



# CYCLOPOID COPEPODS (ASCIDICOLIDAE, NOTODELPHYIDAE) ASSOCIATED WITH *PHALLUSIA NIGRA* SAVIGNY, 1816 (ASCIDIACEA) IN THE RED SEA: A NEW ASCIDICOLID AND FIRST DESCRIPTIONS OF THE MALES FROM TWO NOTODELPHYIDS

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#### ABSTRACT

A new species of copepod, *Styelicola omphalus* n. sp., of the family Ascidicolidae is described as an associate or symbiont of the ascidian *Phallusia nigra* Savigny, 1816 from the Red Sea. As major differential features of the new species, the body is large, more than 4 mm long, the antennule is 5-segmented, the mandibular palp is armed with one or two apical setae, and the maxillary syncoxa, maxilliped, and endopods of legs 1-4 are unarmed. This is the first confirmed ascidicolid reported from the Red Sea. Supplementary descriptions for two additional copepods from the same host, but in the family Notodelphyidae, are provided. Males of *Bonnierilla projecta* Stock, 1967 and *Janstockia phallusiella* Boxshall and Marchenkov, 2005 are detailed for the first time. The occurrence and location of the three species of copepods differed within the host. *Styelicola omphalus* was found in approximately 3% of hosts examined, consistently attached to the visceral mass. *Bonnierilla projecta*, in contrast, occurred in 63% of hosts and was located in the pharyngeal sac, and *J. phallusiella* was found attached to the internal surface of the tunic (atrium) in 11% of the hosts examined. Although the ascidian *P. nigra* has a circumtropical distribution, the copepods discussed above have only been reported from the Red Sea. Approximately 25 species of copepods are known as associates of *Phallusia* worldwide, but these appear restricted to only three (possibly five) of the 20 species currently recognized in this ascidian genus.

KEY WORDS: Bonnierilla projecta, Janstockia phallusiella, Notodelphyidae, Styelicola omphalus n. sp., symbiotic associations

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### INTRODUCTION

Ascidians act as hosts to a large number of copepod associates, mostly belonging to the families Ascidicolidae Thorell, 1859 and Notodelphyidae Dana, 1853 (Gotto, 1979; Monniot, 1990; Marchenkov and Boxshall, 1995). In fact, it has been estimated that approximately 50% of all ascidian species host copepods, with some ascidians showing regional differences in these associations (Monniot, 1990). Many of the ascidian-dwelling copepods are known only from females (e.g., Illg, 1958; Stock, 1967a; Jones, 1979; Boxshall and Marchenkov, 2005; O'Reilly, 2008) and most are considered parasitic, even in cases when heavily "infested" hosts seem to suffer no adverse effects (Monniot, 1990).

The solitary ascidian *Phallusia nigra* Savigny, 1816 is a widely distributed species that lives on shallow marine hard bottoms. It was originally described from the Red Sea, but has been reported since then in many tropical and subtropical locations worldwide although its native range remains unknown (Vandepas et al., 2015). *Phallusia nigra* has been described as an introduced species in the Pacific and Indian Oceans, and in the Mediterranean Sea, but it is considered either cryptogenic or native to the West Atlantic and the Red Sea (Izquierdo-Muñoz et al., 2009; Shenkar, 2012; Vandepas et al., 2015). Eight species of copepods have been reported as associates of P. nigra to date, almost exclusively from Red Sea locations (Table 1): Notodelphys ciliata Schellenberg, 1922 from the Gulf of Suez (Schellenberg, 1922), N. steinitzi, Bonnierilla projecta, Doropygus apicatus, Lonchidiopsis tripes, and Prophioseides brevis, all described by Stock (1967a) from the Dahlak Archipelago (Ethiopia), Janstockia phallusiella Boxshall and Marchenkov, 2005 from the Suez Canal (Boxshall and Marchenkov, 2005), and Bonnierilla yangpoensis Kim and Moon, 2011 from the Korean coast of the Sea of Japan (Kim and Moon, 2011). In addition, Por and Ferber (1972) referred to an undescribed species of Ophioseides, also from the Suez Canal, but no further work on this species is found in the literature. Paranotodelphys phallusiae (Gurney, 1927) from the Suez Canal has also been linked to this ascidian (Gurney, 1927), but this requires confirmation in spite of the species name.

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Table 1. Copepods associated with the ascidian genus Phallusia Savigny, 1816 worldwide. Only the currently accepted scientific names are provided.

Copepod species	Phallusia sp. host	Reference
Family Ascidicolidae Thorell, 1859		
Ascidicola rosea Thorell, 1859	P. mammillata	Illg and Dudley (1980); Pastore (2001)
Styelicola omphalus n. sp.	P. nigra	This study
Family Enteropsidae Thorell, 1859	-	
<i>Enteropsis roscoffensis</i> Chatton and Brément, 1909	P. mammillata	Illg and Dudley (1980); Holmes and Gotto (2000)
Family Lichomolgidae Kossmann, 1877		
Lichomolgus forficula Thorell, 1859	P. mammillata	Holmes and Gotto (1992); Constanzo et al. (1997) Pastore (2001)
Lichomolgus marginatus Thorell, 1859	P. mammillata	Huys et al. (2012)
Family Notodelphyidae Dana, 1853		
Bonnierilla projecta Stock, 1967	P. nigra	Stock (1967a); Por and Ferber (1972); this study
Bonnierilla yangpoensis Kim and Moon, 2011	P. nigra (P. philippinensis?) <sup>1</sup>	Kim and Moon (2011)
Botachus cylindratus Thorell, 1859	P. fumigata, P. mammillata (P. monachus?) <sup>2</sup>	Illg (1958); Monniot (1961); Holmes and Gotto (2000)
Doropygella psyllus (Thorell, 1859)	P. fumigata	Illg and Dudley (1965)
Doropygus apicatus Stock, 1967	P. nigra	Stock (1967a)
Doropygus pulex Thorell, 1859 <sup>3</sup>	P. mammillata	Holmes and Gotto (2000)
Gunenotophorus globularis O. G. Costa, 1838	P. mammillata	Illg (1958)
Janstockia phallusiella Boxshall and Marchenkov, 2005	P. nigra	Boxshall and Marchenkov (2005); this study
Lonchidiopsis tripes Stock, 1967	P. nigra	Stock (1967a)
Notodelphys allmani Thorell, 1859 <sup>3</sup>	P. mammillata, P. fumigata	Illg (1958); Pastore (2001)
Notodelphys ciliata Schellenberg, 1922	P. nigra	Schellenberg (1922)
Notodelphys prasina Thorell, 1859	P. mammillata	Illg (1958); Bocquet and Stock (1960); Illg and
		Dudley (1965); Holmes and Gotto (2000); Huys et al. (2012)
Notodelphys rufescens Thorell, 1859	P. mammillata	Bocquet and Stock (1960); Holmes and Gotto (2000)
Notodelphys steinitzi Stock, 1967	P. nigra	Stock (1967a)
Notodelphys tenera Thorell, 1859	P. mammillata	Illg (1958)
Notopterophorus elongatus Costa O. G., 1838 <sup>3</sup>	P. mammillata	Illg (1958); Illg and Dudley (1965); Holmes and Gotto (2000); Pastore (2001)
Notopterophorus papilio Hesse, 1864 <sup>3</sup>	P. mammillata	Gourret (1887, 1888)
<i>Ophioseides</i> sp.	P. nigra	Por and Ferber (1972)
Paranotodelphys phallusiae (Gurney, 1927)	P. nigra $(?)^4$	Gurney (1927)
Pachypygus gibber (Thorell, 1859)	P. fumigata	Illg (1958)
Prophioseides brevis Stock, 1967	P. nigra	Stock (1967a)

<sup>1</sup> A recent molecular analysis (Vandepas et al., 2015) suggests that the host reported as *P. nigra* in Kim and Moon (2011) might be *P. philippinensis*.

<sup>2</sup> Illg (1958) refers to *Phallusia monacha* (*P. monacha*), which we interpret as *P. monachus* Savigny, 1816, a valid species.

<sup>3</sup> Although subspecies have been recognized in this taxon, we report *Phallusia* host records on the species as a whole.

<sup>4</sup> Gurney assumed this species came from dredged *P. nigra* and stated "the animals no doubt came from *Phallusia nigra*, which was common at El Kantara" (Gurney, 1927: 482).

During studies on the ecology of copepod-host interactions in the Egyptian Red Sea, a previously unreported copepod was observed in *P. nigra* along with other poorly studied species. We herein describe this new species as *Styelicola omphalus* in the family Ascidicolidae. Supplementary descriptions for *Bonnierilla projecta* and *Janstockia phallusiella* (both in Notodelphyidae) are also given. These include the first descriptions of the males of both species. While all these copepods could be parasites of *P. nigra*, we use the term "associates" to emphasize the lack of information on the nature of the interactions between these crustaceans and their ascidian host.

#### MATERIAL AND METHODS

Specimens of *Phallusia nigra* (N = 170) were collected by snorkeling at various reefs near the vicinity of El Gouna on the Egyptian coast of the Red Sea (approximately 27°23′50.4″N, 33°40′30.2″E) during August 2012 and May 2013. Ascidians were carefully dislodged from hard substrates and transported in sealed plastic bags with seawater to the John D. Gerhart Field Station (American University in Cairo). There, ascidians were dissected using a long lateral incision contouring the tunic, and carefully analyzed under a dissecting microscope for associated fauna. The visceral mass (the internal organs), atrium, and branchial sac (pharynx) of *P. nigra* were carefully examined after removal in order to assess the location of different copepod species. Copepods were carefully removed with fine forceps or Pasteur pipettes and fixed in 10% formaldehyde in sea water. Some specimens were photographed under a dissecting microscope to record natural coloration and habit prior to preservation (Fig. 1).

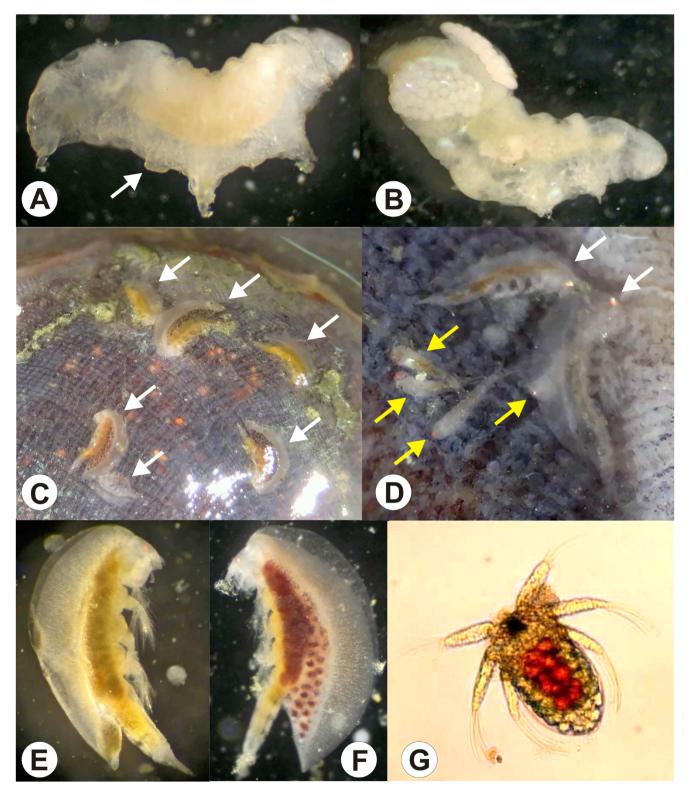


Fig. 1. Appearance of some of the copepods studied: A, *Styelicola omphalus* n. sp., non-ovigerous female showing the tubercle on the fourth pedigerous somite, for which the species is named (white arrow); B, ovigerous female with flattened lateral egg sacs; C, dissected *Phallusia nigra* Savigny, 1816 showing six *Bonnierilla projecta* Stock, 1967 females (white arrows) in the branchial basket of the host; D, different specimen of *P. nigra* showing males (yellow arrows) close to two females (white arrows), one of the males being under the dorsal processes of the larger female; E, isolated mature *Bonnierilla projecta* female without eggs; F, a different female showing the maturing eggs in the brood pouch (brown); G, newly-released swimming nauplius from *B. projecta* showing the darker central region. See scale bars in Figs. 2 and 4 for relative sizes.

Prior to microscopic observation and dissection for taxonomy, preserved copepod specimens were immersed in lactic acid for about 30 minutes. Dissection and observation were done following the reversed slide method (Humes and Gooding, 1964). All illustrations were drawn with the aid of a drawing tube mounted on an Olympus BH-2 microscope.

Roman numerals indicate spines and Arabic numerals represent setae in the formula for the armature of legs 1-4. Authorities for all pertinent taxonomic ranks used are based on the World Register of Marine Species database (WoRMS Editorial Board, 2016).

#### **Systematics**

## Order Cyclopoida Burmeister, 1835 Family Ascidicolidae Thorell, 1859 Genus *Styelicola* Lützen, 1968 *Styelicola omphalus* n. sp. Figs. 1-3

Material Examined.—Seven females from the visceral mass of the solitary ascidian *Phallusia nigra*, Abu Tieg Marina, El Gouna, Red Sea (Egypt), 13 August 2012. Holotype (female, NIBRIV0000440477) and paratypes (4 females, NIBRIV0000440697) deposited in the National Institute of Biological Resources, Incheon, Korea. Dissected paratypes (2 females) are retained in the collection of I.-H. Kim.

Description.—Female. Body (Figs. 1A, B; 2A, B) caterpillar-like, soft, slightly dorsoventrally depressed, gradually broadening from anterior to genital double-somite, consisting of cephalosome, first to fifth pedigerous somites, genital double-somite, 3-segmented abdomen. Body length 4.35 mm. Greatest width of body 1.36 mm across genital double-somite. Prosome-urosome division unclear. Cephalosome nearly triangular, narrower than posterior somites. Each pedigerous somite with weak dorsal tergite. Boundaries between anterior somites obscure, only represented by slightly constricted wrinkled regions. Fourth pedigerous somite with distinct, blunt median tubercle in posterior region of ventral surface (arrowhead in Figs. 1A, 2B). Tergite of fifth pedigerous somite rudimentary, covering only small part of somite (Fig. 2A). Genital double-somite expanded (Fig. 2C), wider than long,  $0.81 \times 1.36$  mm, with indistinct dorsal suture line, rounded lateral margins. Genital aperture positioned dorsolaterally in anterior region of somite. Abdomen distinctly tapering, 3-segmented; segmentation distinct dorsally but incomplete ventrally. Three abdominal somites  $(0.38 \times 0.88, 0.23 \times 0.55, 0.18 \times 0.36 \text{ mm})$ from anterior to posterior). Caudal ramus (Fig. 2D) small,  $158 \times 73 \ \mu m$  (length/width ratio 2.16:1), incompletely articulated from anal somite, with 6 setae consisting of 1 outer lateral, 1 dorso-distal, 4 distal setae; outer lateral seta positioned slightly proximal to midlength of ramus; all setae naked, not longer than width of ramus.

Rostrum absent. Antennule (Fig. 2E) short, stout, tapering, about 180  $\mu$ m long, 5-segmented; armature of segments 2, 10, 5, 2, 12; all setae naked, some short or blunt; aesthetascs, if present, hardly distinguishable from setae; last segment with trace of segmentation in middle; second, third, fifth segments ornamented with 1 to several setules. Antenna (Fig. 2F) small and 3-segmented; first segment (coxobasis) largest, slightly longer than wide, with large, lamellalike seta on projecting inner distal corner; second segment (first endopodal segment) also slightly longer than wide, obliquely inserted on first segment, unarmed, with longer inner margin, shorter outer margin; third segment (second endopodal segment) slightly tapering, armed with small proximal seta on inner margin, 3 small setae distally, and terminal claw, ornamented with minute spinules on inner surface.

Labrum (Fig. 2G) semicircular, with stout slightly incurved process on each posterolateral corner, sclerotized band on lateral sides. Mandible (Fig. 2H, I) consisting of large gnathobase, small palp; cutting edge of gnathobase with 4 major, 6 minor teeth (arranged as 1, 2, 3 between proximal to distal major teeth), with accessory tooth on proximal margin at base of spinulose proximal margin of proximalmost major tooth, palp unsegmented or incompletely 2-segmented, spindle-shaped, armed distally with 1 or 2 thick setae. Paragnath (Fig. 3A) lobate, weakly bifurcate distally. Maxillule (Fig. 3B) consisting of precoxa, palp; precoxa longer than wide, with 6 blunt setae on medial (apical) margin, pointed seta on proximal margin; palp slightly longer than precoxa, with 2 larger setae on distal margin, 9 (3 trinary) shorter setae on medial (apical) margin. Maxilla (Fig. 3C, D) 2-segmented; proximal segment (syncoxa) large but unarmed; distal segment (basis) with 3 (occasionally 2) small, blunt spines on anterior surface, apical, ventral, dorsodistal spine; latter occasionally absent (Fig. 3C). Maxilliped (Fig. 3E) rudimentary, indistinctly 2-segmented; distal segment with small point apically.

Legs 1-4 consisting of clearly defined coxa, basis, 2segmented exopod, endopod. Sizes of legs larger from anterior to posterior pairs. Coxae, basis, endopodal segments with transverse rows of minute spinules on surfaces; endopods broad, unarmed, as long as exopods, with rounded distal margin of distal segment; exopods tapering, much narrower than endopods, with unarmed proximal segment and thick plus small terminal spines on distal segment. Leg 1 (Fig. 3F) with small inner spine on basis, short outer seta at articulation of exopod. Leg 2 (Fig. 3G) similar to leg 1 but lacking inner spine on basis. Legs 3, 4 identical to leg 2 in shape, armature.

Armature formula of legs 1-4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1:	0-0	1-I	0-0; II	0-0; 0
Legs 2-4:	0-0	1-0	0-0; II	0-0; 0

Leg 5 (Fig. 3H) with large, lamelliform protopod, small exopod; protopod 462  $\times$  769  $\mu$ m, with small seta on distal margin; exopod (Fig. 3I, J) 1-segmented, inserted to ventral side of protopod but not articulated with latter, about 106  $\times$  75  $\mu$ m (length/width ratio 1.41:1), armed distally with 5 or 6 (5 being usual) naked setae. Leg 6 (Fig. 3K) represented by 2 thick plus slender spines in genital aperture. Egg sacs external, oblong, flattened, convex laterally, concave medially, varying in size, 1410  $\times$  864  $\mu$ m in largest measured one containing more than 200 eggs (Fig. 1B); each egg 130  $\mu$ m in diameter.

Male. Unknown.

Etymology.—The name *omphalus* is derived from the Greek *omphalos* ("navel"), alluding to the median ventral protuberance on the fourth pedigerous somite in the female of the new species (Figs. 1B, 2B).

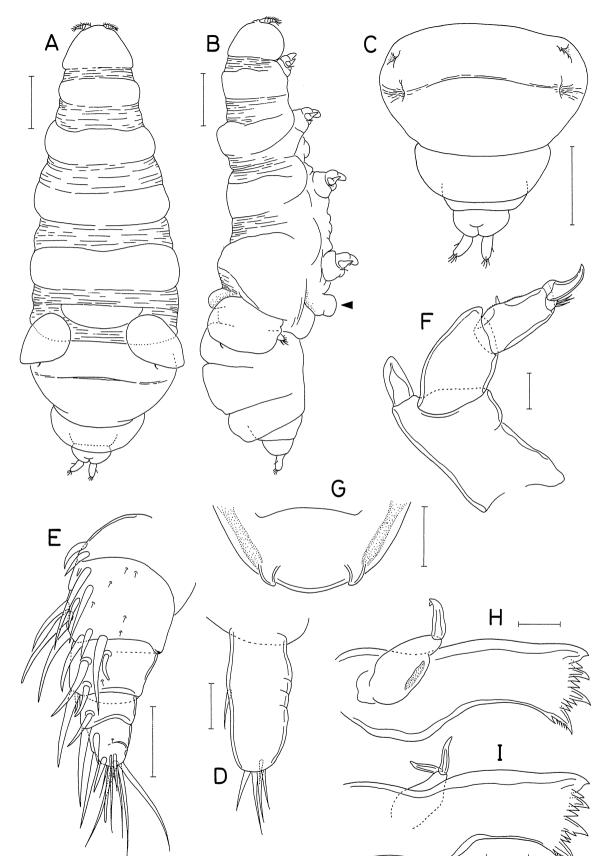


Fig. 2. *Styelicola omphalus* n. sp., female. A, habitus, dorsal; B, habitus, lateral; C, genital double-somite and abdomen, dorsal; D, right caudal ramus, ventral; E, antennule; F, antenna; G, labrum; H, I, mandibles. Scale bars: A-C, 0.5 mm; D, E, G, 0.05 mm; F, H, I, 0.02 mm.

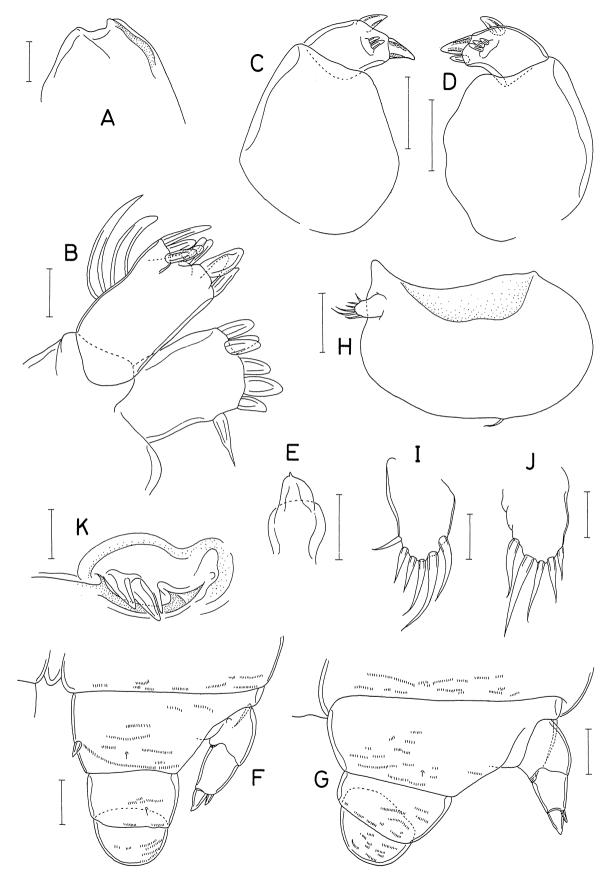


Fig. 3. *Styelicola omphalus* n. sp., female. A, paragnath; B, maxillule; C, right maxilla; D, left maxilla; E, maxilliped; F, leg 1; G, leg 2; H, leg 5; I, J, exopods of leg 5; K, genital area. Scale bars: A, B, E, K, 0.02 mm; C, D, F, G, I, J, 0.05 mm; H, 0.2 mm.

Table 2. Morphological differences in females of the three species of <i>Styelicola</i> Lützen, 1968.
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Character	S. bahusia Lützen, 1968	S. lighti Illg and Dudley, 1980	S. omphalus n. sp
Body form	Caterpillar-like	Slender	Caterpillar-like
Body length	2.0 mm	3.4 mm	4.35 mm
Antennule	8-segmented	7-segmented	5-segmented
1 <sup>st</sup> endopodal segment of antenna	Unarmed	1 seta	Unarmed
Mandibular palp	4 setae	7 setae	1 or 2 setae
Maxillule			
Precoxa	8 setae	7 setae	7 setae
Palp	11 setae	13 setae	14 setae
Maxilla			
Syncoxa	2 setae	3 setae	Unarmed
Basis	7 elements	8 elements	5-6 elements
Maxilliped	1 seta	4 setae	Unarmed
Leg armature formulae			
Leg 1 exopod	I-0; II or IV	I-0; VI, 1	0-0; II
Leg 1 endopod	0-0; II or III	0-0; IV	0-0; 0
Legs 2-4 exopod	0-0; II	I-0; hook	0-0; II
Legs 2-4 endopod	0-0; II or III	0-I; V or VI	0-0; 0

Remarks.—There are two congeners of *Styelicola omphalus* n. sp.: *Styelicola bahusia* Lützen, 1968 associated with the tunicates *Styela sigma* Hartmeyer, 1912 (= *Styella atlantica* (Van Name, 1912)) and *Styela gelatinosa* (Traustedt, 1886) from the Skagerrak coast of Sweden (Lützen, 1968) and the French coast of the Bay of Biscay (Monniot, 1981), and *Styelicola lighti* Illg and Dudley, 1980 associated with the tunicate *Hartmeyeria chinensis* Tokioka, 1967 from Amoy, China (Illg and Dudley, 1980).

*Styelicola omphalus* n. sp. differs from its two congeners in various ways based on female anatomy. The female of the new species has a larger body, a 5-segmented antennule, one or two setae on the mandibular palp, an unarmed maxillary syncoxa, an unarmed maxilliped, and unarmed endopods of legs 1-4. These and other differences compared to *S. bahusia* and *S. lighti* are summarized in Table 2.

Live Coloration.—The copepod appears milky white to the naked eye. The body is translucent under dissecting microscopy, with off-white to yellow developing gonads and some internal organs visible through the integument (Fig. 1A). Eggs are white in ovigerous females (Fig. 1B).

Location in Host.—All individuals were found attached to, and sometimes embedded in, the visceral mass of the host. It was impossible to ascertain whether the copepods were consistently attached to any particular organ. Occurrence in the host was relatively low, with only about 3% of *P. nigra* inhabited by the copepod.

### Family Notodelphyidae Dana, 1853 Genus *Bonnierilla* Canu, 1891 *Bonnierilla projecta* Stock, 1967 Fig. 4

Material Examined.—Two females, 5 males from the branchial sac of the solitary ascidian *Phallusia nigra*, Zeytouna Reef, El Gouna, Red Sea (Egypt), 14 August 2012 (Fig. 4C-F).

Description.—Female. Body (Fig. 4A) 2.63 mm long, consisting of small cephalosome, unsegmented metasome

(brood pouch), indistinctly segmented urosome. Prosome 2.50 mm long. Metasome formed by fusion of first to fourth pedigerous somites, characteristically with tapering posterior part. Dorsal surface of metasome broadened, flat or forming broad longitudinal groove. Other morphological features as described and illustrated in original description by Stock (1967a).

Male. Body (Fig. 4B) 942  $\mu$ m, much smaller than that of female (Fig. 4A). Four metasomal somites well demarcated. Urosome 6-segmented. Fifth pedigerous somite 102  $\mu$ m wide. Genital somite 75 × 102  $\mu$ m. Four abdominal somites 92 × 89, 92 × 174, 68 × 85, 38 × 78  $\mu$ m in dorsal view. Anal somite with deep posteromedial incision. Caudal rami widely divergent; each ramus 80 × 28  $\mu$ m (length/width ratio 2.86:1).

Rostrum (Fig. 4C) consisting of broader proximal part and narrower, semicircular distal part. Antennule segmented, armed as in female. Antenna (Fig. 4D) consisting of coxa, basis, 2-segmented endopod. Coxa short, unarmed. Basis slightly longer than wide, with small lateral seta distally. First endopodal segment with small lateral subdistal seta. Second endopodal segment  $46 \times 13 \ \mu m$ , about 3.5 times as long as wide, with proximal plus middle small setae on medial surface, 2 medial subdistal setae, 3 blunt distal setae. Terminal claw strongly curved, with hyaline lobe on both sides near distal end.

Labrum, maxilla, maxilliped as in female. Mandible (Fig. 4E) with 4 teeth on coxal gnathobase; basis with inner distal seta; exopod with 5 plumose setae, 2 rows of minute spinules, distally on first segment; endopod incompletely articulated to basis indistinctly 2-segmented, with 4 setae on first segment, 7 setae on second segment, with outer 2 setae plumose. Maxillule (Fig. 4F) unsegmented, with 7 inner setae, 2 distal plumose setae.

Legs 1-4 with same armature formula as in female. Inner seta on coxa of legs 1-4 shorter than basis, weakly plumose. Leg 1 (Fig. 4G) with 3-segmented exopod, endopod; endopod segments with rows of minute spinules distally; inner distal spine on basis large, extending to middle of second

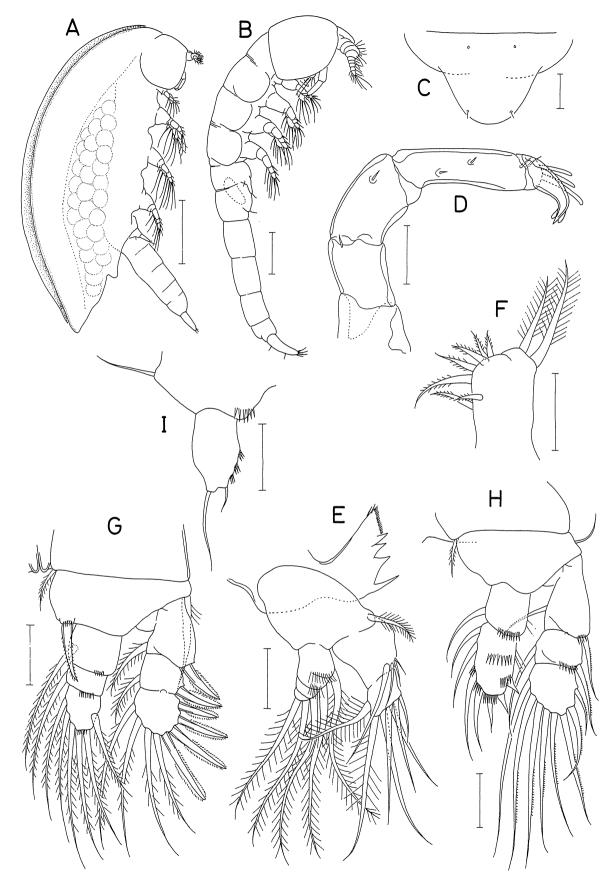


Fig. 4. *Bonnierilla projecta* Stock, 1967. Female: A, habitus, right. Male: B, habitus, right; C, rostrum; D, antenna; E, mandible; F, maxilliped; G, leg 1; H, leg 4; I, leg 5. Scale bars: A, 0.5 mm; B, 0.1 mm; C-F, I, 0.02 mm; G, H, 0.05 mm.

endopodal segment; outer spines on exopod longer than in female, but setae on both rami shorter than in female. Legs 2-4 with 3-segmented exopod, 2-segmented endopod; setae on these legs, especially on endopod of leg 4 (Fig. 4H), shorter than in female. Rows of minute spines distally on segments 1, 2 of endopods, exopods.

Leg 5 (Fig. 4I) protopod with outer seta; exopod 24  $\times$  13  $\mu$ m, 1.71 times as long as wide, with small subdistal seta, large distal seta. Rows of minute spinules distally on protopod, exopod. Leg 6 represented by 2 slender, naked setae on distal margin of genital operculum.

Remarks.—Stock (1967a) described this species based only on females collected from the Red Sea coasts of Massawa (Eritrea) and the islands of Entedebir and Um Aabak (Dahlak Archipelago, Ethiopia). The males examined followed the above description. The male differs from the female as follows: 1) the body is smaller, less than half as long as that of the female, 2) the caudal ramus is 2.86 times as long as wide, contrasting to more than 3 times in females (in the original description, see Stock, 1967a), 3) the plumosity of the setae on the legs and mouth appendages is less developed in the male compared to the female, 4) the setae on the legs are much shorter than in the female, 5) the second endopodal segment of the mandible is armed with 7 setae (6 setae in the female), and 6) the exopod of leg 5 is distinctly shorter than that of the female, 1.71 times as long as wide.

Live Coloration.-The copepods had noticeable red eyes (Fig. 1C-F) when observed under the microscope. The thin dorsal processes arising from the metasoma were finely speckled in yellow, but largely translucent. The developing gonads and parts of the gut could be clearly seen through the integument in mature females (Fig. 1G). Gonads ranged from milky white to dark green. Eggs (Fig. 1F) were yellow to brown and developed into dark nauplii inside the brood pouch. The coloration observed differs from that described by Stock (1967a), who pointed to the carmine color of the eggs. Swimming nauplii were released through a posterior opening of the pouch and had a darker central region rich in what appeared to be oil droplets (Fig. 1G). Females with mature nauplii would immediate push the offspring out upon being removed from the host and placed in an observation dish.

Location in Host.—Concurring with Stock (1967a) and Por and Ferber (1972), all individuals were found in the branchial sac (pharynx) of *P. nigra* (Fig. 1C, D). The smaller males often would move through the pharyngeal stigmata whereas females were mostly found on the external surface of the sac towards the basal portion. Males commonly outnumbered females within the hosts that were dissected, but their small size made them difficult to observe and extract. As many as 44 individuals (males and females) could occur in a single host. At least one individual of *B. projecta* was found in 63% of *P. nigra* examined.

# Genus Janstockia Boxshall and Marchenkov, 2005 Janstockia phallusiella Boxshall and Marchenkov, 2005 Figs. 5, 6

Material Examined.—Two females, 2 males from the internal surface of the tunic (atrium) of the solitary ascidian *Phallusia nigra*, Abu Tieg Marina, El Gouna, Red Sea (Egypt), 4 May 2013.

Female. Body (Fig. 5A) 6.25 mm long in dissected specimen, consisting of cephalosome, long trunk, small abdomen. Body surface covered with fine hairy setules. Cephalosome with distinct, round posterolateral expansions. Area of first pedigerous somite laterally expanded, concealing part of posterolateral swelling in dorsal view (Fig. 5B). Abdomen slightly wider than long, incompletely 2-segmented, with suture line only on lateral sides. Caudal rami absent, leaving 3 or 4 minute caudal setae on distal margin of abdomen.

Rostrum (Fig. 5C) with round posterior apex. Antennule densely covered with setules, with 5 traces of segmentation along posterior margin, 6 groups of armature elements arranged as 3, 6, 2, 1, 4 + aesthetasc, 9 + 2 aesthetascs, armature elements difficult to distinguish from setules. Antenna stout, 3-segmented; coxa short; basis unarmed; endopod with 2 subdistal, 3 distal setae; terminal claw strong.

Labrum elongated, tongue-like, covered with fine setules (setules not shown in Fig. 5C). Mandible consisting of smooth, elongated gnathobase (indicated by arrowhead in Fig. 5C), palp; palp with 7 setae, several setules (setules, some of setae omitted in Fig. 5C). Maxillule with 7 broad, setulose setae, 3 of them lobate (most of setae omitted in Fig. 5C). Maxilla incompletely 2-segmented; proximal segment smooth; distal segment longer than proximal segment, lanceolated, unarmed but covered by dense setules. Maxilliped absent.

Legs and other morphological features as in original description by Boxshall and Marchenkov (2005).

Male. Body (Fig. 6A) gradually tapering posteriorly, 1.16 mm long. Cephalosome well defined from metasome, 277 × 369  $\mu$ m; posterolateral expansions more prominent than in female, tapering, longer than wide. Metasome not articulated but metasomites clearly defined by constrictions between them. Four metasomites 127 × 273, 135 × 265, 115 × 219, 96 × 173  $\mu$ m. Urosome (Fig. 6B) 6-segmented, distinctly articulated. Fifth pedigerous somite small, 57 × 123  $\mu$ m. Genital somite sub-rectangular, 104 × 142  $\mu$ m. Four abdominal somites 52 × 88, 58 × 85, 46 × 71, 48 × 81  $\mu$ m. Caudal rami widely divergent, slightly tapering, 42 × 29  $\mu$ m, 1.45 times as long as wide, armed with 6 naked setae.

Rostrum as small, blunt lobe. Antennule (Fig. 6C) 9segmented, 158  $\mu$ m long; armature formula 2, 5, 10, 4, 1, 0, 2, 3, 10; all setae naked, most of them short; first, third, fourth segments ornamented with few setules. Antenna as in female.

Labrum distinctly tapering, with blunt posterior apex, several setules on distal region. Mandible consisting of gnathobase (Fig. 6D), palp (Fig. 6E) as in female, but palp armed with 6 setae, several setules. Maxillule (Fig. 6F) with 7 setulose setae, apical 2 of them broader than remaining 5.

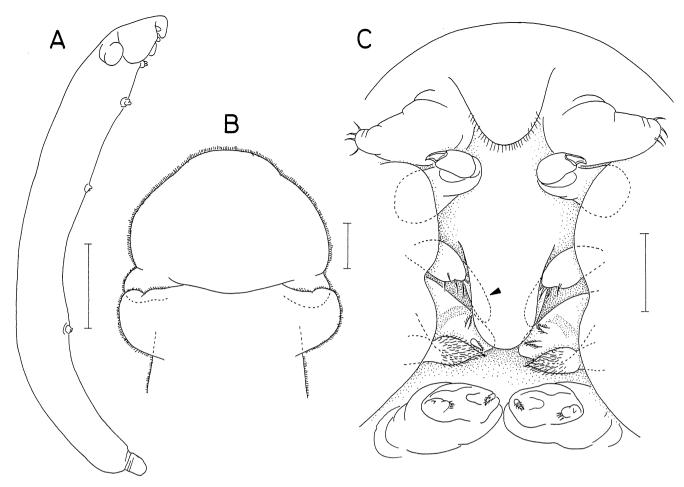


Fig. 5. Janstockia phallusiella Boxshall and Marchenkov, 2005. Female: A, habitus, right; B, cephalothoracic region, dorsal; C, cephalothoracic region, ventral (arrowhead indicates mandibular gnathobase). Scale bars: A, 1 mm; B, 0.2 mm; C, 0.1 mm.

Maxilla (Fig. 6G) unsegmented, but shaped as in female. Maxilliped absent,

Legs 1-4 consisting of coxa, basis, 3-segmented exopod endopod (Fig. 6H-J); all legs with scattered setules on coxa, basis, rami. All setae on legs short. Outer spines on exopods hardly distinguishable from setae. Legs 1-3 without inner seta on coxa. First endopodal segment of leg 1 lacking inner seta. Armature formula for legs 1-4 as follows:

<b>x</b> .	Coxa	Basis	Exopod	Endopod
Leg 1:	0-0	1-I	1-0; I-1; II. I. 3	0-0; 0-1; 1, 2, 2
Legs 2 and 3:	0-0	1-0	I-0; I-1;	0-1; 0-2;
Leg 4:	0-1	1-0	II, I, 5 I-1; I-1;	1, 2, 3 0-1; 0-2;
205 4.	01	10	II, I, 4	1, 2, 2

Leg 5 (Fig. 6K) 2-segmented; protopod with outer seta; exopod about 1.5 times as long as wide, with 2 unequal setae distally. Leg 6 represented by 2 setae on distal margin of genital operculum (Fig. 6B).

Remarks.—*Janstockia* consists of two known species, *J. phallusiella* and *J. truncata* Kim and Moon, 2011. In ventral view of the oral region, the mandibular gnathobase of

Janstockia is hardly visible under the microscope. It originates from the anterior oral region lateral to the proximal part of labrum and extends posteromedially underneath the labrum, and was regarded as the maxilla by Boxshall and Marchenkov (2005) or the lateral process of the labrum by Kim and Moon (2011). We reinterpret it as the mandibular gnathobase. The hirsute last oral appendage, which is lanceolated and called the maxilliped by both Boxshall and Marchenkov (2005) and Kim and Moon (2011), is reinterpreted as the maxilla, based on recent observations that in vermiform notodelphyid copepods the loss of mouth organs occurs generally from a posterior to an anterior location (I.-H. Kim, unpublished).

Live Coloration.—The vermiform females are easy to locate upon dissection of the host due to their large size and milkywhite appearance to the naked eye. The males are virtually transparent and difficult to see against the wet black tunic of the ascidian without the aid of a dissecting microscope.

Location in Host.—The majority of the females collected were found in the atrium of the host, attached to the tunic, closer to the siphons than to the base of the ascidian. A few individuals were found stuck to the host visceral mass, although it is unclear whether those were originally attached to the tunic as well and were dislodged during sample

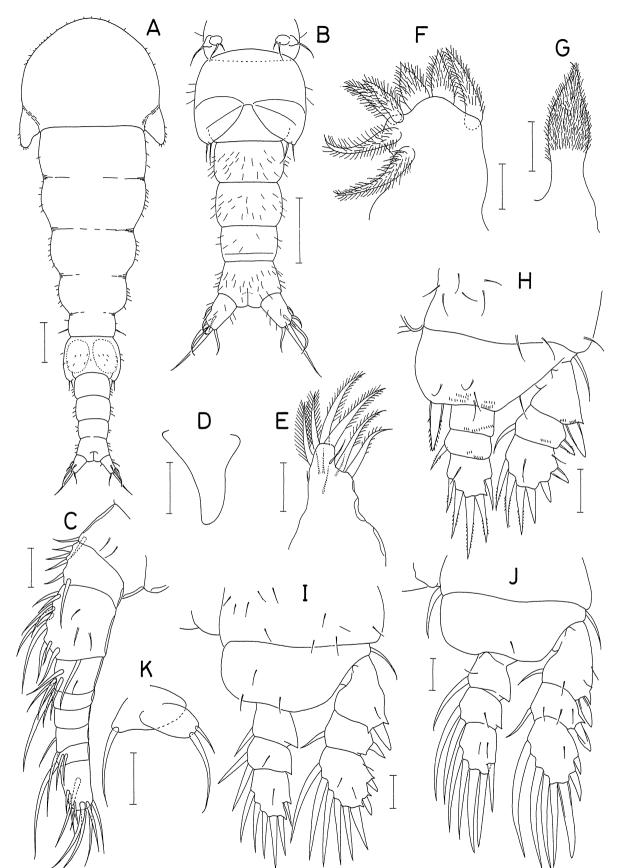


Fig. 6. *Janstockia phallusiella* Boxshall and Marchenkov, 2005. Male: A, habitus, dorsal; B, urosome, ventral; C, antennule; D, mandibular gnathobase; E, mandibular palp; F, maxillule; G, maxilliped; H, leg 1; I, leg 2; J, leg 4; K, leg 5. Scale bars: A, B, 0.1 mm; C-K, 0.02 mm.

manipulation. The males were found on the atrium crawling on the tunic, but their mobility made it impossible to assess the original site of attachment, if any. *Janstockia phallusiella* was uncommon, with only 11% of hosts carrying the copepod. This species was also observed releasing eggs or swimming nauplii upon removal from the host.

### DISCUSSION

Since the pioneering work of J. Stock in the 1960s (see Wagner, 1999), relatively few studies have assessed the diversity of Cyclopoida associated with invertebrates in the Red Sea. Stock (1967a) listed 28 species of ascidian-dwelling copepods in the family Notodelphyidae and described an additional species belonging to a different family (Stock, 1967b). Almost half a century later, only two more species of ascidian-inhabiting copepods have been added to that list: *Janstockia phallusiella* (Boxshall and Marchenkov, 2005) and *Styelicola omphalus* described herein.

Styelicola omphalus n. sp. constitutes at present the sole representative of Ascidicolidae for the Red Sea. The two other species from this area previously grouped in this family have now been removed, as former subfamilies have been revised to family status. They include the ascidian associate Mychophilus fallax Stock, 1967, originally placed in Enterocolidae (Stock, 1967b), but later in Ascidicolidae (López-González and Conradi, 1996), and now in Enteropsidae (Boxshall and Halsey, 2004). Similarly, Enterograthus lateripes Stock, 1966 was originally described as an ascidicolid endoparasitic in three species of Red Sea crinoids (Stock, 1966), but it is now placed in Enterognathidae (Boxshall and Halsey, 2004). Although there are three species of Styelicola (including the one described here), further analysis of this genus may be warranted (Table 2). Styelicola lighti differs from S. bahusia and S. omphalus n. sp. in having markedly apomorphic endopods of legs 2-4, in which the distal segment is transformed into a powerful hook. It also has, like Ascidicola, a slender body in females, a spinose pad between the penultimate and anal somites of abdomen, and an outer spine on the first exopodal segment of legs 2-4. In these respects, S. lighti presumably could represent a separate genus.

The other two copepods studied belong to Notodelphyidae. In both species, the males are independent from females (i.e., not parasitic on them), but considerably reduced in size. *Bonnierilla projecta* was the most abundant and common species, followed by *Janstockia phallusiella* and the rare *S. omphalus* n. sp. Co-occurrence of *B. projecta* with either of these two other species, and with *Doropygus apicatus* Stock, 1967 (which also lives in the ascidian pharyngeal sac) was commonly observed. Furthermore, amphipods of the genus *Leucothoe* Leach, 1814, which also inhabit the ascidian pharynx, were found in most of the *P. nigra* we collected. The nature of the interactions among all these dwellers in *P. nigra* remains poorly understood.

In general, our assessments of the females for both notodelphyids agree with previous works (Stock, 1967a; Boxshall and Marchenkov, 2005), although a reinterpretation of the oral appendages for *J. phallusiella* is presented. While two species of *Janstockia* have been described, a detailed comparison of the two reveals very little difference between congeneric females. Noticeable differences are in

the body length (6.25 mm in our specimen of *J. phallusiella* vs. 7.40-8.75 mm in *J. truncata* according to Kim and Moon, 2011) and in the shape of the cephalosome, the posterolateral expansions of which are prominent in *J. phallusiella* but absent in *J. truncata*. These differences seem not sufficient to consider them as separate species, but conspecificity is doubtful because of discrepancies in host selectivity and zoogeography. *Janstockia truncata* was described from *Chelyosoma siboja* Oka, 1906 in the Sea of Japan, but was not found in *Phallusia* cf. *nigra* from the same region (Kim and Moon, 2011).

It is noteworthy that the relationship between ascidians and copepods in Ascidicolidae and Notodelphvidae is often assumed to be parasitic (Illg, 1958; Stock, 1966, 1967a, b). There is nevertheless little information on the nature and costs and benefits of the interaction in almost all species. It has been noted that, while some copepods induce the formation of cysts or disorganization in the host tissues (suggesting an adverse immune response), other species attain large densities inside a host without seemingly affecting host fitness (Monniot, 1990; but see Hirose et al., 2005). While we did not measure host fitness directly, we observed no deformations or reductions in visceral mass despite the sometimes high numbers of associated copepods (Sherif, El-Sahhar and Cruz-Rivera, unpublished). The position of the copepods within the host could also provide clues to the nature of the association. For example, both S. omphalus and J. phallusiella were found directly attached to host tissues and organs, suggesting that these copepods could be feeding directly on the ascidian and could be classified as parasites. In contrast, B. projecta, which commonly attained 20-40 individuals in a single host, were more mobile, external within the host pharynx, and exposed to filtered particles inhaled into the host branchial sac and the mucus layer produced, both of which represent potential food sources. Furthermore, the large thin dorsal projections in the females of this species could serve to absorb nutrients from the surroundings, as has been suggested for other copepods, even those with functional guts like *B. projecta* (Bresciani, 1986; Østergaard, 1998). This hypothesis, however, must be properly tested.

Associated copepods have been reported only from approximately 12 (Schellenberg, 1922; Gurney, 1927; Stock, 1967a, b; Por and Ferber, 1972; Boxshall and Marchenkov, 2005; Shenkar and Loya, 2008) of the 73 ascidian species known from the Red Sea (Shenkar, 2012). Although Phallusia nigra is a widely-distributed species, and has also been introduced in a number of non-native ecosystems (Izquierdo-Muñoz et al., 2009; Vandepas et al., 2015), symbiotic copepods have been reported almost exclusively from the Red Sea. The one exception is Bonnierilla vangpoensis from Korea (Kim and Moon, 2011), but the host of this copepod species needs to be reconfirmed because a recent molecular analysis (Vandepas et al., 2015) reveals that reports of P. nigra in the West Pacific are likely P. philippinensis Millar, 1975. Besides the apparent geographic specificity of copepods associated with a single host species, the distribution of these copepods in other species of the genus Phallusia seems to be strongly biased as well. There are 20 recognized species of *Phallusia* worldwide, but copepod associates have been reported from only *P. mammillata* (Cuvier, 1815), *P. nigra* Savigny, 1816, and *P. fumigata* (Grube, 1864) (and perhaps *P. monachus* Savigny, 1816 and *P. philippinensis* Millar, 1975; Table 1). These represent a total of 17 copepod genera in four families, with Notodelphyidae accounting for 81% of the studied species. These patterns pose a number of interesting questions. For example, is the geographic distribution a result of evolutionary history or, more mundanely, a matter of copepodologist (or ascidian researcher) distribution worldwide? And, why are some species of *Phallusia* apparently more susceptible to copepods? Considering the importance of many ascidians as aggressive invasive species (Lambert, 2007; Bullard and Carman, 2009; Locke and Hanson, 2011), the study of their copepod associates might provide insights into the origins of introduced populations.

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#### REFERENCES

- Bocquet, C., and J. H. Stock. 1960. Copépodes parasites d'invertébrés des côtes de France. XI. Le genre *Notodelphys*, de la famille des Notodelphyidae. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen (Series C, Biological and Medical Sciences) 63: 123-136.
- Boxshall, G. A., and S. H. Halsey. 2004. An Introduction to Copepod Diversity. The Ray Society, London.
- , and A. Marchenkov. 2005. A new genus of notodelphyid copepod (Crustacea, Copepoda, Cyclopoida) from a compound ascidian host collected in the Suez Canal. Zoosystema 27: 483-497.
- Bresciani, J. 1986. The fine structure of the integument of free-living and parasitic copepods. A review. Acta Zoologica 67: 125-145.
- Bullard, S. G., and M. R. Carman. 2009. Current trends in invasive ascidian research, pp. 57-79. In, C. P. Wilcox and R. B. Turpin (eds.), Invasive Species: Detection, Impact and Control. Nova Science Publishers, New York.
- Costanzo, G., N. Crescenti, and N. Calafiore. 1997. Copepodid stages of Lichomolgus forficula Thorell 1859 (Copepoda, Poecilostomatoida, Lichomolgidae), a copepod associated with *Phallusia mammillata* (Cuvier, 1815). Journal of Natural History 31: 1019-1028.
- Gotto, R. V. 1960. A key to the ascidicolous copepods of British waters with distributional notes. Journal of Natural History 3: 211-229.

. 1979. The association of copepods with marine invertebrates. Advances in Marine Biology 16: 1-109.

Gourret, P. 1887. Sur quelques Crustacés parasites des Phallusies. Comptes Rendus hebdomadaires des Séances de l'Académie des Sciences, Paris 104: 185-187.

——. 1888. Études zoologiques sur quelques Crustacés parasites des Ascidies. Bibliothèque de l'École des Hautes Études, Paris (Section Sciences naturelles) 36: 1-64.

- Gurney, R. 1927. Report on the Crustacea: Copepoda (littoral and semiparasitic), Cambridge Expedition to the Suez Canal, 1924. Transactions of the Zoological Society of London 22: 451-577.
- Hirose, E., A. T. Oka, and M. Akahori. 2005. Sexual reproduction of the photosymbiotic ascidian *Diplosoma virens* in the Ryukyu Archipelago, Japan: vertical transmission, seasonal change, and possible impact of parasitic copepods. Marine Biology 146: 677-682.
- Holmes, J. M. C., and R. V. Gotto. 1992. A list of the Poecilostomatoida (Crustacea, Copepoda) of Ireland. Bulletin of the Irish Biogeographical Society 15: 2-33.
- —, and —, 2000. A checklist of the Cyclopoida (Crustacea: Copepoda) of Ireland. Irish Biogeographical Society Bulletin 24: 2-42.

- Humes, A. G., and R. U. Gooding. 1964. A method for studying the external anatomy of copepods. Crustaceana 6: 238-240.
- Huys, R., F. Fatih, S. Ohtsuka, and J. Llewellyn-Hughes. 2012. Evolution of the bomolochiform superfamily complex (Copepoda: Cyclopoida): new insights from ssrDNA and morphology, and origin of umazuracolids from polychaete-infesting ancestors rejected. International Journal for Parasitology 42: 71-92.
- Illg, P. L. 1958. North American copepods of the family Notodelphyidae. Proceedings of the United States National Museum 107: 463-659.
- , and P. L. Dudley. 1965. Notodelphyid copepods from the vicinity of Naples. Pubblicazioni della Stazione Zoologica di Napoli 34: 373-451.
- , and \_\_\_\_\_. 1980. The family Ascidicolidae and its subfamilies (Copepoda, Cyclopoida), with descriptions of new species. Mémoires du Muséum national d'Histoire naturelle (Nouvelle Série A, Zoologie) 117: 1-192.
- Izquierdo-Muñoz, A., M. Díaz-Valdés, and A. A. Ramos-Esplá. 2009. Recent non-indigenous ascidians in the Mediterranean Sea. Aquatic Invasions 4: 59-64.
- Jones, J. B. 1979. New Notodelphyidae (Copepoda: Cyclopoida) from New Zealand solitary ascidians. New Zealand Journal of Marine and Freshwater Research 13: 533-544.
- Kim, I.-H., and S. Y. Moon. 2011. Eight new species of ascidicolous copepods from the eastern coast of Korea (Crustacea, Copepoda, Cyclopoida). Ocean Science Journal 46: 23-46.
- Lambert, G. 2007. Invasive sea squirts: a growing global problem. Journal of Experimental Marine Biology and Ecology 342: 3-4.
- Locke, A., and J. M. Hanson. 2011. Trends in invasive ascidian research: a view from the 3rd International Invasive Sea Squirt Conference. Aquatic Invasions 6: 367-370.
- López-González, P. J., and M. Conradi. 1996. *Mychophilus palmatus*, a new species (Copepoda: Cyclopoida: Ascidicolidae) associated with the compound ascidian *Botryllus leachi* Savigny, with remarks on congeneric species and related genera. Hydrobiologia 330: 67-72.
- Lützen, J. 1968. *Styelicola bahusia* n. g., n. sp. (family Ascidicolidae), a commensal copepod from *Styela atlantica* and *S. gelatinosa* from the Skagerrak. Crustaceana, Supplement 1: 96-102.
- Marchenkov, A. V., and G. A. Boxshall. 1995. A new family of copepods associated with ascidiaceans in the White Sea, and an analysis of antennulary segmentation and setation patterns in the order Poecilostomatoida. Zoologischer Anzeiger 234: 133-144.
- Monniot, C. 1961. Doropygella spinicauda n. sp. Nouvelle espèce de copépode parasite d'Ascidie de la région de Banyuls-sur-mer. Bulletin de la Société Zoologique de France 86: 186-192.
- . 1981. Description de copépodes ascidicoles (Notodelphyidae et Ascidicolidae) de la pente continentale du golfe de Gascogne. Bulletin du Muséum National d'Histoire Naturelle, Paris, Série 4A 3: 431-454.
- ———. 1990. Diseases of Urochordata, pp. 569-636. In, O. Kinne (ed.), Diseases of Marine Animals. Vol. III. Biologische Anstalt Helgoland, Hamburg.
- O'Reilly, M. 2008. New records of copepods associated with ascidians from Scottish waters, including the description of a new species, *Enterocola ooishiae* n. sp. (Cyclopoida, Ascidicolidae), from a simple ascidian. Glasgow Naturalist 25: 57-74.
- Østergaard, P. 1998. An anatomical and developmental study of an endoparasitic copepod *Parachordeumium amphiurae* (Herouard) living in the brittlestar *Amphipholis squamata* (Delle Chiaje). Zoologischer Anzeiger 236: 189-202.
- Pastore, M. 2001. Copepods associated with *Phallusia mamillata* and *Ciona intestinalis* (Tunicata) in the area of Taranto (Ionian Sea, southern Italy). Journal of the Marine Biological Association of the United Kingdom 81: 427-432.
- Por, F. D., and I. Ferber. 1972. The Hebrew University-Smithsonian Institution collections from the Suez Canal (1967-1972). Israel Journal of Zoology 21: 149-166.
- Schellenberg, A. 1922. Neue Notodelphyiden des Berliner und Hamburger Museums mit einer Übersicht der ascidienbewohnenden Gattungen und Arten. Mitteilungen aus dem Zoologischen Museum in Berlin 10: 219-298.
- Shenkar, N. 2012. Ascidian (Chordata, Ascidiacea) diversity in the Red Sea. Marine Biodiversity 42: 459-469.
- , and Y. Loya. 2008. The solitary ascidian *Herdmania momus*: native (Red Sea) versus non-indigenous (Mediterranean) populations. Biological Invasions 10: 1431-1439.

- Stock, J. H. 1966. Copepoda associated with invertebrates from Gulf of Aqaba. 2. *Enterognathus lateripes* n. sp. a new endoparasite of Crinoida (Cyclopoida Ascidicolidae). Proceedings of the Koninklijke Nederlandse Akademie van Weteschappen (Series C – Biological and Medical Sciences) 69: 211-216.
- . 1967a. Report on the Notodelphyidae (Copepoda, Cyclopoida) of the Israel South Red Sea Expedition. Israel South Red Sea Expedition, 1962, Reports 27: 3-126.
- ——. 1967b. *Mychophilus fallax* n. sp., a new vermiform copeped parasite of a Red Sea tunicate. Israel South Red Sea Expedition, 1967, Reports 24: 9-12.
- Vandepas, L. E., L. M. Oliveira, S. S. C. Lee, E. Hirose, R. M. Rocha, and B. J. Swalla. 2015. Biogeography of *Phallusia nigra*: is it really black and white? Biological Bulletin 228: 52-64.
- Wagner, H. P. 1999. The carcinological work and taxa of Jan Hendrik Stock (1931-1997). Crustaceana 72: 725-759.
- WoRMS Editorial Board. 2016. World Register of Marine Species, available online at http://www.marinespecies.org.

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