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Agarna malayi Tiwari 1952 (Crustacea: Isopoda: Cymothoidae) Parasitising the Marine Fish, *Tenualosa toli* (Clupeidae) from India: Re-description/description of Parasite Life Cycle and Patterns of Occurrence

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Panakkool Thamban Aneesh, Kappalli Sudha, Ameri Kottarathil Helna, and Gopinathan Anilkumar (2018) This paper re-describes the female stage of *Agarna malayi* Tiwari 1952, a protandrically hermaphroditic parasitic cymothoid, and describes the remaining life cycle stages for the first time. The re-description (female phase) of *A. malayi* was made based on the type specimens deposited by Tiwari (1952) in the National Zoological Collections of the Zoological Survey of India (NZC-ZSI) and data obtained from several live specimens collected from Ayyikkara Fish Landing Centre (11°51'N, 75°22'E, of Malabar Coast, India) and Marina Beach (13.0500°N, 80.2824°E, Bay of Bengal, India). General morphology, mouthparts and appendages of the species' life cycle stages such as larvae, juveniles, male and transitional stages are also reported for the first time. We closely observed 80 marine fish species belonging to 35 different families to identify the potential host of *A. malayi*, and only recovered the parasite from one fish, *Tenualosa toli*, signifying *A. malayi*'s oligoxenous host specificity; the prevalence and intensity of parasitisation is 17.3 and 1.86 %, respectively. The present paper also discusses sequential life cycle stages of the species.

Key words: Parasitic cymothoid, Lifecycle, Clupeid fish, Host-parasite interaction, India.

BACKGROUND

Members of the family Cymothoidae Leach 1814 are known to be obligatory parasites of teleost fishes from diverse ecosystems. The adverse effect of its parasitism has generated considerable interest among parasitologists and fish pathologists (Brusca 1981; Aneesh et al. 2016). Cymothoids usually prefer specific parts of the host fish; for instance, the *Nerocila* Leach 1818 and *Anilocra* Leach 1818 are usually found attached to the general body surface, species of

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Cymothoa Fabricius 1793, *Ceratothoa* Dana 1852 and *Glossobius* Schiödte and Meinert 1883 are found in the buccal cavity, while species of *Agarna* Schiödte and Meinert 1884, *Joryma* Bowman and Tareen 1983, *Ryukyua* Williams and Bunkley-Williams 1994 and *Mothocya* Costa and Hope 1851 prefer the branchial cavities (Aneesh et al. 2013 2015; Bruce et al. 2016).

In the literature, Cymothoid-infecting fish have been reported all over the world, particularly in South Africa (Hadfield et al. 2013 2014), South America (Thatcher et al. 2003 2007), Europe (Trilles 1994; Horton and Okamura 2001); America (Brusca 1981; Wiliams and Wiliams 1994), Australia (Bruce 1987; Martin et al. 2015; Bruce et al. 2016), Philippines (Yamauchi et al. 2005), Kuwait (Bowman and Tareen 1983), China (Yu and Li 2003), Malaysia (Seng and Seng 1990) and India (Tiwari 1952; Pillai 1964; Trilles et al. 2011; Aneesh et al. 2015). Our knowledge on cymothoid parasitisation of marine fishes in India still needs to be updated.

Agarna Schiödte Meinert, 1884, a genus in the family Cymothoidae, currently include four valid species: *A. bengalensis* Kumari, Hanumantha, Rao and Shaymasundari 1990, *A. cumulus* (Haller 1880), *A. malayi* Tiwari 1952 and *A. pustulosa* Pillai 1954. Three additional species of *Agarna* (*A. brachysoma* Pillai 1964, *A. engraulidis* Barnard 1936 and *A. tartoor* Pillai 1954) were synonymized with *Joryma* Bowman and Tareen 1983 by Bowman and Tareen (1983).

Agarna malayi Tiwari, 1952, a very poorly known cymothoid, was described 65 years ago by Tiwari, (1952) based on ovigerous female specimens collected from its clupeid host fish, *Nematalosa nasus* (Bloch, 1795), from Kolkata, India. The author illustrated the dorsal and lateral view of the specimen and provided a description of mouth parts and few pereopods. Later, Pillai (1964) transferred this species to the genus *Indusa* Schiödte and Meinert 1884 with a brief description; but Bowman and Tareen (1983) retained the species under *Agarna.*

Cymothoids exhibit protandric hermaphroditism, so a description of their life cycle stages (e.g. larvae and adults) would facilitate precise species identification, irrespective of the infective stages. We therefore 1) re-described females of *Agarna malayi* based on the type specimens identified by Tiwari (1952) extant in the NZC-ZSI and on the data obtained from several live specimens collected recently; 2) describe all other life cycle stages including male, transitionals, juvenile and larvae (manca I and manca II).

Though the occurrence of *A. malayi* has recently been reported from the Malabar Coast, India (Aneesh et al. 2015), the precise information regarding their distribution and pattern of host-parasite interaction are still lacking. At this juncture, this paper addresses the questions of the distribution, prevalence, intensity, host specific and site specific nature of *A. malayi* parasitisation. An attempt was also made to discuss the sequential life cycle stages of *A. malayi*.

MATERIALS AND METHODS

Live and fresh fishes were obtained from the Ayyikkara Fish Landing Centre (11°51'N, 75°22'E, Malabar Coast, India) to check for parasitisation by *Agarna malayi*. Much care was taken to collect the fishes directly from local fishermen and/ or fishing boats, which were just harbored to the landing centres and thereby ensured the reproducibility of sampling of parasites. During the study period of November 2009 to November 2013, we have observed over 80 species of marine fishes belonging to 35 families for the presence of Cymothoids. Additional collections were made from Fish Landing Centre at Marina Beach, Chennai (13.0500°N, 80.2824°E, Bay of Bengal, India) from March to June, 2016. Collections were made twice a week. Immediately after each collection, body parts of the fish were closely examined for the presence of cymothoids using a hand lens. The recovered parasites and their hosts were brought to the laboratory and subjected to detailed examination with the aid of a stereo microscope (Leica-S6D). The live cymothoids were fixed in 5% formaldehyde and preserved in 75% ethanol (Ramakrishna 1980; Aneesh et al. 2016) and subjected to detailed taxonomic identification (Tiwari 1952) according to appropriate taxonomical keys. Total lengths of both host fish and the parasite (*A. malayi*) were measured. The identification of life cycle stages and classification of female stages were done according to Bakenhaster et al. (2006) and Aneesh et al. (2016). Larval stage nomenclature followed the Atlas of Crustacean Larvae by Martin et al (2014). The description and the type series of *A. malayi*, comprising the holotype and paratype, were also thoroughly examined and kept in good condition, extant in the NZC-ZSI. Dissection and mounting of appendages were done according to Aneesh et al. (2016). Mouthparts and appendages of the recovered live and fresh parasite (*A. malayi*) were carefully dissected out using a dissecting needle and a pair of fine forceps, and observed under microscopes (Leica DM-750 and Leica M-205A). The specimens were microphotographed using a multi focusing stereo microscope (Leica-M205A) and image capturing software (Leica Application Suit). The drawings of the observed mouthparts and appendages were performed using a camera lucida attached the microscope (Leica M-205A). Fish taxonomy and host nomenclature were performed according to FishBase (Froese and Pauly 2017) and Catalogue of Fishes (Eschmeyer 2017). The prevalence (P) and intensity (I) of recovered *A. malayi* were calculated by the methods described in Margolis et al. (1982) and Bush et al. (1997). Voucher specimens were deposited in the collections of PCM-CBRL, Sree Narayana College, Kannur, Kerala, India.

Abbreviations

The following abbreviations are used in the text or in the checklist: NZC-ZSI- National Zoological Collections-Zoological Survey of India, PCM-CBRL-Parasitic Crustacean Museum, Crustacean Biology Research Laboratory, Sree Narayana College, Kannur, Kerala, India.

RESULTS

TAXONOMY

Family Cymothoidae Leach 1814
Genus *Agarna* Schiödte and Meinert 1884

Agarna Schiödte and Meinert 1884 - Barnard 1936; Tiwari 1952: p 295-300, pl. iv, text fig. 1-2; Pillai, 1954; Bowman and Tareen 1983, p 21; Aneesh 2014

Type species: *Agarna cumulus* (Haller, 1880)

Agarna malayi Tiwari 1952

Agarna malayi Tiwari 1952: p 295-300, pl. iv, text fig. 1-2; Bowman and Tareen 1983, p 21; Aneesh et al. 2015b: p. 1-8, fig. 1 a-d.
Indusa malayi (Pillai 1964): p 211-223, fig. 3 and 7d; Trilles 1994, 1981; Trilles and Vala 1975
Indusa ophueseni Pillai 1954: p.15.

Host: All the present Indian material is from *Tenualosa toli* (Valenciennes) (Clupeidae). The other host fishes are *Nematalosa nasus* (Bloch, 1795) (Clupeidae) (Tiwari 1952) and *Mugil ophueseni* (Bleeker) (= *Valamugil cunnesius* (Valenciennes)) (Mugilidae) (Pillai 1954, 1964).

Distribution: Kolkata (Tiwari, 1952), Travancore (Pillai 1954), Kayamkulam Lake, Kerala, southwestern coast of India (Pillai 1964), Bay of Bengal and Malabar Coast of Kerala, India (present study).

Material examined: 55 ♀♀, 5 transitionals, 50 ♂♂, 4 juveniles, 28 manca I and 36 manca II.

Type material: Holotype: 1♀, from *Nematalosa nasus*, purchased from Bow Bazar, Kolkata, India; Coll. Shri Malay Kumar Datta, Reg. No. C3121/2 ZSI.

Paratype: 1♀ from *Nematalosa nasus,* purchased from Bow Bazar, Kolkata, India; Coll. Shri Malay Kumar Datta, Reg. No.C3122/2 ZSI (remaining 5 ♀♀ deposited by Tiwari in 1954 were damaged).

Non-type material: All from *Tenualosa toli* Ayyikkara Fish Landing Centre (Lat. 11°51'N, Long. 75°22'E; Malabar Coast, India; Coll. Aneesh deposited in PCM. 1 ♀ Reg. No. PCM AM-01; 1 ♂ Reg. No. PCM AM-02; 1 ♀ Reg. No. PCM AM-03; 1 ♂ Reg. No. PCM AM-04; 1 ♀ Reg. No. PCM AL-05; 1 transitional Reg. No. PCM AL-23; 1 transitional Reg. No. PCM AL-25; 12 manca I Reg. No. PCM AM-17; 7 manca II, Reg. No. PCM AM-27.

Description (Figs. 1-13): *Ovigerous female* (Figs. 1, 2 E and F, 3 and 4): Asymmetrical and hunched body, slightly longer than wide, widest at pereonite 4. One side of the body almost straight and other side strongly recurved. Cephalon symmetrical, 1.8-2 x as wide as long, roughly triangular with narrow round apex, accommodated in the deeply recessed amphicephalic process of pereonite 1. Eyes small, distinct, sub-dorsal and in the posterio-lateral of cephalon. Pereonite 1 without coxa, all the coxae are visible in dorsal view; 2-3 transversely placed, 4-7, triangular, circuitously disposed on antero-lateral surface of the lateral margin and the posterior margin free from the pereonite. All the pereonite except p-1, asymmetrical. Pereonite 1 longest, pereonites 2-3 subequal in length; 4 longer than 3. Pereonite 5-7 decreasing in length progressively, pereonite 7 shortest. The pereonites 1-4 abruptly increase in width towards one side; pereonite 4 widest and gradually decrease the width from 5-7. Pleonites 2-5 visible, increasing progressively in width posteriorly from 1 to 5; pleonite 5 widest and longest. Pleotelson 1.6-1.8 x as wide as long, slightly shorter than pleonite 5, posterior margin

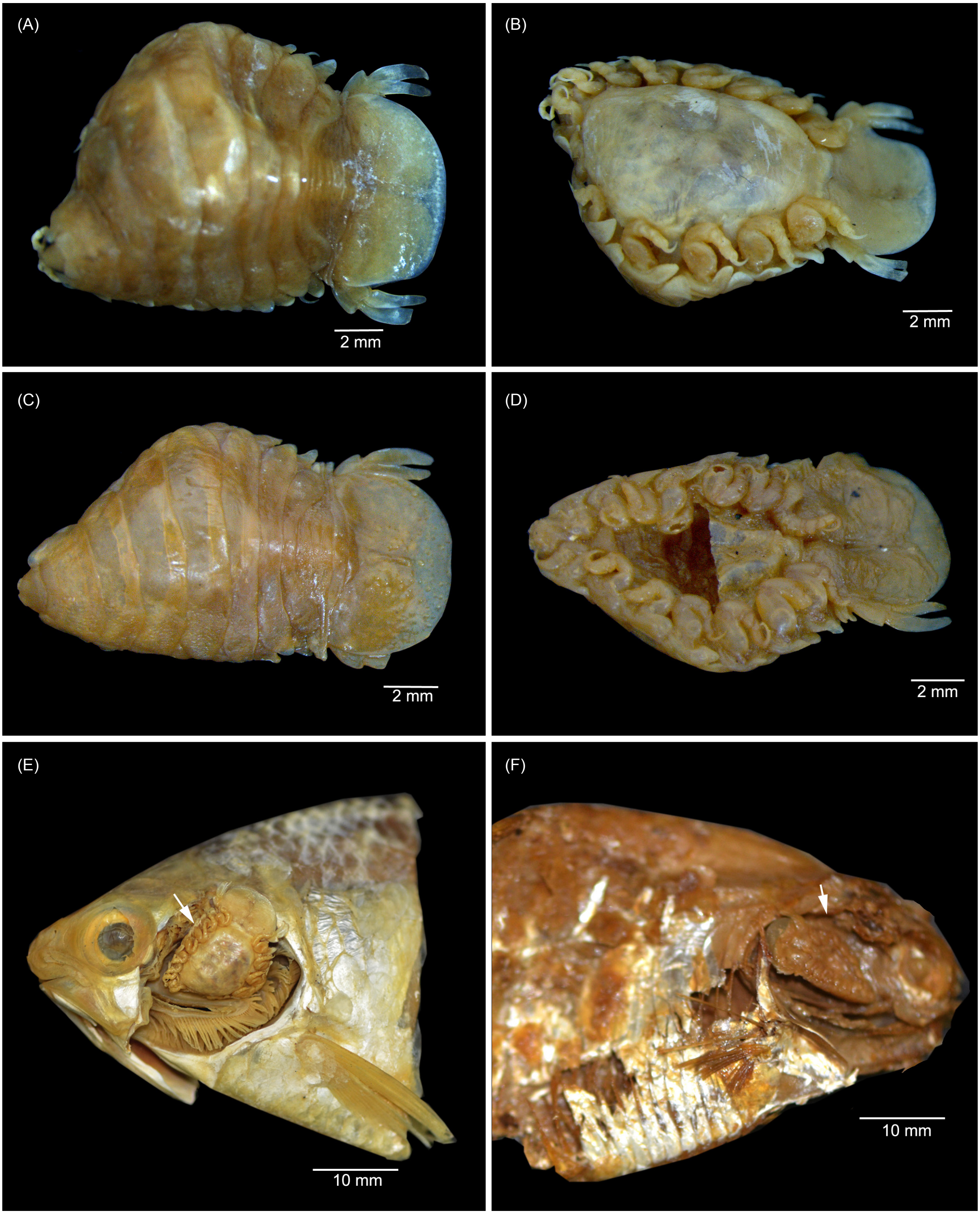

Fig. 1. Photographs of the holotype and paratype of *Agarna malayi* Tiwari 1952 specimens studied: (A) holotype (C3121/2) dorsal view; (B) holotype (C3121/2) ventral view; (C) paratype (C3122/2) dorsal view; (D) paratype (C3122/2) ventral view; (E) branchial cavity of *Nematalosa nasus*, showing the holotype (C3122/2) *in situ*; (F) branchial cavity of *Nematalosa nasus*, showing the paratype (C3122/2) *in situ.*

broadly rounded. Presence of many pustules on dorsal surface of pleotelson.

Antennule stouter than antenna, composed of 8 articles; articles 4-8 with many setae. Antenna with 9-13 articles, longer and stouter than antennule. Mandible with a well-developed incisor process. Mandible palp 3 segmented and without setae in ovigerous female; basal segment border, apical segment short and conical. Maxillule with 4 unequal slightly recurved apical spines; outer pair much shorter than inner. Bi-lobed maxilla with 2 slightly recurved spines on inner median lobe and two spines on outer lateral lobe. Maxilliped without oostegial lobe; article 3 with 3 large terminal recurved spines.

Pereopods 1-7 without spines; pereopod 1 short and robust, gradually increase in size from 1 to 7, P-7 longest. Pleopods not distinctly visible in dorsal view; pleopod 2 without appendix masculina. Exopodite of pleopod 1-5 without setae. Uropod rami short, flattened extend almost up to the distal margin of pleotelson; rami subequal in length, curved and apically rounded.

Brood pouch with 4 pairs of overlapping oostegites arising from the bases of pereopods 2, 3, 4 and 6; 2nd oostegites small, 4th and 6th large and 3rd medium. Number of eggs or larvae per brood pouch ranges from 110 to 320 according to the size of the female.

Male (Figs. 2 A and B, 5 and 6): Relatively smaller than ovigerous female and transitional stage. Body longer than wide and symmetrical, 2.5-2.6 x as long as wide. Cephalon triangular with sub-truncate anterior border. Eyes dorsal and larger than female. Pereonite 1 anteriorly trisinuous, anterio-lateral corners slightly produced. Pereonite 1 longest, 2-6 successively shorter. Pereonites 3-5 subequal in width and widest. All the coxae visible in dorsal view, coxae 2-4 more or less equal to pereonite, 5 and 6 longer than pereonite and 7 shorter than pereonite. All pleonites except pleonite 1 visible, increasing progressively in width posteriorly; pleonite 5 widest and longest. Pleotelson as long as wide, slightly shorter than pleonite 5 and distally semicircular.

8 articled antennule stouter and shorter than antenna. Article 2 slightly wider than others. Antennae 9-11 articled, decreasing gradually in width, terminal article with 2-6 setae. Mandible palp 3 segmented, articles 3 with few setae on terminal margin. Maxillule with 4 apical spines slightly recurved; maxilla bi-lobed, with 1 slightly recurved spines on inner median lobe and two spines on outer lateral lobe. Maxilliped without oostegial lobe and a palp with 3 recurved spines on article 3.

Pereopods 1-7 gradually increasing in size, without spines; basis increasing in width progressively. Penes visible on sternite 7. Pleopods not distinctly visible in dorsal view. Pleopod 2 with appendix masculina. Endopodite of pleopods 4 moderately and 5 strongly folded. Uropod rami, nearly reaching the distal margin of pleotelson; rami subequal in length, curved and apically rounded (Fig. 7).

Pereopods 1-7 gradually increasing in size, without spines; Pereopods 1 short and robust, P-7 longest. Pleopods not distinctly visible in dorsal view; pleopod 2 with appendix masculina. Exopodite of pleopod 1-5 without setae. Uropod rami short, flattened extend up to the distal margin of pleotelson; rami subequal in length, curved and apically rounded (Fig. 7).

Transitional (Figs. 1 C and D and 5): Body 2.35-2.4 times as long as wide; slightly curved towards one side, Eyes small and moderately distinct. Cephalon partially immersed in poorly developed amphicephalic process of pereonite 1, as long as wide, anterior margin truncate. Pereonites, pleonites, antennules, antenna and mandible palp are similar to those of the ovigerous female and maxilla, maxilliped similar to those of the male. A rudimentary penis is visible on sternite 7. Pleotelson 1.8-2 times as wide as long, slightly wider than pleonite 5 and uropods similar to those of the ovigerous female. Pereopods and pleopods similar to those of female and male.

Juvenile (Figs. 1 H and 8): Body approximately 3.3 times as long as wide, relatively narrower and smaller than those of the female, transitional stage and male. Eyes prominent, ovate and conspicuous in dorsal view; anterior margin of cephalon rounded, not immersed in pereonite 1. Pereonite 1 longest, pereonite 2, 3, 4 subequal; pereonite 4 and 5 widest and subequal. Pereonite 5-7 gradually decreasing in length. Pleonites, subequal in length, gradually decreasing in width towards posterior. Pleotelson as long as wide, apical margin rounded with many small reduced setae.

Antennule 8 articled, reaching the anterior margin of pereonite 1; articles 4-8 with few spinules and article 8 with few terminal aesthetics. Antenna longer than antennule, 9 articled, extending to anterior margin of pereonite 2; all the article with few spinules. Article 2 of mandible palp with 2 marginal setae; third article with 3 marginal setae and one apical recurved seta. Maxillule, maxilla and maxilliped similar to those of the male stage.

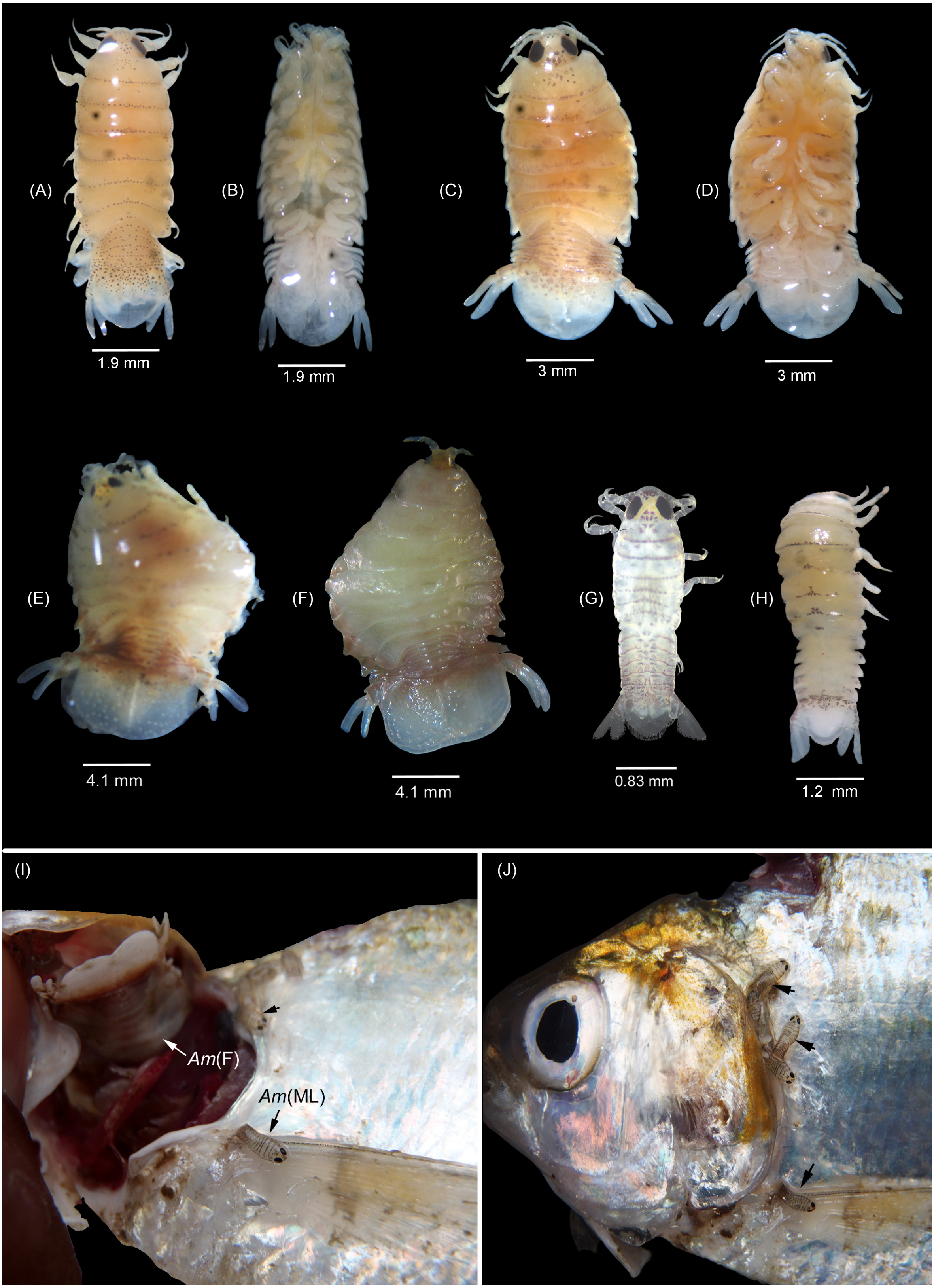

Fig. 2. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes). (A-B) male dorsal and ventral view; (C-D) transitional stage dorsal and ventral view; (E-F) ovigerous female dorsal view; (G) manca II larva; (H) juvenile; (I) ovigerous female on *T. toli*; (J) manca II on *T. toli*.

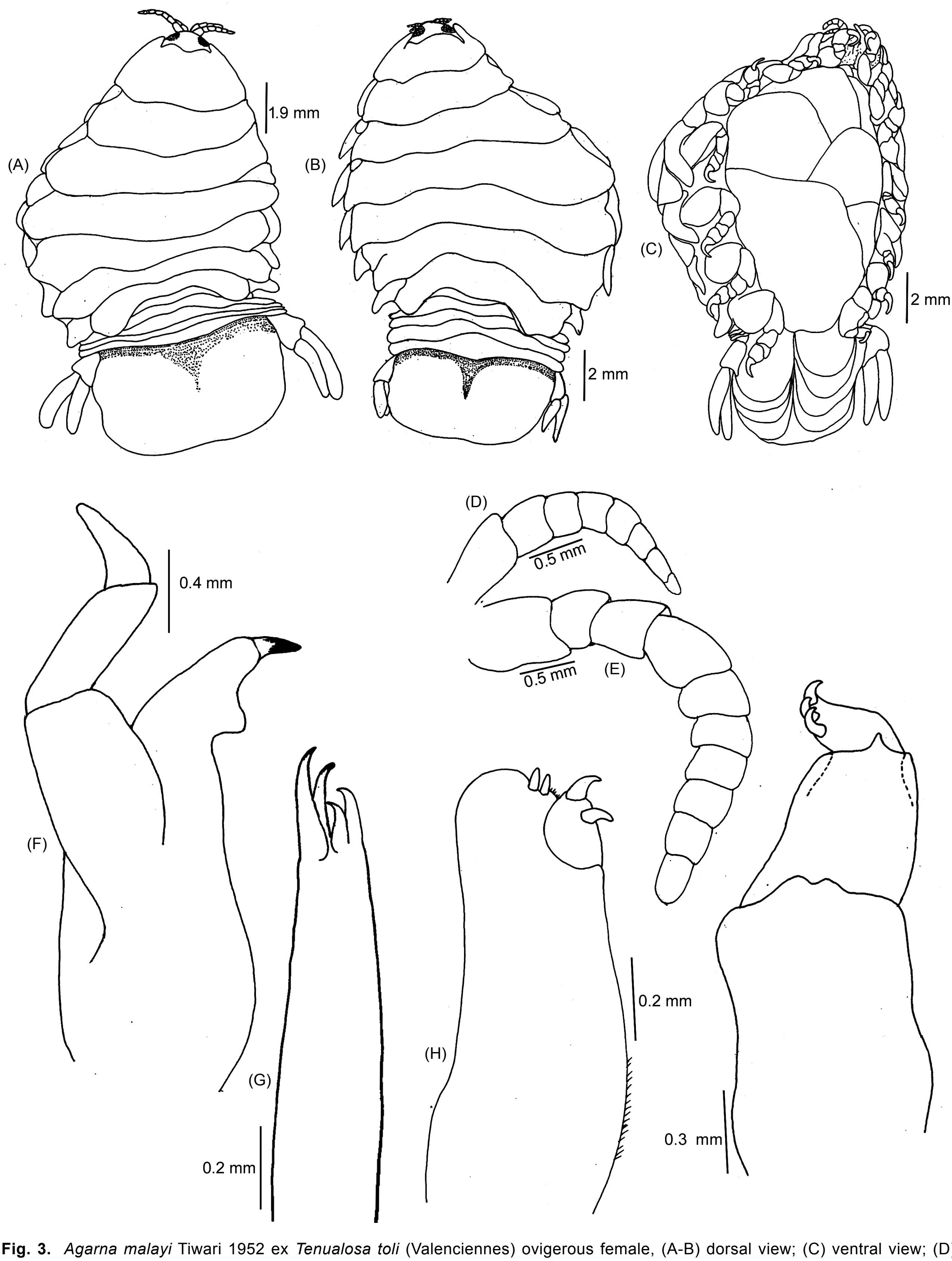

Fig. 3. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) ovigerous female, (A-B) dorsal view; (C) ventral view; (D) antennule; (E) antenna; (F) mandible; (G) maxillule; (H) maxilla; (I) maxilliped.

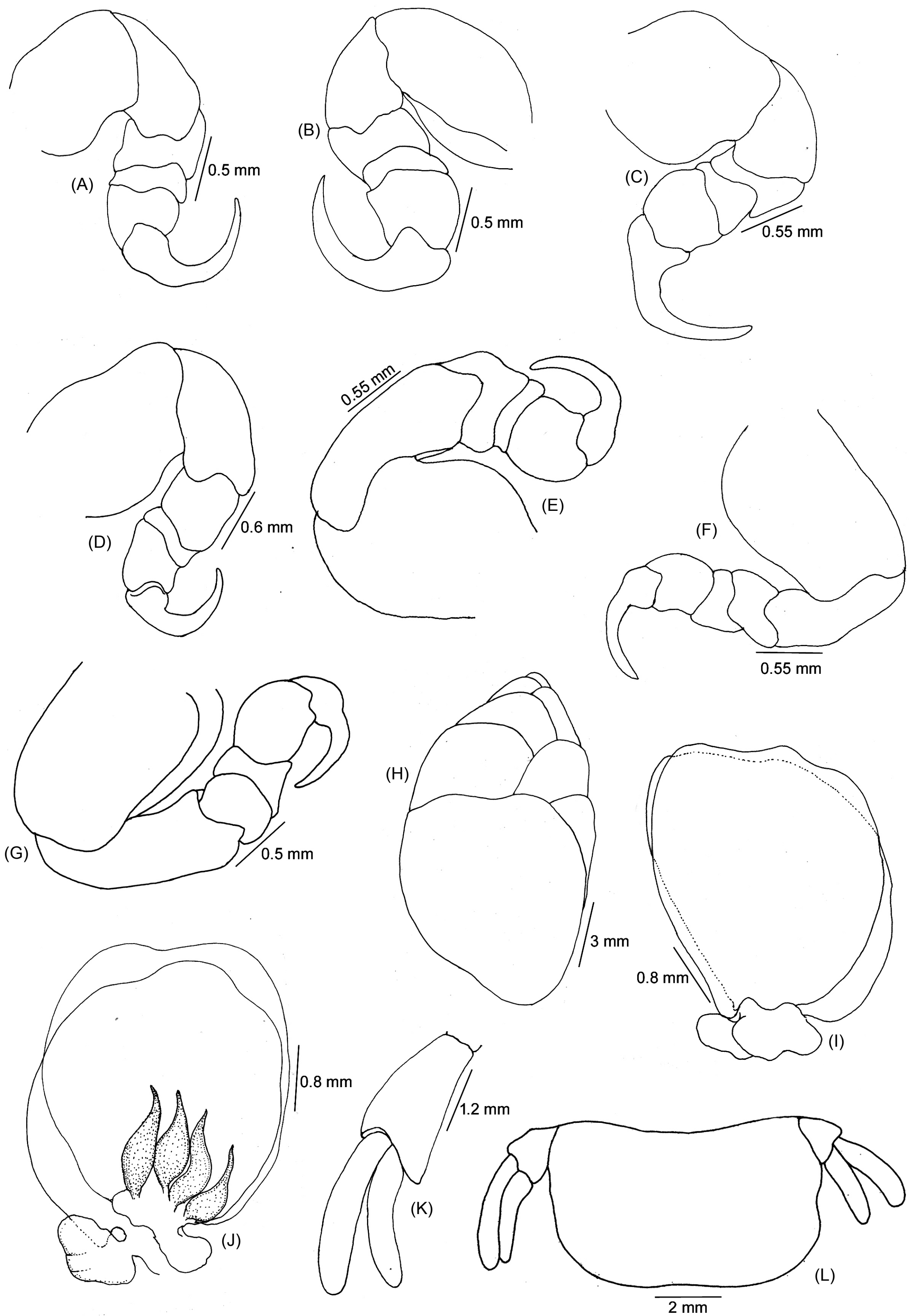

Fig. 4. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) ovigerous female, (A-G) pereopods 1-7; (H) brood; (I) pleopod 2; (J) pleopod 5; (K) uropod; (L) pleotelson and uropods.

Pereopod 1-7 with 2-5 spines on posterior margin of propodus; except pereopod 5 and 7 all other pereopod with one spine on the posterior margin of merus. Dactylus of pereopods without tooth and about two times longer than propodus. Penes not developed. The lateral margin of pleopods without setae. Median margin of protopod with 4/5 retinaculae. Pleopod 2 with thick and vestigial appendix masculina, as long as endopodite. Uropod extending beyond the distal margin of pleotelson, inner margin of basis with few setae; rami slightly curved, endopodite relatively large. Endopodite margin few setae. Exopodite few setae, restricted to the inner margin (Fig. 9).

Manca II (Figs. 1G, 10 and 11): Body elongated and transparent, 3.3 times as long as wide, Eyes black, prominent, ovate and prominent in dorsal view. Cephalon 1.5 times as wide as long and golden yellow in colour. Pereonite 1 relatively long; pereonites 2, 3 and 4 subequal in length; longer than pereonites 5 and 6; pereonites 5 and 6

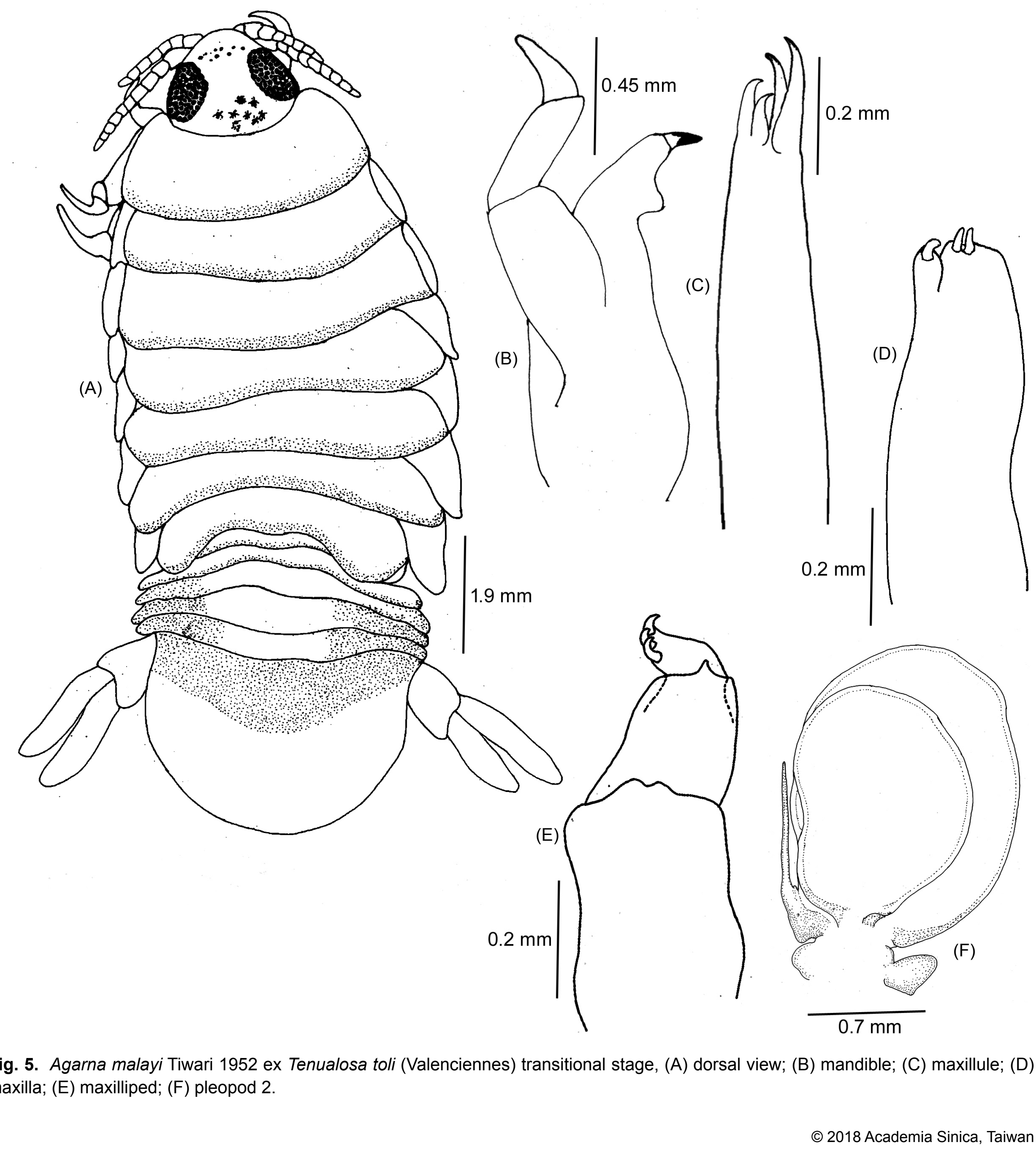

Fig. 5. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) transitional stage, (A) dorsal view; (B) mandible; (C) maxillule; (D) maxilla; (E) maxilliped; (F) pleopod 2.

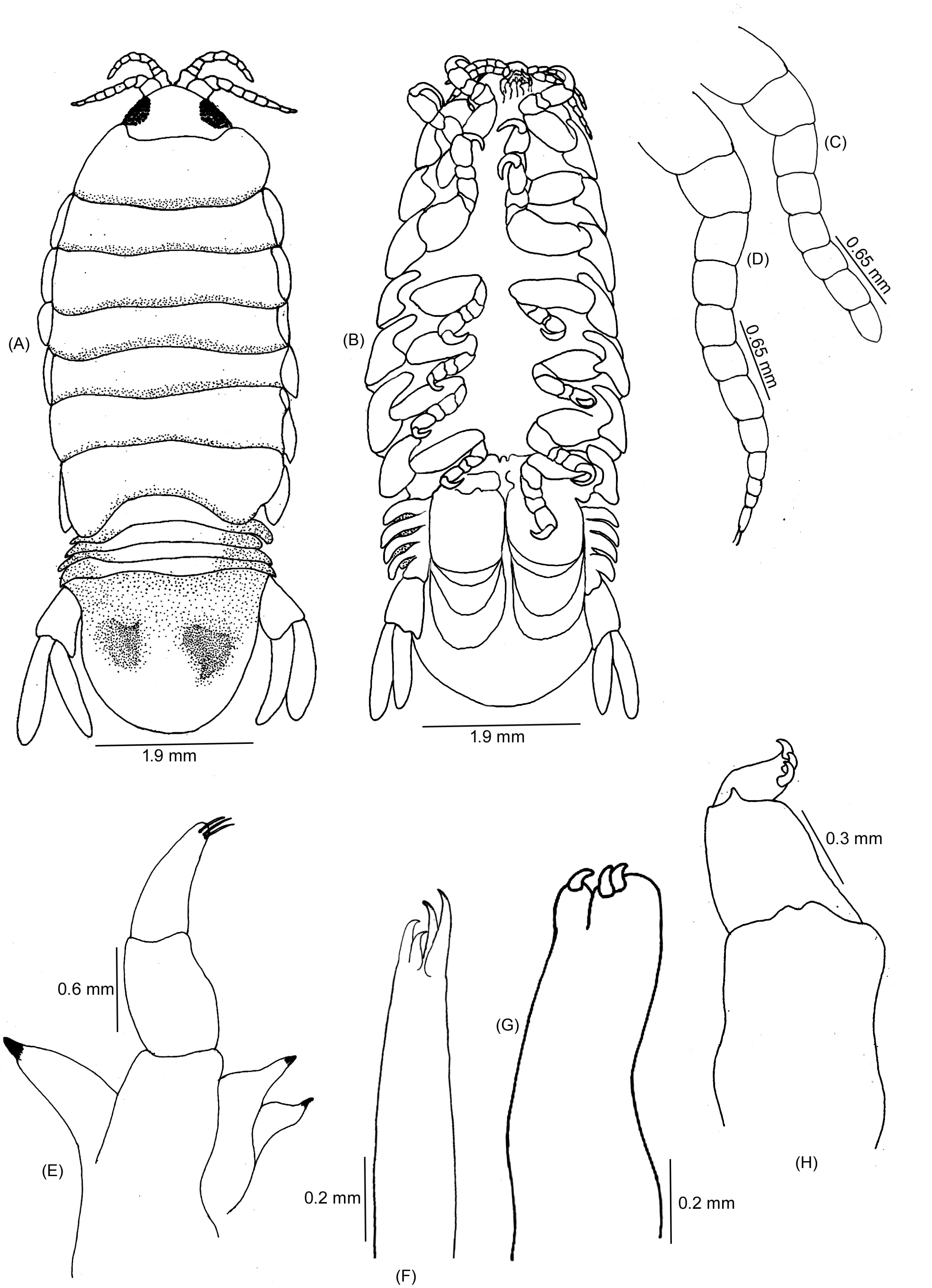

Fig. 6. *Agarna malayi* Tiwari 1952 ex *Tenualosa toil* (Valenciennes) male (A) dorsal view; (B) ventral view; (C) antennule; (D) antenna; (E) mandible; (F) maxillule; (G) maxilla; (H) maxilliped.

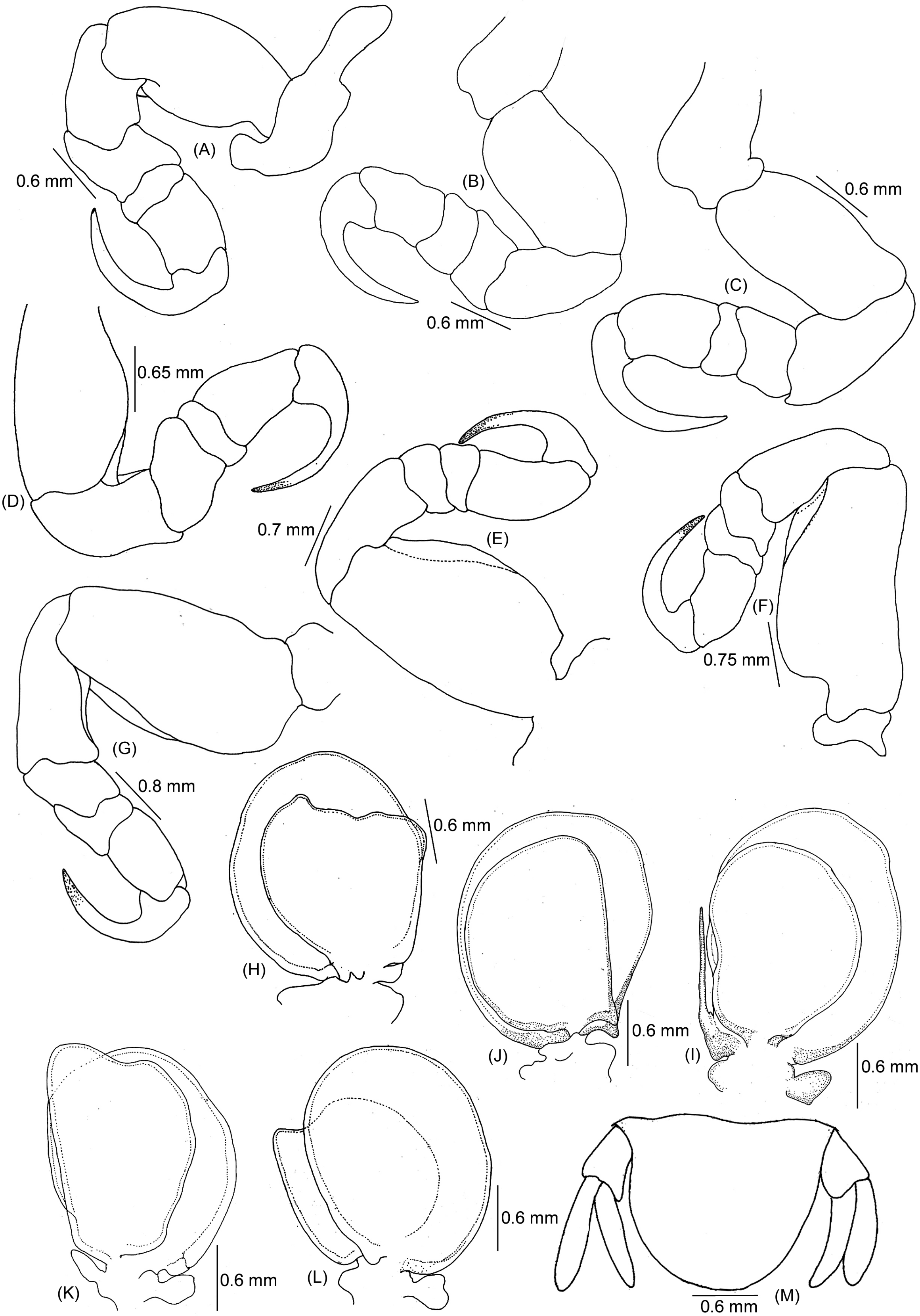

Fig. 7. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) male, (A-G) pereopods 1-7; (H-L) pleopods 1-5; (M) pleotelson and uropods.

subequal in length; pereonite 7 short and narrow; pereonites 5 widest. All pleonites visible and subequal in width and length; 4.5 times as wide as long. Pleotelson 1.3 times as wide as long and the apical margin with 40-42 plumose setae.

Eight articled antennule extends to posterior margin of pereonite 1; all the articles with setae and 3-4 spinules and article 8 with four terminal aesthetascs. Antenna longer than antennule, 11 articled, extending to posterior margin of pereonite 2; all the articles with spinules and article 11 with few terminal aesthetascs. Article 2 of the mandible palp with two seta arising from the disto-lateral margin; article 3 with four marginal setae and one apical recurved seta. Maxillule, maxilla and maxilliped similar to those of the male stage.

Six pereopods; pereopod 1-3, with one spine on merus. Dactyls of all the pereopod without tooth. Propodus of pereopod 1-3 broad with 5-6 tooth, 4-6 with few spines. Pleopods not distinctly visible in dorsal view. Pleopod 1 with 20-28 plumose setae on endopodite and 32-36 plumose setae on exopodite. Pleopod 2-5, apical margin of exopodite with 32-36 plumose setae. Uropod rami sub equal, extending strongly beyond the distal margin of pleotelson, curved and apically rounded. Exopodite with 26-28 plumose setae and apical margin with one small and one larger slightly recurved spine; endopodite with 20-22 plumose setae and apical margin with one slightly recurved spine. Uropods and pleotelson with many dark chromatophores.

Manca I (Fig. 12): Body elongated and transparent, 3.8-4.2 times as long as wide, Eyes

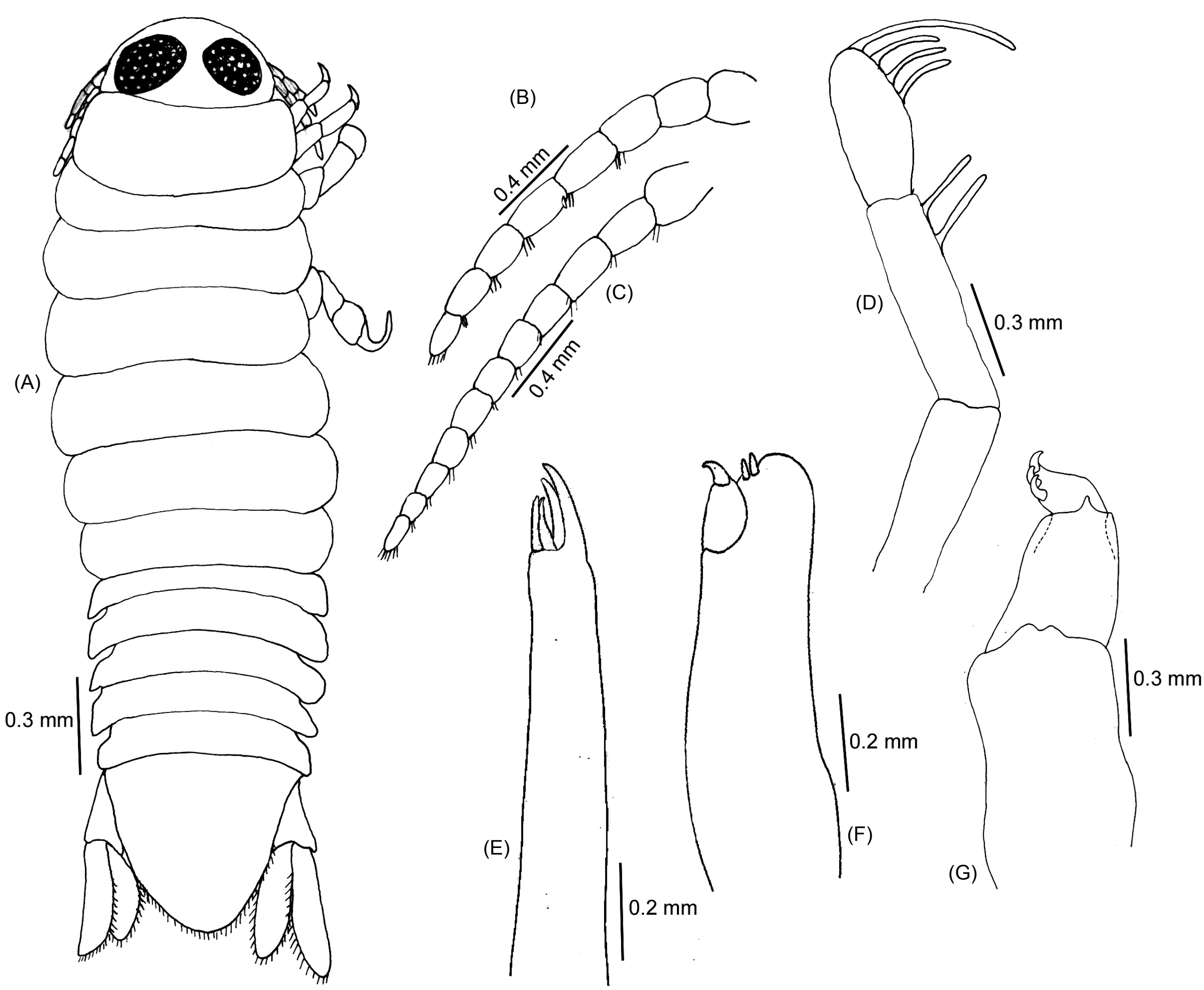

Fig. 8. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) juvenile, (A) dorsal view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped.

black, prominent and conspicuous in dorsal view. Cephalon 1.5 times as wide as long, not immersed in pereonite 1. Yolk globules are visible in the pereon, between the pereonites 2 and 6. Pereonite 1 slightly long, pereonite 7 short and narrow. Pereonites 5 widest. All pleonites visible and subequal in width and length; 3.3 times as wide as long. Pleotelson as wide as long, without plumose

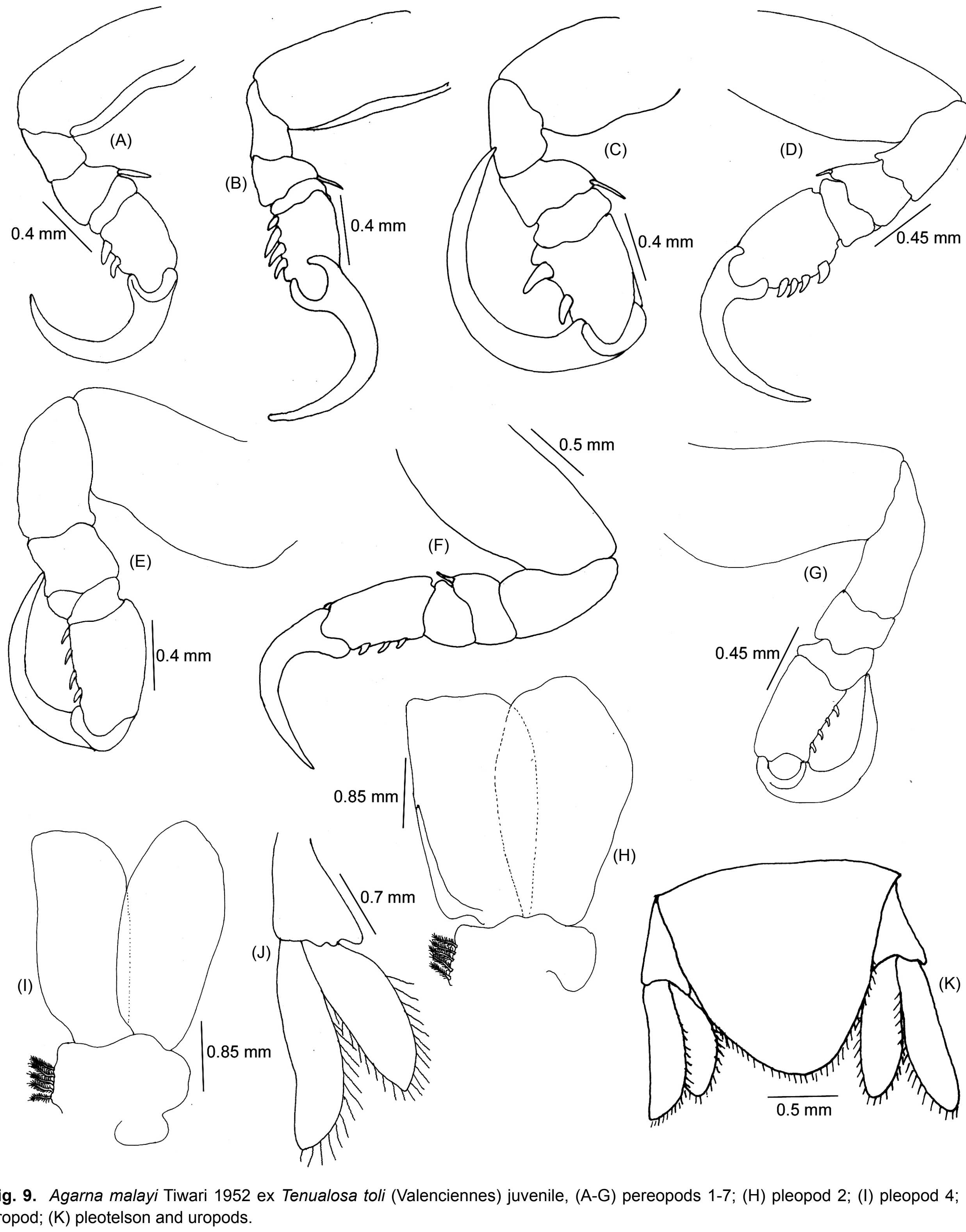

Fig. 9. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) juvenile, (A-G) pereopods 1-7; (H) pleopod 2; (I) pleopod 4; (J) uropod; (K) pleotelson and uropods.

setae.

Antenna larger than antennule. 8 articled antennule extends to anterior margin of pereonite 2; antenna longer than antennule, 11 articled, extending to posterior margin of pereonite 3; all articles without setae and spinules. Mouthparts are not well developed; mandible palp articles without setae and spines; maxillule, maxilla and maxilliped with poorly developed apical spines. Apical spines not recurved.

Six pereopods; pereopods 1-6 without spines. Distal margin of dactylus of pereopod 1-6 slightly indented and then forming a narrow terminal part. Propodus of pereopod not toothed. Pleopods not distinctly visible in dorsal view. Pleopods 1-5 without plumose setae. Uropod rami subequal, extending strongly beyond the distal margin of pleotelson, apically rounded without plumose setae.

Size: Ovigerous female (14-18 mm), transitional (12-15 mm), male (9-13 mm), juvenile (8 mm), manca I (2.4-2.8 mm), manca II (3.4-3.6 mm).

Colour: Male, transitional and female - body white, with scattered faint chromatophores on the dorsal side, more in proximal end of the telson. Juvenile, manca I and manca II- transparent with scattered chromatophores.

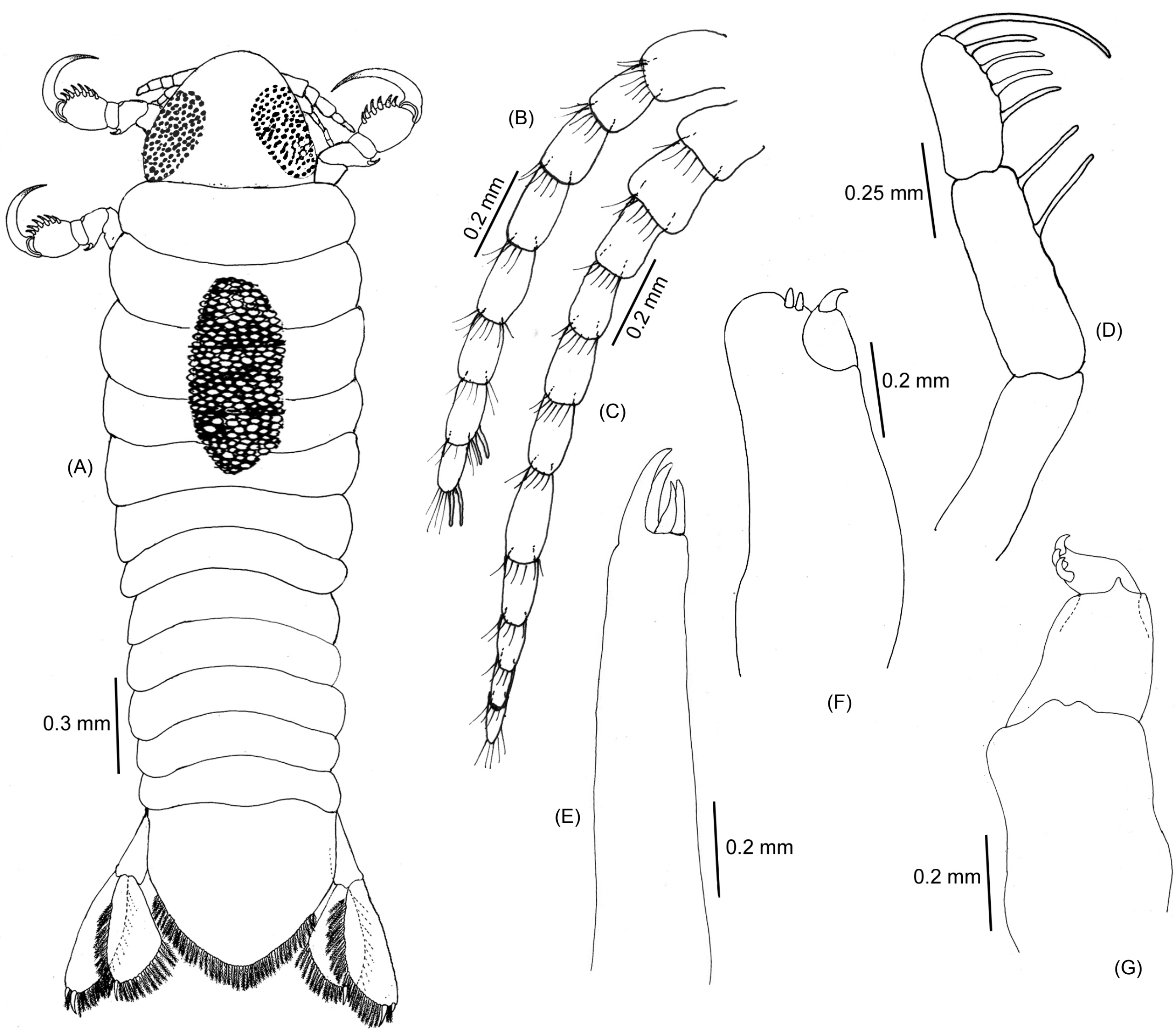

Fig. 10. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) manca II, (A) dorsal view; (B) antennule; (C) antenna; (D) mandible; (E) maxillule; (F) maxilla; (G) maxilliped.

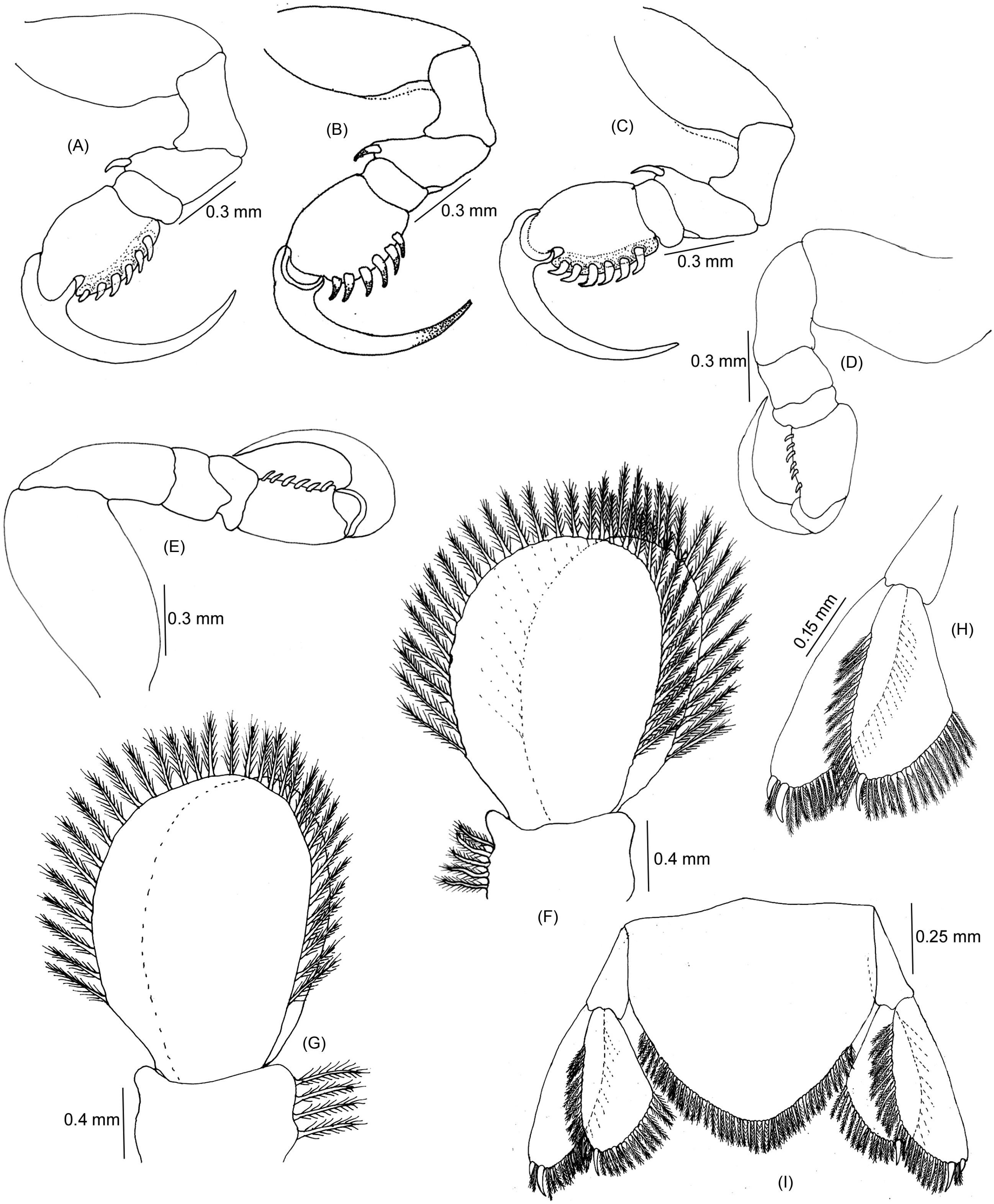

Fig. 11. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes) manca II, (A-C) pereopods 1-3; (D-E) pereopod 5-6; (F) pleopod 1; (G) pleopod 5; (H) uropod; (I) pleotelson and uropods.

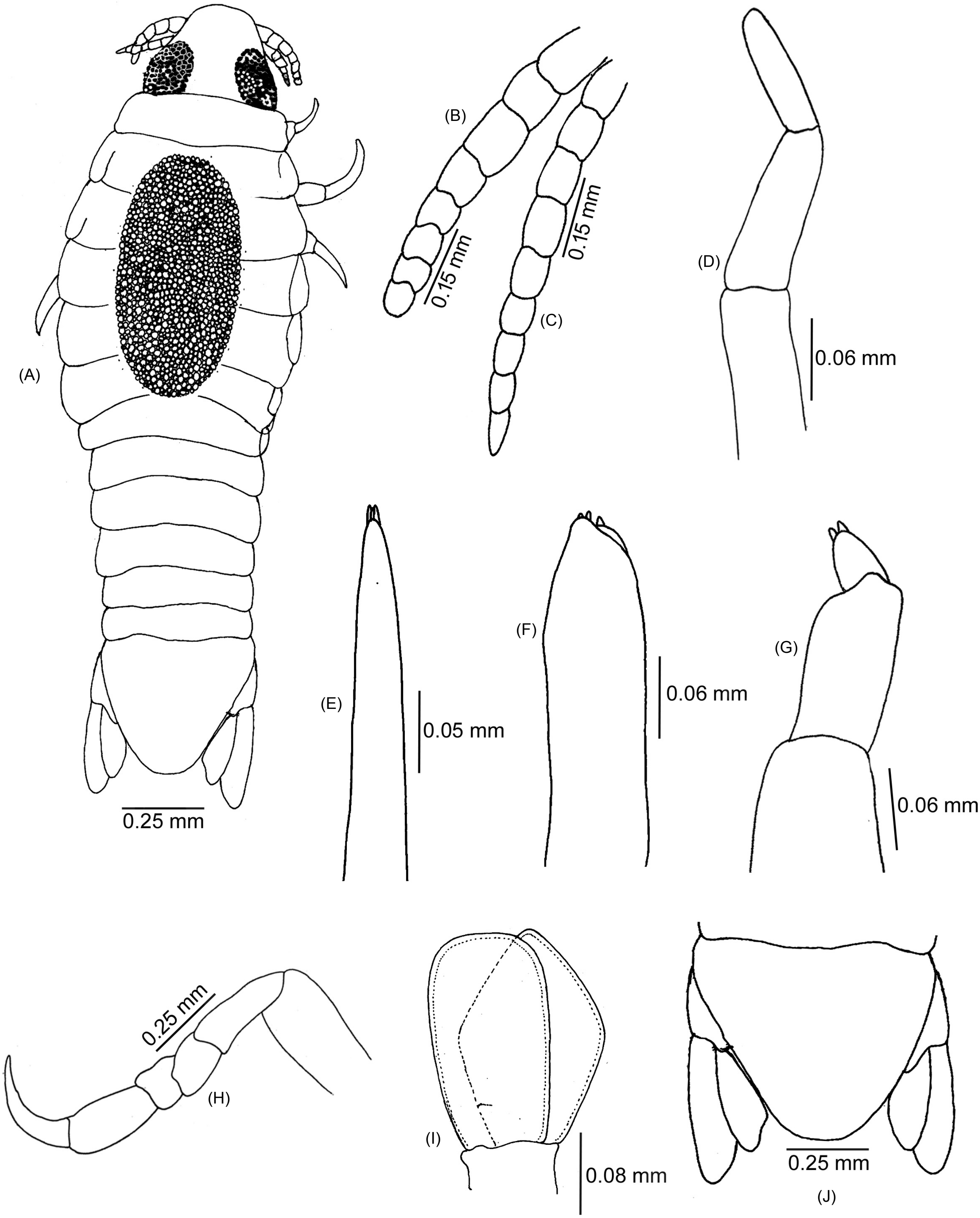

Fig. 12. *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes), manca I, (A) dorsal view; (B) antennule; (C) antenna; (D) mandible palp; (E) maxillule; (F) maxilla; (G) maxilliped; (H) pereopod 1; (I) pleopod 2; (J) pleotelson and uropods.

Agarna malayi* - Pattern of parasitic interaction with its host fish *Tenualosa toli

Eighty species of marine fishes belonging to 35 families were closely examined during the study period (November 2009 to November 2013) for the presence of *A. malayi*, and the parasite was recovered only from *Tenualosa toli*, signifying *A. malayi*'s oligoxenous host specificity. Out of 485 host fish observed, 84 showed infestation with *A. malayi*; a total of 156 parasites including 70 males, 77 ovigerous females, 5 transitionals and 4 juveniles were recovered from the gill chamber of infected host fish, prevalence and intensity being 17.3 and 1.86%, respectively. Parasites were usually found in pairs in the host fish, one in each branchial cavity, and, in 65 (77.4%) instances, male-female pairs were found; the relatively large ovigerous female preferred to attach to the inner wall of the operculum, close to the postero-dorsal corner of the gill chamber, orienting upside down (Figs. 1 E and F and 2 I and J). Small sized males were found to occupy the opposite gill chamber more or less in the same position. In 5 occasions (6%), transitionals were also paired with males. In two cases (2.4%), paired juveniles were also found. In twelve cases (14.2%), unpaired females were found. Interestingly, the bodies of both females and transitionals showed an asymmetry in their shape inasmuch as it is hunched either towards left or right according to their occupation in the right and left branchial cavity, respectively; more than 70% (56 out of 77) of the females were recovered from the right gill chamber (Figs. 1, 2 E and F). The parasite occupying the branchial chamber of the host fish invariably showed the presence of a large depression and the atrophied gills, the severity of which was proportional to the size of the parasite.

In a single instance, apart from *A. malayi*, the host fish (*T. toli*) also showed the simultaneous infestation with another cymothoid, *Anilocra leptosoma* (Bleeker 1857), which was settled on the base of the dorsal fin.

Life cycle of *Agarna malayi*

Three sequential stages in the *Agarna malayi* life cycle were identified: marsupial, free living and infective (Figs. 2 and 13). The marsupial stage essentially comprises residents of marsupium (marsupiumites) consisting of six morphologically distinct ontogenic stages sequentially as a zygotic stage, three embryonic stages (ES-1, ES-II and ES-III), and two larval stages (manca I and manca II) (Fig. 14). The zygotic stage is characterized by a round or oval shape with a size range of 1.1-1.4 mm and a light florescent yellow colour. The ES-1 is a sub-spherical to ovoid embryo, with 1.4-1.6 mm length and 1.2-1.3 mm width and no morphological evidence for organogenesis; it is also a light florescent yellow. The ES-II is 1.6-1.85 mm long and 1.3-1.35 mm wide, it is an elongate or oblong embryo with a cephalic end and early limb bud developed. The eye spot begins to get pigmented in ES-II. The ES-III is characterized by the following features: curved and segmented body with pigmented eyes spots, thoracic and abdominal limb buds appeared, dorsal surface shows chromatophores. The ES-III is 1.9- 2.2 mm long and 1.3-1.40 mm wide. Stage III embryo undergoes further developmental changes and hatches into the marsupium called manca-I. Manca I undergoes moulting to transform into the second larva, manca II (characteristic features are given in the description).

Manca II is found in three stages: final marsupial, free living and first infective stage. Manca II in the marsupium is released into the surrounding medium. After being released, manca II, the final staged marsupiumite, leads a short free swimming life (the free swimming manca II was reared in the laboratory, which survives up to four days) before finding and infecting a specific host fish (*T. toli*). Once the manca II settled in the branchial cavity of the host fish, it undergoes series of biphasic moults to transform from juvenile into subsequent adult stages such as functional male, transitional and ovigerous female (Fig. 13). Reproductively active (ovigerous) females continue the biphasic moult as oostegition and de-oostegition moults in an alternative manner crucial to the formation or replacement of marsupium. In the present study, six sequential stages (Fs-1- Fs-6) of *A. malayi* ovigerous females were noticed (Fig. 13). Fs-1 is an ovigerous female with a fully grown ovary and newly formed brood plates, but no marsupiumites. Fs-1 undergoes oviposition to form Fs-2. The Fs-2 is characterized by the presence of any marsupial stages. After larval release (manca II), the empty brooded ovigerous female with a growing ovary is called Fs-3. Fs-3 is an ovigerous female with a growing ovary that carries an empty old brood pouch, after releasing manca II larva. It undergoes a moult to transform into Fs-4. Fs-4 is an ovigerous female with a growing ovary and no brood pouch/brood plates. It undergoes a moult to transform into Fs-5. Fs-5 is an ovigerous female with newly formed brood plates but no marsupiumites. Fs-5 undergoes oviposition to form Fs-6. Fs-5 is morphologically similar to Fs-1 and Fs-6 is morphologically similar to Fs-2, the only difference is that Fs-5 and Fs-6 are in the second cycle.

DISCUSSION

The present study updated information on the taxonomy of *Agarna malayi*, one of the inadequately studied cymothoid species. The female stage of *A. malayi* can be easily distinguished from other species of *Agarna* by its largely hunched body and the shape of the head and pereonite 1. The antennae, mandible and maxillae are unique in *A. malayi*. In *A. pustulosa*, according to Pillai (1964), prominent pustules are present on the dorsal side of the telson. But, while re-examining the type materials (holotype and paratype) of *A. malayi*, a similar kind of pustules were also found in the telson of *A. malayi*. The presence of this feature was not mentioned in the original description by Tiwari (1952), which raises questions regarding the taxonomic validity of *A. pustulosa*. The validity of another species - *Agarna bengalensis* Kumari, Hanumantha Rao and Shaymasundari, 1990 - is also doubtful and, to our knowledge, information regarding the type materials of *A. bengalensis* is not available.

In the present study, all the freshly collected adult specimens and type specimens of *A. malayi* follow a common pattern: all have a single pair of coxae in pereonite 2-7 and all the pereonites have a pair of pereopods. On the other hand, *A. malayi* larvae (manca I and manca II) have only six pairs of pereopods and these are absent in the 7th pereonite, akin to reports of other cymothoids such as *M. renardi* and *C. frontalis* (Aneesh et al. 2015, 2016). Unlike the common pattern found in *A. malayi*, according to Richardson (1905) the coxal plates and pereopods in *Agarna carinata* were absent in the 7th pereonite and the pereonite 4 was found to have two pairs of coxae and pereopods. Later, the statement by Tiwari (1952)

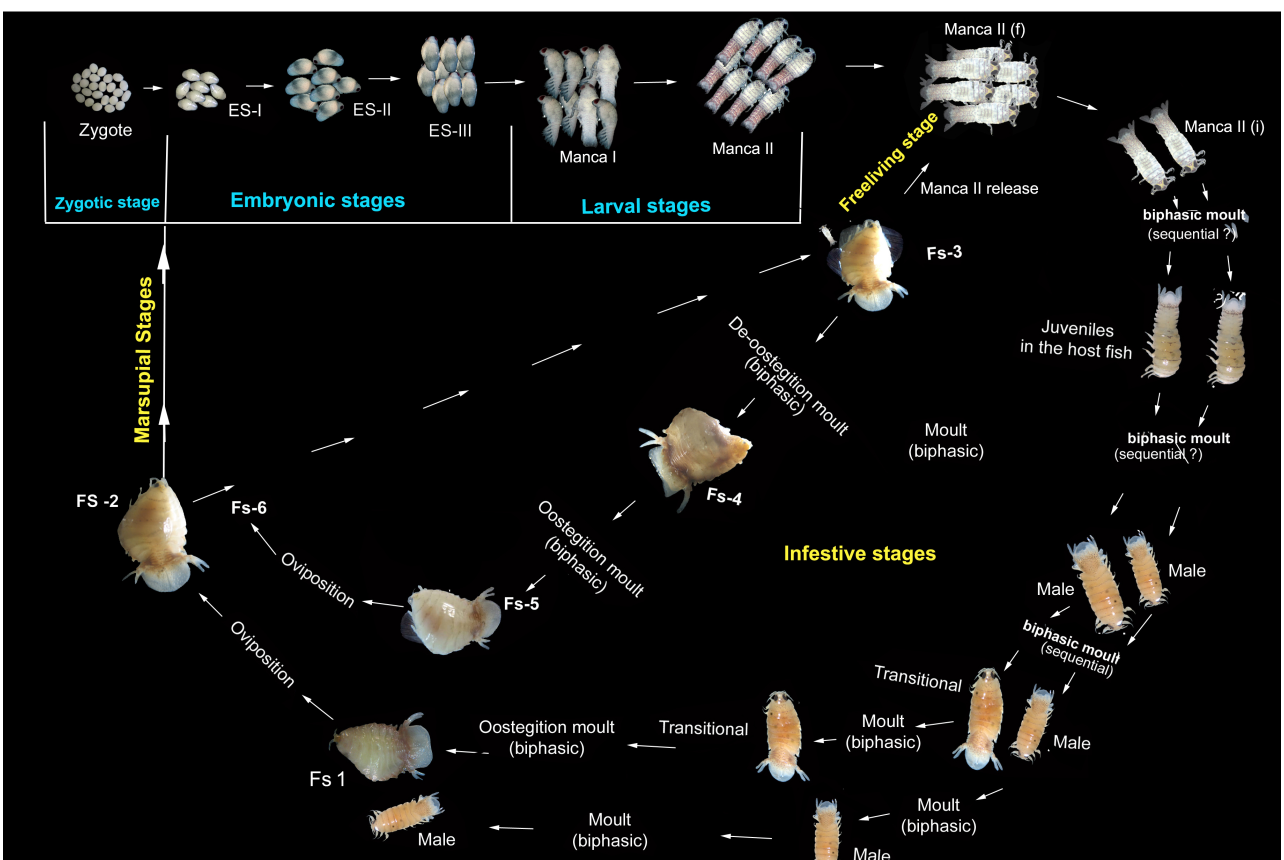

Fig. 13. Schematic representation of the life cycle of *Agarna malayi* Tiwari 1952 ex *Tenualosa toli* (Valenciennes); Abbreviations: Manca II (F), manca II at free living stage; Manca II (I), manca II at infective stage; ES, embryonic stage; Fs, female stage.

showed that there might have been an error in the taxonomic observation of *A. carinata*.

During the transitional stage, *A. malayi* shows an assembly of male and female characters like in all other protandrous cymothoids - *Glossobius hemiramphi* Williams and Williams 1985*, Mothocya renardi* (Bleeker 1857) and *Cymothoa frontalis* H. Milne Edwards, (1840) - reported so far (Williams and Williams 1985; Aneesh 2014; Aneesh et al. 2015, 2016). Moreover, the cymathoid species exhibit remarkable morphological differences right from the larval stage manca II. For instance, the toothed and broad propodus of the first three pairs of pereopods and spines present in the manca II of *A. malayi* appear to be a parasitic adaptation that apparently helps the parasites cling to the body of host fish during their infective stages; in the manca II of *C. frontalis* and *M. renardi*, teeth are present only on the dactylus of the first three pereopods (Aneesh et al. 2015, 2016).

The present study also provides information on the occurrence, host-parasite interaction and life cycle of *A. malayi*. Despite 80 species of fishes closely observed for four consecutive years, it (*A. malayi*) was recovered only from the clupeid fish *Tenualosa toli*, indicating its oligoxenous host specificity; this specificity was also observed in several other cymothoid species, including *C. frontalis*, *Norileca indica* (H. Milne Edwards 1840) and *M. collettei* (Aneesh et al. 2015 2016). The other reported *A. malayi* hosts include the type host *Nematalosa nasus* (Clupeidae) from Kolkata and *Mugil ophueseni* [= *Valamugil cunnesius* (Valenciennes)] (Mugilidae) from Travancore and Kayamkulam Lake, Kerala, southwestern coast of India (Tiwari 1952; Pillai 1954 1964). Admittedly, these previously reported clupeid host fishes were not obtained from the Malabar Coast during the present study period.

The prevalence shown by *A. malayi* on *T. toli* was relatively low (below 20%) compared to that of other cymothoids such as *C. frontalis* - which infests *S. strongylura* - and *M. renardi* - which parasitises *S. leiura* - all of which showed a prevalence above 60%.

Like several other branchial cymothoids, *A. malayi* also shows strict niche specificity inasmuch as it infects only the branchial cavity of its host fish (*T. toli*) and resides in the space between the host's operculum and upper ramus of the first gill arch (Figs. 1E, F, and 2I); in all cases, the head of the parasite is found oriented towards the anterior while the telson and uropods project outwards through the opercular slit (Fig 2I). The belly (ventral

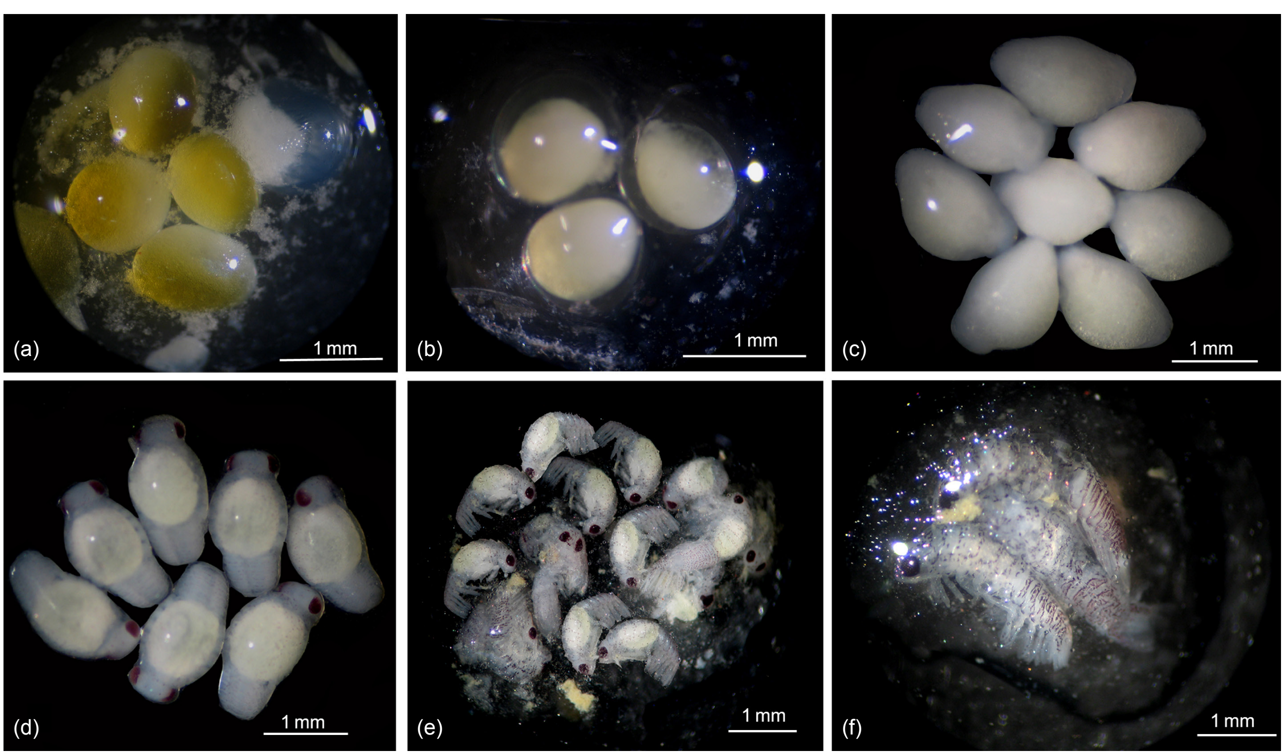

Fig. 14. Marsupiumites showing six morphologically distinct ontogenic stages sequentially as zygotic stage (a); three embryonic stages (ES-1 (b), ES-II (c) and ES-III (d)); and two larval stages (manca I (e) and manca II (f)).

side) of the parasite is pressed against the inner lining of operculum and the hunched dorsal side is pressed against the upper gill rami, irrespective of life cycle stage (male, transitional, female and juvenile). Tiwari (1952) also observed the similar pattern of site specificity in *A. malayi* females recovered from *Nematalosa nasus.* Interestingly, akin to that of several protandrous branchial cymothoids, the female body, to a great extent, is shown to have an asymmetry in its shape and the body is hunched either towards left or right according to its occupation in the right and left branchial cavity, respectively; the body hunching seems to be initiated right from the transitional stages (Fig. 2C and D), as was reported in other branchial cymothoids (Tiwari 1952; Williams and Williams 1994).

The parasitic occupation of the female parasite seems to be damaging the branchial chamber of the host fish, which is evident from the presence of atrophied gills and a deep pit in the floor of the gill chamber. This observation supports the previous studies demonstrating the damage caused in other diverse groups of host fishes such as lutjanids, serranids, sparids and belonids due to the parasitisation of different cymothoid species (Alvarez and Flores 1997; Horton and Okamura 2001; Yamauchi et al. 2012; Aneesh et al. 2015; Aneesh et al. 2016). The size of pit formed due to the occupation of female is visibly larger than that formed by other adult infective life stages (male and transitional). The negative impacts of parasitisation by cymothoids on the host fish could vary according to the site of attachment and also the species. For instance, buccal cymothoids are known to cause tongue degeneration and skull deformations (Romestand and Trilles 1977; Brusca and Gilligan 1983). Body surface cymothoids degenerate the fins and damage the scales and epidermis, leading to pronounced epithelial hyperplasia (Brusca 1978; Rand 1986).

The *A. malayi* life cycle pattern (Fig. 13) shows much resemblance to that which has been reported in other cymothoids, including *M. renardi* and *C. frontalis* (Bakenhaster et al. 2006; Aneesh et al. 2016). Saito et al. (2014) reported that different morphotypes of manca are free swimming forms. In the present study, manca II of *A. malayi* is found to be released into the surrounding water within few days of their development in the marsupium and likely leads a free-swimming life until it infects a specific host. Though the manca stage showed a possible taxonomic affiliation with preferred hosts (Fogelman and Grutter 2008), the mechanism that drives manca II towards its specific host is not defined in detail.

CONCLUSIONS

In conclusion, this supplementary report on taxonomy, host-parasite interaction and life cycle of *Agarna malayi* would provide key information useful not only for the precise identification of the species irrespective of the life cycle stages, but also on the parasitic and protandric hermaphroditic life of this cymothoid.

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Availability of data and materials: Voucher specimens were deposited in the collections of PCM-CBRL, Sree Narayana College, Kannur, Kerala, India.

Consent for publication: Consent is given.

Ethics approval consent to participate: The specimen is not under the listed category of experimental animals which need ethics approval.

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