Zootaxa 1582: 59–68 (2007)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

A new species of *Dactylopusioides* (Copepoda: Harpacticoida: Thalestridae) infesting brown algae, and its life history

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Abstract

A new species of harpacticoid copepod, *Dactylopusioides malleus* **sp. nov.**, belonging to the family Thalestridae is described from central Japan. The species is obligately endophagous in dictyotalean brown algae (*Dictyota dichotoma* (Hudson) J.V. Lamouroux, *Dictyota maxima* Zanardini and *Dictyopteris undulata* Holmes). The species nests by burrowing into the algal tissue during the copepod naupliar stages, while copepodids and adults reside in a dome-shaped, transparent capsule made of mucus and formed on the algal tissue. In laboratory experiments, the new species progressed through its complete life cycle (i.e. from egg to adult) while feeding only on unialgal dictyotalean tissues; this confirmed the obligate relationship with the host. The new species shares the following morphological characters with other species of *Dactylopusioides*: 1) antennary exopod 1-segmented; 2) first segment of P1 endopod in female elongated with a long seta on the inner proximal margin; 3) baseoendopod and exopod of P5 in female with five setae. It differs from other species in the following ways: 1) antennary exopod with six setae; 2) a stout hammer-shaped inner spine on the basis of P1 in male; 3) terminal short seta on third segment of P2 endopod in male is plumose.

Key words: brown algal endophagy, *Dactylopusioides*, mucus capsule, Harpacticoida, life history

Introduction

Members of the harpacticoid family Thalestridae Sars are mostly marine, and include epi(endo)phytic species as well as many benthic species. For example, *Diarthrodes nobilis* (Baird) lives epiphytically on the filamentous red alga *Ceramium rubrum* C. Agardh (Ceramiales) in a capsule made of mucus, and feeds on bacteria and organic debris that adhere to the capsule (Hicks & Grahame 1979). The copepodid and adult stages of *Diarthrodes cystoecus* Fahrenbach live on the thallus of *Fauchea laciniata* J. Agardh (Rhodymeniales) and other red algae in a dome-shaped, transparent capsule made of mucus; this copepod species also feeds on the macroalgal tissues (Fahrenbach 1954, 1962). As many as six species have been reported to excavate galleries or form gall-like swellings within the macroalgal tissue. For example, *Thalestris rhodymeniae* (Brady) lives in the algal tissue of the foliose red alga *Palmaria palmata* (Linnaeus) Kuntze (Palmariales), producing gall-like swellings (Harding 1954). *Diarthrodes feldmanni* Bocquet forms galleries in the tissue of several red algal species from different families (Bocquet 1953). *Amenophia orientalis* Ho and Hong and *Parathalestris infestus* Ho and Hong live in the brown alga *Undaria pinnatifida* (Harvey) Suringar (Laminariales; Ho & Hong 1988), producing galls with pinholes, while *Dactylopusioides macrolabris* (Claus) has been found in galleries between the two epidermal layers of the brown alga *Dictyota dichotoma* J. V. Lamouroux (Dictyotales: Brian 1928; Green 1958).

Recently Shimono *et al.* (2004) described *Dactylopusioides fodiens* Shimono, Iwasaki and Kawai; the second species in the genus. This species also excavates and lives in galleries within *D. dichotoma* and *Pachy-*

Accepted by G. Walker-Smith: 2 Aug. 2007; published: 12 Sept. 2007

dictyon coriaceum (Holmes) Okamura. In the present study, we describe a third species of *Dactylopusioides* (i.e. *Dactylopusioides malleus* **sp. nov.**) and document its feeding behavior and observations of its life history under laboratory conditions.

Material and methods

Harpacticoids found burrowing in the tissues of various macroalgae were collected by snorkelling and SCUBA diving in the intertidal and subtidal zones at Imagoura, Kasumi (35°39'N, 134°38'E) on 17 June 2003; Maiko, Kobe (34°37'N, 135°2'E) on 13 May and 10 August 1999; Oiso, Awaji Island (34°33'N, 134°59'E) on 10 June 2002; and Yura, Awaji Island (34°16'N, 134°57'E) on 13 July 2000 in Hyogo Prefecture. Since the harpacticoids could not be identified with the naked eye in the field, macroalgal thalli with the characteristic holes of harpacticoid burrows were collected. The macroalgal thalli were examined in the laboratory using a dissecting microscope (Nikon SMZ-U) to find burrow-forming harpacticoids. When nauplii and copepodids were found, they were isolated using capillary pipettes, rinsed in sterilized seawater, transferred to a plastic Petri dish containing a unialgal host macroalga in culture medium (PESI; Tatewaki 1966), and incubated under a long-day photoregime of 16:8 (light:dark), illuminated by daylight-type fluorescent tubes (50 μmol/m^{2}s) at 18 °C. To establish unialgal cultures of the host macroalga *Dictyota dichotoma*, clean apical fragments were excised and cultured in PESI medium, which included 1% GeO_2 to inhibit the growth of contaminating diatoms (Kawai *et al*. 2004).

Copepods living in the unialgal thalli were grown in 12-well plastic plates (Falcon, Franklin Lakes, NJ, USA), and their growth and behavior were observed under a dissecting microscope, or inverted light microscope (Olympus IX-50). For taxonomic study, harpacticoid specimens that had been fixed in 2% formaldehyde-seawater were dissected using fine needles. Dissected specimens were mounted on slides in polyvinyl lactophenol, and observed using an Olympus BX-50 compound microscope. Line drawings were produced by tracing the photomicrographs captured by a microscope digital camera (Olympus DP-11).

Throughout this paper the six pairs of pereiopods are denoted by P1–P6.

Results

Order Harpacticoida Sars, 1903

Family Thalestridae Sars, 1905 *sensu* Lang, 1948

Genus *Dactylopusioides* Brian, 1928

Dactylopusioides malleus sp. nov.

(Figs. 1–3)

Type material. Holotype. Adult female (total body length 0.76 mm, measured from anterior margin of rostrum to the posterior margin of caudal rami) deposited in the Natural History Museum, London; registration no. NHM 2006. 1914.

Paratypes. 10 females and 5 males deposited in the Natural History Museum, London, registration no. NHM 2006. 1915-1929. 10 females and 5 males deposited in the National Science Museum, Tokyo, Japan; registration numbers NMST-Cr 16855 and 15856. All type material was collected from the brown alga, *Dictyota dichotoma* in Oiso, Hyogo prefecture (34°33'N, 134°59'E) on 10 June 2002, by T. Shimono. Specimens have been preserved in 70% ethanol.

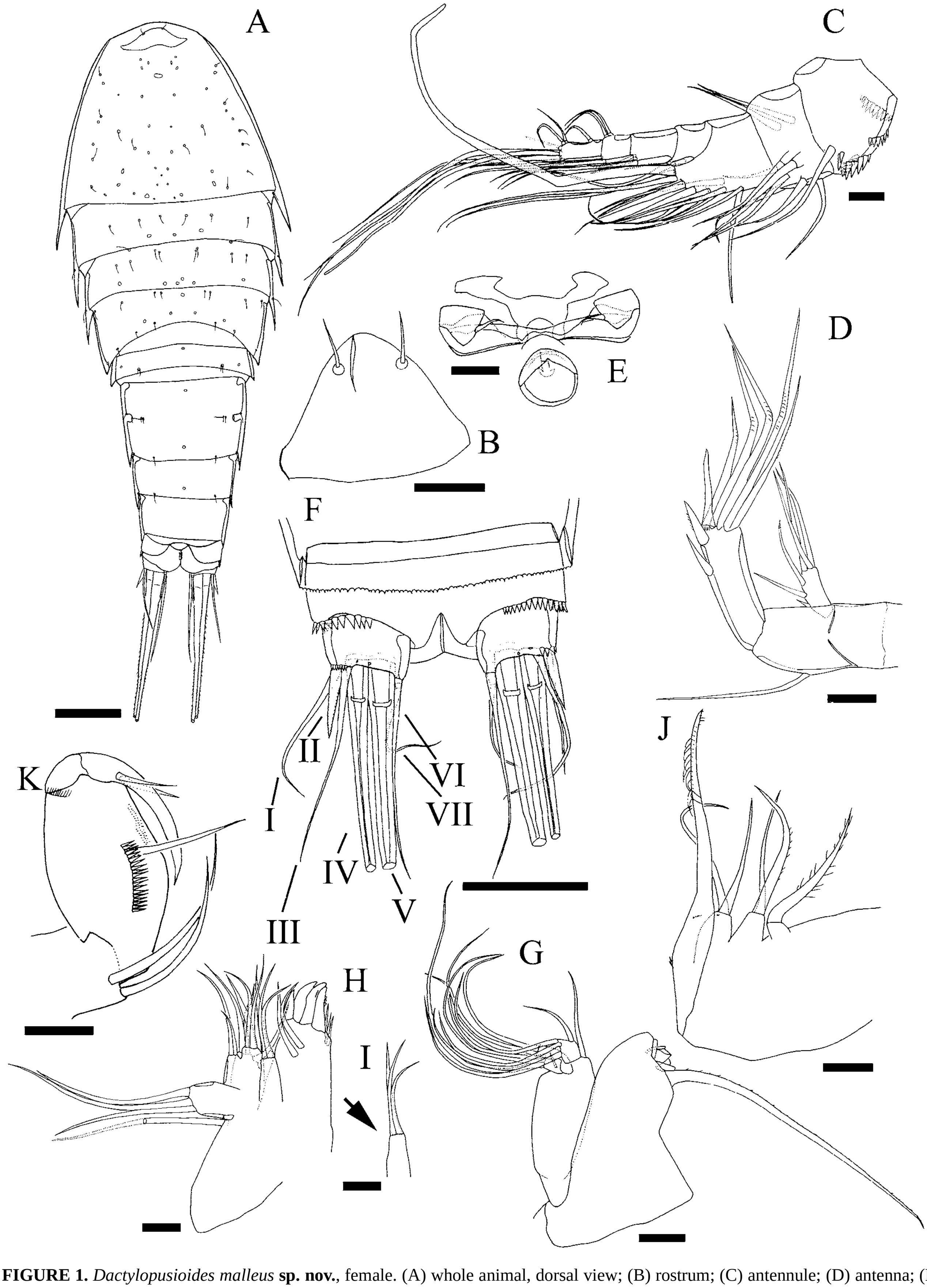

FIGURE 1. *Dactylopusioides malleus* **sp. nov.**, female. (A) whole animal, dorsal view; (B) rostrum; (C) antennule; (D) antenna; (E) genital field and P. 6, ventral view; (F) anal somite and caudal rami, ventral view; (G) mandible; (H) maxillule; (I) coxa of maxillule; (J) maxilla; (K) maxilliped. Scales: A, 0.01 mm; B–E; G–J, 0.02 mm; F, 0.05 mm.

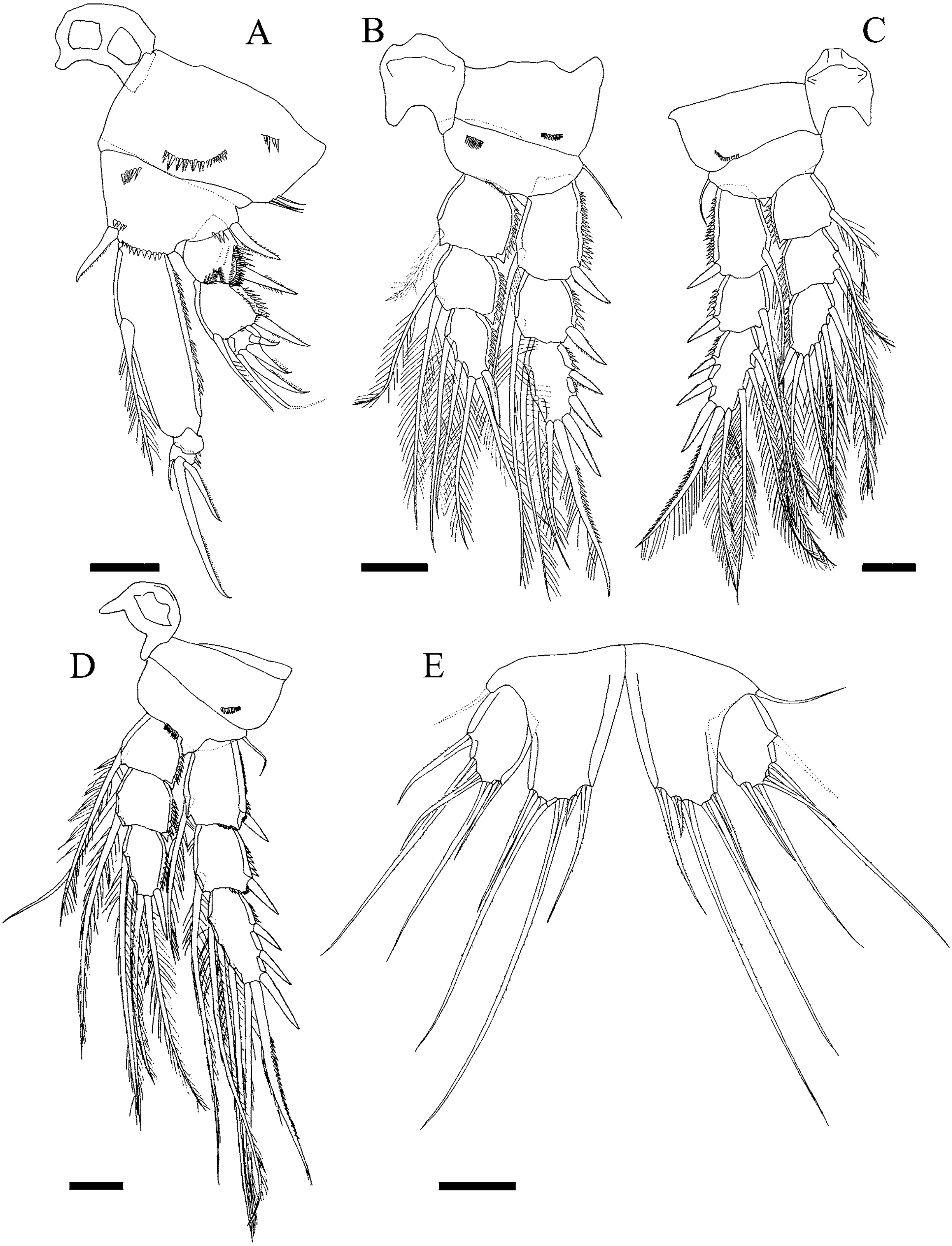

FIGURE 2. *Dactylopusioides malleus* **sp. nov.**, female. (A) P1; (B) P2; (C) P3; (D) P4; (E) P5. Scales: A–E, 0.05 mm.

Other material examined. 10 females and 6 males, dissected and mounted on slides in polyvinyl lactophenol, deposited in the personal collection of TS.

Diagnosis. Antennary exopod 1-segmented with 6 setae. Short, terminal seta on segment 2 of P2 endopod in male is plumose. Maxillule, coxa with 3 terminal setae. Male P5, baseoendopod and exopod with 3 and 5 setae, respectively. Male P6 reduced to a small plate with 2 setae.

Description of female. Mean total body length 0.74 mm (n=16), measured from anterior margin of rostrum to posterior margin of caudal rami. Color from greenish-brown to brownish-green. Body subcylindrical (Fig. 1A). Rostrum (Fig. 1B) articulated, triangular, apex rounded with 2 sensilla. Cephalothorax slightly shorter than wide. Genital double-somite divided dorsally and laterally by subcuticular rib. Genital field (Fig. 1E) with copulatory pore situated medially. Anal somite with small, round anal operculum (Fig. 1A). Caudal ramus (Fig. 1F) shorter than wide; seta I longer than seta II; seta II spinous; seta III longer than seta II; seta IV one-third as long as seta V; seta VI slightly longer than VII; seta VII triarticulate. Egg-sac single.

Antennule (Fig. 1C) short, 7-segmented. Two aesthetascs present, 1 on segment 3, the other on segment 8. Setal formula 1:9:13+a:2:4:4:7+a.

Antenna (Fig. 1D) with small coxa unarmed, basis incompletely fused with first endopod segment forming allobasis, bearing long inner seta. Exopod short, 1-segmented with 3 long distal setae and 1 short and 2 long lateral setae. Endopod with 4 geniculate distal setae and 1 long and 1 short distal seta, and 2 short lateral setae.

Labrum ovoid, carved in anterior margin, equipped with denticles.

Mandibular gnathobase (Fig. 1G) round, with long seta at inner corner. Basis large, with 1 seta. Endopod 1-segmented with 1 lateral and 4 terminal setae. Exopod 1-segmented with short seta and 5 long setae.

Maxillule (Fig. 1H & I), arthrite of praecoxa with 4 stout spines and 3 spinulose setae around distal margin and 2 subapical surface setae. Coxa with 3 terminal setae. Basis with 6 setae. Endopod much smaller than exopod, with 2 setae. Exopod 1-segmented, with 3 setae on distal margin. Epipodite represented by a seta.

Maxilla (Fig. 1J), syncoxa with 3 endites; proximal, middle and distal endites bearing 1 plumose, 1 plumose and 1 naked, and 2 naked setae, respectively. Allobasis represented by a thin elongate claw. Endopod absent.

Maxilliped (Fig. 1K) pedestal. Syncoxa smooth and armed with 2 setae at inner corner. Basis with spinulose seta on inner-lateral margin. Endopod 1-segmented, prehensile with 2 spinulose setae.

P1 (Fig. 2A), coxa large. Intercoxal sclerite semi-oval, wider than long. Basis with stout spine at inner and outer distal corners. Endopod 3-segmented, prehensile; first segment elongated, longer than exopod, armed with long spinulose seta in proximal half of inner margin; second segment asetose, small; third segment short, armed with 2 serrate claws and inner, short, spinulose seta. Exopod 3-segmented; first segment with outer spine; second segment bearing outer spine and inner seta distally; third segment shortest, bearing 4 terminal spines increasing in length towards medial edge.

P2–P4 (Fig. 2B–D) coxa rectangular. Intercoxal sclerite longer than wide. Basis with outer seta. Rami 3-segmented, with copious spinules on outer margin, endopod shorter than exopod. Setal formula as follows:

	Exopod	Endopod
P2	1 : 1 : 2.2.3	1 : 2 : 2.2.1
P3	1 : 1 : 3.2.3	1 : 2 : 3.2.1
P4	1 : 1 : 3.2.3	1 : 2 : 2.2.1

P5 (Fig. 2E), endopodal lobe exceeding exopod slightly, baseoendopod with 5 setae on distal and subdistal, basal seta naked and second innermost seta shortest, exopod bearing 5 setae.

P6 (Fig. 1E) close to genital pores, trapezoidal, inner seta inwardly directed.

Male. Mean total length 0.62 mm (n=10). Differs from the female in the following respects:

Antennule (Fig. 3A) haplocer, 9-segmented, an aesthetasc on segment 3, segment 5 and segment 9.

P1 (Fig. 3B & C), intercoxal sclerite not oval. Inner spine on basis stout, hammer-shaped.

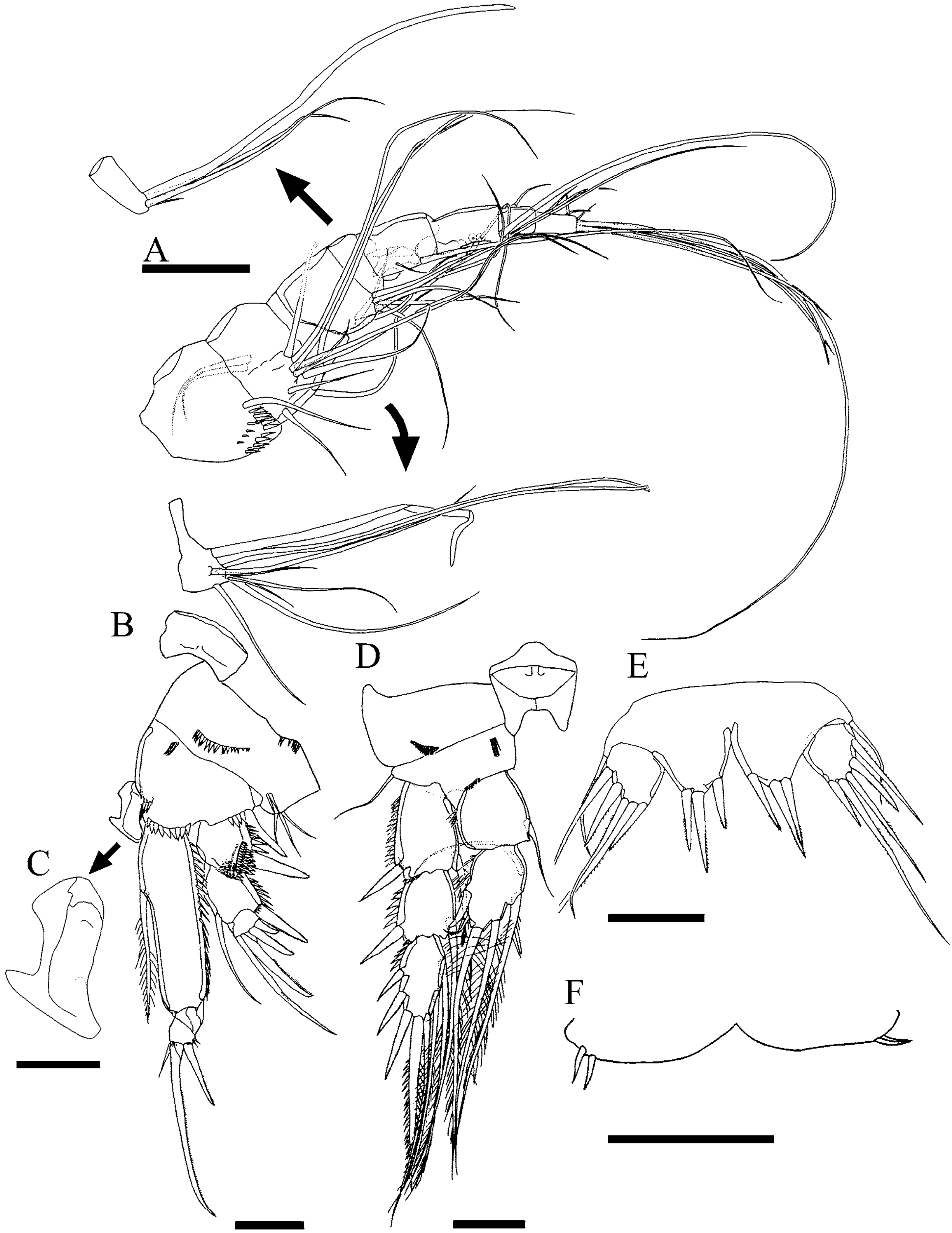

FIGURE 3. *Dactylopusioides malleus* **sp. nov.**, male. (A) antennule; (B) P1; (C) Inner short spine on basis of P1 in male; (D) P2; (E) P5; (F) P6. Scales: A–B, D, E, 0.05 mm. C, 0.02 mm

P2 (Fig. 3D) endopod modified, 2-segmented; outer spine on segment 2 shorter and stouter than that of female; outer terminal plumose spine on segment 2 much smaller than that of female. Setal formula as follows:

	Exopod	Endopod
P2	1 : 1 : 2.2.3	1 : 2.2.2

P5 (Fig. 3E), right and left baseoendopods fused medially, bearing 3 serrate spiniform setae on endopodal lobe and a naked seta at outer corner. Exopod bearing 5 spinulose setae.

P6 (Fig. 3F) reduced to small plate with 2 setae.

Etymology. The epithet *malleus* refers to the stout hammer-shaped inner spine on the basis of P1 in the male; different compared to known species of *Dactylopusioides.*

Life history. In the field, *Dactylopusioides malleus* **sp. nov.** (Fig. 4A) was found forming burrows in the thalli of dictyotalean brown algae *Dictyota dichotoma*, *Dictyota maxima* and *Dictyopteris undulata.* At Imagoura, Maiko, Oiso and Yura, in Hyogo Prefecture (central Honshu), the new species was found from May to August in the intertidal and subtidal zones. All life history stages of the new species were found burrowing into the thalli of *Dictyopteris undulata* at Imagoura and *Dictyota dichotoma* at Yura, on 17 June 2003 and on 13 July 2000, respectively.

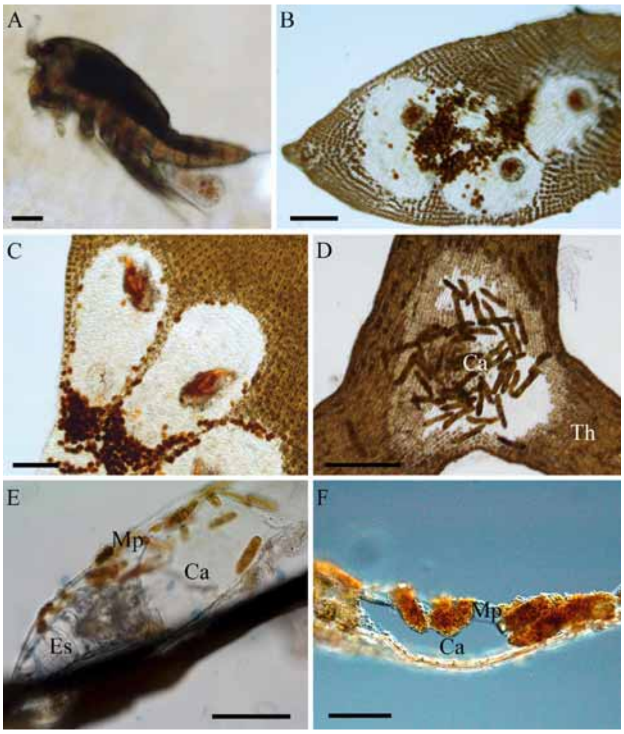

FIGURE 4. The life history of *Dactylopusioides malleus* **sp. nov.** (A) An adult female with a hatched nauplius; (B) The nauplius two days after hatching, within the host alga; (C) copepodid; (D) Exterior view of the capsule; (E) Lateral view of the burrow; (F) The burrow in cross-section. Scales: A–C, 0.01 mm; D, 0.02 mm; E, 0.05 mm. Mp: Mucus production of *D. malleus* **sp. nov.**; Ca: capsule; Es Exoskeleton of a copepodid; Th: Thallus.

In laboratory experiments, *D. malleus* **sp. nov.** progressed through its complete life cycle (from egg to adult to egg) while eating only the tissue of *D. dichotoma,* which was its host in the field. Eggs hatched out in 3–4 (3.6 ± 0.5 S.D.) days. The first stage of the nauplii made burrows (tunnels) by eating the host algal tissue from the surface, and then entering the algal tissue. In the burrows, nauplii ate the algal cells but left the outer cell wall and overlying cuticle of the peripheral cells intact (Fig. 4B); thus the burrow was not exposed to outside. Nauplii molted several times within the burrow and then formed a gelatinous matrix, within which they metamorphosed (Fig. 4C). The copepodids left the burrow and continued development on the surface of the algal tissue, inside a capsule made of mucus they secreted (Fig. 4D–F). At 18 °C, copepodids became adults in 9–13 (11.2 ± 1.5) days. Both nauplii and copepodids occasionally left the burrow or capsules and formed new burrows or capsules. At 18 °C, adult females changed capsules at intervals of 2.2 ± 1.2 days. No mating behavior was observed during the experiments, but eventually the females bore eggs in an egg sac, in an experimental setting where both males and females were present. In contrast, females did not form eggs in the absence of males. Females produced eggs at intervals of 4.3 ± 1.2 days, and retained the egg sac until the eggs hatched. At 18 °C, each egg sac contained 7–24 (17.1 ± 4.1) eggs.

Discussion

Taxonomic characters of the new species agree with the diagnosis of the genus *Dactylopusioides* given by Lang (1948): antennary exopod 1-segmented; first segment of P1 endopod in female elongated with a long seta on the inner proximal margin; second segment of P2–P4 endopods in female with 2 inner setae; baseoendopod and exopod of P5 in female with 5 setae respectively; endopod of P2 in male 2-segmented.

The genus *Dactylopusioides* contains three species, including the new species. *D. malleus* **sp. nov.**, which differs from *D. macrolabris* and *D. fodiens* in several characters (see Table 1). In particular, the new species has a stout hammer-shaped inner spine on the basis of P1 of the male, which is not found in the other two species of the genus.

TABLE 1. Comparisons of anatomical features of the genus *Dactylopusioides*. Characters of *D. macrolabris* and *D. fodiens* are cited from Claus (1866), Brian (1928), Lang (1948), Green (1958) and Shimono *et al*. (2004).

	D. macrolabris	*D. fodiens*	*D. malleus* **sp. nov.**
No. of segments of antennule in female	7	8	7
No. of setae on antennary exopod	3	4	6
No. of setae on maxillular coxa	Unknown	4	3
Inner spine on basis of P1 in male	Unknown	Stout	Stout hammer-shaped
No. of spines on segment 3 of P1 exopod	4	5	4
Terminal short seta on segment 2 of P2 endopod in male	Naked	Naked	Plumose
No. of outer setae on segment 3 of P2–P4 exopods	2	3	3
No. of setae on inner lobe of P5 baseoendopod in male	3	2	3
No. of setae on P5 exopod in male	4	4	5
No. of spines of P6 in male	Unknown	3	2
Distribution	Coast of Mediterranean and England (Lang 1948; Green 1958)	Japan (Shimono *et al*. 2004)	Japan (this study)
Host	Brown alga *Dictyota dichotoma* and calcareous algae (Brian 1928)	*D. dichotoma* and *Pachdictyon coriaceum* (Shimono *et al*. 2004)	*D. dichotoma*, *D. maxima* and *Dictyopteris undulata* (this study)

The species belonging to *Dactylopusioides* are obligately endophagous in macroalgae, spending most of their lives in their burrows, eating only host algal tissues: *D. macrolabris*, *D. fodiens* and the third species *D. malleus* **sp. nov.** all live on dictyotalean brown algae. Therefore, the obligate endophagy is considered to be a common feature of the genus. However, *D. malleus* differs from *D. macrolabris* and *D. fodiens* in forming a dome-shaped capsule during the copepodid and adult stages, whereas the others two species stay in within the tissue for their entire life (Green 1958, Shimono *et al*. 2004).

In the genus *Diarthrodes* G.M. Thomson, 1883 which is phylogenetically close to *Dactylopusioides*, two marine parasitic species are reported: *Diarthrodes feldmanni* and *D. cystoecus*. According to Bocquet (1953), *D. feldmanni* lives within the algal tissues from nauplius to adult, like *Dactylopusioides fodiens* and *D. macrolabris*. In contrast, *Diarthrodes cystoecus* lives in the algal tissue in the nauplius stage, not in a burrow but a dome-shaped capsule, as seen in *Dactylopusioides malleus* **sp. nov.** Thus it would appear that there is a diversity of life history patterns within *Diarthrodes* and *Dactylopusioides*.

Furthermore, *Diarthrodes nobilis* lives on the algal tissue forming a mucus capsule, but does not eat the algal tissues (Hicks & Grahame 1979). Some differences are apparent between *D. nobilis* and *D. malleus* **sp. nov.** in the function of mucus capsule. Hicks and Grahame (1979) reported that *D. nobilis* used the capsule to trap food (e.g. bacteria and organic debris). In contrast, *D. malleus* **sp. nov.** feeds on the host algal tissue and therefore only uses the capsule as a physical covering, to it protect from wave action or predators. Such use of the capsule is also seen in *Diarthrodes cystoecus*, which also feeds on marine macroalgae (Fahrenbach 1954, 1962). *Dactylopusioides malleus* **sp. nov.** forms a mucus matrix even within the burrow in the algal tissues during the metamorphosis, and this is considered to have a protective function. Other species of Thalestridae such as *Amenophia orientalis* and *Parathalestris infestu*, excavate and dwell within macroalgal tissue, do not form mucus structures (Ho & Hong 1988).

Acknowledgements

We thank Dr Eric Henry for reviewing an earlier draft of this paper.

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