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RESEARCH ARTICLE

A new symbiotic scale worm (Polychaeta: Polynoidae) living in association with burrowing callianassid shrimps in the Black Sea

Новая симбиотическая полихета (Polychaeta: Polynoidae), ассоциированная с роющими креветками-каллианассидами в Черном море

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Abstract. A new scale worm tentatively assigned to the genus *Malmgrenia* McIntosh, 1874 (Annelida: Polynoidae) is described from the burrows of callianassid shrimps belonging to the genus *Gilvossius* Manning et Felder, 1992 (Decapoda: Axiidea: Callianassidae). This is the first association involving callianassid shrimps with a polynoid scale worm, known from the Black Sea (northeastern basin) and even for the Atlantic Ocean basin. The taxonomy of the new species, as well as the previously known associations of scale worms with callianassid shrimps are also discussed.

Резюме. Новая симбиотическая полихета, предварительно отнесенная к роду *Malmgrenia* McIntosh, 1874 (Annelida: Polynoidae), описана из нор креветок-каллианассид рода *Gilvossius* Manning et Felder, 1992 (Decapoda: Axiidea: Callianassidae). Это первая симбиотическая ассоциация для креветок-каллианассид с полихетами-полиноидами из Черного моря (северо-восточной часть) и даже для бассейна Атлантического океана. В статье обсуждается таксономия нового вида, а также известные ассоциации симбиотических полихет с роющими креветками-каллианассидами.

Key words: diversity, associations, Black Sea, Polychaeta, Polynoidae, Crustacea, Callianassidae, *Malmgrenia*, *Gilvossius*, new species

Ключевые слова: разнообразие, ассоциации, Черное море, полихеты, ракообразные, новый вид

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Introduction

The diversity of the Black Sea benthic fauna represents about 20–25% of the neighboring Mediterranean Sea (e.g., Zaitsev & Mamaev, 1997; Oğuz & Öztürk, 2011), mainly due to its less saline water (15–20‰) and the high concentration of hydrogen sulfide in deepest zones, which restricts suitable habitats to the upper water layers (Mutlu et al., 1993; Zaitsev et al., 2002). Moreover, there are few biodiversity studies dealing with highly cryptic infaunal species, for example, those living in crevices, under rocks or in deep burrows (e.g., Surugiu, 2011; Bat et al., 2011; Boltachev & Karpova, 2014; Evcen et al., 2016; Marin, 2018b, 2021). Collecting animals living in burrows requires special equipment (for example, hand pumps), since their captures are quite rare when using standard collecting and fishing gears (e.g. trawls of dredges). Moreover, such cryptic animals are usually fragile and often became severely damaged in trawl samples. Thus, only careful manual sampling allows collecting appropriate specimens for scientific descriptions, while having the additional benefit of allowing to assess their ecology and relationships with other organisms. Among these fragile animals, polychaete worms are one of the most important taxa of marine organisms in benthic ecosystems (Zaitsev & Mamaev, 1997).

The Black Sea polychaete fauna from the shallow waters down to 200 m depth is rather well studied, especially along the Crimean, Georgian, Bulgarian and Romanian coasts (Surugiu, 2011). Among them, only six species belong to the family Polynoidae (so called scale worms), namely: *Polynoe scolopendrina* Savigny, 1822 (syn. *Parapolynoe sevastopolica* Czerniavsky, 1882), *Harmothoe extenuata* (Grube, 1840), *H. imbricata* (Linnaeus, 1767), *H. impar* (Johnston, 1839), *Malmgrenia lilianae* (Pettibone, 1993), and *Malmgreniella* sp. (e.g., Czerniavsky, 1882; Surugiu, 2005, 2011; Kurt-Sahin & Çinar, 2012; Kurt-Sahin et al., 2019; Ciftcioglu et al., 2020). At the same time, the diversity of polynoid worms in the Black Sea seems to be underestimated, particularly taking into account the much higher diversity of the family in the Mediterranean Sea. Moreover, no symbiotic scale worms have been reported to date, despite the presence of potential hosts inhabiting the Black Sea (e.g. Kurt-Sahin et al., 2019; Ciftcioglu et al., 2020).

An intensive sampling of the shallow waters of the northeastern Black Sea down to 10 m depth revealed a fauna of burrowing decapods more diverse than previously known, as well as the presence of some red-coloured scale worms living inside their deep burrows (Marin, 2021). Identification by morphology has shown that these worms can be tentatively assigned to the genus *Malmgrenia* McIntosh, 1874, the taxonomy of which is quite complicated due to the lack of a comprehensive revision. Nevertheless, comparison with currently known congeners revealed that the discovered scale worms belong to an undescribed species, which is fully described and illustrated here.

Material and methods

Sample collection and treatment

Samples were collected in the shallow waters of the northeastern part of the mainland coast of the Crimean Peninsula (northeastern part of the Black Sea) from 0 to 10 m depth using a bait suction (yabby pump), following Eleftheriou & McIntyre (2005) and Dworschak (2015). The hosts and symbionts were photographed alive in situ using a Canon G16 digital camera and then fixed in 90% ethanol. Drawings are based on preserved specimens and made with a camera lucida attached to an Olympus SZX10 stereomicroscope. Scanning electron microscope (SEM) micrographs were made on a Tescan Mira3 SEM after critical point drying and coating with 300Å of gold, in the Joint Usage Center "Instrumental methods in ecology" at the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences. All symbiotic polychaetes were measured to the nearest 0.1 mm using a calibrated ocular micrometer. Body length (L in mm, defined as the dorsal length from the distal prostomium margin to the distal margin of the most posterior segment) and body width (W in mm, defined the width of the widest segment including the parapodia but excluding chaetae in the middle of the body) of polychaetes are used as standard measurements. Species names and taxonomic position were checked in the World Register of marine Species (WoRMS) (Read & Fauchald, 2022). The number of observed symbionts (n) associated with each host shrimp species, as well as the extensity and intensity of settlement by symbionts was calculated during the collections in 2020, when 30 individuals of each host shrimp species were collected simultaneously.

Type material is deposited in the collections of the Zoological Museum of the Moscow State University (ZMMU) and the Laboratory of Ecology and Evolution of Marine Invertebrates (LEMMI) of the A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (Moscow, Russia).

Molecular study

Total genomic DNA was extracted from muscle tissue of the collected polychaetes using the innuPREP DNA Micro Kit (AnalitikJena, Germany) following the manufacturer's protocol. The gene markers were amplified with the following primers: (1) mitochondrial cytochrome c oxidase subunit I (COI mtDNA gene marker), "m13polylco" (TGTAAAACGACGGCCAGTGAYTATWTTCAACAAATCATAAAGATATTGG) and "m13polyhco" (CAGGAAACAGCTATGACTAMACTTCWGGGTGACCAAARAATCA) (Carr et al., 2011); (2) mitochondrial 16S small subunit rRNA (16S rRNA), +16SA ('CGCCTGTTTATCAAAAACAT') and –16SH ('CCGGTCTGAACTCAGATCACG'); and (3) nuclear 28S large subunit r-RNA (28S rRNA), +C1 ('ACCCGCTGAATTTAAGCAT') and –D2 ('TCCGTGTTTCAAGACGG'). Each 10µL of the reaction mixture contained 1µL of total DNA, 2uL of 5xPCR mix (Dialat, Russia) and 1uL of each primer. Polymerase Chain Reaction (PCR) procedures were performed on a T100 amplifier (Bio–Rad, USA) under standard protocols and conditions. Amplification products were separated by gel electrophoresis on a 1.5% agarose gel in 1xTBE, and then stained and visualised with 0.003% EtBr using imaging UV software. DNA nucleotide sequences were determined using the genetic analysers ABI 3500) and BigDye 3.1 (Applied Biosystems, USA) with direct and reverse primers. All obtained sequences were deposited in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Phylogenetic analysis was performed for COI using RAxML v.8.0.0 with GTR+I+G evolutionary model for Maximum–Likelihood (ML) analysis (Electronic supplementary material, see the section "Addenda"). Additional dataset of COI mtDNA sequences of the representatives of the family Polynoidae and outgroup taxa was taken from GenBank (NCBI) database. Accession numbers for all species used in the analyses are included in the Electronic supplementary material (see the section "Addenda").

Results

Class **Polychaeta** Grube, 1850

Order **Phyllodocida** Dales, 1962

Family **Polynoidae** Kinberg, 1856

Genus ***Malmgrenia*** McIntosh, 1874

Type species. *Malmgrenia andreapolis* McIntosh, 1874.

Diagnosis (based on Barnich et al., 2019). Body flattened dorsoventrally, short, up to 46 segments, more or less covered by elytra or short tail uncovered (large specimens). Elytra 15 pairs on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, 32. Prostomium bilobed, without cephalic peaks, with three antennae; ceratophore of median antenna in anterior notch, ceratophores of lateral antennae inserted termino-ventrally; two pairs of eyes, anterior pair usually dorsolaterally in front of widest part of prostomium, posterior pair dorsally near hind margin of prostomium. Parapodia biramous, noto- and neuropodia with elongate acicular lobe; tips of noto- and neuroacicula penetrating epidermis; neuropodia with or without supra- and sub-acicular process. Notochaetae with rows of spines of variable shape: either stout with blunt or pointed tip, or tapering to fine entire or bidentate tip. Neurochaetae more numerous, with rows of spines only distally and tips bidentate and/or entire with pointed or knob-like tip.

Remarks. The species described below is assigned to the genus *Malmgrenia* based on the lateral antennae inserted termino-ventrally and the bilobed prostomium without cephalic peaks. These morphological characters distinguish the species from the closely related genus *Harmothoe* Kinberg, 1856, which has distinct cephalic peaks and the lateral antennae clearly located ventral to the median antennae (Barnich & Fiege, 2001).

Malmgrenia callianassidophila **sp. nov.**
(Figs 1A, 2–6)

Holotype. 1 complete specimen (ZMMU PL4415), **Black Sea**, *Crimean Peninsula*, Sevastopol, Kruglaya Bay (Omega), 44°35′51.8″N 33°26′40.8″E, 14.VI.2020, sea grass bottom, 1.0–1.5 m depth, collected from callianassid burrows in sand by I. Marin.

Paratypes. 1 complete specimen, 2 broken up specimens (LEMMI), **Black Sea**, *Crimean Peninsula*, Sevastopol, Kazach'ya Bay, 44°34′10.8″N 33°24′47.1″E, 10.VI.2020, sea grass bottom, 1.0–1.5 m depth, collected from callianassid burrows in sand by I. Marin.

Additional material. 1 specimen, incomplete (anterior part), same data as for holotype; 2 complete specimens (LEMMI), same locality and collector as for paratypes, 15.VII.2022, 1.5–2.0 m.

Diagnosis. Elytral surface more or less completely covered with microtubercles, outer lateral elytral margin with many long papillae, posterior margin with shorter papillae. Neuropodia with subconical prechaetal lobe without supraacicular process. Up to 90 stout notochaetae with distinct rows of spines and blunt tips. Up to 50 thin neurochaetae, upper and lower unidentate, middle bidentate, with thin and long secondary tooth.

Description. Holotype 19 mm long, 7 mm wide, with 36 chaetigers. Body dorso-ventrally flattened. Prostomium bilobed, about 1.3 times as wide as long, without cephalic peaks, with three antennae and a pair of palps (Figs 3A, 4A, 6A, B). Two pairs of ovate black eyes, similar in size; anterior pair lateral in position, on widest part of prostomium, posterior pair near posterior margin of prostomium, dorsally orientated (Figs 3A, 6A). Median antenna with ceratophore in anterior notch, style papillate, tapering to filiform tip, 3–4 times as long as lateral styles. Lateral antennae with short papillate tapering styles and ceratophores, inserted termino-ventrally. Palps minutely papillate, longer or as long as lateral antennae, tapering.

First segment invisible dorsally, tentaculophores long, inserted laterally to prostomium, without chaetae; two pairs of tentacular cirri papillate, tapering to filiform tips, dorsal and ventral tentacular cirri similar in length or dorsal slightly longer than ventral (Figs 3A, 4A, 6A, B). Second segment with biramous elytrophorous parapodia and long ventral buccal cirri, as long as ventral tentacular cirri.

Elytra 15 pairs on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, and 32, almost completely covering dorsum except for the last few segments

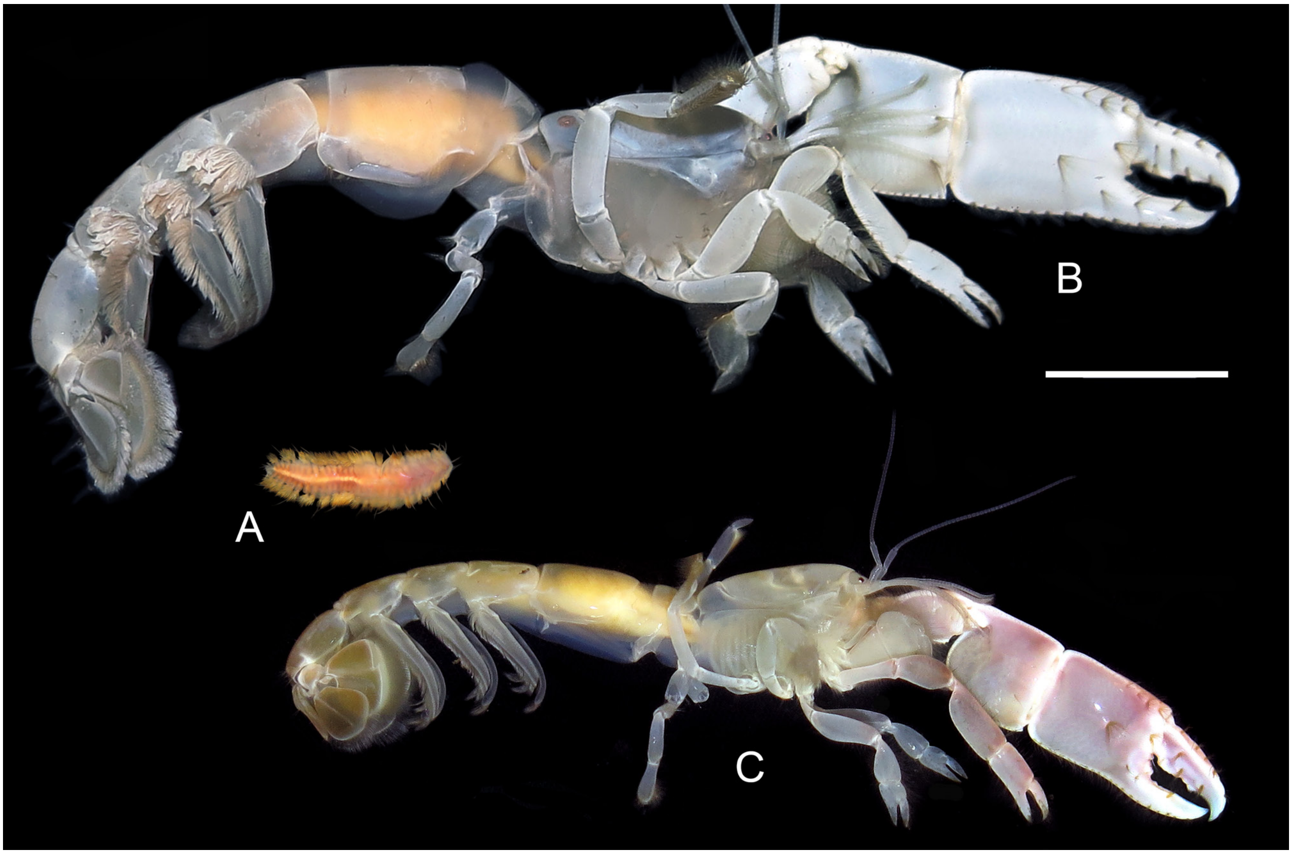

Fig. 1. *Malmgrenia callianassidophila* **sp. nov.** (A) and its hosts, callianasid shrimps *Gilvossius candidus* (Olivi, 1792), male (B) and *G. tyrrhenus* (Petagna, 1792), male (C). Scale bar: 10 mm.

Fig. 2. *Malmgrenia callianassidophila* **sp. nov.**, general appearance of paratype from Kazach'ya Bay, Crimean Peninsula, northwestern Black Sea. Dorsal (A) and ventral (B) view. Scale bar: 1 mm.

(Figs 1A, 2). Elytra thin, with surface covered more or less completely with conical microtubercles, outer lateral elytral margin with many long papillae, posterior margin with rare shorter papillae also occurring on elytral surface near outer lateral margin (Figs 3C, D, 4B).

Parapodia biramous, with single noto- and neuro aciculum penetrating epidermis (Fig. 3B). Notopodia with short rounded prechaetal lobe and longer pointed acicular lobe; neuropodia with subconical prechaetal lobe without supraacicular process and shorter rounded postchaetal lobe (Figs 3B, 4C). Dorsal cirri with large cylindrical cirrophores and long papillate styles, extending beyond chaetae (Fig. 3B). Smooth ventral cirri short, tapering, shorter than neuropodia (Figs 3B, 4C).

Notochaetae (up to 90) thick, both short and long with distinct rows of spines and blunt tips (Figs 3E, F, 5A, B). Neurochaetae (up to 50) thin, with rows of spines only in distal part, upper unidentate (Figs 3J, 5C), middle neurochaetae bidentate, with long thin secondary tooth (Figs 3H, 4D), often broken off in anterior part of body (Fig. 5D, E); lower neurochaetae usually unidentate (Figs 3I, 5F), rarely bidentate, with secondary tooth.

Coloration. Dorsal side of live scale worms transparent pink to yellowish, pale in preserved specimen; ventral side red; setae yellowish orange (Figs 1A, 2, 6B).

Size. Largest specimen (holotype) 19 mm long, 7 mm wide, with 36 chaetigers. Other specimens, including paratypes, slightly smaller, with L 15–18 mm, W 6–7 mm, with 30–36 chaetigers.

Etymology. The specific name is a noun in ap-

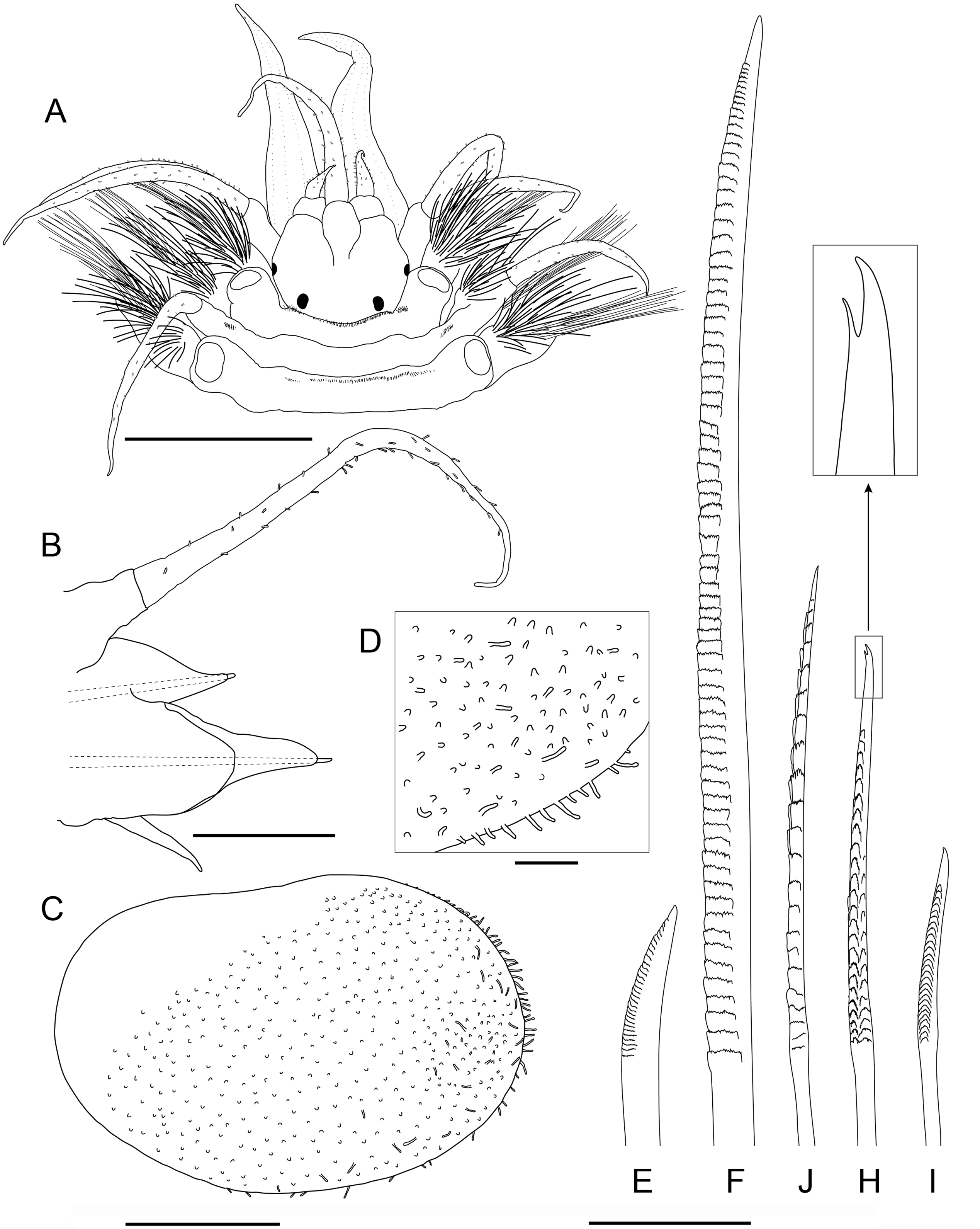

Fig. 3. *Malmgrenia callianassidophila* **sp. nov. A**, dorsal view of anterior end; **B**, posterior view of right parapodium of chaetiger 14; **C**, elytron from midbody; **D**, detail of posterior margin of same elytron. Chaetae of parapodia from chaetiger 14–15: **E**, upper notochaeta; **F**, lower notochaeta; **J**, distal part of upper neurochaeta; **H**, distal part of middle neurochaeta; **I**, distal part of lower neurochaeta. Scale bars: 1 mm (A, C), 500 μm (B), and 100 μm (D, E, F, G, H, I).

position, derived from the name of the family Callianassidae, which includes the genus *Gilvossius* associated with the new species, and the suffix *-phila*.

Distribution. Known only from two neighboring bays, Kruglaya Bay (Omega), 44°35′51.8″N 33°26′40.8″E, and Kazach'ya Bay, 44°34′10.8″N 33°24′47.1″E (Crimean Peninsula, Black Sea); upper sublittoral, 1–2 m depth.

GenBank accession numbers. COI mtDNA – OP804503, OP804504; 16S mitochondrial rRNA – OP806306, OP806307; 28S nuclear rRNA – OP806514, OP806515.

Comparison. So far 15 species of the genus *Malmgrenia* McIntosh, 1874 are known: *M. andreapolis* McIntosh, 1874, *M. arenicolae* (Saint-Joseph, 1888), *M. bicki* Barnich, Dietrich, Hager et Fiege, 2017, *M. castanea* McIntosh, 1876, *M. darbouxi* (Pettibone, 1993), *M. lilianae* (Pettibone, 1993), *M. ljungmani* (Malmgren, 1867), *M. louiseae* Jourde, Sampaio, Barnich, et al., 2015, *M. lunulata* (Delle Chiaje, 1830), *M. marphysae* (McIntosh, 1876), *M. mcintoshi* (Tebble et Chambers, 1982), *M. perspicua* Intes et Le Loeuff, 1975, *M. polypapillata* (Barnich et Fiege, 2001), *M. thomsonae* Barnich, Dietrich, Hager et Fiege, 2017, and *M. uschakovi* Ozolinsh, 1990 (Barnich et al., 2019; Read & Fauchald, 2022). Most of described species occur in the Atlantic Ocean and the Mediterranean Sea, with the exception of one Pacific species, *M. uschakovi*, recorded from the Sea of Japan (Ozolinsh, 1990; Pettibone, 1993; Barnich & Fiege, 2001; Jourde et al., 2015; Barnich et al., 2019).

Malmgrenia callianassidophila **sp. nov.** can be clearly distinguished from all other known species of *Malmgrenia*, except for *M. polypapillata*, *M. ljungmani* and *M. mcintoshi*, by: (1) the presence of conical microtubercles covering the elytral surface almost completely, (2) numerous papillae on the lateral and posterior elytral margin, and (3) the absence of neuropodial supraacicular process on the subconical prechaetal lobe of neuropodia.

The Mediterranean *M. ljungmani*, similar to the new species, also has the elytra covered with microtubercles, but only few scattered papillae present on the elytral margin in this species (*vs.* almost completely covered in the new species), the neuropodia with digitiform supraacicular process (*vs.* without process), the papillate ventral cirri (*vs.* smooth), and all the neurochaetae are usually bidentate (*vs.* only middle neurochaetae bidentate) (see Barnich & Fiege, 2001).

Malmgrenia mcintoshi, recorded from the Atlantic Ocean and the Barents Sea, can also be confused with the new species due to the presence of a distinct fringe of papillae on the elytral margin and also the absence of the neuropodial supraacicular process and has bi- and unidentate neurochaetae. In addition, *M. mcintoshi* has only a small patch of microtubercles in the anterior part of elytra (*vs.* the elytra are almost completely covered with microtubercles) and papillae on the ventral cirri (*vs.* smooth ventral cirri). Moreover, this species has never been recorded from the Mediterranean Sea.

The Mediterranean *M. polypapillata* is mostly morphologically similar to the new species and also can be clearly distinguished from all other known *Malmgrenia* species by the conical microtubercles almost completely covering the elytral surface and by numerous papillae on the lateral and posterior elytral margins. However, *Malmgrenia callianassidophila* **sp. nov.** can be distinguished from *M. polypapillata* by the absence of the neuropodial supraacicular process and papillae on the ventral cirri, and by the presence of both uni- and bidentate neurochaetae, while *M. polypapillata* has exclusively unidentate neurochaetae. Moreover, *Malmgrenia callianassidophila* **sp. nov.** has very numerous notochaetae and neurochaetae in the middle part of the body, up to 90 notochaetae and 50 neurochaetae, respectively. The description of the *M. polypapillata* does not indicate the number of chaetae; however, based on the figure, it can be assumed that notochaetae and neurochaetae are not so numerous (Barnich & Fiege, 2001: fig. 6a).

Molecular analysis. Our phylogenetic hypothesis (based on COI mtDNA gene marker) showed that the new species forms a sister clade to *Polynoe scolopendrina* Savigny, 1822 (a species also known from the studied area) and is closely related to the Mediterranean *Harmothoe oculinarum* (Storm, 1879), being well separated from the related *M. mcintoshi* and *Malmgreniella nigralba* (Berkeley, 1923) (described as *Malmgrenia nigralba* Berkeley, 1923) (Electronic supplementary material, see the section "Addenda"). At the same time, combining all COI, 16S rRNA and 28S rRNA

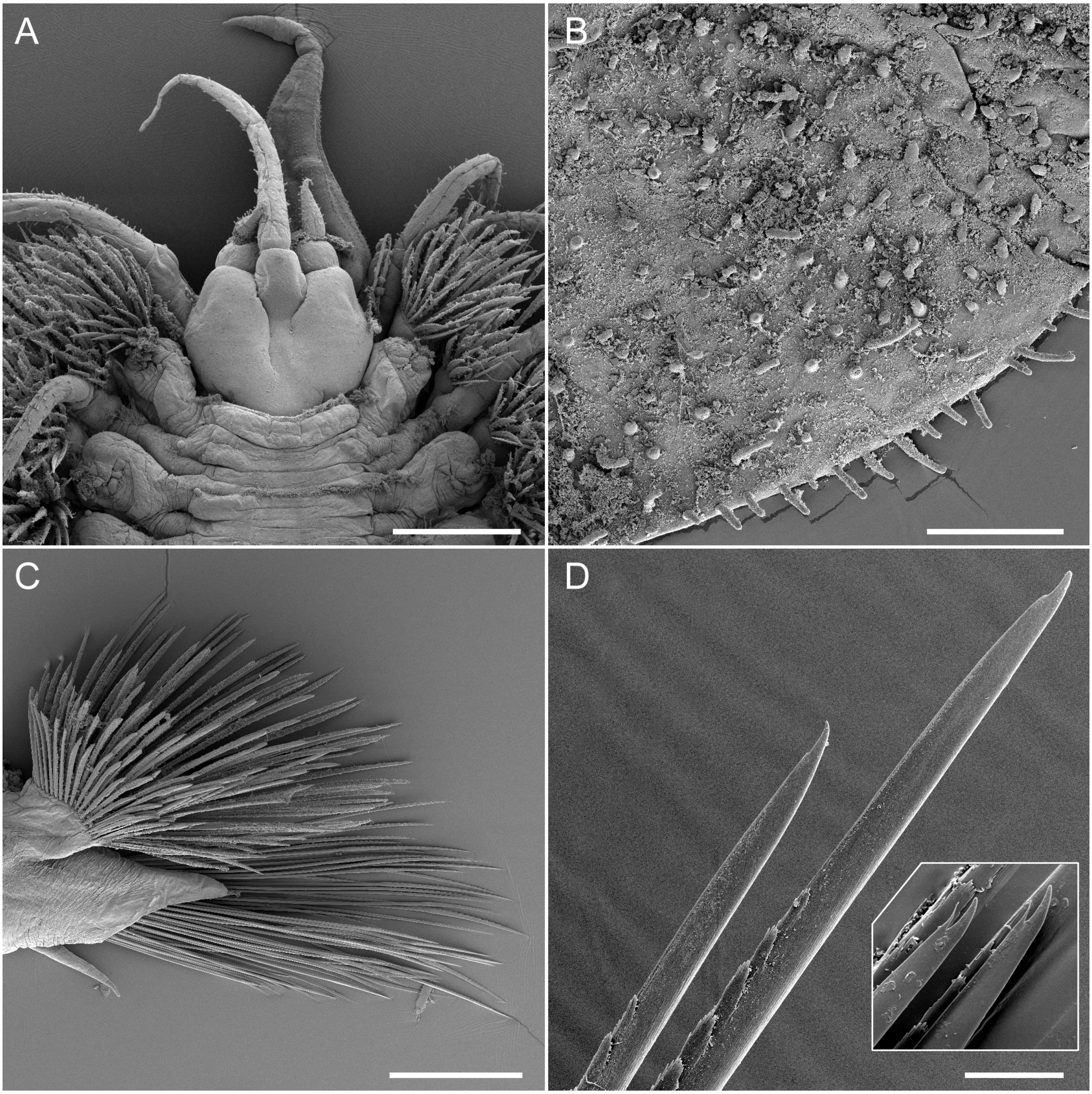

Fig. 4. *Malmgrenia callianassidophila* **sp. nov. A**, dorsal view of anterior end of body; **B**, detail of posterior margin of elytron (detached from middle of body after fixation); **C**, anterior view of left parapodium of segment 15; **D**, distal part of middle neurochaeta, showing bidentate tips. Scale bars: 500 µm (A, C), 100 µm (B), and 20 µm (D).

markers into a single analysis showed ambiguous results. Thus, the obtained data on 16S rRNA and 28S rRNA markers are just added to the GenBank (NCBI) database for further molecular genetic researches.

Ecology. *Malmgrenia callianassidophila* **sp. nov.** (n=7) was collected from the burrows of callianassid shrimps of the genus *Gilvossius* Manning et Felder, 1992 (Decapoda: Axiidea: Callianassidae), *G. candidus* (Olivi, 1792) (n=30) and *G. tyrrhenus* (Petagna, 1792) (n=30), with the infestation prevalence of about 5%. At the same time, the new scale worm was never found in association with *Necallianassa truncata* (Giard et Bonnier, 1890) (Decapoda: Axiidea: Callianassidae) (n=30) and *Upogebia pusilla* (Petagna,

1792) (Decapoda: Gebiidea: Upogebiidae) (n=30), nor free-living.

All collected scale worms were found solitary inside the burrows of the host shrimps, suggesting the presence of territorial behavior shown for other symbiotic polychaetes (Martin & Britayev, 1998, 2018; Britayev & Martin, 2021). Moreover, some specimens seem to show traces of regeneration in the posterior segments (Figs 1A, 2), which are commonly found in many territorial species (Britayev & Zamyshliak, 1996; Britayev & Mekhova, 2014; Britayev et al., 2007) and have recently been demonstrated to occurs as a result of territorial skirmishing among individuals (Britayev & Martin, 2021).

Other associated invertebrates found in the same burrows are unidentified opossum shrimps, possibly belonging to the genus *Hemimysis* G.O. Sars, 1869 (Crustacea: Mysida: Mysidae), and the parasitic copepod *Clausidium* cf. *apodiformis* (Philippi, 1839) (Crustacea: Copepoda: Clausidiidae) living attached to the body of the host shrimps (Marin & Turbanov, 2016).

Discussion

Phylogeny

Malmgrenia callianassidophila **sp. nov.** (together with the Mediterranean *M. polypapillata*) can be well distinguished from other representatives of *Malmgrenia* by the arrangement of microtubercles and marginal papillae on the elytra (see above). At the same time, our COI phylogenetic results indicate that the new species is more closely related to species of the genus *Polynoe* Lamarck, 1818 and another one of *Harmothoe* Kinberg, 1856 than to any *Malmgrenia* species. Similar ambiguous results were obtained when combining all gene markers in a single analysis, which generally cast some doubts on the assignment of the new species to the genus *Malmgrenia*.

The relatively small number of available sequences for species of the family Polynoidae available in the GenBank database does not provide strong support for any possible phylogenetic hypothesis based on these data. Therefore, in our opinion, assessing the real phylogenetic position of *M. callianassidophila* **sp. nov.** will require further analyses. Nevertheless, we expect that the availability of our data will contribute to further clarification of its taxonomic position.

Ecology

Large tubicolous polychaetes, such as parchment tube worms (Chaetopteridae), spoon worms (Echiura), lugworms (Arenicolidae), and ornate worms (Terebellidae), among others, can host numerous associates, from protists to fishes (reviewed by Martin & Britayev, 1998, 2018; Hutchings & Fauchald, 2000; Anker et al., 2005; Marin & Antokhina, 2020). In turn, polychaetes can be symbionts (cohabitants) of other large burrowing animals (see Martin & Britayev, 1998, 2018). Fossil unidentified burrowing polychaetes seem to be the most probable candidates for infauna associated with tiny tubes of the fossil callianassid genus *Egbellichnus* Hyžný, Šimo et Starek, 2015 (Callianassidae), found in the Upper Miocene sublittoral deposits of Lake Pannon (Vienna Basin, Slovakia) (Hyžný et al., 2015). At present, four species of the North Pacific scale worm genus *Hesperonoe* Chamberlin, 1919 (Polynoidae), namely *H. complanata* (Johnson, 1901), *H. hwanghaiensis* Uschakov et Wu, 1959, *H. coreensis* Hong, Lee et Sato, 2017 and *H. japonensis* Hong, Lee et Sato, 2017, are known as specialised infaunal associates (commensals) living inside burrows of gebiidean and axiidean shrimps (Martin & Britayev, 1998, 2018; Sato et al., 2001, 2016; Hong et al., 2017; Marin & Antokhina, 2020).

The genus *Malmgrenia* is probably symbiotic, as several its species are known to live associated with other larger invertebrates, mostly echinoderms (e.g., Fauvel, 1923; Pettibone, 1993; Martin & Britayev, 1998). However, for most of the described species of the genus, there are no data on associations with hosts. Our data suggest that the new species is specifically associated with the burrowing callianassid shrimps of the genus *Gilvossius*, which is the first case know

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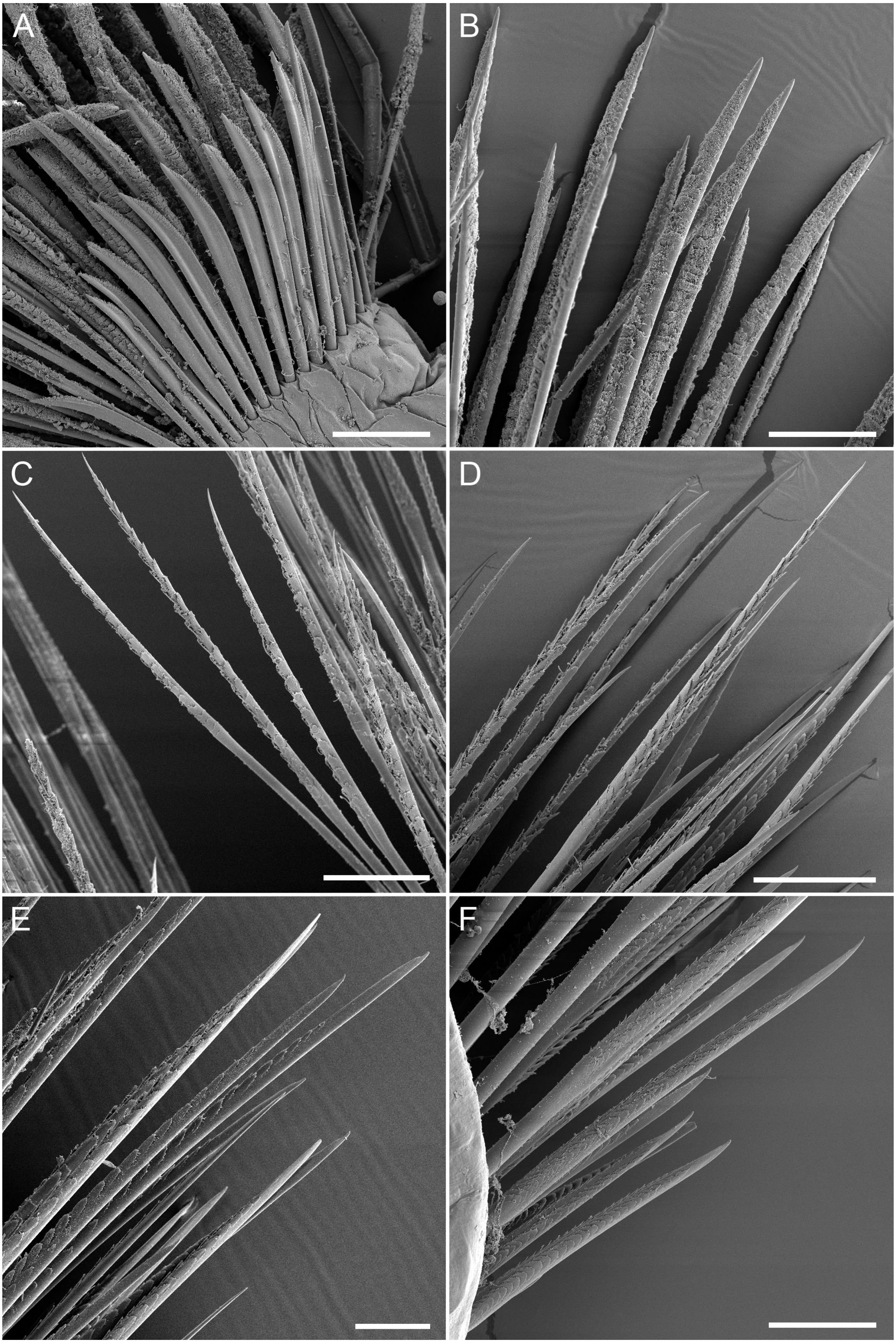
Fig. 5. *Malmgrenia callianassidophila* **sp. nov.** Chaetae of parapodia from chaetiger 14–15. **A**, upper and middle notochaetae; **B**, lower notochaetae; **C**, upper neurochaetae; **D**, middle neurochaetae; **E**, middle neurochaetae; **F**, lower neurochaetae. Scale bars: 100 µm (A, B, C, D) and 50 µm (E, F).


A
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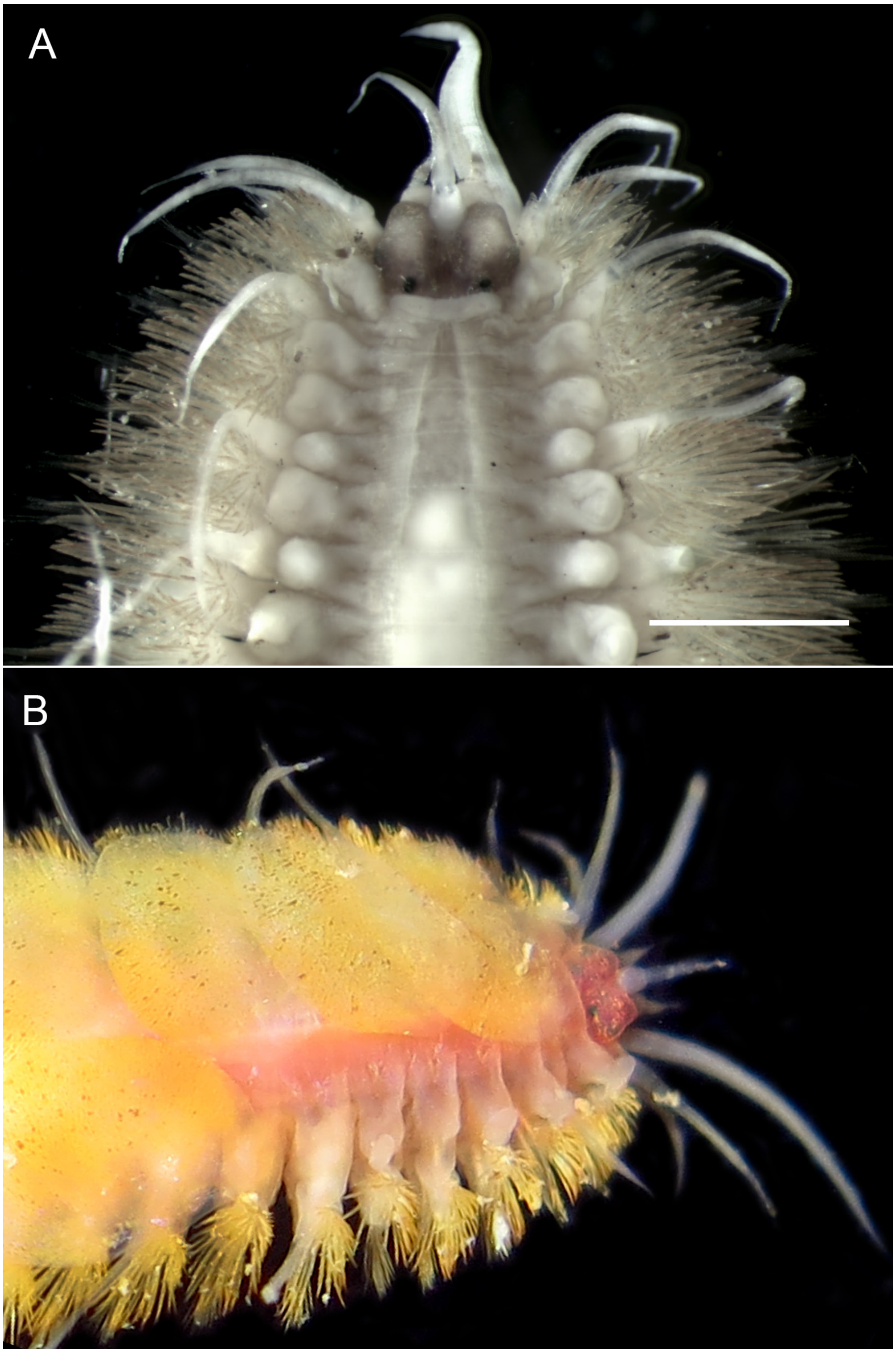

Fig. 6. *Malmgrenia callianassidophila* **sp. nov.** Dorsal view of anterior end of body. **A**, preserved specimen (under light microscope); **B**, living worm. Scale bar: 1 mm.

for the genus *Malmgrenia*, as all previously described symbiotic congeners are known to live in association with echinoderms (i.e., holothurians, ophiuroids, echinoids and asteroids), annelids (sipunculans and other polychaetes) and hemichordates (Barel & Kramers, 1977; Pettibone, 1993; Granja-Fernández et al., 2013, Martin & Britayev, 1998, 2018).

The finding of only seven more or less complete specimens of *M. callianassidophila* **sp. nov.** cast some doubts on the obtained infestation prevalence and, certainly, more precise estimates will require further studies. However, the species shows: (1) a regular distribution, with all specimens living solitary inside the burrows of the host shrimps, and (2) traces of posterior ends regeneration, often resulting from territorial skirmishing of individuals, strongly suggestive of territorial aggressive behavior, as demonstrated for other symbionts including polychaetes (Britayev & Zamyshliak, 1996; Martin & Britayev, 1998, 2018; Britayev et al., 2007; Britayev & Mekhova, 2014; Britayev & Martin, 2021).

The association of *M. callianassidophila* **sp. nov.** with burrowing callianassid shrimps is also the first example of such symbiosis for the Atlantic basin. We also assume that the discovery of our new species can be expected in Mediterranean brackish habitats like the Venice lagoon since the Black Sea harbors brackish waters are inhabited by macroinvertebrates characteristic for brackish Mediterranean lagoons (see Marin, 2018a; Engin et al., 2016; Marin & d'Udekem d'Acoz, 2019; Boltacheva & Lisitskaya, 2019; Spiridonov et al., 2020) and the same species of callianassid burrowing shrimps are present there.

Addenda

Electronic supplementary material

Molecular phylogenetic (COI mtDNA gene marker) reconstruction (tree). Next to the nodes, support based on ML algorithm is indicated. Codes are GenBank accession numbers.

File format: JPEG. Available from: https://doi.org/10.31610/zsr/2022.31.272

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