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First record of a Rhynchomolgid Copepod associated with Common Cuttlefish, *Sepia officinalis* L. from the Levantine Sea

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Abstract: A species of the family Rhynchomolgidae, *Doridicola longicauda* (Claus, 1860) collected from Cuttlefish, *Sepia officinalis* Linnaeus, 1758 captured from north eastern part of the Levantine Sea, is reported for the first time. Cuttlefish (n=20) were caught on July 2015 by sole trammel nets. Seventeen adult females and three male specimens of *D. longicauda* were collected from the ventral surface of the lateral fins of cuttlefish examined. General morphology and the key diagnostic characters of the collected specimens were photographed by using light and scanning electron microscope. The specimens were identified to species level according to Claus (1860).

Keywords: *Doridicola longicauda*, *Sepia officinalis*, Mediterranean, copepod, Turkey.

Introduction

Cephalopods play a crucial role in marine food webs as major prey of many predators. This situation makes them ecologically pivotal. Additionally, cephalopods have economic importance. Cuttlefish *Sepia officinalis*, one of the highly consumed species among cephalopods, is widely distributed in Atlantic and Mediterranean waters (Rosas-Luis et al., 2008; Soufi-Kechaou et al., 2017)

The genus *Doridicola* Leydig, 1853 is the most specious rich taxon ranked within the copepod family Rhynchomolgidae established by Humes and Stock (1972). Species belonging to this genus can be found on various marine invertebrates such as Porifera, Cnidaria, Mollusca and Echinodermata (Humes and Boxshall, 1996; Boxshall and Halsey, 2004; Kim, 2007). With the recently discovered species, *D. indistinctus* Ho and Ivanenco, 2013, the number of the species within the genus *Doridicola* reached up to 52 valid species (Walter and Boxshall, 2016). Among these valid species, only two species have been found on two different species of the genus *Sepia* (Cephalopoda: Sepiidae). These are; *D. sepiae* (Izawa, 1976) found on *S. esculenta* Hoyle, 1885 reported from Japan (Izawa, 1976) and *D. longicauda* (Claus, 1860) on *S. officinalis* Linnaeus, 1758 have been reported from France (Mediterranean and North Atlantic coasts), Netherlands (Wadden sea), England (The English Channel), Italy (Adriatic Sea) (Claus, 1860; Wierzejski, 1877; Stossic, 1880; Graeffe, 1902; Pesta, 1909; Cuenot, 1927; Stock, 1956; Dollfus, 1958; Ho, 1983; Costanzo et al., 1994; Faasse, 2003). To our knowledge, none of the valid species of the genus *Doridicola* have been reported from the Turkish seas so far. In addition, brief description of the general morphology, key diagnostic characters and morphometric values of the newly collected material of *D. longicauda*, are presented. However, description of presently reported specimens were mainly based on the females as the three male specimens were damaged during sampling.

Materials and Methods

Cuttlefish, *Sepia officinalis* (mantle length ranging from 14 to 23 cm) (n=20) were caught on July 2015 by sole trammel nets in the northeastern Mediterranean waters off Iskenderun Bay, Turkey. Seventeen adult females and three damaged male specimens of *Doridicola longicauda* were collected from the ventral surface of the lateral fins of *S. officinalis* (Fig. 1C). Prevalence was 70% (14 of 20 examined cuttlefish carries *D. longicauda*). The specimens deposited in the collections of the Parasitology Museum of Çukurova University in Adana, Turkey (CUPM-COP/2015-4). The collected copepod samples were preserved in 70% ethanol and cleaned in lactic acid

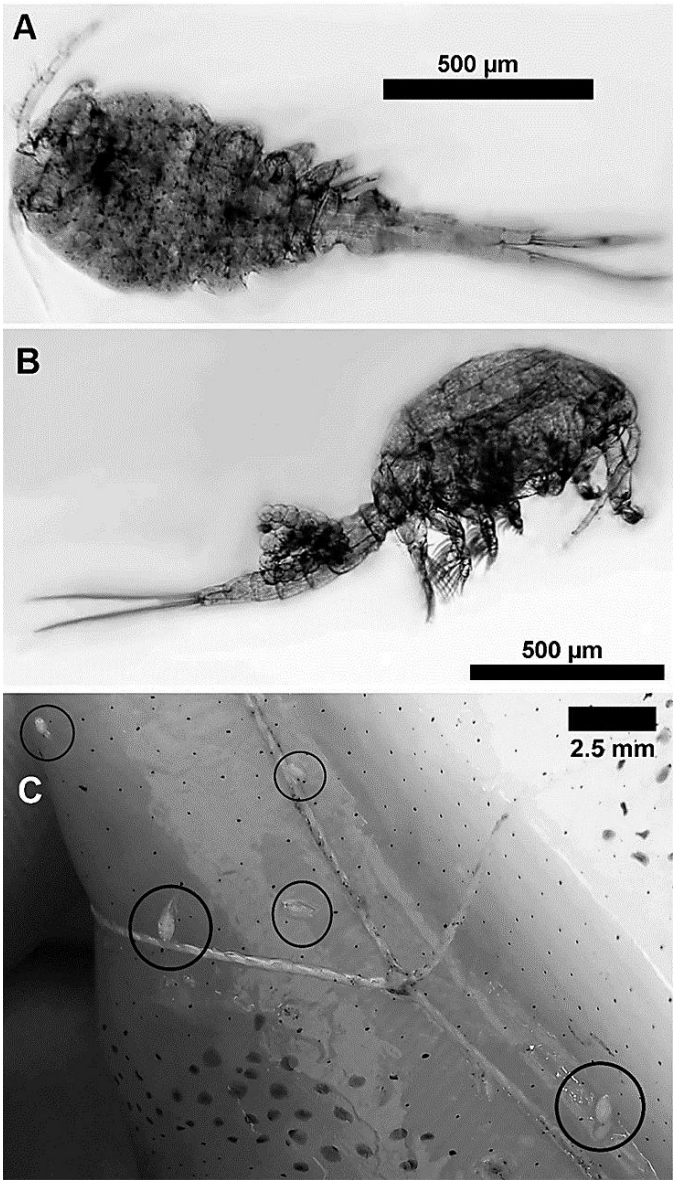

Figure 1. *Doridicola longicauda* (female). (A) Habitus (dorsal), (B) habitus (lateral) and (C) specimens of *D. longicauda* on ventral surface of the lateral fins of *S. officinalis*.

for 2 h. General morphology and the key diagnostic characters of the collected specimens were examined, measured with a stereo microscope equipped with a digital camera. In addition, some of the key diagnostic features were photographed by using scanning electron microscope. The scientific and common names of cephalopods follow Walter and Boxshall (2017), the morphological terminology for the copepods follows Ho and Ivanenko (2013) and Huys and Boxshall (1991). The protocols for preparing crustaceans for scanning electron microscopy (SEM) outlined by Felgenhauer (1987) were followed. Ethanol-fixed specimens were hydrated to distilled water and post-fixed in 1–2% osmium tetroxide (OsO4) in buffer for 2 h, washed in distilled water, dehydrated through graded acetone series, critical point dried using liquid carbon dioxide as the exchange medium, mounted on aluminium stubs and sputter coated with platinum. Coated specimens were examined on a Zeiss Supra 55 (FE-SEM, Germany) field emission scanning electron microscope at 1-3 kV.

Results

Subclass Copepoda

Order Cyclopoida

Family Rhynchomolgidae Humes and Stock (1972)

Genus *Doridicola* Leydig, 1853

Doridicola longicauda (Claus, 1860)

Synonyms: *Lichomolgus longicauda* (Claus, 1860); *Lichomolgus sepicola* Claus, 1860; *Metaxymolgus longicauda* (Claus, 1860); *Sepicola longicauda* Claus, 1860

Description:

Female: Body (Figs. 1A, B) 1.23±0.5 mm long (excluding setae on caudal rami) and 0.44±0.2 mm wide (greatest width of cephalothorax). First pediger distinctly separated from cephalosome and broadly protruded at posterolateral corners. Free thoracic somites decreasing in size from anterior to posterior. Genital-double somite (Fig. 1A) as long as wide (181×181 µm). Female specimens carrying pair of egg sacs. Antennule (Fig. 2A) 7-segmented, with armature formula: 4, 13, 6, 3, 4+1 aesthete, 2+1 aesthete, and 7+1 aesthete. Rostrum (Fig. 2B) broadly rounded. Labrum (Fig. 2C) consists of two large platelike processes with deep central incision. Antenna (Fig. 2D) 4-segmented; with formula of armature: 1, 1, 2+1 claw, and 5+2 claws; claw on third segment small and claws on terminal segment unequal. Maxilla (Fig. 2E) 2-segmented; proximal segment unarmed; distal segment carrying small outer setule at base and simple seta located posteriorly, and larger seta with long spinules on inner, distal margin. Maxiliped (Fig. 2E) 3-segmented; proximal segment largest and unarmed; middle segment carrying two unequal setae; distal segment carrying long spiniform outer seta and short, simple, medial seta at base. Legs 1-4 (Fig. 3A) biramous, with 3-segmented rami, only leg 4 with 2 segmented endopod, all legs (leg1-4) bearing spines on segments with serrations (Fig. 3B). Endopodal segments bearing tiny, denticle like projections at outer distal corner (Fig. 3C) Formula of spines (in Roman

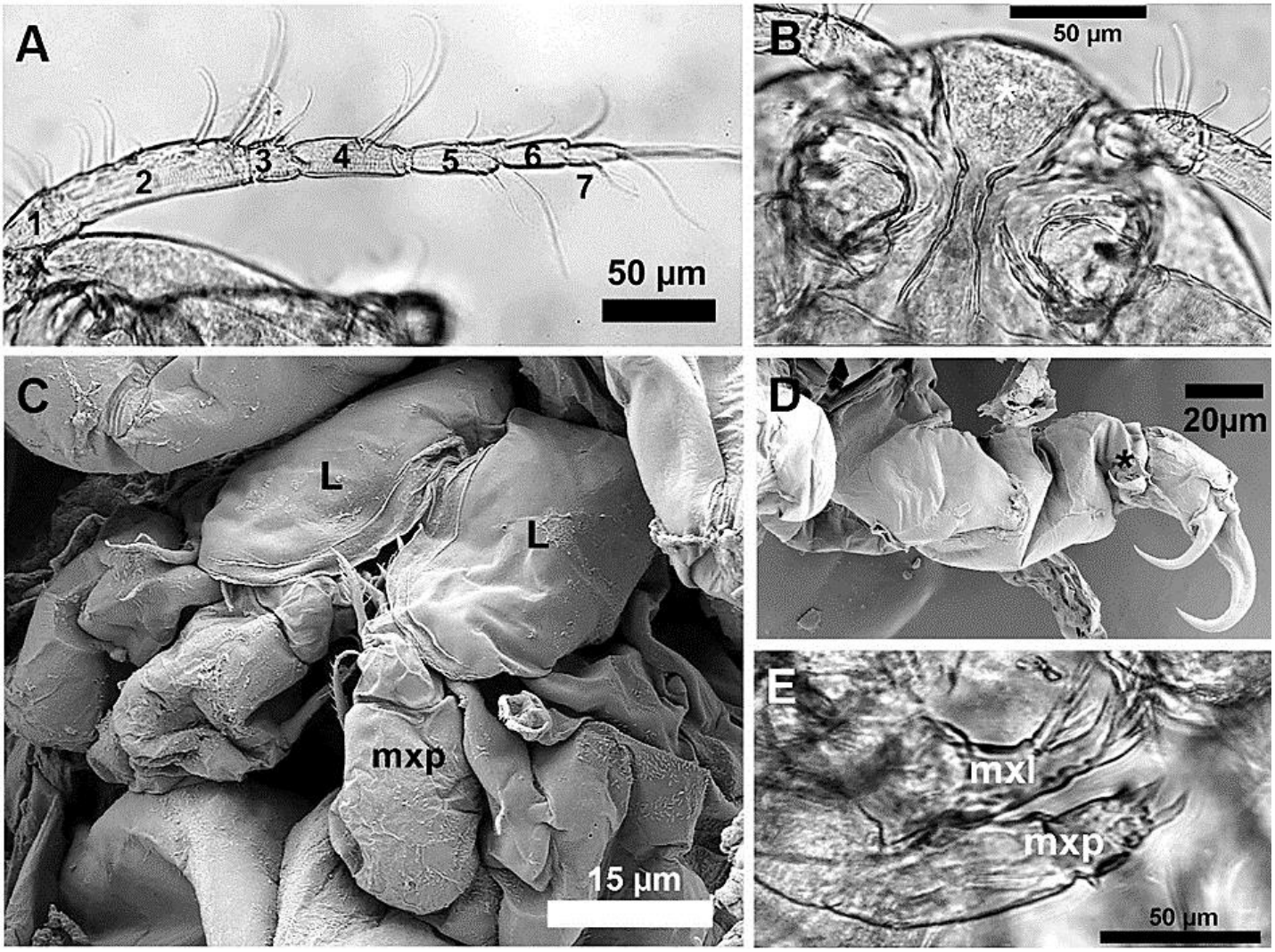

Figure 2. *Doridicola longicauda* (female) (A) 7-segmented antennule, (B) rostral area (white asterisk), (C) SEM images of labrum (L) and maxiliped (mxp), (D) SEM image of antenna showing small claw on third segment (under black asterisk) and two unequal terminal claws and (E) LM images of maxilla (mxl) and maxiliped (mxp).

numerals) and setae (in Arabic numerals) as follows:

	Exopodite	Endopodite
Leg 1	I-0; I-1; I-4	0-1; 0-1; I-5
Leg 2	I-0; I-1; III, I-5	0-1; 0-2; III-3
Leg 3	I-0; I-1; III, I-5	0-1; 0-2; III-2
Leg 4	I-0; I-1; II, I-5	0-1; II-0

Leg 5 (Fig. 3D) with elongate free segment (83×16.6 µm) carrying two unequal, simple setae located apically. Three free abdominal somites (Fig. 3E) 28×51, 25×44, and 11×43 µm, respectively. Caudal rami (Fig. 3F) about 2.21 times longer than wide, measuring 31×14 µm and bearing usual six setae in terminal area.

Male: Maxiliped (Figs. 4A, B) 4-segmented; first segment unarmed; second segment armed with rows of spinules and bearing two inner setae; third segment smallest and unarmed; terminal claw longer than the combined length of other three segments, with terminal lamellae and two very unequal setae in basal region.

Remarks: The most distinguishing feature of *D. longicauda* is the presence of a small medial claw on the distal end of the third segment of the antenna (Fig. 2D, black asterisk). To our knowledge, this unusual feature is shared with only six of the 52 valid species within the genus *Doridicola*. These species are: *D. agilis* Leydig, 1853; *D. inflatiseta* (Humes and Stock, 1973); *D. similis* (Ho and Kim, 2001); *D. scuriger* (Humes, 1964); *D. sensilis* (Humes, 1964); and *D. sepiae* (Izawa, 1976). Nevertheless, four of these six species, *D. agilis*, *D. inflatiseta*, *D. scuriger*, and *D. sensilis*, can easily be distinguished from *D. longicauda* by the following three

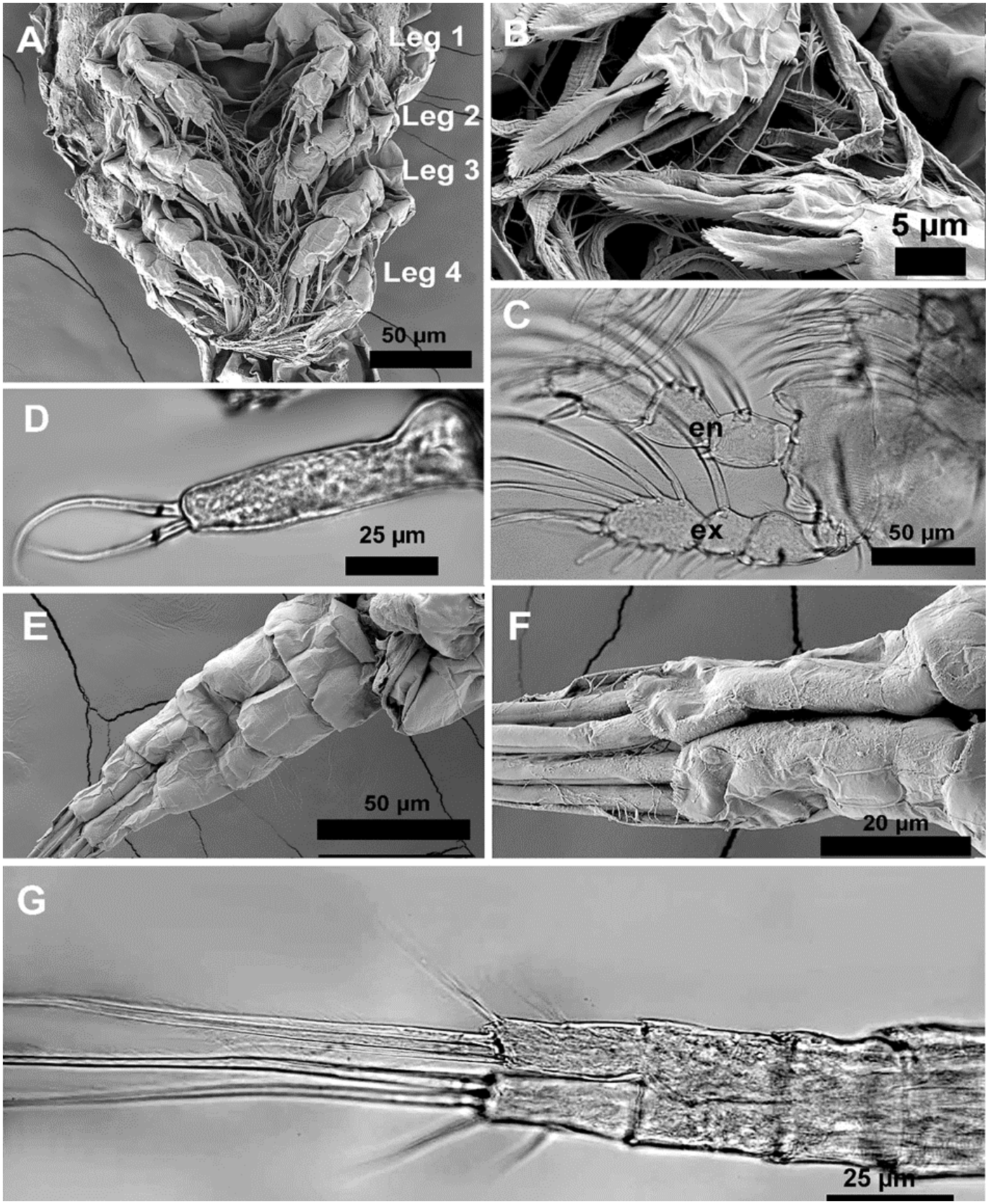

Figure 3. *Doridicola longicauda* (female) (A) Legs 1-4 (ventral view), (B) serrated spines on legs 1-4, (C) leg 2 exopod and endopod, (D) LM image of Leg 5, (E) three free abdominal somites, (F) SEM image of caudal rami and (G) LM image of caudal rami.

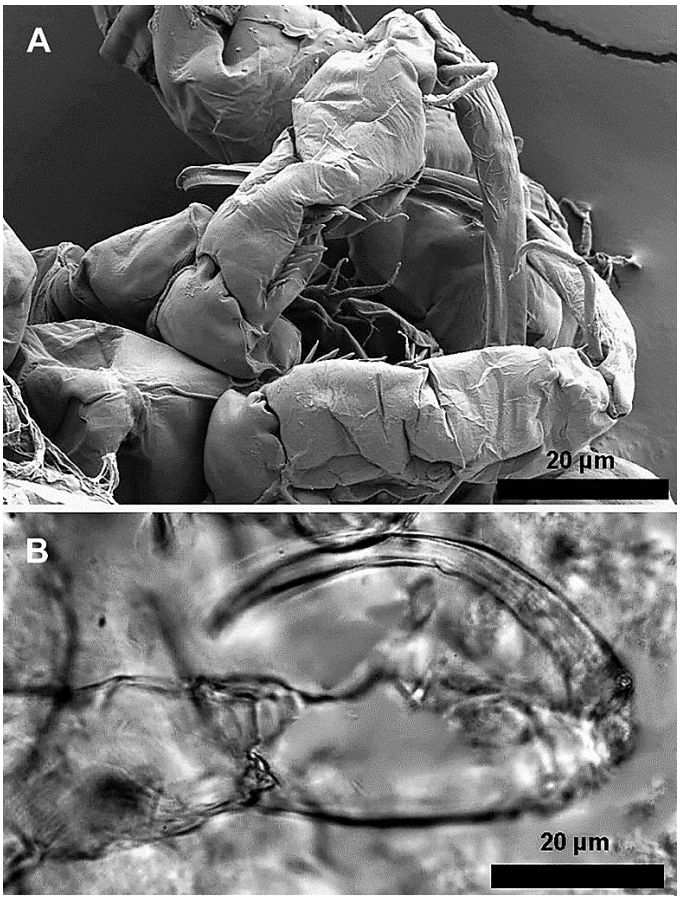

Figure 4. *Doridicola longicauda* (female). (A) SEM image of the maxiliped and (B) LM image of the maxiliped.

characters: (1) short caudal ramus that is either quadrate or wider than long, (2) absence of tuft of spinules distal to the scale of the mandible, and (3) a spinulate seta II on the terminal segment of the maxilla. Therefore, of these six species only *D. similis* and *D. sepiae* revealed closest similarity to our specimens. However, one of the most significant differences observed between presently reported *D. longicauda* and the other two closest species, *D. similis* and *D. sepiae*, is the variation in size and ratio (length to width) of the caudal ramus. The ramus is shortest in *D. longicauda* whereas it is longest in *D. similis*. The size of the caudal ramus of *D. sepiae* is in between *D. longicauda* and *D. similis*. The ratio (length to width) of the female caudal ramus is 2.21 in *D. longicauda* (see Fig. 3F, G), 3.18 in *D. sepiae* (Izawa, 1976), and 4.22 in *D. similis* (Ho and Kim, 2001). In addition, the length of the free segment of leg 5 (Fig. 3D) in *D. longicauda* is smaller (83 µm) than those of reported for *D. sepiae* and *D. similis* (vs. 150 µm and 114 µm, respectively) (Izawa, 1976; Ho and Kim, 2001). Our specimens also revealed close similarity to *D. virgatus*, recently described by Kim (2007), however, it differs from our specimens in having a rod-shaped setae (vs. none) on the terminal segment of the antenna.

In the previous studies, *D. longicauda* was reported from gill (Claus, 1860; Graeffe, 1902) and also mantle cavity (Stock, 1956; Costanzo et al., 1994) of cuttlefish. In the present study, specimens were collected from lateral fins of the host.

Discussion

Presently reported morphological features of our adult females revealed similarities both in shape and morphometrics to *D. longicauda* as described by Claus (1860) and Stock (1956). The body proportions of our females are also in the range given by Stock (1956). Therefore, our observations confirm that the parasitic copepods we found on *S. officinalis* are clearly identifiable as *D. longicauda* (Claus, 1860) which is new to the Turkish copepod fauna. In addition, this is the first report of a species of copepod belonging to the family Rhynchomolgidae Humes and Stock, 1972 in the Levantine Sea.

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