



Description of the life cycle of *Nerocila orbignyi* (Guérin, 1832): a cymothoid isopod parasitizing *Plotosus canius* Hamilton, 1822 of Chilika Lagoon, India

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Abstract

This research aims to examine the morphological characteristics of all the life stages of the isopod *Nerocila orbignyi* (Guérin, 1832), which parasitizes the host fish *Plotosus canius* Hamilton, 1822 (Gray eel-catfish) in Chilika Lagoon, Odisha, India. A detailed examination of the morphology, life cycle, and prevalence of the isopod *N. orbignyi*, was conducted following the standard protocols. The observations included 104 females, 32 males, 11 juveniles, and 47 embryonic stages. This study enhances our understanding of this species' biology, making identification clear and straightforward. This study may be helpful in developing effective management strategies that could prevent isopod infections in aquaculture.

Keywords Cymothoidae · Morphology · Life stages · Parasitism · India

Introduction

The Cymothoidae Leach, 1814 are protandric isopod parasites, feeding on the blood and mucous of freshwater, marine, and estuarine fishes (Roy et al. 2024). This family comprises around 380 species distributed worldwide, belonging to 40 genera (Boyko et al. 2024). They can be found attached to different host fishes' buccal, branchial cavities, and body surfaces (Smit et al. 2014). The typical consequences of an attack by these parasitic isopods include gill lacerations, paleness of the gills, and hemorrhaging at

the attachment sites (Mohapatra et al. 2022a). Such infections can lead to muscle damage and loss of nutrition in the juvenile host fish ultimately leading to their death. The negative consequences of these factors hinder fish growth, compromise aquaculture productivity, and threaten the economic viability of the fisheries sector (Keys 1928; Rajkumar et al. 2005; Rameshkumar and Ravichandran 2013, 2014; Bharadhirajan et al. 2014; Aneesh et al. 2019).

The life cycle of parasitic isopods in the family Cymothoidae includes eight developmental stages: zygotic, embryonic, premanca, manca, juvenile, male, transitional, and female. Among these, the premanca and manca stages are non-infectious. Each stage exhibits unique morphological structures personalized to attach to their fish hosts, which complicates species-level identification. In most studies, the description of cymothoid isopods relies on the morphology of the ovigerous female, making other life stages challenging to identify (Aneesh et al. 2016, 2018; Roy et al. 2024). These isopods can disrupt fish populations in aquaculture when larvae enter farms and infest fish stocks (Smit et al. 2014). Understanding the life cycle stages and their impact on fish health can inform effective management strategies, boosting aquaculture productivity and reducing fish loss. The life stages of various cymothoid species, including *Agarna malayi* Tiwari, 1952; *Brucethoa bharrata* Aneesh, Hadfield, Smit and Kumar, 2020; *B. isro* Aneesh et al. 2024a,

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b; *Cymothoa frontalis* H. Milne Edwards, 1840; *C. indica* Schioedte and Meinert, 1884; *Mothocya renardi* (Bleeker, 1857); *Norileca indica* (H. Milne Edwards, 1840); *Ryukyua circularis* (Pillai, 1954) and *Sandythoa tiranga* Aneesh, Bruce, Helna and Biju Kumar, 2024, have been documented in the coastal waters of India (Aneesh et al. 2015, 2016, 2018, 2020, 2024a, b; Vigneshwaran et al. 2018; Kottarathil et al. 2019; Roy et al. 2024).

The genus *Nerocila* Leach, 1818 has 65 species worldwide with 14 species reported from the Indian coast (Trilles et al. 2013; Mohapatra et al. 2022b; Roy et al. 2022). The genus is characterized by a dorsoventrally flattened, non-vaulted body, a trilobed posterior margin of the cephalon, antennae larger than antennules, posterolateral margin of pereonites 6 and 7 extend outward, and uropod rami extends beyond the pleotelson margin, with the endopod being smaller than the exopod (Trilles et al. 2013; Ravichandran et al. 2019). Currently, the Odisha coast is home to seven species of *Nerocila* genus (Dev Roy and Rath 2017; Mohapatra et al. 2022b). The species *N. orbignyi* (Guérin, 1832) was reported from the Mediterranean Sea, the East and West Atlantic Oceans (Kayis and Ceylan 2011; Horton and Baillie 2019), South-East and South-West Indian Ocean (Hale 1940; Kensley 1978; Bruce 1987; Gonz lez 2019) and India (Mohapatra et al. 2022a). However, there is no literature detailing with the various life stages of this species.

The isopod fauna of Chilika Lagoon was first documented in the early twentieth century (Chilton 1916, 1924), with subsequent contributions by Pillai (1961); Sarma et al. (1979, 1981) and Rao (1987). However, studies focusing on parasitic isopods in Chilika Lagoon remain limited compared to other groups (Chilton 1924; Mohapatra et al. 2022b, c; Mohapatra et al. 2024a, b; Nayak et al. 2024; Roy et al. 2024). This study aims to address this gap by investigating different aspects of isopod species in Chilika Lagoon. The objective of the present study is to establish the different life stages of *N. orbignyi* based on specimens collected from Chilika Lagoon, aiming to improve its identification and understanding of its biology.

Materials and methods

Study area

During the periodic surveys from August 2022 to July 2024, 147 individuals of a dark coloured cymothoid isopod belonging to the genus *Nerocila* were collected at Balugaon fish landing centre (N 19°44'25.03"; E 85°12'37.52"); Langaleswar (N 19°39'57.89"; E 85°09'59.39"), and Pathara (N 19°38'34.00"; E 85°10'12.04") of Chilika Lagoon, Odisha, India (Fig. 1).

Collection procedure

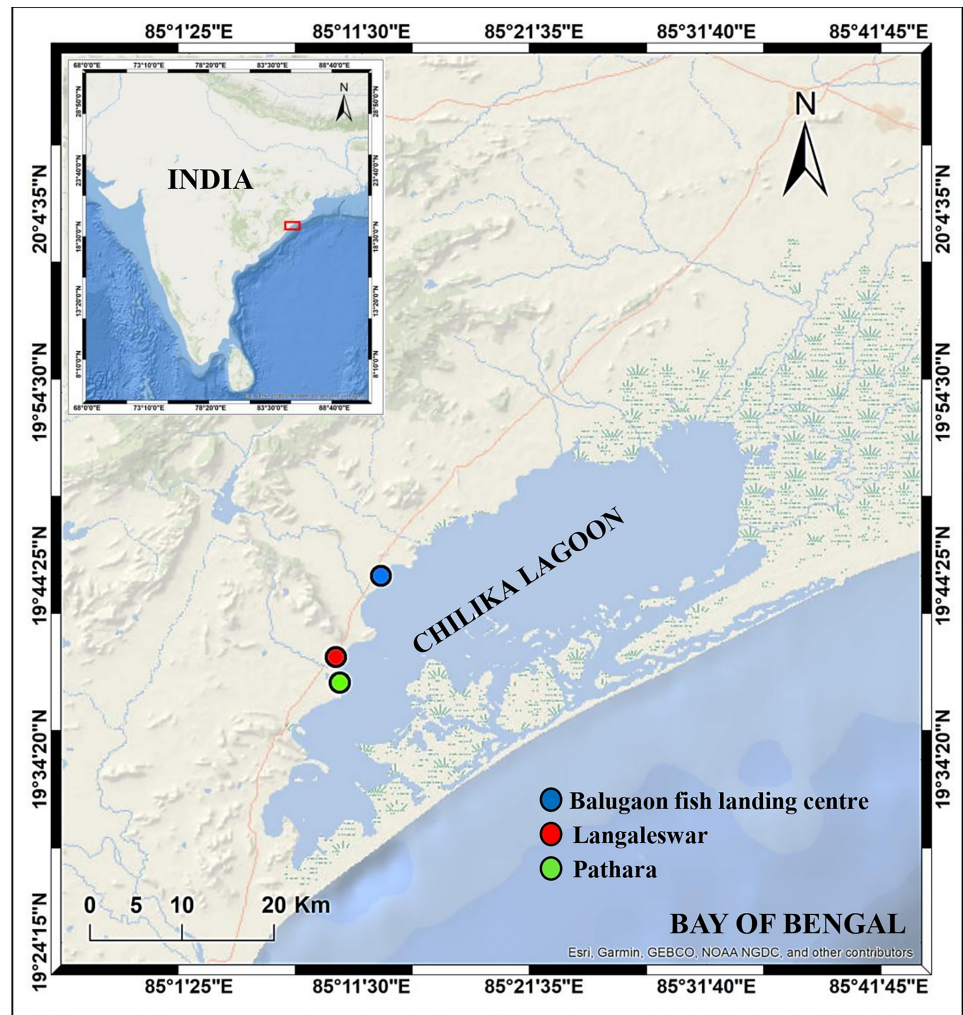
All the individual isopods were found to be parasitising the host fish *Plotosus canius* Hamilton, 1822 (Gray eel-catfish), attaching to the base of either its pectoral or dorsal fins. These host fish were captured using traditional bamboo traps and trammel nets and were part of the regular catch brought to the landing centre for sale. Fresh images of the isopods attached to their host were captured on-site with a Nikon D5600 DSLR camera. The parasites were carefully removed by a pair of forceps and preserved in 70% ethanol for further analysis.

Identification and morphological description procedure

The identification of the parasites was carried out based on the standard keys and description provided by Bruce (1987) and Mohapatra et al. (2022a). The parasitic isopods were found to be *N. orbignyi* (Guérin, 1832). The host fish was identified with the help of necessary pieces of literature (Gomon 1984; Talwar and Jhingran 1991). Appendages of the different life stages were dissected carefully using a dissecting needle and a fine pair of forceps and observed under a stereozoom microscope (Leica S9i) to study the difference in the structure according to the techniques described in Aneesh et al. (2019). The moulting changes of the parasite were determined through microscopical observations of the characteristic changes occurring in the carapace and the appendages following Kappalli et al. (2012). Drawings were digitally inked using the Inkscape v 1.3, vector graphics editor. All the figures were constructed using the Photoshop CS6 image editing software. To collect the eggs, embryonic, premanca and manca stages, each marsupium was dissected, and a fine brush was used to retrieve them. The isopod specimens and the two specimens of the host species were registered separately and deposited in the Molecular Systematics and Evolutionary Biology Laboratory, Post Graduate Department of Zoology, Berhampur University, Odisha, India as MSEBCR103A–MSEBCR103E (104 examples of female); MSEBCR104A–MSEBCR104C (32 examples of male); and MSEBCR105 (11 examples of juvenile). Digital photographs along with hand drawings of different life stages of *N. orbignyi* are provided in the manuscript for better understanding.



Fig. 1 Study area map of isopod collection sites



Results

A total of 147 parasitic isopods, collected after analysing 658 host fish. The descriptions of their life stages are based on observations of 104 females, 32 males, 11 juveniles, and 47 embryonic stages (embryonic stages were observed after dissecting the marsupial sac of the ovigerous female stage).

Description of marsupial stages

Zygotic stage: Approximately 106 to 301 eggs were observed in the brood pouch or marsupium. Each egg is round with a flattened portion on one side, light yellow in fresh specimens, and has a volume ranging from 0.3 to 0.8 mm³ (Fig. 2a).

Embryonic stage I: The embryos are subspherical to ovoid in shape, with a light cream coloration, measuring 0.9 to 1.3 mm in length (Fig. 2b).

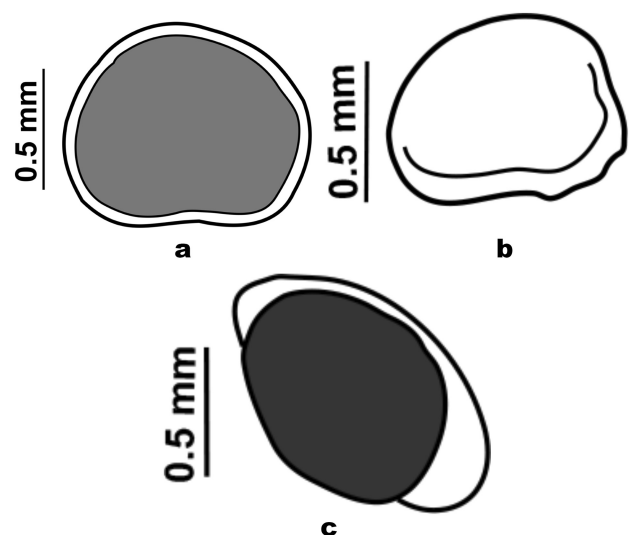


Fig. 2 *Nerocila orbigny*, embryonic stages **a** Zygote **b** Embryonic stage I **c** Embryonic stage II

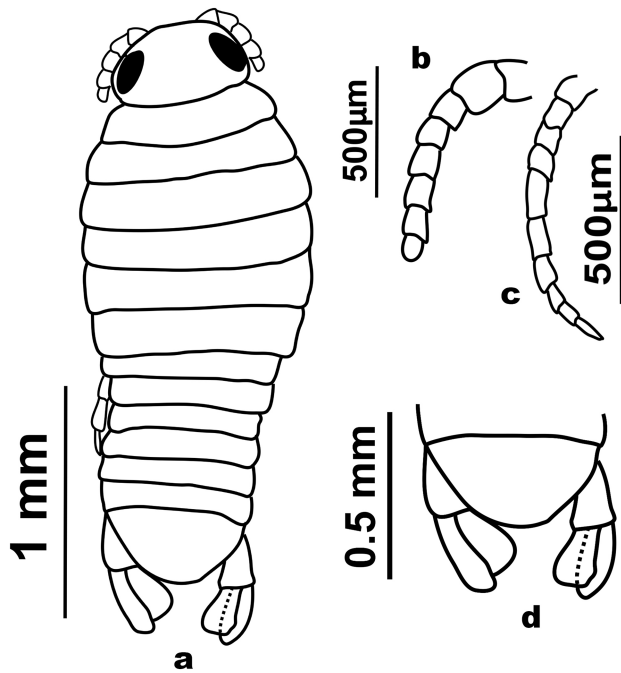


Fig. 3 *Nerocila orbignyi*, premanca **a** Dorsal view **b** Antennule **c** Antenna **d** Dorsal view of uropods and pleotelson

Embryonic stage II: The embryos are ovoid in shape, featuring a transparent sac with a centrally located, round yolk mass (Fig. 2c).

Description of premanca stage

The body is elongated and semitransparent with a total length (TL) nearly 2.5 times the total width (TW) (Fig. 3a). The cephalon is subtriangular, without any lobes. Eyes black, ovate, and prominently developed. Few chromatophores scattered between eyes on the dorsal surface of the cephalon. The antenna and antennule small with 10 and 8 segments respectively, barely reaching till posterior margin of cephalon (Fig. 3b–c). Pereonite, size increases gradually from 1–5 and again decreases in segments 6 and 7. Rows of chromatophores confined to dorsolateral and mid-dorsal region of the body. Pleonites five in number and subequal in size. Chromatophores distributed on mid-dorsal and dorsolateral region, in continuous with that of pereonites. Ventral margins of pleonites also with chromatophores. Uropod extend beyond the lower margin of the triangular pleotelson. Uropodal exopod slightly longer than endopod. Endopod wider than exopod. Exopod and endopod without any setae.

Description of manca stage

Body elongated with yellowish pereonites and pleonites (Greyish in fresh condition). TL: 2.3–2.7 mm; TW:

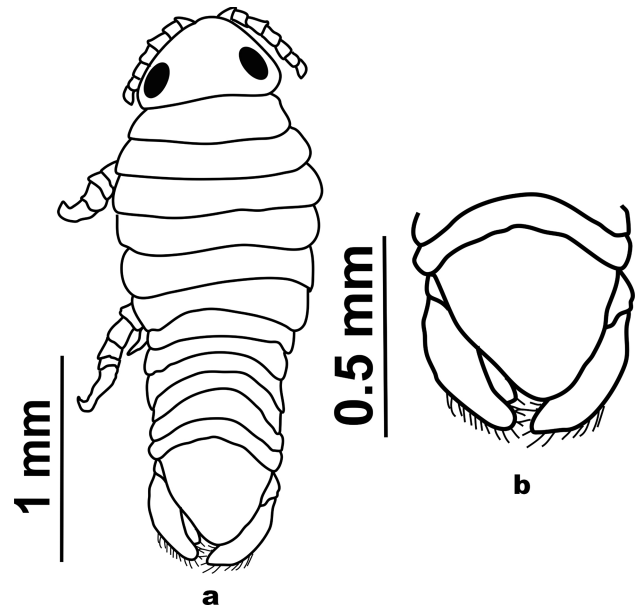


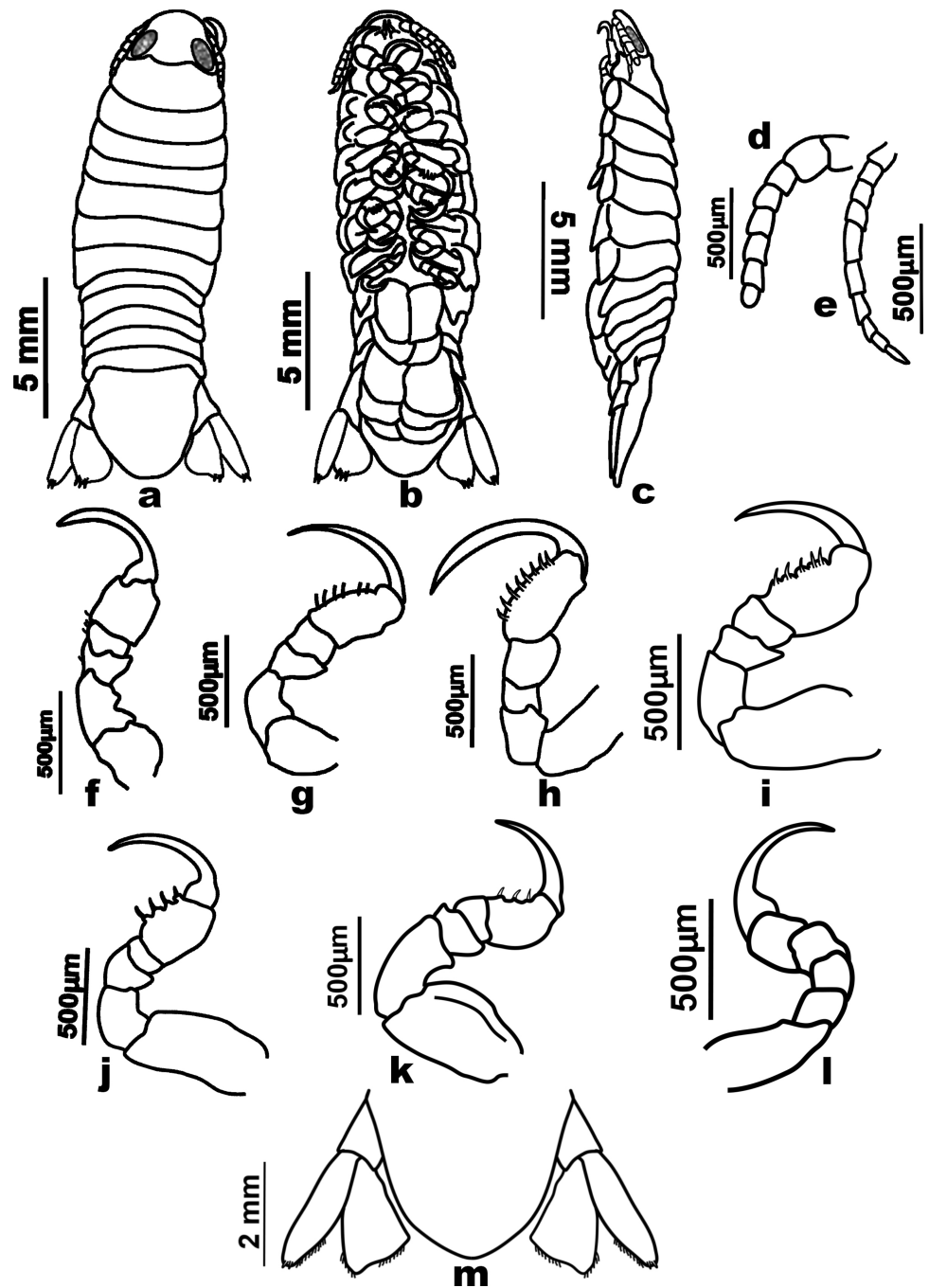
Fig. 4 *Nerocila orbignyi*, manca **a** Dorsal view **b** Dorsal view of pleotelson and uropods

0.7–0.9 mm. Cephalon subtriangular, without any lobes. Eyes developed and positioned dorsolaterally. Antennule short, its length, same as that of the premanca stage, reaching midportion of cephalon. Antenna longer to antennule, extending to posterior margin of the cephalon (Fig. 4a). Mouthparts underdeveloped. Pereon consists of 7 segments, with pereonite 1 being widest, followed by pereonite 4. Anterolateral margins of all pereonites poorly defined. Width of pereonites decreases progressively from 1st to 3rd, while pereonite 7 relatively narrower. Pleotelson subtriangular in shape. Uropods significantly larger, extending beyond margin of pleotelson, both uropods and pleotelson equipped with numerous fine setae, aiding in swimming (Fig. 4b). Propodi of pereopods 1–3, provided with 5–6 fine setae.

Description of juvenile stage

Body elongated, its TL: 6.3–6.6 mm and TW: 2.0–2.2 mm (Fig. 5a–c). Cephalon subtriangular in shape, anterior margin convex. Cephalon width nearly 2 times its length. Eyes large and prominent. Chromatophores on cephalon appears as trident mark between eyes. Eyes black and prominent. Antenna and antennule extend beyond posterior margin of cephalon. Antenna with 10 segments and antennule 8 segments (Fig. 5d–e). Posterolateral corners of pereonites smooth, and blunt. First two coxal plates smaller compared to other and sub rectangular in shape. Posterior margins of the last three coxae are slightly pointed, propodi of pereopods 2–5, with 4–6 fine setae. The propodus of pereopod 6

Fig. 5 *Nerocila orbignyi*, juvenile **a** Dorsal view **b** Ventral view **c** Lateral View **d** Antennule **e** Antenna **f–l** Pereopods 1 to 7 (m) Dorsal view of pleotelson and uropods



with 4 setae while propodi of pereopods 1 and 7 devoid of any setae (Fig. 5f–l). Pleotelson subtriangular with numerous soft setae on posterior distal margin. Uropods extend beyond tip of pleotelson. Uropodal exopod teardrop shaped while endopod subtriangular. Both exopod and endopod sub equal in size with setae on distal margin (Fig. 5m). Chromatophores extends mid-dorsally from pereonite 1 to distal margin of pleotelson.

Description of adult male

Males elongated, with sleek appearance with TL 9.0–14.0 mm, approximately three times body width, TW 3.1–5.2 mm (Fig. 6a–b). Cephalon trilobed posteriorly, width 1.5 times its length. Eyes well-developed. Antennule and antenna contain 8 and 10 segments respectively (Fig. 6c–d); the antennule more robust but shorter than

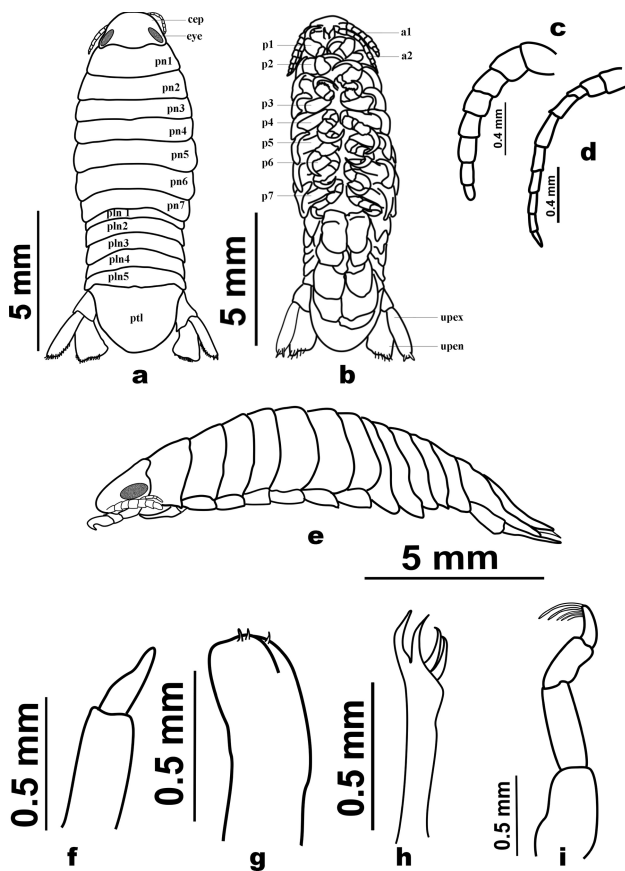


Fig. 6 *Nerocila orbignyi*, male **a** Dorsal view **b** Ventral view **c** Antennule **d** Antenna **e** Lateral view **f** Maxilliped **g** Maxilla **h** Maxillule **i** Mandible (Abbreviation and their full form: cep: cephalon; a1: antennule; a2: antenna; pn1: Pereonite 1; pn2: Pereonite 2; pn3: Pereonite 3; pn4: Pereonite 4; pn5: Pereonite 5; pn6: Pereonite 6; pn7: Pereonite 7; pln1: Pleonite 1; pln2: Pleonite 2; pln3: Pleonite 3; pln4: Pleonite 4; pln5: Pleonite 5; ptl: Pleotelson; p1: Pereopod 1; p2: Pereopod 2; p3: Pereopod 3; p4: Pereopod 4; p5: Pereopod 5; p6: Pereopod 6; p7: Pereopod 7; upex: Uropodal exopod; upen: Uropodal endopod)

antenna, both structures reaching up to the mid portion of pereonite 1. The mouth parts well developed (Fig. 6f–i). A prominent mid-dorsal brown line runs from optical region to tip of pleotelson. A pair of dark lines run along dorso-lateral side of body, extending from pereonite 1 to tip of uropodal exopods. Width of pereonite 1 large followed by pereonite 5, while pereonite 4 smallest. The posterolateral corners of pereonites 1, 2, and 3 undeveloped. Pereonites 4 and 5 exhibit slightly developed posterolateral angles, while pereonites 6 and 7 sharply pointed posterolateral angles that curve downwards. Size of coxal plate increases gradually from pereonite 2–7. The propodi and carpus of pereopods 1–7 with 5–6 fine setae (Fig. 7a–g). A bilobed penis is present on anterior region of the pleopods. All pleonites visible in dorsal view. Length of pleopods gradually decreases. Peduncles of pleopods 1–4 with coupling hooks. Pleotelson

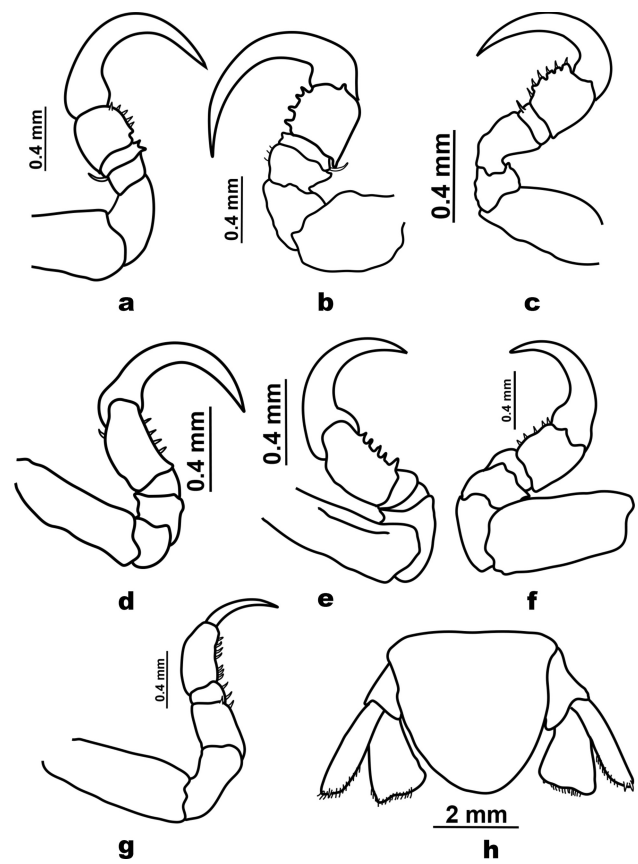


Fig. 7 *Nerocila orbignyi*, male **a–g** Pereopods 1 to 7 **h** Dorsal view of pleotelson and uropods

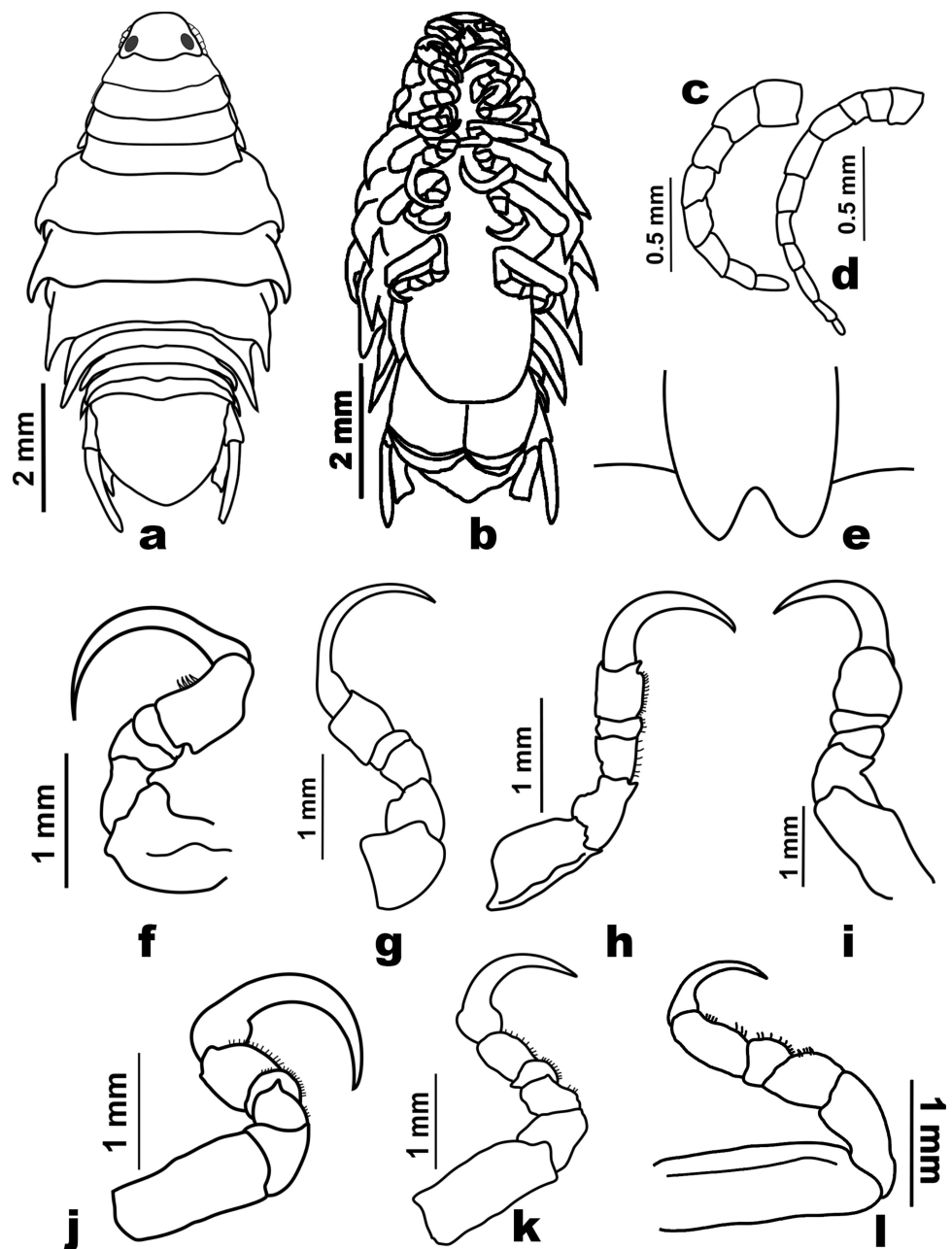
subtriangular, shield-like. Uropodal exopod longer than endopod. Endopod depressed than exopod.

Description of transitional stage

Body elongated, 1.5–2.0 times longer than its width (Fig. 8a–b). Cephalon width roughly two-thirds of its length, with a pair of indistinct eyes occupying about half of cephalon width. Antenna with 10 segments, antennule with eight parts, reaching to midpoint of first pereonite. Antennule more robust than antenna, with first three segments thicker than remaining five distal segments. Pereonite 1 longest, posterolateral angles blunt. Posterolateral angles of pereonites 5–7, pointed and ascending in size from the fifth to seventh pereonite. Coxal plates on pereonites 2–4 not pointed, but on pereonites 5–7 pointed. Ventrolateral margins of first and second pleonites extend posteriorly, aligning with fifth pleonite's margin. Pleotelson shield-shaped, featuring a caudomedial point. Exopod is nearly 0.2–0.4 times the size of endopod. Dactyli of first pereopod more hoot-shaped and robust, while pereopod 7 has three spines on carpus and 5 spines on propodal palm (Fig. 8).



Fig. 8 *Nerocila orbignyi*, transitional stage **a** Dorsal view **b** Ventral view **c** Antennule **d** Antenna **e** Penis **f–l** Pereopods 1 to 7



Description of ovigerous female

Body white with 3 clear dark brown stripes (Fig. 9a–c). TL: 16.3–18.9 mm; TW: 8.9–10.2 mm; cephalon length, CL: 2.0–2.4 mm; cephalon width, CW: 2.7–3.4 mm. Two dark stripes positioned on either side at the dorso-lateral margin, and one located on mid-dorsal region. Cephalon is trilobed with two lateral lobes horn like and middle lobe flat on top. Eyes well developed. Antennule shorter than antenna. Antennule with 8 segments and reaching up to the posterior margin of cephalon. Antenna with 10 segments and reaches up to the middle of pereonite 1. Mouth parts well developed (Fig. 9f–i). Posterolateral angles

of pereonites 2 and 3 are produced but the edges are not pointed. Posterolateral angles of pereonite 4 and 5 are little pointed and those of 6 and 7 are fully developed and pointed. Posterolateral angle of pereonite 7 reaches up to pleonite 2. Pereonite 7 is concealed under that of sixth. The propodi and carpus of pereopods 1–7, excepting pereopod 4 and 5, are provided with 5–6 fine setae (Fig. 10a–g). Width of pleonites 1–4 subequal. Pleonite 5 wider than other pleonites. Spines present on the propodus and carpus of each pereopods. Lobe-like structure present on the merus. Marsupium large, extending between the bases of pereopods 3–7 and consisting of 5 overlapping flaps. Each marsupium harbors around 106–301 eggs.

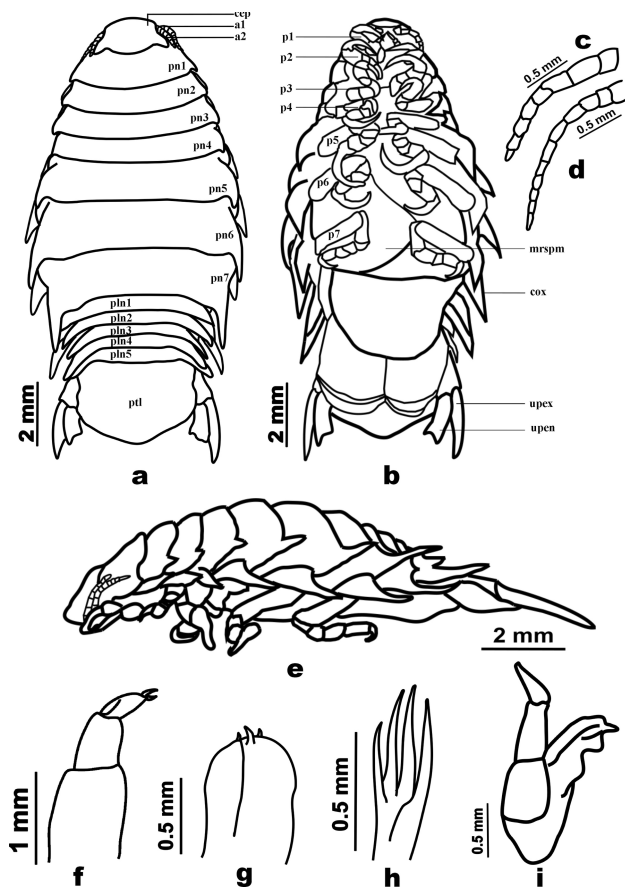


Fig. 9 *Nerocila orbignyi*, ovigerous female **a** Dorsal view **b** Ventral view **c** Antennule and antenna **d** Maxilliped **e** Lateral view **f** Maxilliped **g** Maxilla **h** Maxillule **i** Mandible (Abbreviation and their full form: cep: Cephalon; a1: Antennule; a2: Antenna; pn1: Pereonite 1; pn2: Pereonite 2; pn3: Pereonite 3; pn4: Pereonite 4; pn5: Pereonite 5; pn6: Pereonite 6; pn7: Pereonite 7; pln1: Pleonite 1; pln2: Pleonite 2; pln3: Pleonite 3; pln4: Pleonite 4; pln5: Pleonite 5; p1: Pereopod 1; p2: Pereopod 2; p3: Pereopod 3; p4: Pereopod 4; p5: Pereopod 5; p6: Pereopod 6; p7: Pereopod 7; mrspm: Marsupium; cox: Coxa; upex: Uropodal exopod; upen: Uropodal endopod)

Life cycle of *N. orbignyi*

The marsupial phase typically encompasses a zygotic stage, three embryonic stages (ES I, ES II, and ES III), and two larval stages (premanca and manca), each characterized by distinct morphological features (Papapanagiotou and Trilles 2001; Aneesh et al. 2015, 2024a, b). This study reveals that the life cycle of *N. orbignyi* consists of three main phases: marsupial, free-living, and infective (Fig. 11). Within the marsupium of adult females, only the zygotic stage, two embryonic stages (ES I and ES II), and two larval stages are observable. During ES I, early tissue differentiation is evident, with the embryo adopting a subspherical or ovoid shape and displaying a dark yellow coloration. After this stage, the eggs are ready to hatch, producing premanca larvae. These larvae then undergo moulting to transition into

the manca stage within the marsupium. Shortly afterward, the manca larvae are released into the surrounding seawater, initiating a free-swimming phase before eventually infesting their host fish.

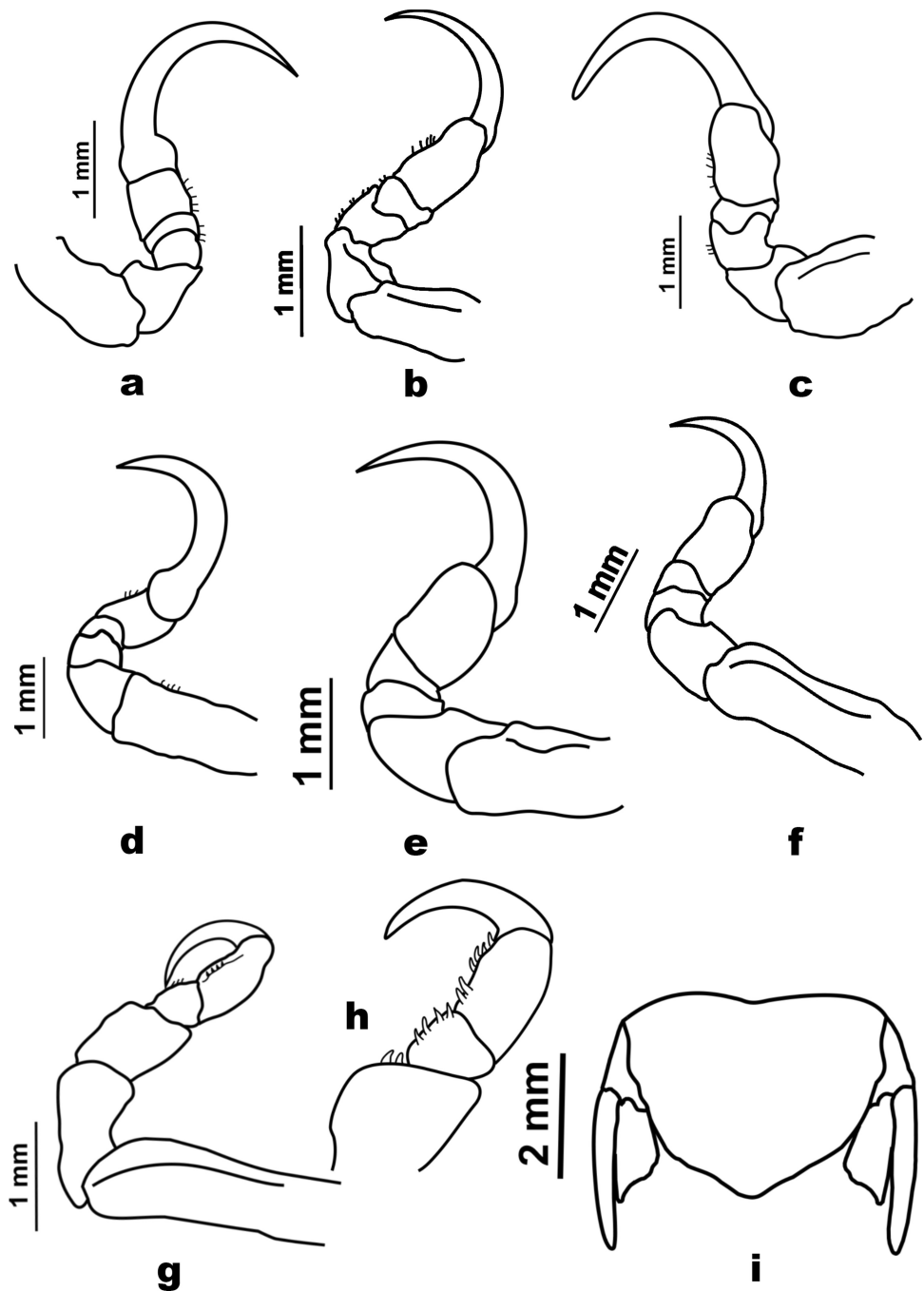
During the free-living phase, juveniles attach to the pectoral and dorsal fins of the host fish, where they moult further into males and then transition into adult females, with an intermediate transitional stage. Adult females mate with adult males to become ovigerous females. Each ovigerous female possesses a marsupial sac containing eggs and provides parental care for the eggs, embryos, and larval stages, ensuring protection and support until they are ready to be released into the surrounding environment.

Discussion

This study presents the first complete documentation of the life cycle of *N. orbignyi*, detailing the transitional, juvenile, manca, and premanca stages essential for accurate identification across growth stages. With this new understanding, the juveniles, adult males and females of *N. orbignyi* can now be reliably distinguished from other species within the same genus. Furthermore, this is the first comprehensive description of all life stages for any species within the genus *Nerocila*. The observation of fewer embryonic stages in *N. orbignyi* compared to other parasitic isopods may reflect an adaptation specific to body surface-dwelling isopods, as opposed to buccal or branchial isopods. Both buccal (*C. frontalis*) and branchial (*A. malayi*) show four embryonic stages (Aneesh et al. 2015, 2018).

Interestingly, the mid-dorsal chromatophore distribution in *N. orbignyi* aligns with that of similar looking species like *N. arres*, *N. sigani* and *N. serra*, though notable differences exist in pereonite structure and endopod serration. In *N. arres* and *N. serra*, all pereonite posterolateral angles are pointed (Ravichandran et al. 2019), whereas in *N. orbignyi*, only the pereonites 5–7 have pointed posterolateral angles (Bruce 1987). Additionally, the lateral margin of uropodal endopod in *N. serra* and *N. arres* is serrated, while in *N. orbignyi* it is smooth (Bowman and Tareen 1983; Bruce 1987). The serrations are shallow in case of *N. serra* while *N. arres* have deep serrations. Moreover, *N. orbignyi* possess an evenly rounded posterior portion of telson posteriorly (vs. telson with distinct caudomedial lobe in both *N. serra* and *N. arres*) (Bowman and Tareen 1983; Ravichandran et al. 2019). The species *N. acuminata* and *N. japonica* come under *N. orbignyi* group (Bruce 1987). But both the species have produced and pointed posterolateral margins of all the pereonites (vs. only the pereonites 5–7 have pointed posterolateral angles in *N. orbignyi*). The endopod is wedge-shaped in *N. acuminata*, while it is obliquely inward in *N. orbignyi* (Mohapatra et al. 2022a). In the juveniles of *N. orbignyi*, the

Fig. 10 *Nerocila orbignyi*, ovigerous female **a–g** Pereopods 1 to 7 **h** Enlarged view of pereopod 7 **i** Pleotelson with uropods



antenna and antennule are small, reaching only the posterior margin of the cephalon. In contrast, in *Cymothoa indica*—a buccal cavity-attaching species whose life cycle was previously described from the Chilika Lagoon—the antenna reaches up to pereonite 2. Additionally, the antenna of *N. orbignyi* consists of 10 articles, while that of *C. indica* comprises 11 articles (Roy et al. 2024). The size of manca of *N. orbignyi* is larger as compared to *C. indica*. The antenna and antennule are shorter, not reaching the posterior margin of cephalon (vs. antenna and antennule are longer in *C. indica*

vs. antenna only longer in *Agarna malayi*, a branchial cavity attaching species reported earlier from the Chilika Lagoon). The pereonite 4 is second widest in *N. orbignyi* but pereonite 2 is second widest in *C. indica*. The juveniles of *N. orbignyi* are larger as compared to *C. indica*. The cephalon is having convex anterior margin, like the cephalon of *A. malayi* (vs. flattened and ventrally bend in *C. indica*). Chromatophore distribution is seen in between the eyes in *N. orbignyi* but not in *C. indica*. Pleotelson is triangular in *N. orbignyi* (vs. elliptical in *C. indica* and *A. malayi*). Uropods are tear drop

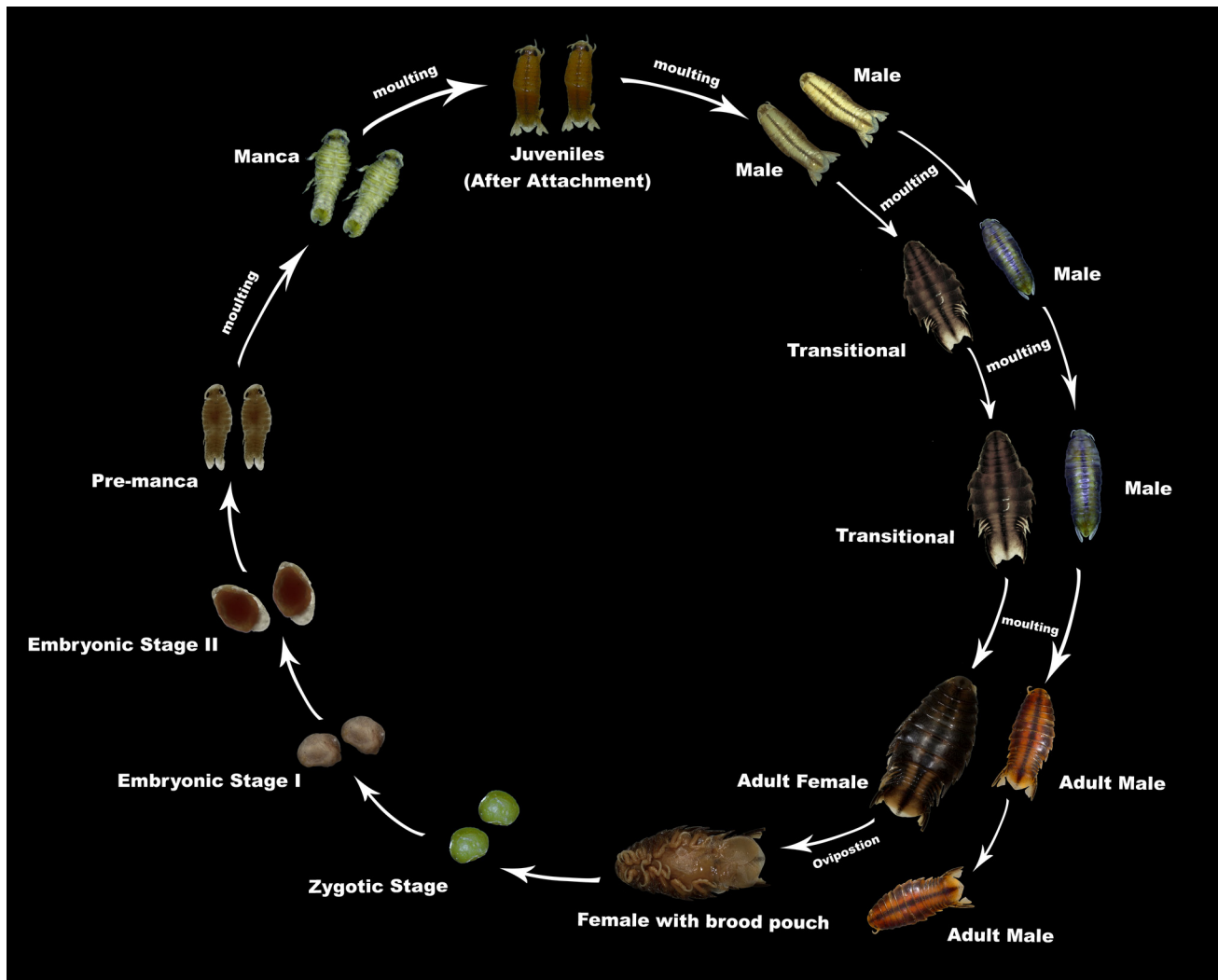


Fig. 11 Schematic representation of the life cycle of *Nerocila orbignyi*

shaped in *N. orbignyi* (vs. paddle shaped in *C. indica* and *A. malayi*) (Aneesh et al. 2018; Roy et al. 2024).

Conclusion

The present study provides valuable insights into the biology of Cymothoids and their effects on host populations. Additionally, the findings of this study have potential applications in improving aquaculture management practices, promoting better productivity and healthier aquatic systems. To enhance understanding of infection dynamics and prevalence, future studies should consider assessing the mean infection intensity per host. However, further research is needed to explore the life stages of other skin attaching cymothoid isopod species to enhance our understanding of their development and ecological roles.

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Author contributions All authors contributed to the conception and design of the work. SR and SKM: Designing the study, sampling, identification, and preparation of the draft manuscript; CP: Study on the mouth parts; HKS: Critical analysis and updating the manuscript; JKS: Conceptualisation, Fund acquisition, updating the manuscript, and critical analysis.

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Data availability All data used in this study are available at the Molecular Systematics and Evolutionary Biology Laboratory, Post Graduate



Department of Zoology, Berhampur University, Odisha, India, and can be verified upon request by contacting the corresponding author.

Declarations

Competing interests Authors don't have any conflict of interest.

Ethical approval The isopod species and host fish examined in this study are not listed under any protection category of the Wildlife (Protection) Act of 1972, India. Furthermore, ethical clearance was not required as the samples were collected from dead host fish, which were obtained with the assistance of local fishermen.

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