas of tropical and subtropical countries. The following species have been accepted as valid: *Apocyclops borneoensis*, *A. dengizicus dengizicus*, *A. dengizicus pelamicus*, *A. dimorphus*, *A. distans*, *A. japonensis*, *A. panamensis*, *A. procerus*, *A. ramkhahaengi*, *A. royi* and *A. spartinus* (Chullasorn *et al.*, 2008; Hołynska *et al.*, 2016; Pesce, 2016). Recently one new species has been identified from fossil samples (*Apocyclops californicus* Hołynska Leggitt and Kotoy, 2016) from the middle Miocene Barstow Formation in Southern California (Hołynska *et al.*, 2016). *Apocyclops* Lindberg, 1942 differ from *Metacyclops* Kiefer, 1927 and all other related genera in the nature of fifth pleopod (P5). It has a short spine (smooth or serrate), smaller than twice the length of the segment and inserted away from the seta. The spine is lateral in position (Lindberg, 1942; Iepure and Defaye, 2003). Plesa (1981) considered this as a subgenus of *Metacyclops* but at present it is accepted as a distinct genus closely related to *Metacyclops* (Karanovic *et al.*, 2011; Pesce, 2016).

Copepods such as calanoids, harpacticoids and cyclopoids are widely used as live feed in fish and shrimp hatcheries (Doi *et al.*, 1997; Nanton and Castell, 1998; Toledo *et al.*, 1999; Rajkumar and Kumaraguru, 2006). Among cyclopoid copepods, *Apocyclops* spp. is the most commonly used live feed in most shrimp hatcheries due to their higher nutritional value in terms of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and vitamin B1 compared to rotifers and *Artemia* (Farhadian, 2006, 2007, 2009; Sung and Sung, 2014). The present paper describes a new species of copepod under the genus *Apocyclops*. The species is being successfully cultured in captivity in the hatchery at Karwar Research Centre of the ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI) and is also being used as live feed in fish and shrimp larval rearing. The present report of new species is the third confirmed report under the genus *Apocyclops* from India.

Materials and methods

Copepod samples were collected from the Arabian Sea off Karwar near the marine cage farm (14° 49' 914" N; 74° 06' 002" E) along the south-west coast of India using plankton net (100 µm mesh size) and were transported live to the laboratory for further isolation and identification. The dominant species was a cyclopoid copepod and the same was isolated for identification and culture. Water quality parameters *viz.*, temperature, salinity, pH and dissolved oxygen recorded at the collection site during sample collection were: 29°C, 20‰, 7.9 and 4.8 mg l⁻¹ respectively. In the laboratory, copepods were isolated

by a series of filtrations using bolting silk of different mesh sizes. Ten brooders of the probable new species were separated and inoculated in pure stock culture of the marine microalga, *Chaetoceros calcitrans* for successive generations for use as live feed in the hatchery. Samples from the wild collection as well as from culture were fixed in 5% neutral buffered formalin and dissected in lactic acid following the method by Humes and Gooding (1964) using stereo zoom microscope (Leica S6). Line drawings were made using a compound microscope with drawing tube (Leica DM 1000) and measurements were taken using a Leica DFC290 camera. Type specimens were deposited in the Designated National Repository of ICAR-CMFRI, Kochi, Kerala, India. General terminology and morphological descriptions were carried out following Huys and Boxshall (1991).

DNA extraction and amplification of the cytochrome C oxidase I (COI) gene

DNA extraction was carried out using a standard phenol/chloroform extraction protocol (Sambrook and Russel, 2001). A 650 bp region of the COI gene was amplified using copepod specific primers (Cop-COI-1498F and Cop-COI-2189R; Bucklin *et al.* 2010). PCR reaction mixture consisted of 10mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 200 µM of each dNTP, 0.2 µM of each oligonucleotide, 1 unit of Taq DNA polymerase and 50 ng of template DNA. PCR reactions were carried out in Biorad T100 thermocycler (Biorad, USA) with the following PCR conditions: initial denaturation at 94°C for 4 min followed by 33 cycles of denaturation at 94°C for 30 sec, annealing at 42°C for 30 sec, extension at 72°C for 40 sec and a final extension at 72°C for 7 min. The PCR products were purified using PCR purification kit (Qiagen, USA) and sequencing was carried out employing BigDye Terminator Sequencing Ready Reaction v3.0 kit (Applied Biosystems).

Sequence alignment and analysis

The COI sequence of *Apocyclops* sp. was aligned with sequences (Gen Bank) of other valid species belonging to *Apocyclops* genus *viz.*, *A. borneoensis* and species belonging to other closely related genera *Mesocyclops*, *Thermocyclops* and *Eucyclops* using Clustal W in MEGA 6. A phylogenetic tree was constructed using UPGMA method with 1000 bootstraps. Tree topology was also tested using maximum likelihood, maximum parsimony and neighbour-joining methods. The tree was then rooted with COI sequence of *Acartia pacifica* retrieved from GenBank. Genetic divergence between all the species was analysed using Kimura 2 p distance values in MEGA 6.

Results

Taxonomy

Apocyclops cmfri sp. nov. Loka and Santhosh (Fig. 1A-R)

Order Cyclopoida Burmeister, 1834

Family Cyclopidae Rafinesque, 1815

Genus *Apocyclops* Lindberg, 1942

Holotype: CMFRI EF.6.7.2.1, ♀ specimen measuring 1009 µm in length (permanent mount), collected from Karwar near marine cage farm (14° 49' 91" N; 74° 06'

002" E) along the south-west coast of India during August 2012. Gen Bank Accession no. KX263726

Paratypes: CMFRI. EF.6.7.2.1, 40 nos. (20 ♀ and 20 ♂), length range 850-1260 µm, collected from the same locality (14° 49' 91" N; 74° 06' 002" E) (undissected preserved in ethanol)

Diagnosis

Female (Fig. 1A - M)

Body length (Fig. 1A) excluding caudal setae 1009 µm (910-1071 µm), robust with prosome/urosome ratio 1.8.

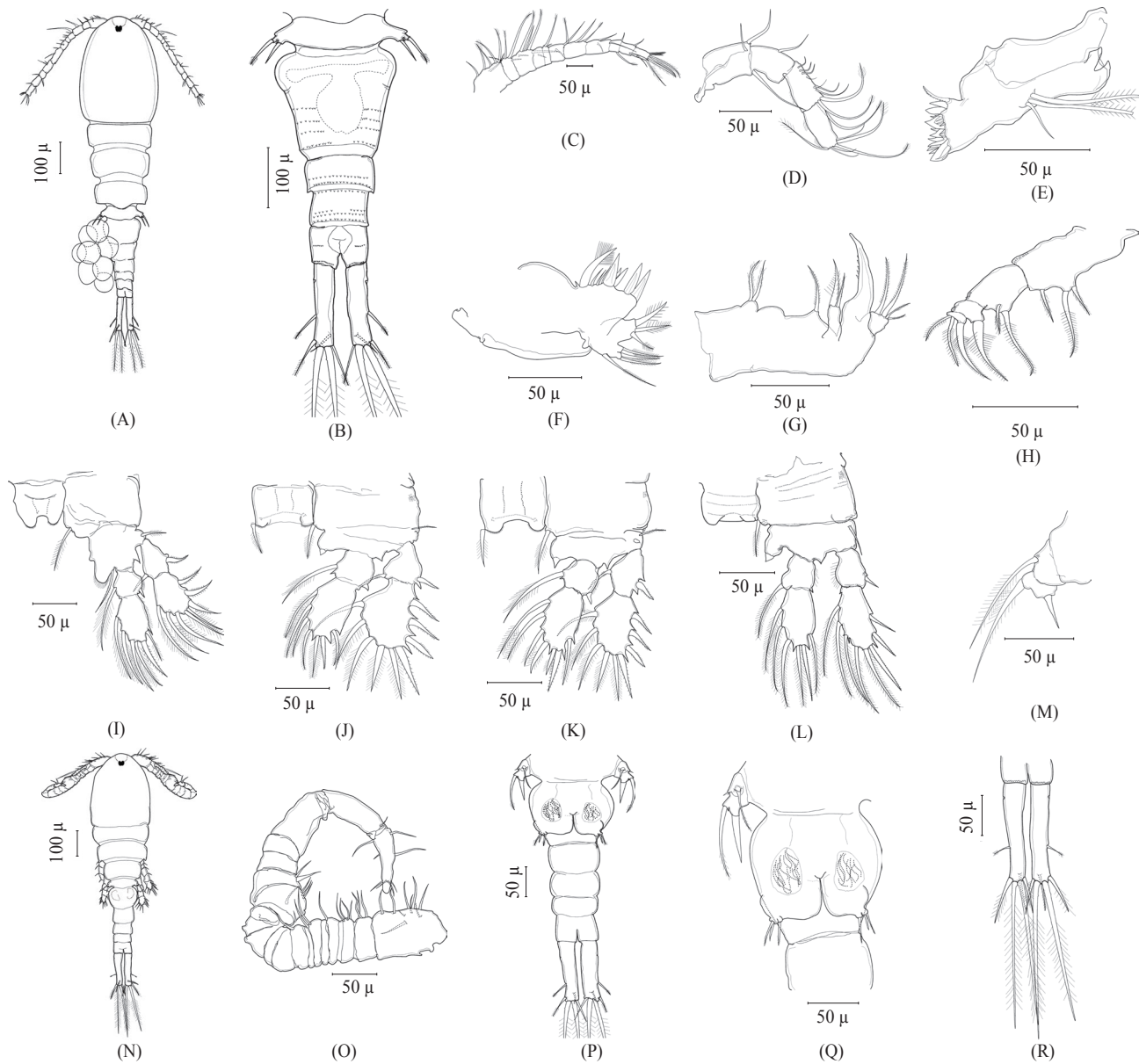


Fig. 1. (A) *Apocyclops cmfri* sp. nov. ♀ habitus, dorsal, (B) Urosome ventral, (C) Antennule, (D) Antenna, (E) Mandible, (F) Maxillule, (G) Maxilla, (H) Maxilliped, (I) P1, (J) P2, (K) P3, (L) P4, (M) P5, (N) *Apocyclops cmfri* sp. nov. ♂ habitus, dorsal, (O) Antennule, (P) Urosome ventral, (Q) P5, P6, (R) Caudal ramus

Cephalothorax swollen, almost semispherical and well separated from thoracic segments. All pedigeral segments distinctly separated dorsally and sequentially arranged in a line. Fifth pedigeral somite clearly demarcated from others and urosome. Body length/width ratio 3.6, cephalothorax 2.4 times of genital double somite in width. Free pediger somites well separated without any lateral expansion. Rostrum not demarcated; dorsal surface of prosomites and urosomites without any ornatation. Genital double somite wider anteriorly, reduced to half posteriorly (Fig. 1B). Urosomites highly ornated ventrally; 4 rows of spinules on the postero-ventral surface of genital double somite, 2 rows of spinules on the second urosomal somite and 3 rows on the third urosomal somite. Anal somite with two rows of spinules. Bifurcation of caudal ramus extends to half of the anal somite.

Caudal rami (Fig. 1 B) slightly diverged, elongated 4 times as long as wide, ornated with two long terminal setae of almost equal length. Anterior quarter of the caudal ramus with a lateral demarcation of a dormant seta/spine which is not visible externally. Each ramus bears 6 setae; 2 large setae with a small knob at base and one small seta each on either side of the large seta in its terminal region, one small seta each on lateral and dorsal middle surface of posterior region. Posterior end of the caudal rami with a small knob like projection between the large seta and the small seta on outer side. Antennule (Fig. 1 C) 11 segmented, almost reaches the posterior margin of the cephalothorax, the segments 1, 7, 8, 10 and 11 longer than wide, 3, 6 and 9 squarish, 2, 4 and 5 wider than long. Length ratio of antennular segment from proximal to distal 1.8 : 0.5 : 0.9 : 0.5 : 0.3 : 0.6 : 1.2 : 1 : 0.5 : 0.8 : 0.9. Setal formula: 6, 4, 5, 1, 2, 2, 3, 3, 2, 2, 8.

Antenna (Fig. 1 D) 4 segmented, comprising of a large coxobasis, cylindrical and 3 segmented endopod with setose margin in the posterior side; coxobasis about 1.4 times as long as wide with two terminal setae dorsally and one large setae terminally representing exopod on ventral side. Endopod 3 segmented, first segment with one seta, second with 3 groups of setae, posterior group with 3 small setae, middle group with 3 setae of medium size and the third group with 3 large terminal setae. The distal segment carries 3 large setae and 3 small setae distally. Length ratio of antennal segments - 1.1 : 1 : 1 : 1.

Mandible (Fig. 1 E) composed of coxa and small reduced palp. Gnathobase cutting edge with 13 strong teeth along distal margin, a row of small 6-7 sharp spinules in the distal margin and outer long subapical pinnate seta. Mandibular palp small with 2 very long finely plumed setae and one naked seta distally.

Maxillule (Fig. 1 F) with well developed praecoxa and 2 segmented palp. Praecoxa bearing 9 elements along inner face, 2 strong dentate spines distally, one large and 2 small dentate spines in the middle, 3 naked setae and one large bipinnate spine basally. Palp consists of coxobasis with one basal seta representing exopod, endopod distally bifurcated, distal segment with one large and 2 small bipinnate spines, proximal segment with 3 long bipinnate setae.

Maxilla (Fig. 1 G) 4 segmented praecoxa partially fused with coxa. Proximal endite with 2 plumose setae, distal end small and unarmed. Proximal endite of coxa with one pinnate seta, distal endite highly movable and carries one strong pinnate seta and one plumose seta. Basis form a large, dentate, strong claw with strong denticles in the inner margin. Endopod single segmented with two terminal bipinnate spines and 2 plumose setae.

Maxilliped (Fig. 1 H) 4 segmented, composed of syncoxa, basis and 2 segmented endopod. Syncoxal endite with few minute spines on outer distal corner, one bipinnate spine and 2 plumose setae in inner distal margin. Basis bearing one bipinnate spine, one plumose setae distally and few minute spinules on outer distal corner. Endopod 2 segmented, proximal segment with one long and stout bipinnate spine, distal segment armed with one large and one small bipinnate spine and one plumose seta distally.

All swimming legs P1- P4 (Fig. 1 I-L) with 2 segmented exopods and endopods of similar size. All intercoxal sclerites with paired small lobes produced posteriorly with few small spinules on inner distal margin, fragmentary ornatation on ventral surface of all sclerites except the fourth one with 3 transverse rows of small spinules. Setal arrangement in exopod 2 of P1 - P4 : 5, 5, 5, 5. Spine formula of exopod 2 of P1- P4 : 3, 4, 4, 3.

Table 1. Armature of pleopods (P1- P4) of *Apocyclops cmfri* sp. nov

Pleopod	Coxa	Basis	Exopod	Endopod
1	0 - 1	1 - I	1 - I ; III, 1, 4	1 - I; 1, I, 4
2	0 - 1	1 - 0	1 - I ; IV, 1, 4	1 - I; 1, I, 5
3	0 - 1	1 - 0	1 - I ; IV, 1, 4	1 - I; 1, I, 5
4	0 - 1	1 - 0	0 - I ; III, 1, 4	1 - I; 1, I, 4

P1 (Fig. 1 I) coxa with two groups of minute spinules on the outer margin, one plumose seta on inner distal margin. Coxa, characteristically expanded in the disto-medial part and with a seta originate from the middle. Basis with disto-medial part wide and protruding with setose margin, armed with one pinnate spine on inner side and one seta distally. Median part of basis between exopodite and endopodite spiniform.

Coxa of P2 and P3 (Fig. 1 J and K) with one group of spinules on the proximal end of outer margin on ventral side and one plumose seta on disto-medial part. Basis with one outer seta, spiniform process on disto-medial part, the median margin between exopods and endopod produced in to one spiniform process.

P4 (Fig. 1 L) coxa with 3 rows of surface ornatation with small spinules and several rows of small spinules proximally. Both corners of distal margin also bear several rows of small spinules. Medio-distal part of basis produced into a blunt spiniform process with several rows of small spinules basally.

P5 (Fig. 1 M), two segmented, basal segment with one large seta, distal segment bifurcated distally. The lateral segment ends with one large seta and the inner lobe with one smooth strong spine. The spine and seta always perpendicular in position, spine larger than the segment, widely separated from seta.

Colour creamish brown in live condition and greyish white after preservation. Egg sacs paired, multiseriata with 5-10 round eggs.

Male (Fig. 1 N-R)

Body (Fig. 1 N) 911.4 µm (851 - 969 µm) in length excluding furcal setae, width 253 µm (208 - 289 µm), shorter and more slender than female. Arrangement of cephalothorax and thoracic segments similar to female. Body length width ratio 3.6. Cephalothorax twice as wide as genital double somite and slightly longer than wide. Colour and ornatation, similar to that of females. Genital somite (Fig. 1 P) 1.4 times wide as long. Anal somite (Fig. 1 R) ornated distally with one row of small spinules on posterior margin. Caudal rami similar to female.

Caudal ramus (Fig. 1 R) 4.2 times wider as long. Setal distribution as similar to that of female.

Antennule (Fig. 1 O) larger than cephalothorax, 8 segmented, highly modified, thicker, geniculate with double geniculation between 8 and 12 segments as well as between 1.p and q segments; armature 9, 3, 4, 1, 2, 1, 0, 0, 0, 0, 1, 4, 2, 0, 0, 0, 1, 8.

Antennae, mouth parts, pleopods 1-5, similar to that of females. Pleopod 6 (Fig. 1 Q) represented by lateral plates on ventral side of genital somite, with one small spine in the middle and two outer setae.

Etymology

The specific epithet *cmfri* is named after the organisation, Central Marine Fisheries Research Institute (CMFRI), where this organism was isolated, identified and is being reared as a live feed in hatchery. The letters in the acronym *cmfri* should be pronounced individually.

Sexual dimorphism

The males differ from females in size, antennules, shape of genital double somite and sixth leg. Males smaller than females, both antennule highly modified, large, extending beyond the cephalothorax. Genital double somite spherical and bears the 6th leg.

Genetic analysis

Partial sequence of a 495bp region of the COI gene from the newly described species was deposited in GenBank with the accession no: KX263726. The phylogenetic tree constructed using 13 sequences of the four closely related genera with *Apocyclops cmfri* sp.nov. showed distinct clustering among genera (Fig. 2) with significant bootstrap values. Similar tree topology was

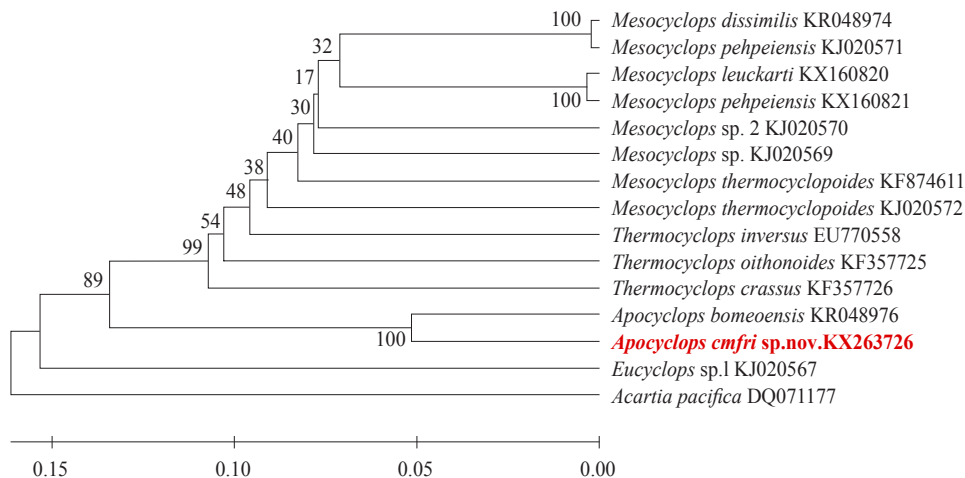


Fig. 2. UPGMA tree based on COI sequences of different species of *Apocyclops*, *Mesocyclops*, *Thermocyclops* and *Eucyclops*

also obtained when phylogenetic analyses were conducted using maximum likelihood, maximum parsimony and neighbour joining methods. Kimura 2 p distance values (Table 2) between species showed 10.2% divergence between *A. cmfri* sp. nov. and *A. borneoensis* and 26.6, 27.5 and 34.9% divergence between *Apocyclops* genus and the genera *Mesocyclops*, *Thermocyclops* and *Eucyclops* respectively.

Life cycle and culture of *Apocyclops cmfri* sp. nov.

Life cycle of *A. cmfri* sp. nov. is very similar to that of common cyclopoid copepod comprising of six naupliar stages and five copepodite stages. It is comparatively hardy and easy to rear in hatchery using live microalgae as feed. Shortest life cycle of captive reared *Apocyclops cmfri* sp. nov. was recorded as 8 days when fed with *C. calcitrans* (Fig. 3) with fecundity of 30 eggs per female per day. Hatching rate observed was 90%. Naupliar stages lasted for two days and the survival was 90%. Further development of copepodite stages into adults (male and female) and brooders took four days and the survival rate was 95%. Pure stock culture of *A. cmfri* sp. nov. is being maintained on live algal culture of *C. calcitrans* at a density of 2-5 numbers ml⁻¹. Mass culture of this species is being carried out for use as live feed for fish and shrimp larvae in

the Marine Hatchery Complex of Karwar Research Centre of ICAR-CMFRI.

Discussion

Eleven valid and accepted species excluding one extinct species (fossil) are reported till date under the genus *Apocyclops* which are widely distributed in tropical and subtropical waters. The following species have been accepted as valid: *A. borneoensis* (Lindberg, 1954), *A. dengizicus dengizicus* (Lepeshkin, 1900), *A. dimorphus* (Kiefer, 1934), *A. distans* (Kiefer, 1956), *A. japonensis* (Ito, 1957), *A. panamensis* (Marsh, 1913), *A. procerus* (Herbst, 1955), *A. ramkhahaengi* Chullasorn *et al.*, 2008, *A. royi* (Lindberg, 1940) and *A. spartinus* (Ruber, 1968) (Chullasorn *et al.*, 2008; Holynska *et al.*, 2016; Pesce, 2016). *A. californicus* (Holynska *et al.*, 2016) was reported from fossil records of middle Miocene Barstow Formation in Southern California.

There are 5 valid species of *Apocyclops* reported from continental Asia including three species from India; *A. royi* (Lindberg), as *Cyclops (Metacyclops) royi* and two sub species *A. dengizicus dengizicus* (Lepeshkin, 1900), *A. dengizicus elamicus* (Lindberg) as *Cyclops (Metacyclops) dengizicus dengizicus* and *Cyclops*

Table 2. Kimura 2p distance values based on COI sequences and standard error values between different genera (k2p values on the left side of the diagonal and standard error on the right side of the diagonal)

Species	<i>Apocyclops</i>	<i>Mesocyclops</i>	<i>Thermocyclops</i>	<i>Eucyclops</i>
<i>Apocyclops</i>	-	0.023	0.023	0.031
<i>Mesocyclops</i>	0.266	-	0.016	0.026
<i>Thermocyclops</i>	0.275	0.201	-	0.026
<i>Eucyclops</i>	0.349	0.298	0.300	-

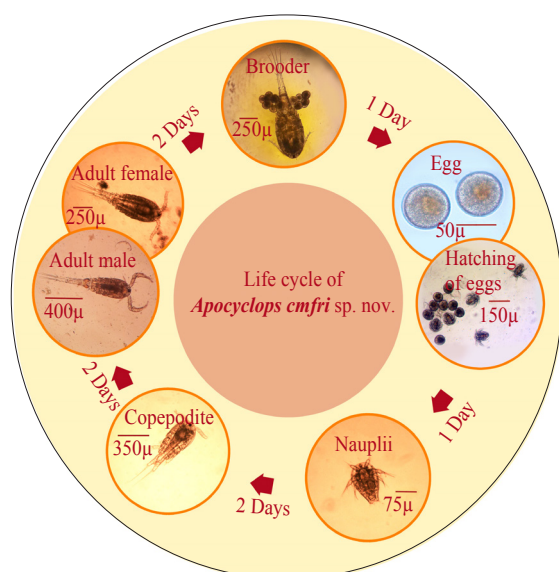


Fig. 3. Life cycle of *Apocyclops cmfri* sp. nov.

(*Metacyclops*) *dengizicus elamicus* respectively from an island in the Arabian Sea off the Mumbai coast of India (Lindberg, 1940). Lindberg (1955) described *A. borneoensis* from an island situated in the eastern Celebes Sea off the north-eastern coast of Borneo. Ito (1957) described *A. japonensis* from eel ponds on Ise Bay in Japan. Later, Botelho, (1999) synonymised *A. japonensis* Ito with *A. borneoensis* Lindberg but it is an accepted taxon now. Chullasorn *et al.* (2008) described *A. ramkhahaengi* Chullasorn, Kangtia, Pinkaew and Ferrari, 2008 from brackishwater area of Trat Province, Thailand.

The new species described here shows greater similarities to the previously reported species from India, *A. dengizicus* (Lindberg) and *A. royi* (Lindberg). Caudal rami of *A. dengizicus* (Lindberg) 7 times as long as width, while caudal rami of *A. cmfri* sp. nov. is only 4 times longer than wide, lateral setae originate from posterior

portion of the caudal rami, P5 serrated in accepted subspecies *A. dengizicus dengizicus* spine curved and obtuse in *A. dengizicus elamicus* and P6 is present in female representing 2 small spine, but P5 spine of *A. cmfri* sp. nov. is straight and P6 is completely absent in females. Moreover, average size of adult females and males are much larger in *A. dengizicus dengizicus* (1287 and 974 µm respectively) and *A. dengizicus elamicus* (1340 and 1045µm µm respectively) (Lindsberg, 1942) as compared to *A. cmfri* sp. nov. (1009 and 911.4 µm respectively). In comparison to the other Asian redescription of *A. dengizicus dengizicus* (Lepeschkin, 1900) from Uzbekistan and Kazakstan (Mirabdullayev and Stuge, 1998) and from Ukraine and Russia (Monchenko, 2003), *A. cmfri* sp. nov. differs in size of caudal rami as well as in the characteristic shape of coxa and basis. Both coxa and basis, characteristically expanded in the disto-medial part and the seta of coxa originate from the middle and the basis form a tongue like expansion in P1. Disto-medial part of basis of P2-P4 form a broad and stout spine with a pointed dentate tip. There is specific single broad spinous expansion with a pointed tip in between exopod and endopod in basis of P1to P4. Only one terminal spine is present in second segment of endopod of P1. Both exopod and endopod of P2 to P4, terminate in a spine and a seta of almost equal size. Maxillule highly dentate and maxilla basis forms a large dentate claw with inner serration. All these characters form distinct identification features compared to all the 12 accepted species descriptions of the genus *Apocyclops* (Lindberg, 1942; Ruber, 1968; Dussart, 1982; Lim and Fernando, 1985; Defaye and Dussart, 1988; Dumont and Maas, 1988; Mirabdullayev and Stuge, 1998; Reid *et al.*, 2002; Monchenko, 2003; Chullasorn *et al.*, 2008; Yoon and Chang, 2008; Holynska *et al.*, 2016).

The inner setae of caudal ramus of *A. royi* (Lindberg) is longer than all setae except the terminal setae the base of which appears to be segmented, basal lamina of P4 rounded, apical spine of endopod of P4 is much smaller than the apical seta, outer dorsal seta of P6 long in male and reaches first abdominal segment. Whereas in *A. cmfri* sp. nov., the inner setae of caudal ramus is the smallest among all setae, the terminal setae base has a small protuberance in lateral margin, basal lamina of P4 modified into a flat stout spine, apical spine and seta of endopod of P4 are equal in length, all setae of P6 of male are small and equal in size.

Six species of *Apocyclops* viz., *A. dimorphus*, *A. panamensis*, *A. spartinus*, *A. japonensis*, *A. borneoensis* and *A. ramkhamhaengi* are having antennae with 2 segmented endopod. *A. cmfri* sp. nov., clearly differs from these species in having 3 segmented endopodite.

A. dimorphus, *A. panamensis*, *A. spartinus* and *A. distans* have P6 of male with setae extending beyond 3rd abdominal somite (Ruber, 1968; Dussart, 1982).

Inner margin of the basis of P4 of *A. spartinus*, *A. panamensis*, *A. borneoensis*, *A. distans* and *A. ramkhamhaengi* are not modified (Ruber, 1968; Chullasorn *et al.*, 2008) whereas in *A. cmfri* sp. nov., it is modified as a broad spine with a dentate tip. *A. californicus* is more similar to *A. panamensis* and differ from the present species in many aspects including, spinulation on the basal segment of P5, scattered spinules on the ventral surface of P5, large protuberance in the inter-coxal plate of P4 and also setation in many swimming legs (Lim and Fernando, 1985; Chullasorn *et al.*, 2008; Holynska *et al.*, 2016).

Together with this new species, the genus *Apocyclops* currently includes 12 valid species for which a key for identification is proposed here:

1. Endopodite of second antennae of female 3 segmented 2
Endopodite of second antennae of female 2 segmented 5
2. Terminal endopodal seta of fourth swimming leg is 4 times or more longer than the spine
A. procerus (Herbst, 1955)
Same setae 2 times or shorter than spine 3
3. Caudal rami more than 6 times as long as wide 4
Caudal rami around 4 times as long as wide
A. cmfri sp. nov. Loka and Santhosh, 2017
4. P6 present in females representing two small setae
A. dengizicus dengizicus (Lepechkin, 1900)
P6 completely absent in females
A. dengizicus elamicus (Lindberg, 1940)
5. Armature of the terminal segment of endopodite of 4th leg of female - I,5 6
Same segment with I, 3 (a fossil species)
A. californicus, Holynska, Leggit & Kotov, 2016.
6. Caudal rami around 5 or less than 5 times as long as wide 7
Caudal rami around 10 times as long as wide
A. japonensis Ito, 1958
Caudal rami around 7 times as long as wide
A. borneoensis Lindberg, 1954
7. Terminal endopodal seta of fourth swimming leg is between 3-4 times or more longer than the spine in females 8
Same setae above 5 times longer than spine
A. distans (Kiefer, 1956)
Same setae below 3 times or more shorter than spine
A. royi (Lindberg, 1940)

8. Antennule extends beyond the first thoracic segment 9
 9
 Antennule extends less than the cephalothorax
 *A. ramkhamhaengi* Chullasorn, Kangtia,
 Pinkaew & Ferrari, 2008.
9. Distal segment of maxilla with 5 spines and dorso-lateral
 lobes of 5th thoracic segment with tiny hairs
 *A. panamensis* (Marsh, 1913)
 Distal segment of maxilla with 4 spines and dorso-lateral
 lobes of 5th thoracic segment without tiny hairs
 *A. spartinus* (Ruber, 1966)

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References

- Botelho, M. J. C. 1999. *Revisao do genero Apocyclops Lindberg, 1942 (Copepoda: Cyclopoida)*. Ph. D. dissertation, Instituto de Biociencias, Universidade de Sao Paulo, Sao Paulo, 106 pp.
- Chullasorn, S., Kangtia, P., Pinkkaew, K. and Ferrari, F. D. 2008. *Apocyclops ramkhamhaengi* sp. nov. (Copepoda: Cyclopoida) in a culture originating from brackishwaters of Chang Island, Trat Province, Thailand. *Zool. Stud.*, 47(3): 326-337.
- Defaye, D. and Dussart, B. H. 1988. Complements a la faune des Crustaces Copepodes des eaux interieures de Guyane française. *Rev. Hydrobiol. Trop.*, 21: 109-125.
- Defaye, D. and Dussart, B. H. 2003. Description of two new species. *Rec. West. Aust. Mus.*, 22: 193-212.
- Doi, M., Ohno, A., Taki, Y., Singhagraiwan, T. and Kohno, H. 1997. Nauplii of the calanoid copepod, *Arcartia sinjiensis* as an initial food organism for larval red snapper *Lutjanus argentimaculatus*. *Suisan Zoshoku*, 45: 31-40.
- Dumont, H. J. and Maas, S. 1988. Copepods of the lagune Ebrie (Cote d'Ivoire). *Rev. Hydrobiol. Trop.*, 2 : 3-7.
- Dussart, B. and Defaye, D. 2006. *World directory of crustacea: Copepoda of Inland Waters II - Cyclopiformes*. Backhuys Publishers, Leiden, 354 pp.
- Dussart, B. and Defaye, H. D. 1985. *Repertoire mondial des Cope-podes Cyclopoides*. C.N.R.S., Paris, 236 pp.
- Dussart, B. H. 1982. Copepodes des Antilles frangaises. *Hydrobiol. Trop.*, 15(4): 313-324.
- Farhadian, O. 2006. *Culture of planktonic cyclopoid Apocyclops dengizicus (Lepeshkin, 1900) and its suitability as live feed for giant black tiger shrimp, Penaeus monodon Fabricius (1798), postlarvae*. Doctoral thesis. Faculty of Science, Universiti Putra Malaysia.
- Farhadian, O., Yusoff, F. M. and Arshad, A. 2007. Ingestion rate of post-larvae of *Penaeus monodon* fed *Apocyclops dengizicus* and *Artemia*. *Aquaculture*, 269: 265-270.
- Farhadian, O., Yusoff, F. M. D., Mohamed, S. and Saad, C. R. 2009. Use of cyclopoid copepod *Apocyclops dengizicus* as live feed for *Penaeus monodon* post-larvae. *J. World Aquac. Soc.*, 40(1): 1-11.
- Fiers, F. 2001. *Meridiocyclops* gen. nov., a new Cyclopoid genus (Crustacea : Copepoda : Cyclopidae) from southern Australia. *Invertebr. Taxon.*, 15: 893-908.
- Herbst, H. V. 1955. Cyclopoida Gnathostoma (Crustacea: Copepoda) von der brasilianischen Atlantikküste. *Kieler Meeresforschung*, 11: 214- 229.
- Holyńska, M., Leggitt, L. and Kotov, A. A. 2016. Miocene cyclopoid copepod from a saline paleolake in Mojave, California. *Acta Palaeontol. Pol.*, 61(2): 345-361.
- Humes, A. G. and Gooding, R. V. 1964. A method for studying the external anatomy of copepods. *Crustaceana*, 6: 238-240.
- Huys, R. and Boxshall, G. A. 1991. *Copepod evolution*. The Ray Society, London, 468 pp.
- Iepure, S. and Defaye, D. 2003. Redescription of *Teratocyclops cubensis* Plesa, 1981 (Copepoda, Cyclopoida, Cyclopidae) from Cuba. *Crustaceana*, 76(5): 591-604.
- Ito Tak. 1957. A new cyclopoid copepod from brackishwater fish ponds in Mie Prefecture, Japan, *Apocyclops japonicas* sp. nov. *Rept. Fac. Fish. Perf. Univ. Mie.*, 2: 463-169.
- Karanovic, T., Eberhard, S. M. and Murdoch, A. 2011. A cladistic analysis and taxonomic revision of Australian *Metacyclops* and *Goniocyclops*, with description of four new species and three new genera (Copepoda, Cyclopoida). *Crustaceana*, 84: 1-67. <http://dx.doi.org/10.1163/001121610X546698>.
- Karanovic, T. 2004 a. Subterranean Copepoda from arid Western Australia. *Crustaceana Monographs*, 3: 1-366.
- Karanovic, T. 2004 b. The genus *Metacyclops* Kiefer in Australia (Crustacea: Copepoda: Cyclopoida), with description of two new species. *Rec. West. Aust. Mus.*, 22: 193-212.
- Kiefer, F. 1934. Neue Ruderfusskrebse von der Inseln Haiti. *Zool. Anz.*, 108: 227-233.
- Kiefer, F. 1956. Freilebende Ruderfusskrebse (Crustacea, Copepoda). Calanoida und Cyclopoida. In: Gessner, F., Vareschi, V. (Eds.), *Ergebnisse der Deutschen Limnologischen Venezuela-Expedition, 1952*, 1: 233-268.
- Kołodziejczyk Andrzej 2015. A new species of *Metacyclops* from a hyporheic habitat in North Vietnam (Crustacea, Copepoda, Cyclopidae). *Zookeys*, 522: 141-152.

- Lee, J. and Chang, C. Y. 2015. *Metacyclops woni* n. sp., a new cyclopoid species (Copepoda : Cyclopoida : Cyclopidae) from Cambodia. *Anim. Syst. Evol. Diversity*, 31(4): 247-256. <http://dx.doi.org/10.5635/ASED.2015.31.4.247>.
- Lepeshkin, V. D. 1900. K faune Copepoda Akmolinskoi oblasti. Obshch. Lyubit. Estestv., *Antropol. Etnogr. Imp. Mosk. Univ.*, 3: 21-30.
- Lim, R. P. and Fernando, C. H. 1985. Review of Malaysian freshwater Copepoda with notes on new records and little known species. *Hydrobiologia*, 128: 71-89.
- Lindberg, K. 1938. Cyclopides (Crustacea copepodes) nouveaux de L'Inde. *Bulletin de la Societe zoologique de France*, 63: 288-302.
- Lindberg, K. 1940. Cyclopoïdes (Crustacea Copepodes) de l'Inde. *Rec. Indian Mus. Calcutta*, 42: 519-526.
- Lindberg, K. 1942. Cyclopides (Crustacea Copepodes) de l'Inde. XI-XIII. *Rec. Indian Mus. Calcutta*, 44: 73-94.
- Lindberg, K. 1954. Cyclopides (Crustacea Copepodes) d'iles du Pacifique Sud (Melanesie et Micronesie) et de Borneo. *Kungl. Fysiogr. Sal. Lund Forh.*, 24(18): 1-14.
- Lindberg, K. 1955. Contribution a l'etude de la faune d'eau douce de corse Cyclopides *Vie Milieu*, 6: 241-247.
- Lindberg, K. 1961. Remarques sur le genre *Metacyclops* (Kiefer, 1927) et description d'un *Metacyclops* nouveau du Portugal. *Kungl. Fysiogr. Sallsk Lund Forh.*, 31(14): 133-145.
- Marsh, C. D. 1913. Report on freshwater Copepoda from Panama, with descriptions of new species. *Washington Smifhson Insf. Misc. Collecf.*, 61(3): 1-30.
- Mirabdullayev, I. M. and Stuge, T. S. 1998. Redescription of *Apocyclops dengizicus* (Lepeschkin, 1900) from Central Asia. *Spixiana*, 21: 173-178.
- Monchenko, V. I. 1975. *Eurycyclops* (Crustacea, Copepoda) in fauna of the Soviet Union. *Dok. Akad. Nauk Ukra*, 5: 471-477.
- Monchenko, V. I. 2003. New north records, redescription and halopathy of *Apocyclops dengizicus* (Copepoda, Cyclopoida). *Vestnik Zoologii*, 37(6): 79-84.
- Nanton, D. A. and Castell, J. D. 1998. The effect of dietary fatty acids on the fatty acid composition of the harpacticoid copepod, *Tisbe* sp., for use as a live food for marine fish larvae. *Aquaculture*, 163: 251-261.
- Pesce, G. L. 2016. *Copepod Web Portal: Cyclopoida*. <http://www.luciopesce.net/copepods/allo.htm> and An update world review of the genus *Metacyclops*. www.luciopesce.net/copepods/metacy.htm (Accessed 6th March 2017).
- Plesa, C. 1981. Cyclopides (Crustacea, Copepoda) de Cuba. *Result at sdes Expeditions Biospeologiques Cubano-Roumainesa, Cuba*, 3: 17-34.
- Rajkumar, W. and Kumaraguru, K. P. 2006. Suitability of the copepod, *Acartia clausi* as a live feed for seabass larvae (*Lates calcarifer* Bloch): compared to traditional live food organisms with emphasis on the nutritional value. *Aquaculture*, 261: 649-658.
- Reid, J. W., Hamilton, R. I. V. and Duffield, R. M. 2002. First confirmed new world record of *Apocyclops dengizicus* (Lepeshkin), with a key to the species of *Apocyclops* in North America and the Caribbean region (Crustacea: Copepoda: Cyclopidae). *Jeffersoniana*, 10: 1-25.
- Ruber, E. 1968. Description of a salt marsh copepod, *Cyclops (Apocyclops) spartinus* n. sp. and a comparison with closely related species. *Trans. Amer. Micro. Soc.*, 87: 368-375.
- Sambrook, J. and Russell, D. W. 2001. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2028 pp.
- Sung, J. Y. and Sung, B. H. 2014. Selection of Copepods as live food for marine fish larvae based on their size, fecundity and nutritional value. *Ocean Polar Res.*, 36(2): 199-208.
- Toledo, J. D., Golez, M. S., Doi, M. and Ohno, A. 1999. Use of copepod nauplii during early feeding stage of grouper, *Epinephelus coioides*. *Fish. Sci.*, 65: 390-397.
- Yoon, H. J. and Chang, C. Y. 2008. Two brackish cyclopoid copepods from southern coast of Korea. *Korean J. Syst. Zool.*, 24(3): 241-250.
- Walter, T. C. and Boxshall, G. 2016. *World of copepods database*. <http://www.marinespecies.org/copepoda/aphia.php?p=taxdetails&id=106441> (Accessed 9th March 2017)

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