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First record of two sabelline fan-worms in the tunic of the exotic sea squirt *Cnemidocarpa amphora* (Kott, 1992) (Stolidobranchia, Styelidae) from the Mediterranean Sea off Alexandria, Egypt

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ABSTRACT

The sea squirt *Cnemidocarpa amphora* was collected from Ras El-Tin beach in the Mediterranean Sea off Alexandria, Egypt. This study identifies two small sabelline fan-worms, *Parasabella minuta* and *Parasabella leucaspis*, inhabiting the tunic of the sea squirt. The identification was made on live and fixed worms using stereoscopic and scanning electron microscopes based on body size, radiolar crown, ciliation in the radioles, lenticular, pygidial and peristomial eyes, thorax and abdomen, morphology of lips, noto- and neuropodia, uncini, setae, secondary denticles, manubrium and the peristomial collar. *P. minuta* measures up to 5.5 mm in length and up to 1 mm in width, bearing lenticular radiolar eyes. Dorsal lips are triangular and the ventral lips are curved. Thoracic neuropodia are provided with uncini aviculares and three rows of secondary denticles. The manubrium is well-developed and slightly short. Abdominal neuropodia are provided with 2–6 elongated setae and the abdominal ones are provided with uncini aviculares covered by 3–4 rows of secondary denticles. *P. leucaspis* measures 5–41 mm long and up to 5 mm wide, with radiolar crown measuring 1–10 mm long. Dorsal lips are triangular and are provided with radiolar appendages while the ventral lips are curved. The thorax consists of 5–9 segments. The first segment has a narrow margin and is provided with elongated setae. From the second segment, the notopodia are provided with elongated upper setae. Thoracic neuropodia are provided with uncini aviculares and five rows of secondary denticles with elongated manubrium. Abdominal neuropodia are provided with 2 rows of bristles and the abdominal notopodia are provided with uncini aviculares and 5–6 rows of secondary denticles.

Introduction

Cnemidocarpa amphora is an alien species collected from the Mediterranean Sea of Alexandria, Egypt for the first time. It is possibly brought as a hitchhiker on the hulls of ships. This species is spread by anthropogenic activity and globalization from its main habitats to the new ecosystem near Alexandria harbor. It was recorded previously in the Red Sea (Sanamyan, 2015). Marine Polychaetes inhabit the intertidal region to abyssal depths, and from tropical regions to the poles (Castelli et al., 2008). The symbiotic relationship between the marine invertebrates and their microorganisms from the Bacterial and Archaea Domains makes it difficult to grow in the absence of the host (Abdel-Gawad and Mola, 2014, Erwin et al., 2014, López-Legentil et al., 2015). Sabelline larvae search vigorously for a substrate rich with biofilm and settle upon to metamorphose (Johansson, 1927, Castelli et al., 2008). Within Ascidiacea class, numerous species of the Didemnidae family are symbiotic with the single-celled *Prochloron didemni* (Tatsuya et al., 2019). Cyanobacteria release photosynthates and the sea squirt uses these materials as an extra source of nutrients (Erwin et al., 2014). In the sea squirt *Ecteinascidia turbinata*, *E. frumentensis* is present inside the cells of the pharynx (Tatsuya et al., 2019). Epibiotic bacteria form always a biofilm on sea squirts (Erwin et al., 2014, Tatsuya et al., 2019). The giant tubeworm *Riftia pachyptila* is symbiotic with the chemoautotrophic *gammaproteobacterium C* and *Endoriftia Persephone* (Klose et al., 2016). The sea squirts *Ascidia ahodori* and *Ascidia sydneiensis* accumulate vanadium where *Pseudomonas* and *Ralstonia* were extremely abundant in their branchial sacs (Tatsuya et al., 2019). The Sabellidae family was constructed by Latreille (1825) to encompass sessile poly-

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chaetes which have tentaclular crowns and built mucous tubes or use sediment particles in their tubes (Johansson, 1927, Mikac et al., 2013, Abdel-Gawad and Mola, 2014). The monophylogeny of this family is currently supported by three synapomorphies: thoracic neuropodium consists of uncini with a main tooth covered by minor secondary denticles; a manubrium lies in the proximal region of uncini and the abdominal neuropodium bears spine-like setae (Faulwetter et al., 1989, Capa and López, 2004). Sabelid worms have a very wide distribution, being found associated with freshwater and marine environments. Some species as *Clymeneis stigmosa* Rathke, 1843 are symbionts of gastropods and bivalves, perforating their shells (Castelli et al., 2008). All sea squirts host polychaetes (Abdel-Gawad and Mola, 2014). All species in this family are suspensivorous, showing great efficiency in particle selection (Tovar-Hernandez and Salazar-Vallejo, 2006). The head of sabelid worms is composed of a prostomium and a peristomy. The former is only a hint of a radiolar crown and is composed of two halves comprising a certain number of radioles (Fauchald, 1977). The number of radioles varies enormously between genera and species, ranging from two pairs as in *Monroika africana* (Monro, 1939), to few hundred pairs, as in *Sabella pavonina* Savigny 1820 (Faulwetter et al., 1989, Mikac et al., 2013). The radiolar crown of sabelid worms is a structure homologous to the grooved palps found in other polychaetes (Wong et al., 2014). The remainder of the prostomium is limited to the tissue area posterior to the mouth (Johansson, 1927, Castelli et al., 2008). The peristomy forms a complete ring in the anterior portion of the body and is usually associated with a necklace. The body of these worms have a conspicuous distinction between the thorax and the abdomen, which can be noticed due to the inversion of the setal pattern between notopodium and neuropodium and y the presence of the fecal leak (Grube, 1950). Normally, eight thoracic segments are found, but this number varies to four, as in *Pseudobranchiomma longa* (Kinberg, 1866), up to 12, as in *Amphiglena terebro* Rouse 1993 (Fauchald, 1977, Grube, 1950). The number of abdominal segments ranges from two to four, as in *Fabriciola phuketensis* (Faulwetter et al., 1989) and up to more than 100 as in *Sabella pavonina* Savigny 1820 (Wilson, 1936, Knight-Jones, 1983). The location of the bristles and uncini is differentiated in the thorax and abdomen. In the thorax, the notopodium is composed of bristles and the neuropodium is composed of a row of uncini while in the abdomen it is the opposite (Faulwetter et al., 1989). The body ends in a structure called pygidium. In most sabellid worms, the tube is constructed using the radiolar crown appendages to actively select particles available in the sediment and combine them with mucus (Fauchald, 1977). Some species occupy their tubes permanently (Castelli et al.2008), while others can abandon their tubes due to unfavorable conditions and build new ones (Chughtai and Knight-Jones, 1988, Abdel-Gawad and Mola, 2014). The main characteristics used in the group's taxonomy include: presence of accompanying bristle in the thoracic uncini; shape of the thoracic uncini, which can be acicular or avicular and the types of bristles and radiole structure (Johansson, 1927, Grube, 1950). The first significant subdivision of the Sabellidae family was made by Rioja (1923), when the family was subdivided into three subfamilies: Myxicolinae, Fabriciinae and Sabellinae. Recently, a detailed phylogenetic study of the Sabellidae family concluding that only two subfamilies constituted monophyletic groups: Fabriciinae and Sabellinae (Faulwetter et al., 1989, Ehlers, 2010). The present study aims to identify and characterize two sabelline fan-worms associated with the tunic of the sea squirt *Cnemidocarpa amphora* (Kott, 1992).

Material and methods

The sea squirt *Cnemidocarpa amphora* (Kott, 1992) was collected from Ras El-Tin beach in the Mediterranean Sea off Alexandria, Egypt for one year during June-August 2018–2019 to evaluate cadmium and lead detoxification. During spring and summer, in each month 15 squirts were collected while in autumn and winter months 5–10 squirts were found. It was noticed during field study that tubicolous polychates live onto the tunic of the sea squirt. Collections were performed in the intertidal zone by scraping substrata with spatulas to extract the sea squirt and the associated tubicolous polychates. In the laboratory this sea squirt is harvested in glass aquaria, continuous aeration is provided and the sea water is changed every other day. The fan-worms were removed from the tubes and analyzed while still alive with the aid of a stereoscopic microscope. Afterwards, the fan-worms are anesthetized in seawater with menthol crystals, fixed in 4% formaldehyde and finally washed and transferred to 70% alcohol. The identification was made with the aid of stereoscopic, optical and scanning electron microscopes. In addition, a digital camera (Olympus Camedia C-7070) coupled with stereoscopic (Olympus SZX9) and optical (Olympus BX51) microscopes were used to obtain photographs. For study under scanning electron microscope, the material was dehydrated, initially in ethanol solutions with progressively higher concentrations, between 70 and 100% and, then, using the critical point equipment. The specimens were placed in the stubs, covered with a 25–30 ηm gold foil and observed under the electron beam. For analysis, scanning electron microscopes from the faculty of science was used. The measurements of the total length of the bodies do not include the radiolar crown, and the width was always measured at the widest point of the thorax. The measures of the width of the margin of the bristles were always taken at the widest point of the bristle and the measures of the width of the rod were taken immediately below the beginning of the margin; the measurement of the distance between the thorax and the thoracic uncini was made from the top of the crest to the end of the thorax; measurements of the uncini and the manubrium length were taken from the end of the thorax to the end of the manubrium. The two fan-worms were identified according to Johansson (1927), Grube (1950), Fauchald (1977), Knight-Jones (1983), Perkins (1984), Faulwetter et al. (1989), Mikac et al. (2013), Wong et al. (2014).

Results

The adult *Cnemidocarpa amphora* measures 10–13 cm in length and 4–6 cm in width. Both oral and atrial siphons bear four-lobes. Usually four folds on each side of the pharyngeal sac are present. Pharyngeal stigmata are straight and longitudinally parallel to the endostyle. This squirt possesses a thick brown and irregularly wrinkled tunic. The oral orifice is present terminal and the atrial one and lies is about the midline of the dorsal aspect. There are 4–9 gonads on the right side and 1–5 on the left side. This is the first report of this alien species in the Mediterranean Sea of Alexandria, Egypt. *Parasabella minuta* Treadwell (1941) is a small sabelline, collected from the wrinkles of the squirt tunic, being in excess near both siphons. During warm months 4–6 polychaetes were collected from each squirt and 3–4 during cold months. This fan-worm is considered as an alien species in the Mediterranean Sea of Alexandria and it is the first record for Egypt. It measures up to 5.5 mm in length and up to 1 mm in width, with radiolar crown measuring up to 2 mm in length. Radiolar crown is provided with brown spots in the fixed material while in living material it has pink and colorless bands on the radioles, interspersed with green bands on the pinnacles; radiolar fringes present (Fig. 1a); lenticular radiolar eyes are present as independent units, and irregularly distributed along both sides of the radioles (filiform projections of the prostomium, longitudinally furrowed, with double rows of pinules, originating from the inner margin of the radioles, they may have an internal cellular skeleton) (Fig. 1b), 2–30 per radiole. Long, triangular dorsal lips (Fig. 1c); curved ventral lips,

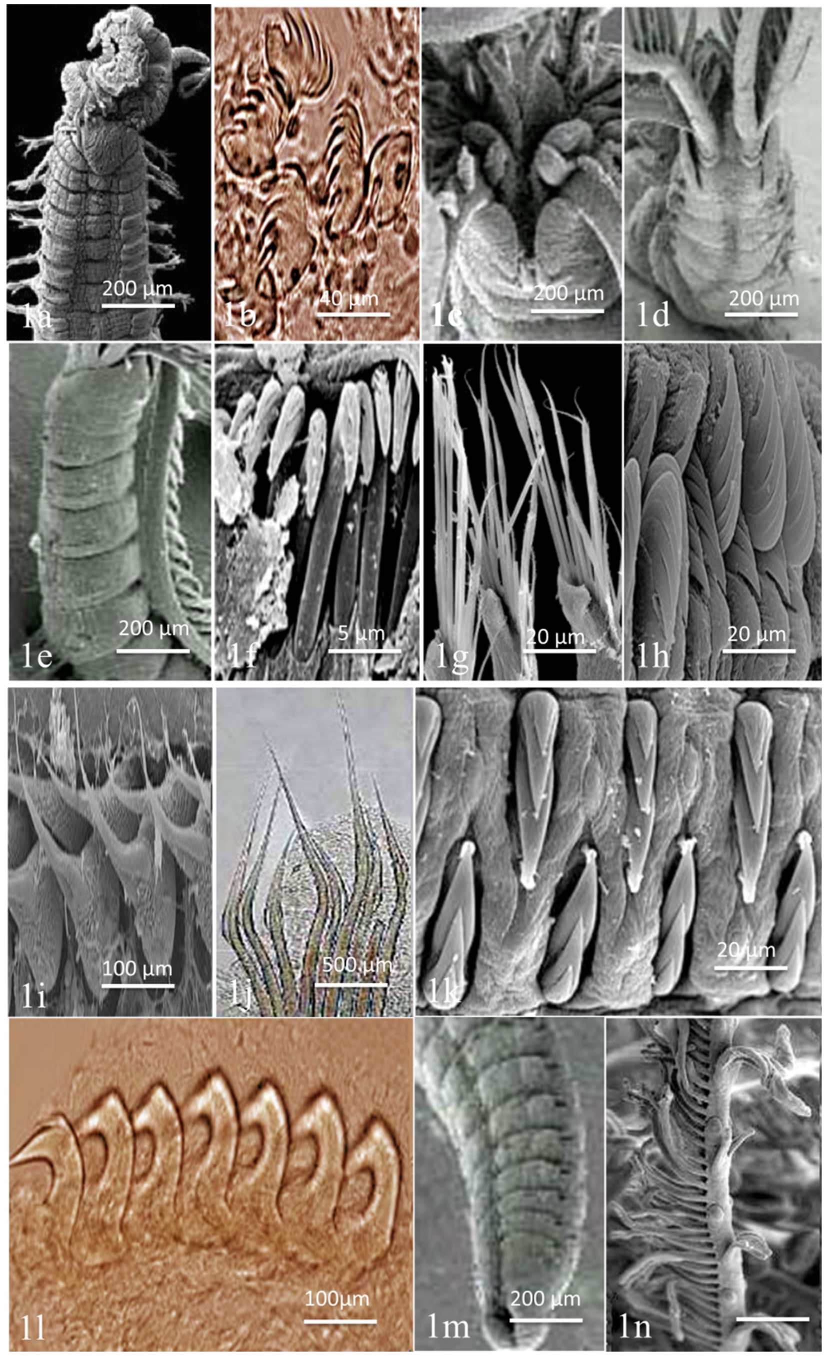

Fig. 1. photomicrographs and SEM images of *Parasabella minuta*: **1a.** radiolar crown and fringes. **1b.** lenticular radiolar eyes are distributed along both sides of the radioles. **1c.** triangular dorsal lips present. **1d.** curved ventral lips with a pair of ventral sacs in the proximal part. **1e.** collar is with non-overlapping ventral lamellae and ends away from the faecal dorsal groove. **1f.** thorax is with 4–5 segments. Segment1 is with bristles like spines. **1g.** from segment 2, thoracic notopodia are with upper setae like spines and thoracic neuropodia with uncini with 3 rows of secondary teeth. **1h.** well developed thorax and manubrium slightly short. **1i.** bristles accompanying the uncini with denticulate cap in the shape of a drop and long tapering tip progressively. **1j.** abdominal neuropodia with 2–6 elongated setae, with narrow margin arranged in two rows. 1k. Abdominal notopodia with uncini aviculares covered by 3–4 rows of secondary denticles. **1l.** manubrium is similar in size to the thoracic ones. **1m.** simple and round pigment presents. **1n.** ocelli.

forming parallel lamellae which protrude between the ventral lamellae of the collar; with a pair of ventral sacs in the proximal part (Fig. 1d). The collar is provided with non-overlapping ventral lamellae, slightly oblique laterally, ending away from the faecal dorsal groove (Fig. 1e). The thorax consists of 4–5 segments (Fig. 1e). Segment 1 is provided with bristle-like spines (Fig. 1f); from segment 2, thoracic notopodia are provided with upper seta-like spines (Fig. 1g), arranged in dorsal arches, the lower 4–8 setae bear broad limb (Fig. 1g) and aligned in 2 rows partially included in the arch. Thoracic neuropodia are provided with uncini aviculares and 3 rows of secondary teeth, occupying approximately half of the surface of the main tooth, well developed thorax and the manubrium is slightly shorter than the distance between the ridge and the thorax (Fig. 1h); bristles accompanying the uncini are provided with denticulate cap in the shape of a drop and long tapering tip progressively (Fig. 1i). Abdominal neuropodia are provided with

2–6 elongated setae, with narrow margin arranged in two rows (Fig. 1j). Abdominal notopodia are provided with uncini aviculares covered by 3–4 rows of secondary denticles (Fig. 1k), occupying approximately half of the surface of the main tooth, the manubrium similar in size to the thoracic ones (Fig. 1l). Simple and round pigment presents (Fig. 1m) with ocelli (Fig. 1n).

Parasabella leucaspis (Kinberg, 1867) is a small to median sized sabelline fan-worm measuring 5–41 mm long and up to 5 mm wide, with radiolar crown measuring 1–10 mm long. During warm months 5–7 ones were collected from each squirt and 2–3 during cold months. Radioles are provided with 2–17 dark brown to orange bands, extending to adjacent pinnacles; up to 2 irregular rows of ocular spots are present along the radiolus margins (Fig. 2a); very conspicuous ciliation is found in the radioles (lateral and internal tracts), pinules (paired projections, originating from the inner margin of the radiolar axis, lined by ciliary tracts) and dorsal and ventral lips (Fig. 2b). Bare tip of the radioles are present with an approximate length of 1.5–2 pinules (Fig. 2c). Juveniles may have pygidial and peristomial eyes. Long, triangular dorsal lips, with radiolar and pinular appendages; curved ventral lips (Fig. 2d) forming parallel lamellae which protrude between the ventral lamellae of the collar (Fig. 2e). Peristomial collar covers the base of the radioles, ventral lamellae are not overlapping; presence of a ciliated band after the end of the parallel lamellae. Thorax consists of 5–9 segments but 8 in most specimens (Fig. 2f), with conspicuous ciliation in the faecal groove and dorsally, on the anterior margin of the thoracic segment (Fig. 2g); the first ventral segment is twice as wide as it is height and is indented by the parallel lamellae (Fig. 2e). Segment 1 is provided with elongated setae and possesses a narrow margin (Fig. 2h); from segment 2, thoracic notopodia are provided with elongated upper setae and possess narrow margins, with 5–6 setae per row (Fig. 2i); lower bristles are provided wide limb, with variable width (Fig. 2j), arranged in 2 rows with 3–7 bristles each (Fig. 2k). Thoracic neuropodia are provided with uncini aviculares and 5 rows of secondary denticles, occupying half the surface of the main tooth (Fig. 2l), long manubrium, greater than the distance from the ridge to the thorax; accompanying bristles of uncini are provided with bulbous and denticulated head, with main tooth, from where the hood protrudes (Fig. 2l). Abdominal neuropodia are provided with 2 rows of bristles with narrow margin, 5–7 per row. Abdominal notopodia are provided with uncini aviculares and 5–6 rows of secondary denticles, occupying about 1/2 of the surface of the main tooth (Fig. 2m), a medium sized manubrium (Fig. 2n). Variation: the color of the radiolar bands varies from dark brown to orange, in general small specimens have a paler color pigmentation of the body, radiolar crown and phosphorescent green radiolar spots were observed. The number of bands per radiolus varies widely in the same worms

Discussion

Cnemidocarpa amphora dwells Australia, Eastern Indian Ocean, Alaska, north Washington, northwestern Pacific, the Arctic, Antarctic Atlantic, the Japanese sea bass, Red sea and the North Sea coasts (Sanamyan, 2015). The authors of this study collected this alien sea squirt from the Mediterranean Sea of Alexandria, Egypt (first record). Identification of this species was according to Sanamyan (2012). Members of family Sabellidae have three synapomorphies: disposition of secondary denticles over the main tooth in the thoracic uncini, forming a crest, presence of a manubrium in the thoracic uncini and abdominal bristles (Johansson, 1927, Faulwetter et al., 1989, Capa and López, 2004, Wong et al., 2014). According to Faulwetter et al. (1989) and Belalal and Ghobashy (2012) most of the family's characteristics are inconsistent, separation of Sabellidae and Serpulidae is done because the second family produces limestone tubes. The absence of an operculum has been already considered a characteristic of Sabellidae, however, it is known that an absence of character does not serve to define a group, in addition to their being serpulids, they do not have an operculum, apparently, a secondary loss (Ehlers, 2010, Mikac et al.2013, Belalal and Ghobashy, 2012, Wong et al., 2014). *Parasabella* Treadwell, 1941 has characteristics in common with *Bispira* (Johansson, 1927) such as short palmar membrane, radiolar fringes and ventral sacs, interramal ocelli, lower thoracic notopodia and presence of accompanying uncini bristles. However, the two genera differ in many ways. The radiolar crown in *Bispira* is arranged in two semicircles and the ventral end ranging from little involute to spiral, while in *Parasabella* it is organized in two semicircles, without involution. *Bispira* has paired compound radiolar eyes and *Parasabella* has odd lenticular radiolar eyes, irregularly distributed along the radioles. Specimens of *Parasabella* genus are smaller than all known species of *Bispira*, the thorax has only 4–5 segments, while *Bispira* has at least 8 thoracic ones (Banse, 1956, Tovar-Hernande and Salazar-Vallejo 2006, Bok et al., 2016, Keppel et al., 2020); and the body length varies between 3 and 5.5 mm, while *Bispira melanostigma* is 11 mm (Bok et al., 2016) and without a crown (Johansson, 1927). After consulting the character matrix used by Faulwetter et al. (1989), specimens of *Parasabella* do not possess most of the characteristics of *Sabella-Bispira-Sabellastarte-Branchiomma-Pseudobranchiomma*, such as lower thoracic notopodia; neuropodia anterior abdominal bristles, in the anterior segment and in the posterior ones. After examination, it was found that the species collected from the tunic of *Cnemidocarpa amphora* (Kott, 1992) from the Mediterranean Sea off Alexandria have many characters in common with *Parasabella minuta*. Studied the genus *Parasabella* collected from the Patagonian Shelf and Humboldt Current System. Chughtai and Knight-Jones (1988) report that *P. minuta* has very small brown spots on the sides of the radioles. All this evidence led us to consider that the worm belongs to *Parasabella*, instead of *Perkinsiana* since the presence of radiolar fringes, palmar membrane and radiolar lenticular ocelli, reduced number of thoracic segments, lower peristomial collar and with smaller ventral lamellae, inter-oral ocelli and morphology of the bristle cap accompanying the uncini differentiate it from *Perkinsiana* species. *Parasabella leucaspis* (Kinberg, 1867) is a median to large sabeline. Radiolar crown is with 4 or more rows of radiolar skeletal cells. Palmar membrane is provided with fringes. Dorsal lips with radiolar and pinular appendages; ventral lips present, continuing through a pair of parallel lamellae projecting between the ventral lamellae of the collar. Peristomial necklace presents. The thorax is provided with up to 9 segments. Thoracic notopodia are provided with lower bristles and broad limbs are arranged in several transversal rows. Thoracic neuropodia are provided with avicular uncini and secondary denticles of uniform size, median sized manubrium; accompanying bristles of uncini, denticulated tip, with upper tooth from which membranous cap protrudes. Abdomen is provided with variable number of segments. Abdominal neuropodia are provided with bristles organized in two transverse rows, in the anterior abdominal segments, both rows bear elongated bristles, in the posterior segments, the anterior row is provided with elongated bristles, the posterior row is provided with elongated bristles, with narrow limbs. Abdominal notopodia are provided with uncini aviculares and secondary denticles, well developed thorax and long manubrium (Perkins, 1984, Keppel et al., 2020). These authors state that the previous definition of the genus given by the presence of radioles arranged in a spiral was not consistent, since *Sabella* and *Bispira* also present the same organization of the radioles and not all *Sabella* species present such an organization of the radiolar crown. Thus, the fundamental character for the diagnosis of the genus today is the mor-

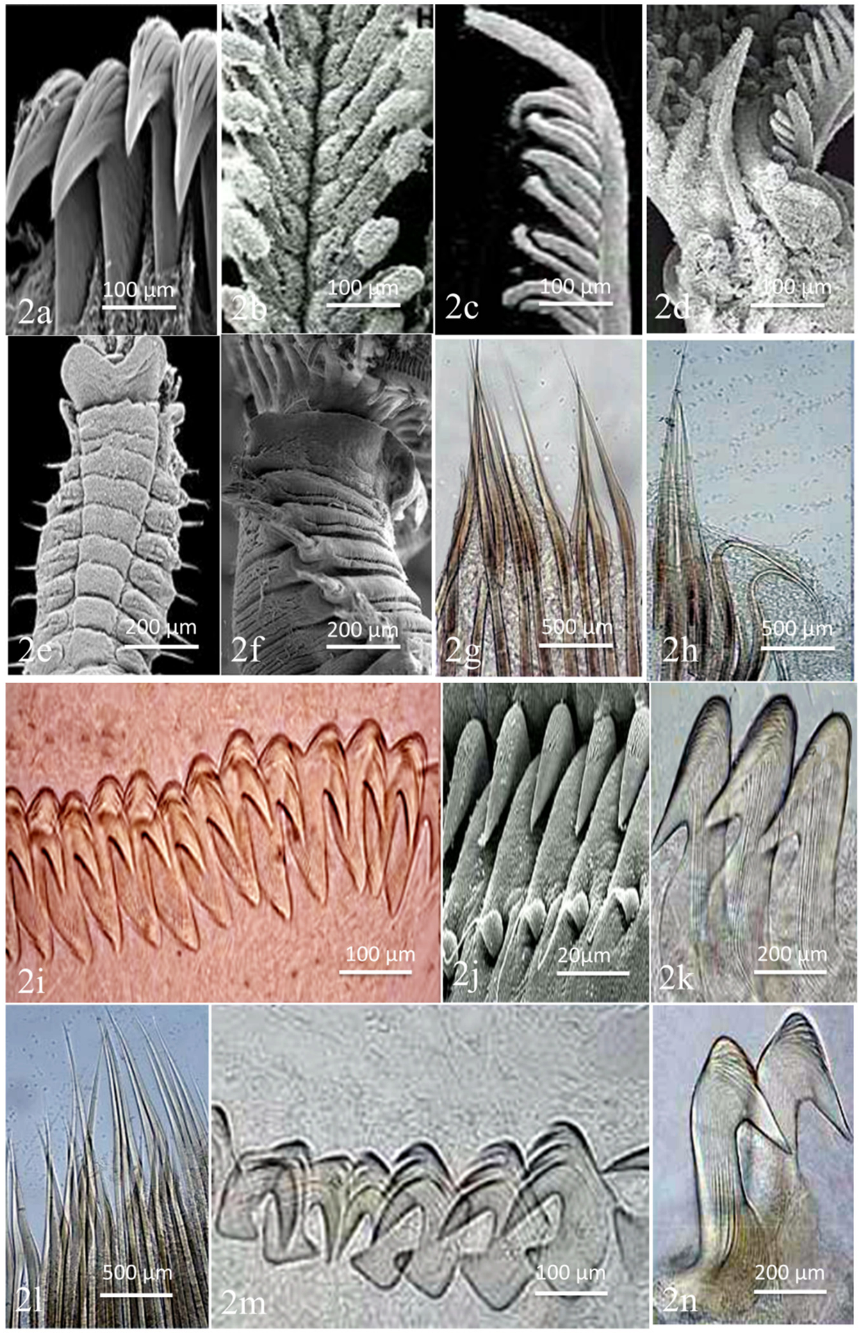

Fig. 2. photomicrographs and SEM images of *Parasabella leucaspis*. **2a**. radioles with dark bands. **2b.** ocular spots along the radiolus margins with very conspicuous ciliation. **2c.** the tip of the radioles are present with pinules. **2d.** pygidial and peristomial eyes. triangular dorsal lips, with radiolar and pinular appendages; curved ventral lips. **2e.** parallel lamellae protrude between the ventral lamellae of the collar. **2f.** thorax with 5–9 segments, 8 in most specimens. **2g.** conspicuous ciliation in the faecal groove are present. **2h.** segment1 is with elongated setae and a narrow margin. **2i.** from segment 2, thoracic notopodia with elongated upper setae, with narrow margin, 5–6 setae per row. **2j.** lower bristles with wide limb, and variable width. **2k.** setae are arranged in 2 rows with 3–7 bristles each. **2l.** thoracic neuropodia are with uncini aviculares with 5 rows of secondary denticles, occupying half the surface of the main tooth. **2m.** abdominal neuropodia are with 2 rows of bristles with narrow margin, 5–7 per row. **2n.** abdominal notopodia with uncini aviculares with 5–6 rows of secondary denticles, occupying about 1/2 of the surface of the main tooth.

phology of the bristles accompanying the uncini. According to Faulwetter et al. (1989), the closest genus to *Demonax* is *Megalomma* (Johansson, 1927), as both have lower thoracic notopodia with a broad limb. The two genera differ in the shape of the bristles accompanying the uncini, and in the fact that *Megalomma* has radiolar compound eyes. According to Faulwetter (2017) the genus was already known as *D. microphthalmus* (Verrill, 1873), identified as *Sabella microphthalma* Verrill, 1873. Rullier and Amoureux (1979). *Parasabella leucaspis* also named as *Demonax krusensterni* has a great variation in their more specific characters, such as thoracic and abdominal uncini accompanying bristles, they coincide with those described by Perkins (1984). Further study of specimens is still needed to determine whether they are one or more species. The analysis of our material points to the possibility that both characteristics are variable in *D. microphthalmus* and that, therefore, these species are synonymous. However, the redescription of *D. microphthalmus* by Perkins (1984) concludes that this species has lenticular radiolar eyes, which does not occur in our material, and has dorsal radiolar appendages much longer than that found in *D. cf. microphthalmus*.

Conclusion

The sea squirt *Cnemidocarpa amphora* is a stable habitat potentially favorable for feeding, shelter, brooding and reproduction for many zooplankton. In addition, the sea squirt has unknown ecological relationships with many sessile and benthic invertebrates. Larvae of *Parasabella minuta* and *Parasabella leucaspis* prefer to settle on the tunic of sea squirts. This habitat enable the tubeworms find virtually all food resources. The tunic of the sea squirt is made of a polysaccharide tunicine and harbor many species of bacteria. The tubeworms can assimilate photosynthates, Moreover, zooplankton like minute invertebrates and larvae of entomostracans, gastropods and bivalves live in excess inside the wrinkles of the sea squirt tunic (own observation). There is a balanced tripartite symbiotic relationship among the sea squirt, its biofilm and the sabelline fan-worms which increases the benthic diversity.

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Ethical clearance and experiments on animals

Ethical clearance for this study was obtained from the university of Alexandria ethical committee. Authors respected the value of the exotic sea squirt *Cnemidocarpa amphora* (Kott, 1992). Authors took into consideration that sea squirts are sentient creatures with the capacity to feel pain. Our treatment of the exotic sea squirt, including the use of animals in research, is an expression of our attitudes and influences us as moral actors. Minimal number of sea squirts as possible was used in the experimentation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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