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Disentangling cryptic species in Parastenocarididae (Copepoda: Harpacticoida) with an integrative approach: the case of *Stammericaris similior* sp. nov. and *Stammericaris destillans* Bruno & Cottarelli 2017

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ABSTRACT

Stammericaris similior **sp. nov.** is described combining light microscopy, scanning electron microscopy, and genetic barcoding. The new species was collected from rimstone pools in Scrivilleri Cave, a cave in Sicily with so far unexplored microcrustacean fauna. The new species is particularly interesting because it is morphologically very similar to *Stammericaris destillans*, an epikarstic parastenocaridid endemic to a different Sicilian cave; however, the phylogenetic analysis based on the mitochondrial COI gene of sixteen parastenocaridids shows that these two *Stammericari*s are two distinct species, with an uncorrected p-distance of 22.9, and the sequences of *Stammericaris similior* **sp. nov.** cluster together in a well-supported monophyletic clade, with two different haplotypes. To our knowledge, the presence of different species of almost identical morphology had not been recorded before for the genus *Stammericaris*. The integrated molecular and morphological analysis, the latter conducted with the support of SEM, allows disentangling the affinities of the new species and identifying a few distinctive characters: the males of the new species are characterized by the caudal rami shorter than the anal somite; the morphology of the P3, which is thin and slightly arched, with three proximal spinules on exp-1; the peculiar structure of the P4 enp; the P4 basis ornamented with two spinules of different length, the one closest to the endopod being the shortest one, and a half-moon shaped lamella. The new species differs from *S. destillans* for its larger size, the presence of: three spinules, instead of two, on the P3 exp-1; the half-moon shaped lamella on the P4 basis; a row of spinules along the inner margin of P4 exp-1. We also provide data on the ecology and distribution of the new species, a list of the other copepod species collected, and a dichotomic key for the males of all species presently assigned to the genus.

Key words: COI gene, epikarst, cave fauna, crustacean stygofauna

INTRODUCTION

Several biospeleological investigation of microcrustaceans of caves conducted in Sicily and Calabria (Southern Italy) in the last 10 years have focused on the epikarstic fauna (Cottarelli *et al.* 2012; Bruno *et al.* 2017, 2018, 2020). Aim of those investigations was to broaden "the patchy and scanty knowledge of cave microcrustaceans of these regions and increase public awareness of the fragility and vulnerability of groundwater assemblages, which are severely impacted by human activities and by the effects of climate change" (Cottarelli *et al.* 2012). The results

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were the record of several new or rare taxa of Copepoda (Crustacea, Maxillopoda) and Bathynellacea (Crustacea, Malacostraca); the highest number of newly recorded species was ascribed to the genus *Stammericaris* Jakobi 1972 and all of the recorded species of this taxon were new to Science, except *Stammericaris trinacriae* (Pesce, Galassi & Cottarelli 1988), which was previously know from phreatic waters of Sicily (Pesce *et al.* 1988), and reported for the first time from a karstic cave by Bruno *et al.* (2017).

The genus *Stammericaris* (subfamily Parastenocaridinae Chappuis 1940) was originally described by Jakobi (1972) and revised and redefined by Schminke (2013) who included *Phreaticaris* Jakobi 1972 in *Stammericaris*. Bruno *et al.* (2017) slightly emended Schminke's (2013) diagnosis of the genus, based on a taxonomic and molecular study of some species of *Stammericaris*. The genus currently includes the following thirteen known species: the type-taxon *S. stammeri* (Chappuis 1937), *S. acherusia* (Noodt 1954), *S. amyclaea* (Cottarelli 1969), *S. destillans* Bruno & Cottarelli in Bruno *et al.* 2017, *S. diversitatis* (Cottarelli & Bruno 2012 in Cottarelli *et al.* 2012), *S. lorenzae* (Pesce, Galassi & Cottarelli 1995), *S. orcina* (Chappuis 1938), *S. palmerae* (Reid 1992), S. *pasquinii* (Cottarelli 1972), *S. phreatica* (Chappuis 1936), *S. remotaepatriae* Cottarelli & Bruno 2021, *S. trinacriae* (Pesce, Galassi & Cottarelli 1988), *S. vincentimariae* Bruno & Cottarelli 2020 in Bruno *et al.* 2020; plus one still undescribed species from Northern Italy and one from Anatolic Turkey, presently under study. *Stammericaris destillans* and *S. diversitatis* are endemic of two different Sicilian caves, *S. trinacriae* of a cave and two phreatic systems in Sicily, and *S. vincentimariae* of a Calabrian cave; *S. acherusia*, *S. amyclaea S. orcina* are known only for Italy (Chappuis 1938; Cottarelli & Drigo 1972; Bruno *et al.* 2017); *S. stammeri* and *S. phreatica* were collected in Spain and the Balkanic area and Czech Republic, respectively (Chappuis 1936, 1937; Rouch 1986); *S. remotepatriae* and *S. palmerae* are the only species known from the Nearctic region (Cottarelli & Bruno 2021) (Figure 1).

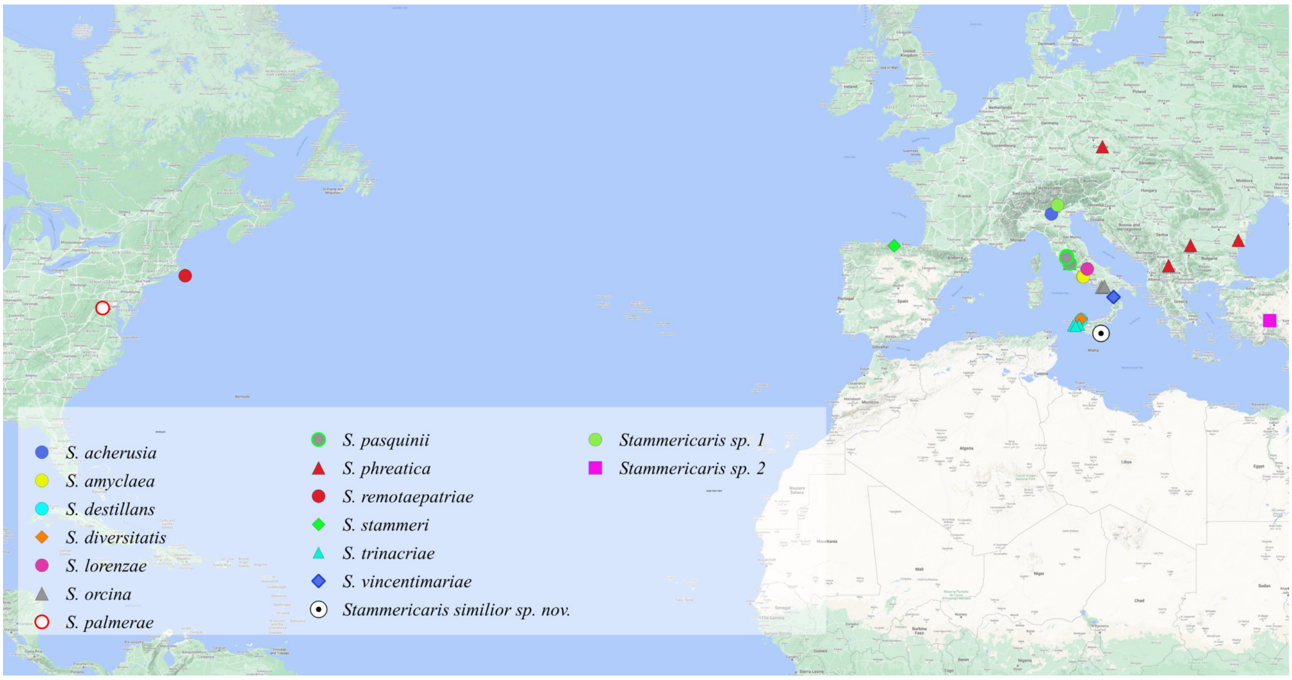

FIGURE 1. Distribution map of all known species of *Stammericaris* (including unpublished records)

Some of the diagnostic characters of the genera *Stammericaris* and *Cottarellicaris* are similar (Schminke 2013), and these two genera were discussed as sister taxa by Bruno *et al.* (2017, 2020), based on the endopod P4 of the male, being represented by a complex two-branched hyaline structure, with the distal outgrowth transformed differently in the two genera: this outgrowth is an autapomorphic elongate lamella in *Cottarellicaris,* and a plesiomorphic tip in *Stammericaris.* Recent morphological and molecular studies confirmed that, notwithstanding the morphological similarities, the two genera are well-defined and valid (Bruno *et al.* 2020).

Stammericaris similior **sp. nov.** was collected from the epikarstic water of Scrivilleri Cave, a karstic cave located in Siracusa province (Italy, see below). The collection and description of this new species has great faunistic and taxonomic relevance: the record of a new species increases the available knowledge on groundwater biodiversity of Sicily and Southern Italy, which is still poorly known compared to surface water one, due to the Racovitzan impediment (Ficetola *et al.* 2018), and a better knowledge of groundwater biodiversity is the foundation for conservation and protection actions. From the taxonomic perspective, *S. similior* **sp. nov.** is morphologically very close to *S. destillans*, a species endemic to the epikarst of a different Sicilian cave, the Molara Cave in Palermo province. Since, to the best of our knowledge, there is no published information on extremely similar *Stammericaris* species, this work is the first one dedicated to this topic.

In this paper we will: i) describe in details the morphology of the new species based on light and scanning microscopy, aiming at confirming the generic status of the new taxon, also by comparing it with *S. destillans*; ii) integrate the morphological and molecular analysis to obtain a more robust taxonomic assessment and to understand species delimitations; iii) assess the phylogenetic relationships between the species of *Stammericaris*; iv) provide information on the ecology of *Stammericaris similior* **sp. nov.** and on the geonemy of the genus; v) propose an up-to-date taxonomic key for the identification of the known species of *Stammericaris.*

MATERIALS AND METHODS

Site description and sampling methods

The "Grotta Scrivilleri" (Scrivilleri Cave, cadastral number: Si SR 7003) is one of the largest Sicilian limestone caves. The cave opening is located near Pirolo Gargallo (Siracusa province), in southeastern Sicily, 4.2 km from the coast. The area is characterized by a Mediterranean climate ('Csa' in the Köppen climate classification) with hot, dry summers and mild to cool, wet winters (see Cottarelli *et al.* 2012 for details). The cave opens at 152 m a.s.l. at the foot of a paleo-cliff (Figures S1, S2) which represent the Eastern border of the Climiti Massif. The origin of most of the outcrops forming the Climiti Massif dates to the Oligo-Miocene and in the western sector are represented by a marly-calcarenitic succession forming the Ragusa Formation (Rigo & Barbieri 1959), and in the eastern sector by the Monti Climiti Formation (Pedley 1981), where white calcarenite and Foraminifera-rich limestones form the lower member (Melilli Member). During the Pliocene, the karstification of the emerged limestone formations lead to the formation of natural cavities such as Grotta Scrivilleri: the Climiti Massif and the whole area (the Iblei Mountains plateau) are characterized by karst morphologies and horizontal caves, created by the oscillations of the sea level and the modification of the coastline occurred during the Quaternary (Cavallaro 1998). The analysis of 60 km^2 of the area around Scrivilleri cave has shown the presence of an extensive cave development, for a total karstic system of about 4 km (Cavallaro 1998), and Scrivilleri Cave developing for a 2.2 km total length (Figure S3), mainly in the horizontal direction (the total level difference of the cave is -21 m). In the whole Iblei Mountains plateau, most of the streams are temporary and, even during rainfall events, about 30-40% of the water quickly infiltrates in the karstic system due to the high permeability of the limestone layer, averaging 10^{-2}-10^{-4} cm s^{-1} (Regione Sicilia 2016). Very few caves in the area have an active underwater flow, and most of the caves of the system, included Scrivilleri Cave, are fossil (i.e., partly filled with speleothemes, alluvial deposits and/or partly collapsed cave roof). In Scrivilleri Cave, the largely-developed carbonatic speleothemes (Figure S5) partly occlude the cavities and have radically modified the original morphology (Cavallaro 1998). Due to the karstic conditions, all the water of Scrivilleri Cave water originates from rainfall, which percolates into the epikarst and drops to saturate the soil of the cave floor, and the rimestone pools are filled with water only during intense rainfall periods.

For this study, 6 pools were selected (Figures 2, S4). On four sampling occasions (one in summer and one in winter, in 2020 and 2021, Table 1), all water was collected from each pool with a syringe following Brancelj (2003). Samples were stored in cooled containers and transported to the laboratory where they were sorted within 48 hours.

Morphological methods

All specimens were sorted alive under a stereomicroscope, placed individually in 70% or 100% ethanol, and stored refrigerated until further morphological and/or molecular analyses. Before morphological analyses, specimens were rinsed in distilled water, dissected and mounted in Faure's or glycerine jelly medium solution between two cover slips to allow observations from both sides. Illustrations were made at different magnifications up to a maximum of 1250 X, using drawing tubes mounted on a Zeiss Axioskop® phase-contrast microscope and a Polyvar Reichert-Jung® interferential-contrast microscope.

Four females and four males of *Stammericaris similior* **sp. nov.** were observed using a JEOL JSM 6010LA scanning electron microscope. The specimens were rehydrated in a graded ethanol series (90%, 70%, 50%, 30%, 10% at 4 °C for 10 minutes each step), fixed with gluteraldehyde in cacodylate solution (at 4 °C for 1 hour), washed in cacodylate buffer (pH 7.2, 4 °C for 1 hour), post-fixed in 1 % osmium tetraoxide in the same buffer (4 °C for 1 hour), washed in cacodylate buffer (pH 7.2, 4 °C for 1 hour), dehydrated in a graded ethanol series (20%, 30%, 50%, 70%, 80%, 90%, 100% ethanol at 4 °C for 10 minutes each step), critical-point-dried in a Balzers Union H CPD 020 apparatus, and coated with gold in a Balzers Union HMED 010 sputter coater. The stubs are deposited at the Interdepartmental Center for Electron Microscopy, Tuscia University.

Specimens of the type series are deposited at La Specola Museum of Natural History, Zoology Section Florence, Italy (MZUF). The taxonomic descriptions and the authority of the new species are the sole responsibility of MCB and VC. Authorship of the new species should be cited as "Bruno & Cottarelli" in Bruno *et al.*, 2023 (following the International Commission on Zoological Nomenclature, Recommendation 51E).

The following abbreviations are used throughout the text and figures: enp: endopod; exp: exopod; A1: antennule; A2: antenna; P1-P5: first to fifth pereiopod; P6: rudimentary sixth pereiopod. The nomenclature and descriptive terminology follow Huys & Boxshall (1991), terminology and homologisation of maxillary and maxillipedal structures follow Ferrari & Ivanenko (2008).

TABLE 1. Pool morphological and physical and chemical characteristics. For position of the pools, see Figure 2. N. m. = not measurable (pool dry or water level too low)

Pool number	Characteristics	Hydrological characteristics				Physico-chemical parameters (measured on 20/II/2021)			
		23/ VIII/2020	08/ XII/2020	20/ II/2021	06/ VIII/2021	pH	EC (μS cm^{-1})	TDS (ppm)	T (°C)
1	Clay pool, partly filled by drip. Size not measurable	water	dry	dry	dry	n. m.	n. m.	n. m.	n. m.
2	Clay pool, filled by water flowing on the cave walls and collecting on the floor. Size not measurable	dry	dry	water	dry	8.19	655	330	14.6
11	Rimestone pool, filled by drip. Size, 20/ II/21: 32 cm X 16cm	water	water	water	water	7.61	489	245	14.8
12	Rimestone pool, filled by drip. Size, 20/ II/21: 97 cm × 84 cm; depth 1-4 cm	water	water	water	water	7.44	604	300	15.1
14	Rimestone pool, filled by drip. Size, 20/ II/21: 46 cm × 40 cm; depth ca. 1 cm	water	water	water	water	7.63	605	303	15.6
16	Rimestone pool, filled by drip. Size, not measurable	water	water	water	water	n. m.	n. m.	n. m.	n. m.

DNA extraction, PCR amplification and sequencing

Three specimens of *Stammericaris similior* **sp. nov.** were used for the molecular analysis. The specimens of *Stammericaris* were identified morphologically without dissection under a 100X magnification using a MOTIC SMZ-168 stereoscope and were stored individually in 100% ethanol at -20°C until DNA extraction.

Prior to DNA extraction, the preserved specimens were carefully rehydrated and rinsed 10 times with MilliQ water, and then processed for DNA extraction using the BIORON GmbH "Ron's Tissue DNA Mini Kit", following the manufacturer's instructions. The selective amplification of the cytochrome c oxidase subunit I (COI) mitochondrial fragment (hereafter "mtDNA COI") and the 18S ribosomal DNA nuclear fragment were carried out by polymerase chain reaction (PCR) using the primer pairs “ZplankF1_t1” and” ZplankR1_t1” (Prosser *et al.* 2013) and the “18SFnew” and “9r” (Tang *et al.* 2012). The COI PCR mix consisted of 15 µl of distilled water, 2.5 µl of Buffer 10X which includes 25 mM of $MgCl_2$, 1 µl of dNTPs (10 mM for each), 1µl of each of the primers (10 µM), 0.5 µl of Taq polymerase (5 U / µl) and 4 µl of template DNA, for a total volume of 25 µl. After an initial denaturation at 95°C for 5 min, the thermal cycle consisted of 40 cycles of denaturation (94°C for 40 sec), annealing (45°C for 40 sec) and extension (72°C for 1 min), followed by 5min at 72°C for the final extension step. The 18S PCR mix consisted of of 12 µl of distilled water, 2.5 µl of Buffer 10X which includes 25 mM of $MgCl_2$, 2 µl of dNTPs (10 mM for each), 2µl of each of the primers (10 µM), 0.5 µl of Taq polymerase (5 U / µl) and 4 µl of template DNA, for a total volume of 25 µl. After an initial denaturation at 95°C for 5 min, the thermal cycle consisted of 35 cycles of denaturation (95°C for 45 sec), annealing (48°C for 45 sec) and extension (72°C for 1 min), followed by 5min at 72°C for the final extension step. After PCRs, 5 µl of each PCR product were used to perform electrophoresis on 2% agarose gel at 90 V for 20 min and then visualized with a UV transilluminator.

When PCR products showed a clear single band of the expected length, they were purified using the Exo-SAP-IT® kit (Affymetrix USB). Sequencing was performed by Macrogen Inc. (Madrid, Spain; https://dna.macrogen.com/eng/) using an ABI 3130xL (Applied Biosystems) sequencer. The same primers used previously for PCR were used for direct sequencing of the PCR products.

The quality of the obtained chromatograms was checked through the measurement of their “Phred score” (Richterich 1998). Only those chromatograms that showed continuous high quality base readings (QV ≥20) were used. Chromatograms were analysed and manually proofread using the software Chromas software v. 2.6.2 (Technelysium, Pty. Ltd. 1998, Queensland, Australia).

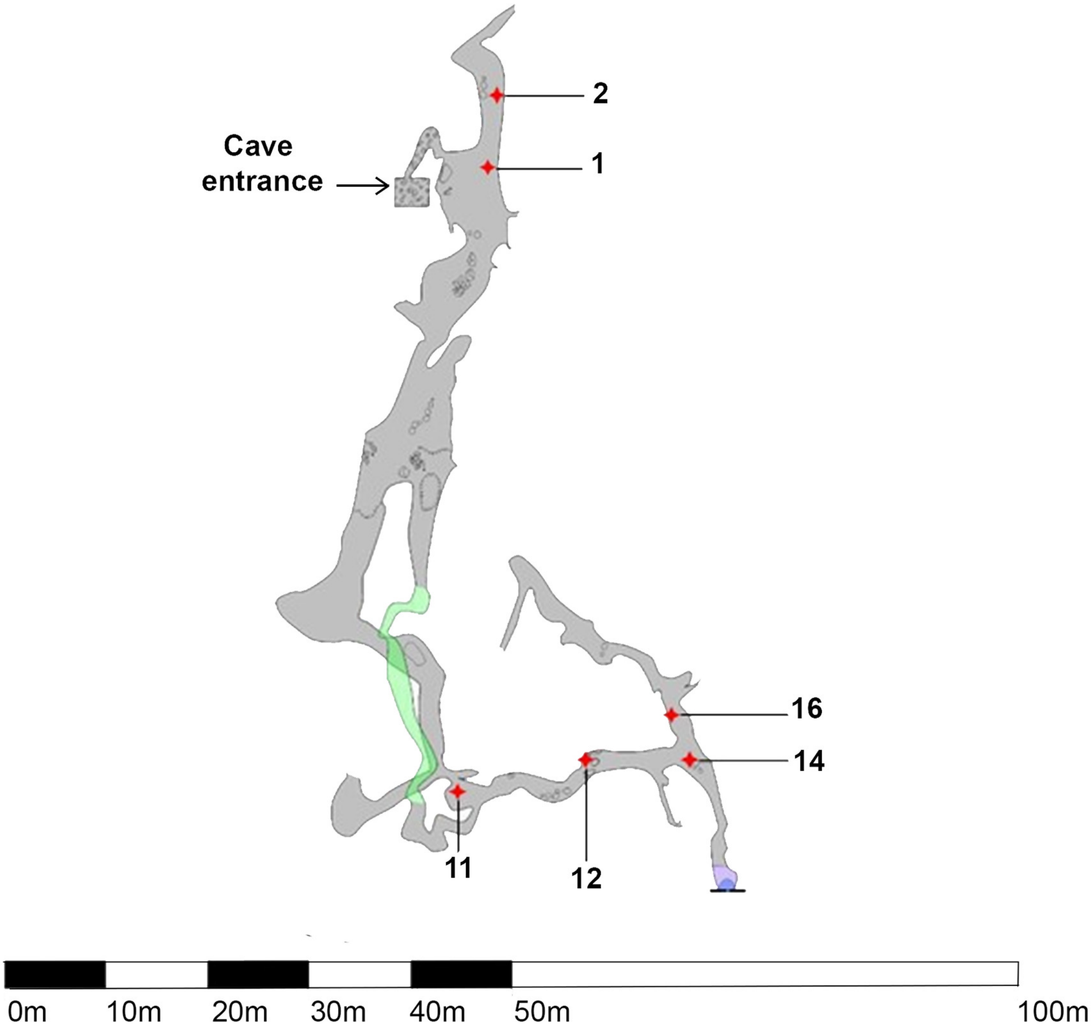

FIGURE 2. Planimetry of the first branch of the Scrivilleri Cave, and location of the sampling pools (1, 2, 11, 12, 14, 16). Green: upper bypass; blue: flooded area. For the full planimetry, see supplementary material.

Phylogenetic analysis

Overall, three novel mtDNA COI sequences of *Stammericaris similior* **sp. nov.** were produced. Novel sequences were deposited in GenBank (Accession Number, A.N., OP972572-OP972574). Conversely, no 18S rDNA sequences of the novel species were obtained by selective amplification of this nuclear fragment, despite the numerous attempts made during the whole amplification process (i.e., enhancing/adjusting PCRs thermal cycle; changing PCRs mix; using different 18S primer pairs).

In order to compare the new sequences with those publicly available, nine *Stammericaris* spp. and three *Cottarellicaris sanctiangeli* COI sequences were downloaded and included in the analyses (see Figure 9 for its A.N.). Furthermore, a COI sequence of *Proserpinicaris amalasuntae* (Bruno & Cottarelli 1998) was downloaded from GenBank to be included in the analysis (see Figure 9 for its A.N.). All sequences were aligned with MEGAX software (Kumar *et al.* 2018) using the ClustalW method (Thompson *et al.* 1997). In addition, MEGA X was used to translate the sequences into amino acids to check for the possible presence of frameshifts or stop codons. Average mtDNA COI sequence divergence between parastenocaridid species was estimated based on uncorrected *p*-distance. The molecular identification of the studied specimens and the inference of the phylogenetic relationships among the taxa was performed following the procedures described in Vecchioni *et al.* (2019).

Taxonomy

Subclass Copepoda H. Milne Edwards

Order Harpacticoida G. O. Sars

Family Parastenocarididae Chappuis, 1940

Subfamily Parastenocaridinae Chappuis, 1940

Genus *Stammericaris* Jakobi, 1972

Stammericaris similior sp. nov. (Figures 3-8)

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Diagnosis. Male characterized by: antennule of the "pocket-knife" type *sensu* Schminke (2010), seventh segment with a small apical tip; anal operculum well-developed, with the distal margin reaching past the distal margin of the anal somite; inner margin of P1 basis with hook; P2 endopod very small, with few apical spinules and distal seta; P3 thin and curved, with a proximal group of three spinules on exp-1; P4 endopod small and of peculiar shape, P4 basis ornamented with two spinules of different length, the one closer to the enp being the shortest one, and a half moon shaped lamella; P4 exp-1 with a long row of longitudinal spinules on the inner margin. Females characterized by one distal seta on the 7th antennular segment; inner margin of P1 basis with a thin seta; P2 endopod very small, with apical spinules and seta as in male.

Type locality. Sicily, Siracusa province (Italy), Scrivilleri Cave (37°08'28.7" N, 15°09'35.9" E), from five different rimstone pools (pools 1, 2, 11, 12, 14, 16).

Type material. Holotype: male, dissected, mounted on one slide labelled: "*Stammericaris similior* holotype: male" (MZUF 673), pool number 11, 23/VIII/2020.

Paratypes: two females dissected, each mounted on one slide labelled: "*Stammericaris similior* paratype: female", same date and pool as holotype. (MZUF 674, 675). One female, dissected, mounted on one slide labelled: "*Stammericaris similior* paratype: female" (MZUF 676), pool number 14, 8/XII/2021. One female, undissected, mounted on slide labelled: "*Stammericaris similior* paratype: female" (MZUF 677), pool number 11, 20/II/2021. One female undissected, mounted on one slide labelled: "*Stammericaris similior* paratype: female" (MZUF 678), pool number 2, 20/II/2021. One female undissected, mounted on one slide labelled: "*Stammericaris similior* paratype: female" (MZUF 679), same date and pool as holotype. Two females and one male, undissected, mounted together on one slide labelled "*Stammericaris similior* paratypes: 2 females, 1 male" (MZUF 980), pool number 14, 8/XII/2021. Five males, dissected (MZUF 681-685), and two males (MZUF 686, 687), undissected, each mounted on one slide labelled: "*Stammericaris similior* paratype: male", same date and pool as holotype. Three females and one male (20/II/2021, pool 11) and one female and three males (8/XII/2021, pool 16) prepared for scanning electron microscopy, on two stubs (CIME). Three specimens (23/VIII/2020, pool 1) used for molecular analysis. All material collected by Rosario Grasso, Maria Teresa Spena, Daniele Vincenzo Caccamo, and Diana Sapuppo.

Etymology. The species epithet is the masculine comparative form of the Latin adjective *similis* = similar, meaning "the most similar", and referring to its strong similarity to another species in the genus (i.e., *S. destillans*).

Description of male. Body unpigmented, nauplius eye absent. Total body length, measured from tip of rostrum to posterior margin of caudal rami (excluding caudal setae) from 316 to 342 µm, mean 330 µm (n = 6), holotype length 333 µm. Habitus (Figure 3A) cylindrical and slender, without any demarcation between prosome and urosome; prosome to urosome ratio: 0.88. Free pedigerous somites without any lateral or dorsal expansions, all connected by well–developed arthrodial membranes. Integument weakly sclerotized, without cuticular pits, ornamented with sensilla on all somites except preanal one. Round cuticular window on cephalothorax (Figures 3A, 4A). Cephalothorax representing about 18 % of total body length. Anal somite (Figures 3A, 4B, 4C, 8A) with pair of large dorsal sensilla at base of anal operculum, one pair of cuticular lateral pores (one pore on each side) on proximal margin and one on the distal margin underneath the operculum. Anal operculum (Figures 3A, 4B, 4C, 8A) well–developed, with slightly concave margin, extending past the distal margin of the anal somite. Anal sinus wide open. Spermatophore as in Figure 4D.

Caudal rami (Figures 3A, 4B, 4C, 8A): shorter than anal somite, approximately cylindrical, length to width ratio: 3.0. Anterolateral accessory seta (I) much shorter of anterolateral seta (II); posterolateral seta (III) slightly shorter than seta II, all setae inserted together distally at 3/4 length of caudal ramus. Outer terminal seta (IV) long and unipinnate (length seta/length caudal ramus: 0.9), inserted subterminally; inner terminal seta (V) without fracture plane, with large pore near its insertion. Terminal accessory seta (VI) short (length seta/length caudal ramus: 0.4) and smooth. Dorsal seta (VII) articulated, inserted distally at 3/4 length of the caudal ramus (length seta/length caudal ramus: 0.49).

Rostrum (Figure 4A): small, not demarcated at base, almost reaching distal margin of first antennulary segment, ornamented with two dorsal sensilla.

A1 (Figures 4E, 4F, 4G, 8B): prehensile, eight–segmented, pocket–knife type *sensu* Schminke (2010). First segment short, with distal, longitudinal spinular row; second segment longest, with six setae, the longest seta unipinnate; third segment with four distal, bare setae of similar length; fourth segment reduced to a small bare sclerite. Fifth segment enlarged, dorsally on the inner margin one basal small pointed apophysis carrying one short spine and one distal large roundish apophysis; ventrally, a distal tubercle with one large aesthetasc fused with long seta, and two subequal setae inserted above the insertion of distal tubercle, reaching past the end of eighth segment. Sixth segment bare, partially fused to previous one. Seventh segment bare, distal anterior corner protruding as a curved apophysis ending in a very small tip. Eighth segment with seven setae and apical acrothek represented by two setae and slender long aesthetasc. Armature formula: 1–[0], 2–[1 uniplumose + 5 bare], 3–[4 bare], 4–[0], 5–[3bare+ae], 6–[0], 7–[1bare], 8–[7 bare + (2 bare + ae)].

A2 (Figure 4H): coxa unarmed; inner margin of allobasis with one basal short seta and one transversal row at mid-length. Exp represented by a small segment, with bipinnate apical seta. Enp bearing two spines along inner margin, one apical inner unipinnate spine, one distal long spiniform unipinnate seta, two geniculate unipinnate setae, one outer transformed seta; all distal elements with long spinules near their insertions; a transversal row of spinules inserted at ¼ of the inner margin.

Mdb (Figure 4I): coxal gnathobase with lateral pinnate short seta, cutting edge with apical teeth. One–segmented palp, with two distal setae of different length.

Mx1 ((Figure 4J): praecoxal arthrite with three apical curved robust spines apically tufted, one subdistal curved seta. Coxal endite long, with one apical seta. Basis cylindrical, with one short and two longer distal bare setae. Enp and exp absent (fused to basis without trace).

Mx2 (Figure 4K): basis with two endites, proximal endite short, with one thin, bare seta; distal endite cylindrical, longer, armed apically with two subequal thin bare setae and one transformed, leaf–like seta; proximal endopodal segment drawn into apical unipinnate claw; distal endopodal segment with two long setae of equal length.

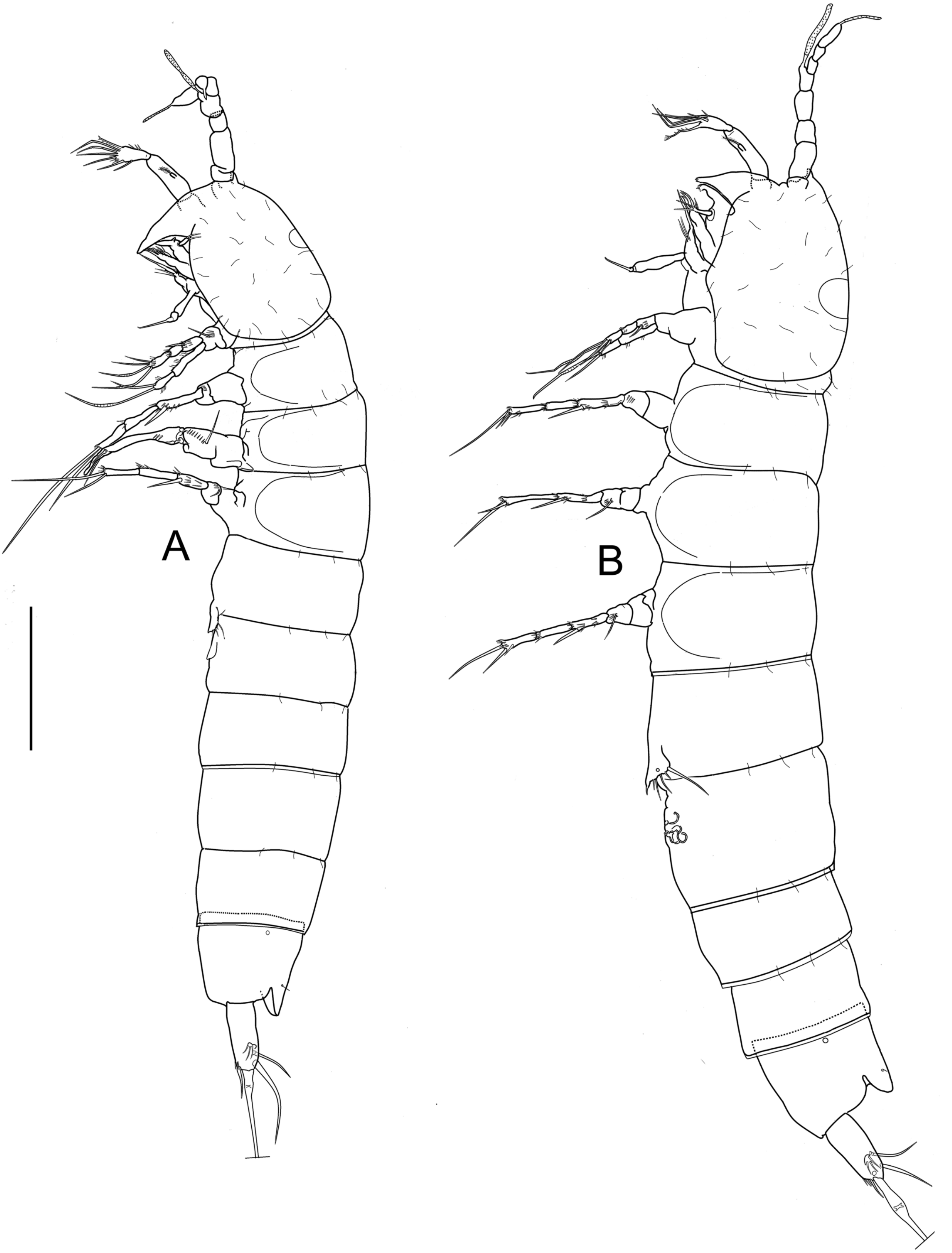

FIGURE 3. *Stammericaris similior* **sp. nov.** Habitus, lateral view. A) male; B) female. Scale bar: 50 micrometers.

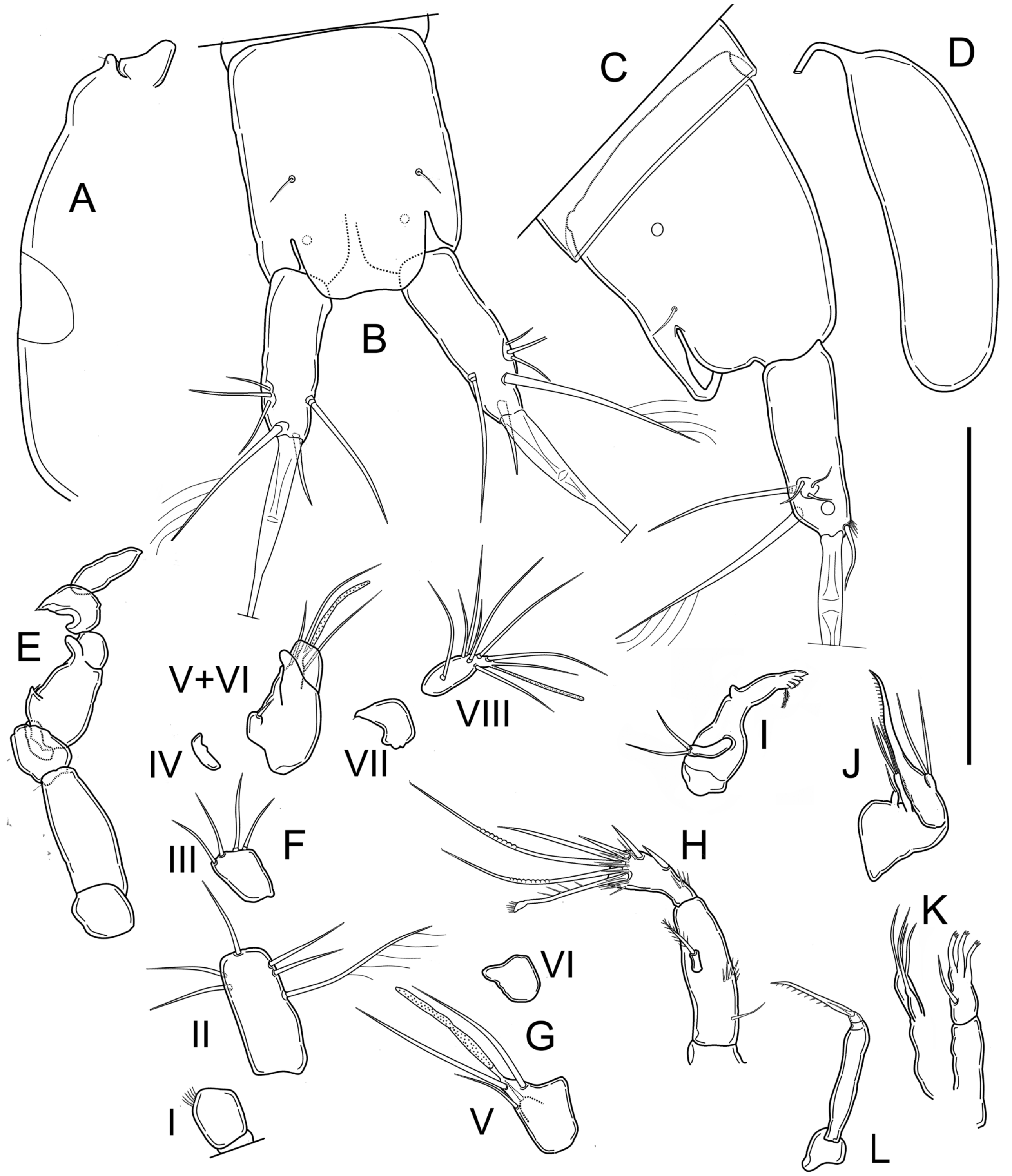

FIGURE 4. *Stammericaris similior* **sp. nov.** Male. A) rostrum, cephalothorax and cephalic windows, lateral view; B) anal somite, anal operculum and caudal rami, dorsal view; C) anal somite, anal operculum and caudal rami, outer view; D) spermatophore; E) antennule, schematic (armature not depicted); F) antennule, disarticulated, dorsal view (antennular segments marked with roman numerals); G) antennule segments V and VI, ventral view (antennular segments marked with roman numerals); H) antenna; I) mandible; J) maxillule; K) maxilla (partly disarticulated); L) maxilliped. Scale bar: 50 micrometers.

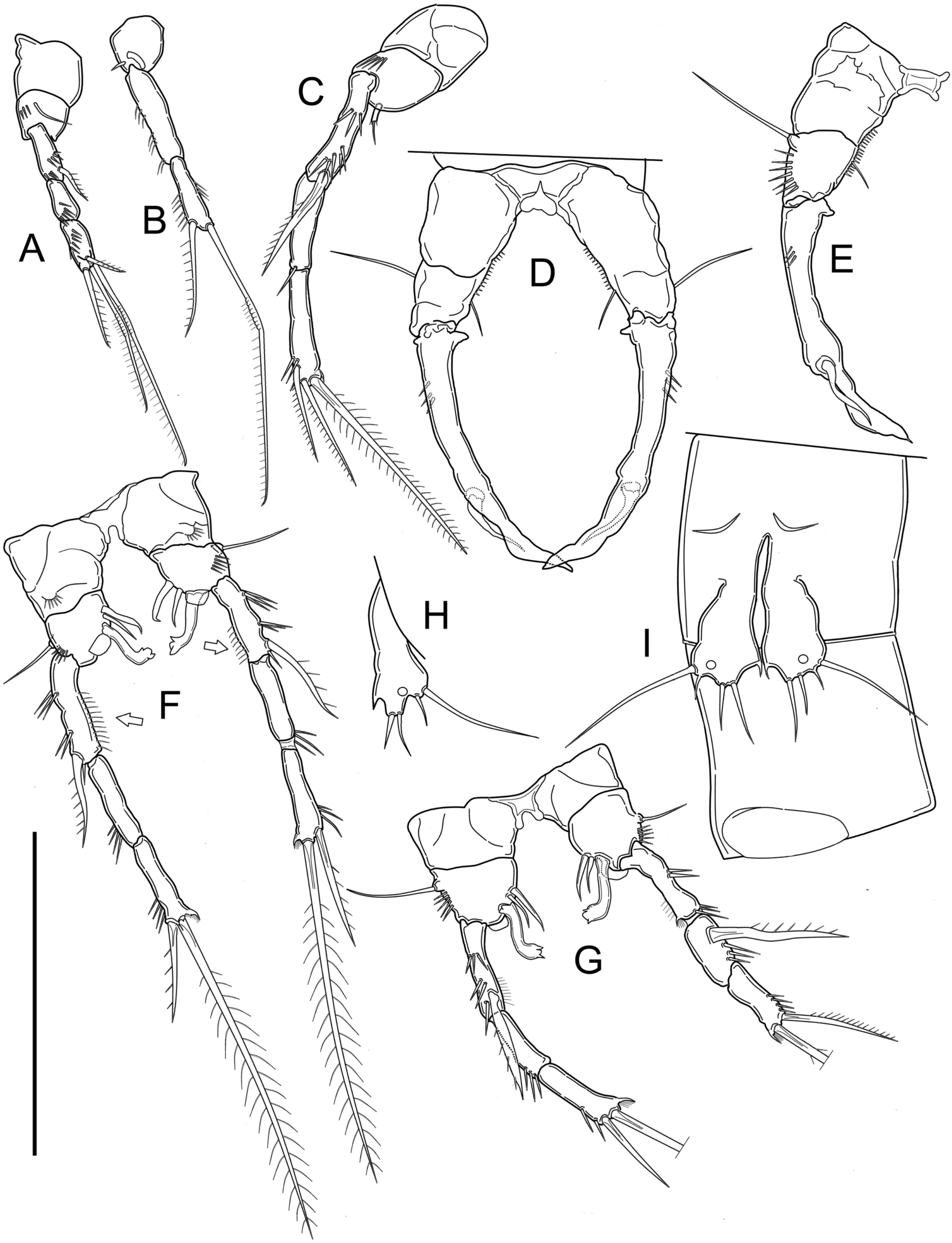

FIGURE 5. *Stammericaris similior* **sp. nov.** Male. A) P1, basis and exopodite, outer view; B) P1, basis and endopodite, inner view; C) P2; D) P3; E) P3 (variability); F) P4 (arrows pointing at longitudinal row of spinules along the inner margin of exp-1); G) P4 (variability); H) P5; I) P5, P6, first and second urosomites, ventral view. Scale bar: 50 micrometers.

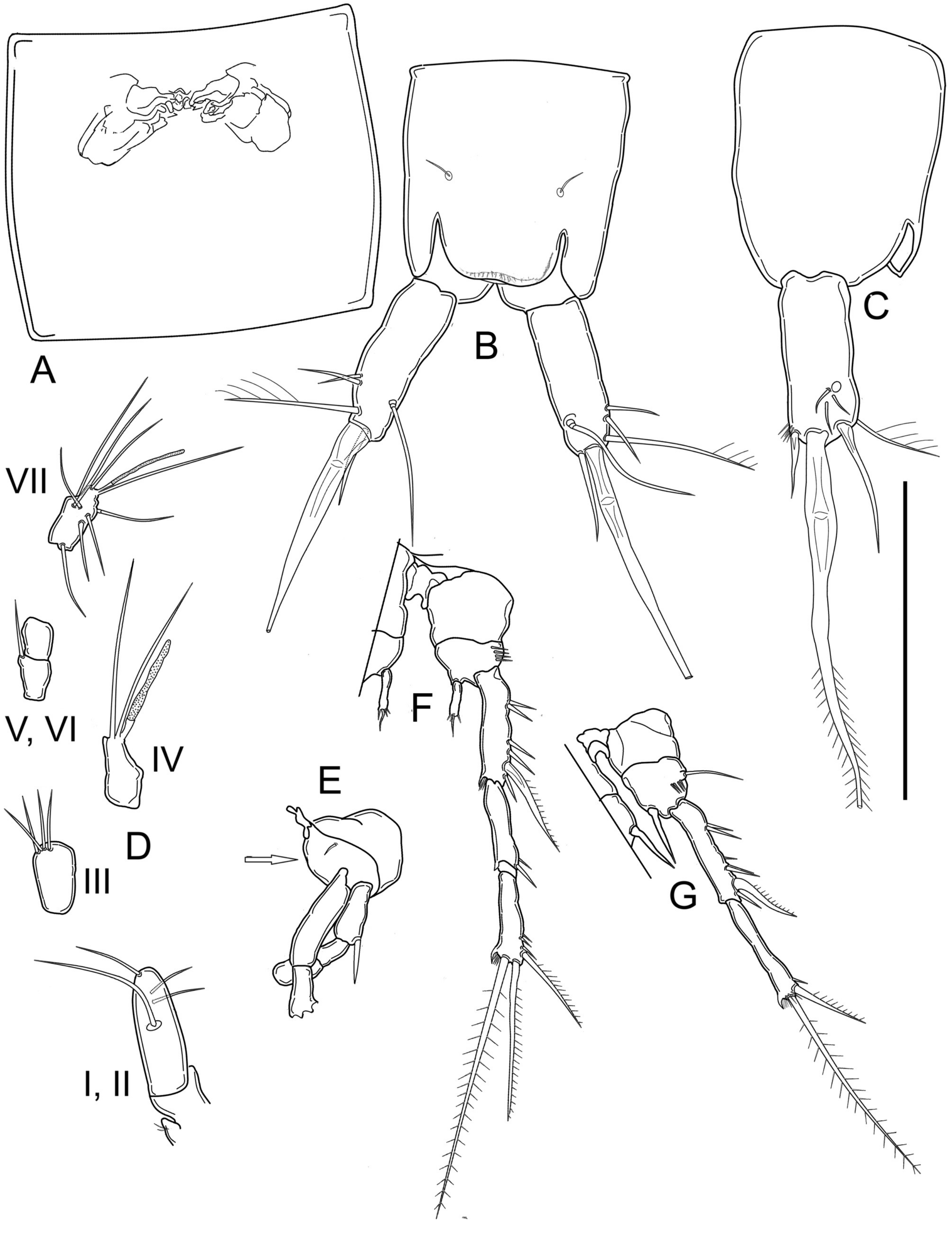

FIGURE 6. *Stammericaris similior* **sp. nov.** Female. A) genital double somite, genital field, P6; B) anal somite, anal operculum and caudal rami, dorsal view; C) anal somite, anal operculum and caudal rami, outer view; D), antennule, disarticulated, dorsal view (antennular segments marked with roman numerals); E) basis P1 (arrow pointing at the inner seta); F) P2; G) P3. Scale bar: 50 micrometers.

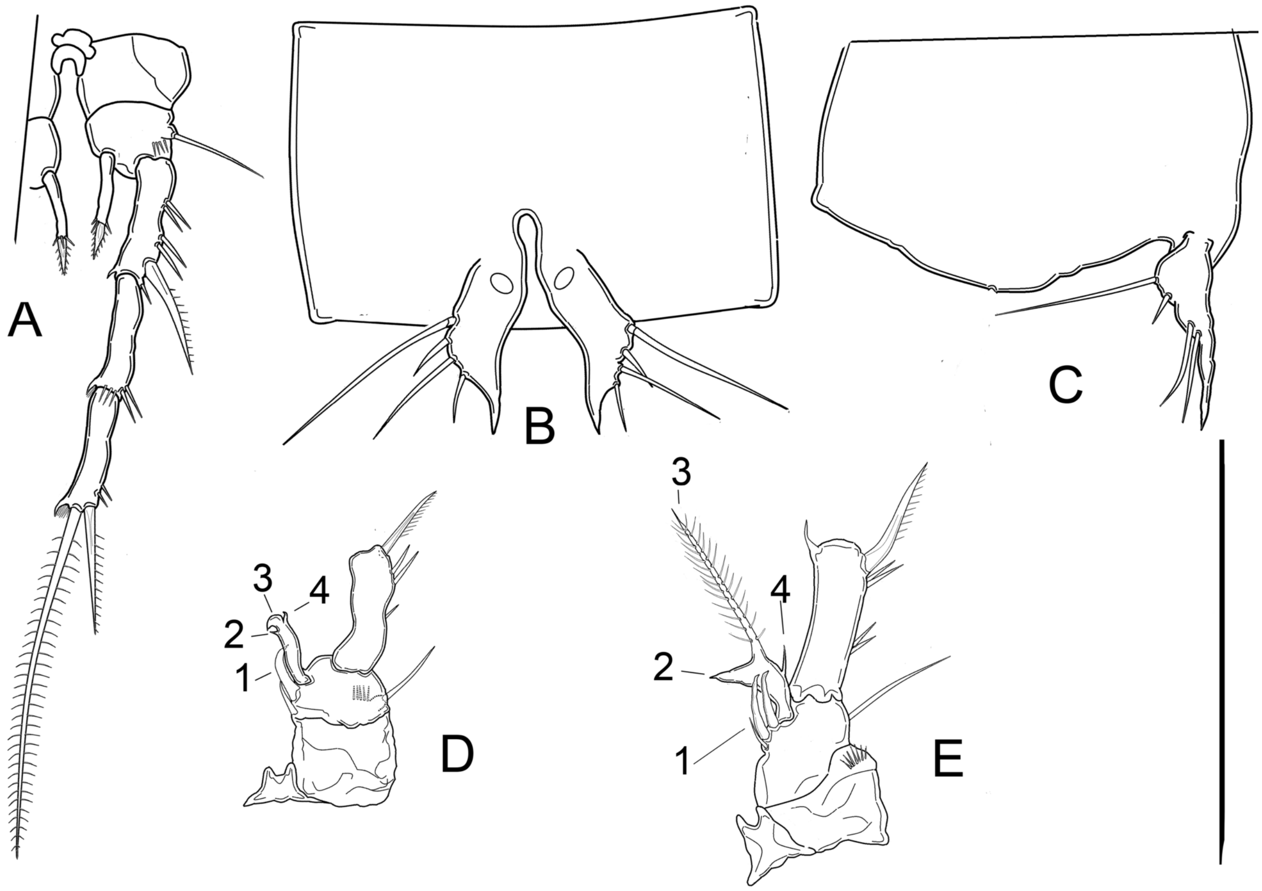

FIGURE 7. *Stammericaris similior* **sp. nov.** Female. A) P4; B) P5; C) P5, lateral view. D) **Stammericaris destillans*, male P4 intercoxal sclerite, coxa, basis, endopodite and exp-1; E) **Stammericaris trinacriae*, male P4 intercoxal sclerite, coxa, basis, endopodite and exp-1. Scale bar: 50 micrometers. * Redrawn from Bruno *et al.* 2017; numbers 1 to 4 refer to the genus diagnostic features as described in Bruno *et al.* 2017. 1 = basis inner row of spinules; 2 = endopod pointed inner tip; 3 = endopod distal outgrowth (in most cases a feathered or plain seta); 4 = endopod proximal outgrowth.

Mxp (Figure 4L): subchelate, composed of small and unarmed syncoxa, basis slim and elongate, unarmed, 1-segmented enp fused to the claw-like apical seta.

P1 (Figures 5A, 5B): with smooth and small intercoxal sclerite; coxa bare. Basis large, with single slender seta and a hook (Figure 8C) with rounded tip on outer margin, and one spinule on inner margin; ornamented with transverse row of minute spinules at base of outer seta. Exp (Figure 5A) three–segmented, slightly shorter than enp; exp–1 with thin unipinnate spine on outer distal corner; exp-2 shortest and unarmed; exp–3 with two geniculate and one normal unipinnate apical setae, and one subapical unipinnate spine. Enp (Figure 5B) two–segmented; enp–1 longer than the first two segments of the corresponding exp, with three transversal rows of spinules on the outer margin. Enp-2 thinner and shorter than enp-1, with three spinules at 1/2 of the inner margin and spinular row along the outer margin; long, geniculate pinnate seta, and shorter pinnate seta on apex.

P2 (Figure 5C): with smooth intercoxal sclerite, twice as wide as tall. Basis unarmed, with row of four spinules on outer margin. Exp three–segmented, exp–1 longest, with rows of spinules at ½ of the outer margin, subdistal strong unipinnate spine with spinules near its insertion. Second and third segments of same length, exp–2 unarmed, with distal row of spinules; exp–3 armed with subapical outer unipinnate spine, apical bipinnate seta and unipinnate spine, ornamented with distal row of spinules and inner hyaline frill. Endopod one-segmented, about 1/4 the length of the corresponding exp-1, represented by small cylindrical segment, with apical seta about as long as segment and apical spinule.

P3 (Figure 5D): intercoxal sclerite narrow and tall, trapezoidal, unornamented, with slightly concave distal margin. Coxa with outer spinular rows. Basis strong, with long, slender, smooth outer seta and transverse spinular row above it (Figure 8D). Inner margin of coxa and basis with longitudinal row of short spinules. Enp reduced to thin seta. Exp–1 outer margin with one proximal group of three spinules, inner margin with conical proximal tubercle fused to exopod. Exp–2 fused with exp–1, without ornamentation, prolonged into long apophysis slightly bent inwards, with pointed tip surrounded. Distal thumb represented by thin and pointed segment, shorter than apophysis.

P4 (Figure 5F): intercoxal sclerite smaller than in P1 or P2, with concave, smooth distal margin. Coxa with spinular row on outer margin. Basis armed with single slender seta on outer margin; ornamented with row of spinules at base of outer seta; two spiniform processes of different size, slightly curved inwards, aligned along inner margin, the smallest one close to endopod; a half-moon shaped hyaline lamella is inserted between endopod and exopod. Exopod three-segmented, slender, all segments approximately of the same length, with inner hyaline frill; exp-1 slightly curved inwards, with distolateral pinnate spine and longitudinal row of spinules along the inner margin (arrowed in Figure 5F and 8D); exp-3 armed with outer pinnate spine and very long apical pinnate seta, spine length less than 1/3 of seta length. Endopod one-segmented (Figure 8E), slightly longer than 1/2 of than corresponding exp-1, represented by a cylindrical element with distal part bent at 90° and enlarged in three protrusions.

P5 (Figures 5H, 5I, 8E): fused to intercoxal sclerite, represented by two trapezoidal cuticular plates with inner-distal corner produced into small, pointed tip, one large pore on the distal inner corner of each plate. Armature on free distal margin, from inner to outer: two bare setae and one spine decreasing in length, and long basipodal seta.

P6 (Figure 5I): vestigial, fused into simple asymmetrical cuticular plate, unornamented and unarmed.

Description of female. Habitus (Figure 3B) cylindrical and slender, without any demarcation between prosome and urosome. Free pedigerous somites without any lateral or dorsal expansions, all connected by well–developed arthrodial membranes. Integument weakly sclerotized, without cuticular pits, ornamented with sensilla on all somites except preanal one. Round cuticular window on cephalothorax. Body length, excluding caudal setae, from 329 to 355 µm, mean 346 µm (n = 6); length of paratype in Figure 3B: 352 µm. Ornamentation of cephalothorax, somites, pigmentation, and lack of nauplius eye as in male, except genital and first urosomite fused into double somite. Cephalothorax representing about 20% of the total body length. Prosome/urosome ratio: 1. Genital double–somite (Figure 6A) without any trace of subdivision. Genital field (Figure 6A) broader than tall, occupying anterior ventral 1/3 of genital double–somite, with pair of ventrodistal pores; single genital aperture covered by fused vestigial sixth legs; median copulatory pore located medially at 1/4 of double–somite length; morphology as typical for the genus. Anal operculum and anal sinus (Figures 6B, 6C) as in male. Caudal rami (Figures 6B, 6C): shape, ornamentation and armature similar to those of male, length/width ratio: 2.7.

Rostrum, A2, oral appendages, as in male.

A1 (Figure 6D): seven–segmented, aesthetasc on fourth segment shorter and thinner than in male, reaching below end of seventh segment. First segment bare. Second segment longest. Apical acrothek represented by two setae of different length and slender aesthetasc. Armature formula: 1-[0], 2-[1 pinnate +3 bare], 3-[4 bare], 4-[2 + ae], 5-[1], 6-[0], 7-[7 bare + (2 + ae)].

P1: intercoxal sclerite, coxa, exp and enp similar to the male's ones in shape, ornamentation and armature. Basis with outer seta and spinular row near its insertion as in male; lamellar hook on inner margin missing but small spinule present as in male (arrowed in Figure 6E);

P2 (Figure 6F): intercoxal sclerite, coxa, basis and exp as in male. Enp similar in shape and ornamentation to that of the male but proportionally larger, and apical seta shorter.

P3 (Figure 6G): intercoxal sclerite small, trapezoidal, with concave margin, bare. Coxa bare. Basis with outer spinular row. Exp two–segmented: exp–1 as long as exp–2, with distolateral curved unipinnate spine, transversal row of spinules at 1/3 and 2/3 of outer margin, distal spinular row, hyaline frill on inner distal corner (Figure 8F); exp–2 with subapical outer unipinnate spine and apical bipinnate seta, spine length about 1/3 of seta, with distal spinular row and hyaline frill on inner distal corner. Enp represented by a thin and pointed pinnate segment (Figure 8F), as long as half of corresponding exp–1.

P4 (Figure 7A): intercoxal sclerite, coxa, and exp as in male. Basis bare. Enp represented by a cylindrical segment, slightly longer than 1/2 the length of corresponding exp–1, ending in a strong spiniform seta with spinules around the insertion.

P5 (Figures 7B, 7C, 8G): fused to intercoxal sclerite, represented by cuticular plate more elongate than in the male, with large proximal pore and inner spiniform process larger than in male; remaining ornamentation represented by three setae, the middle one longest, and long basipodal seta.

P6 (Figure 6A, 8G): vestigial, fused into simple cuticular plate, covering gonopore, unornamented and unarmed.

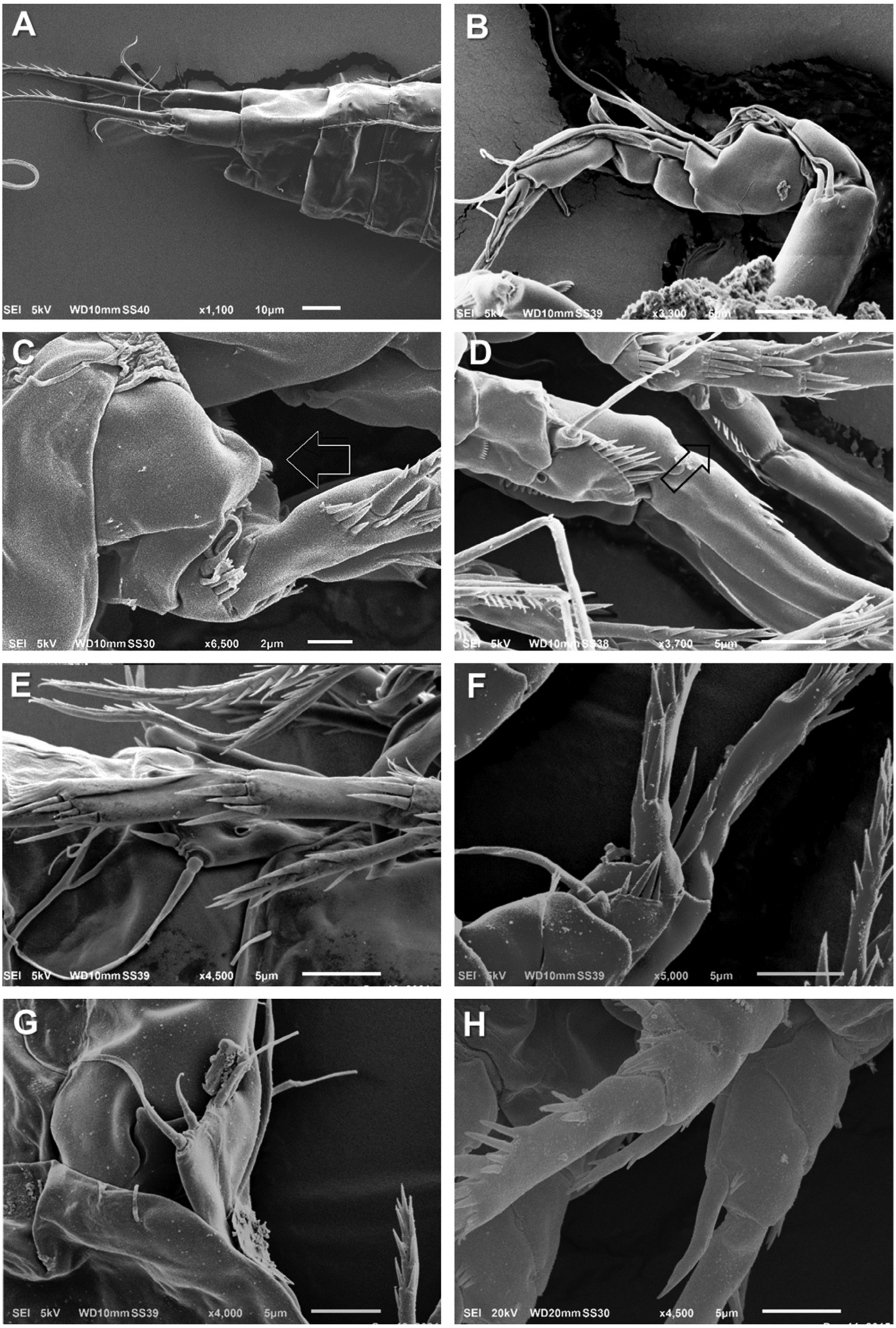

FIGURE 8. *Stammericaris similior* **sp. nov.** A–E: male; F–G: female. A) anal somite, anal operculum and caudal rami, outer view; B) antennule, dorsal view; C) P1 basis, outer view (arrow pointing at hook); D) P3 basis, outer view, and P4 exp-1, inner view (arrows pointing at longitudinal row of spinules along the inner margin of exp-1); E) P4 endopod and P5, latero-ventral view; F) P3 endopod, latero-ventral view; G) P5 and P6, outer view. *Stammericaris destillans*, female: H) P3 basis and endopod, anterior view (from Bruno *et al.* 2017).

Variability. One male with the left P3 with four spinules on the exp-1 instead than three (Figure 5E). One male with malformed P4 exopod: the distal outer spine of exp-1 is inserted basally on exp-2, this segment is short and enlarged, the other exopod is normal (Figure 5G).

Molecular results

Overall, sixteen specimens of parastenocaridid were included in the analyses (see Figure 9). After trimming out the tails of the sequences, which were not present for all the individuals, we obtained a properly aligned 564bp-long COI fragment. The translation of the COI nucleotide sequences into amino acids revealed the absence of stop codons and the existence of a conserved amino acid sequence shared by all the investigated harpacticoids taxa and specimens. The Bayesian inference of phylogeny (BI) and Maximum likelihood (ML) trees based on the mtDNA COI dataset and rooted on *Proserpinicaris amalasuntae* showed a congruent topology, in agreement with the current morphology-based taxonomy, with a well-supported clade where the *Stammericaris* spp. samples constitute the sister group of a clade including the *Cottarellicaris sanctiangeli* samples (Figure 9). Within the *Stammericaris* spp. clade, our novel sequences belonging to *Stammericaris similior* **sp. nov.** cluster together in a well-supported monophyletic clade, with two different haplotypes (Figure 9).

Based on the mtDNA COI dataset, uncorrected p-distances (Table 2) between *Stammericaris similior* **sp. nov.** and the other *Stammericaris* species ranged between 11.5% (*S. pasquinii* vs. *S. similior* **sp. nov.**) and 22.9% (*S. diversitatis* vs. *S. similior* **sp. nov.**).

TABLE 2: Pairwise distances (*p*-distance model) among COI sequences between the analysed species of Parastenocarididae

Species	1	2	3	4	5	6	7	8
1. *Stammericaris vincentimariae*	-							
2. *Stammericaris pasquinii*	0.115	-						
3. *Stammericaris destillans*	0.169	0.225	-					
4. *Stammericaris diversitatis*	0.210	0.229	0.221	-				
5. *Stammericaris trinacriae*	0.194	0.207	0.210	0.146	-			
6. *Stammericaris similior* **sp. nov.**	0.207	0.200	0.217	0.220	0.217	-		
7. *Cottarellicaris sanctiangeli*	0.219	0.196	0.209	0.194	0.196	0.207	-	
8. *Proserpinicaris amalasuntae*	0.256	0.241	0.235	0.237	0.252	0.248	0.245	-

DISCUSSION

Taxonomic definition

The following discussion on the taxonomy and affinities of the new *Stammericaris* is based on the available descriptions, illustrations, scanning microscopy images, and the re-examinations of specimens in our collections, of the following twelve taxa: *S. amyclaea, S. destillans, S. diversitatis, S. lorenzae, S. orcina, S. palmerae, S. pasquinii, S. remotaepatriae, S. trinacriae, S. vincentimariae, Stammericaris* sp. (Trento, Northern Italy, unpublished), *Stammericaris* sp. (Eğirdir Lake, Turkey, unpublished).

Stammericaris similior **sp. nov.** fits well with the emended diagnosis of the genus (Bruno *et al.* 2017, 2020, but see also Schminke 2013) for the following characters: the P1 basis carries an inner hook in males and an inner seta in females; the outer margin of male P3 exp-1 carries a proximal group of spinules; the male P3 apophysis is about twice as long as the thumb; the male P4 basis carries an inner row of 1-4 curved spinules decreasing in size laterally and the endopod is a curved plate with a pointed inner tip carrying at its outer border two outgrowths, in most cases the distal one is a feathered or plain seta; caudal rami cylindrical, almost as long as anal somite, group of lateral setae located at end of rami. Among these characters, the enp P4 of male is the main character for the definition and discrimination of the genus, as it was remarked by Schminke (2013) and *S. similior* **sp. nov.** displays all the features characteristic of the genus (see above) except the typical male P4 endopod, which however can be considered a "simplification" of the typical morphology of this appendix (as already recorded by Bruno *et al.* 2017 for *S. destillans*, the only congeneric species with a similarly unusual endopod). In *S. similior* **sp. nov.**, as in *S. destillans*, "the distal seta is reduced to a tubercle and the inner tip and proximal outgrowth are transformed into the two (inner and outer) protrusions, which are proportionally smaller than those of other species in the genus because the entire endopod is smaller" (Bruno *et al.* 2017). The reduced length of the caudal rami of the new species (shorter than the anal somite), differs from the character which is diagnostic for the genus: "Caudal rami cylindrical almost as long as anal somite, group of lateral setae located at end of rami" (Bruno *et al.* 2017). Nevertheless, the reduced length of the caudal rami does not preclude the attribution to the genus *Stammericaris*, because the insertion of the setae I-III and VII corresponds to the diagnostic position, and the caudal rami are shorter than the anal somite also in other species of the genus (specifically*, S. destillans*, *S. lorenza*e, *S. phreatica*, *S. remotaepatriae, S. vincentimariae*). This character is present also in the females, which, however, display all the remaining diagnostic features of the genus.

Stammericaris similior **sp. nov.** can be easily teased apart from all the other known *Stammericari*s except *S. destillans,* for the shape of the male P4 endopod, which is very similar to the one of the latter species, representing, as already discussed, the main affinity between the two taxa. The new species is further characterized by two autoapomorphic character: the half-moon shaped lamella originating near the P4 endopod insertion (a lamella is not present in all other *Stammericaris*, including *S. destillans*) and the long row of longitudinal spinules on the inner margin of the P4 exp-1.

There are a few more relevant differences in morphological characters which separate the new species from *S. destillans*: i) the inner side of the P1 basis in males carries one hook and one spinule in *S. similior* **sp. nov.** and only one hook in *S. destillans*; ii) the male P3 is proportionally thinner and longer in *S. similior* **sp. nov.**: length exopod (including apophysis)/length basis = 5.5 in *S. similior* **sp. nov.** and 3.4 in *S. destillans*; iii) the P3 exp-1 is ornamented with a row of 3 spinules in *S. similior* **sp. nov.** and with 2 spinules in *S. destillans*; iv) the distal part of the P4 endopod is bent at 90° in the new species ad it is straight in *S. destillans*; v) the P4 basis carries two spiniform processes of different length, apically curved on the inner margin: in *S. similior* **sp. nov.** the process closer to the endopod is the smallest of the two, whereas in *S. destillans* it is the largest one (two spiniform processes are present also in *S. trinacriae*, but the shape of the endopod is very different); vi) the P5 of both sexes carries the inner tip and 4 setae, the second outermost one is very reduced and spiniform, as recorded in *S. destillans* and also in S. *pasquinii*. The P5 of both sexes of *S. similior* **sp. nov.** and *S. destillans* has a pore, but the position of such pore differs; vii) both sexes in the new species are longer than *S. destillans* of about 30 micrometers, although the number of individuals analyzed (six males and six females) are too few to assess the significance of this difference; viii) *Stammericaris similior* **sp. nov.** shares with *S destillans* the presence of an elliptical dorsal integumental window on the cephalothorax of both sexes and the absence of dorsal elliptical windows on the urosomites; this is a very rare condition in Parastenocarididae, where the most common feature is the presence of dorsal windows on cephalothorax and urosomites (Galassi & de Laurentiis 2004, Corgosinho *et al.* 2007) as it occurs, for instance in *S. diversitatis, S. lorenzae*, *S. trinacriae*, *S. remotaepatriae*; in the other species of the genus (*S. vincentimariae*, *S. pasquinii*, *S. orcina*, *S. amyclaea*, *S. acherusia*, *S. stammeri*, *S. phreatica*), there are no windows at all, although windows were rarely taken into account in the earlier descriptions of several species of Parastenocarididae; ix) the male antennule of *S. similior* **sp. nov.** are of the pocket-knife type as in all the recently-described congeners, but the fourth segment is bare in the new species whereas it carries two small setae in *S. destillans* and in all the other Sicilian species. The morphology of the caudal rami in both sexes of *S. similior* **sp. nov.** is similar to those of *S. destillans*, as both species have the caudal rami shorter than the anal somite; in all the other Sicilian species the caudal rami are longer than the anal somite, caudal rami are shorter than the somite also in *S. vincentimariae*, a species endemic to Calabria (Southern Italy).

As in most Parastenocarididae, it is difficult to distinguish the females of *Stammericaris* species, if the males are not co-occurring in the same locality. The females of the new species can be teased apart from those of *S. destillans* by the ornamentation of the fifth segment of the A1, which in *S. similior* **sp. nov.** carries a long seta, whereas it is bare in *S. destillans*. Moreover, SEM images show that *S. destillans* has a spinular row near the enp P2-P4 insertions (Figure 8H), such rows are missing in the new species.

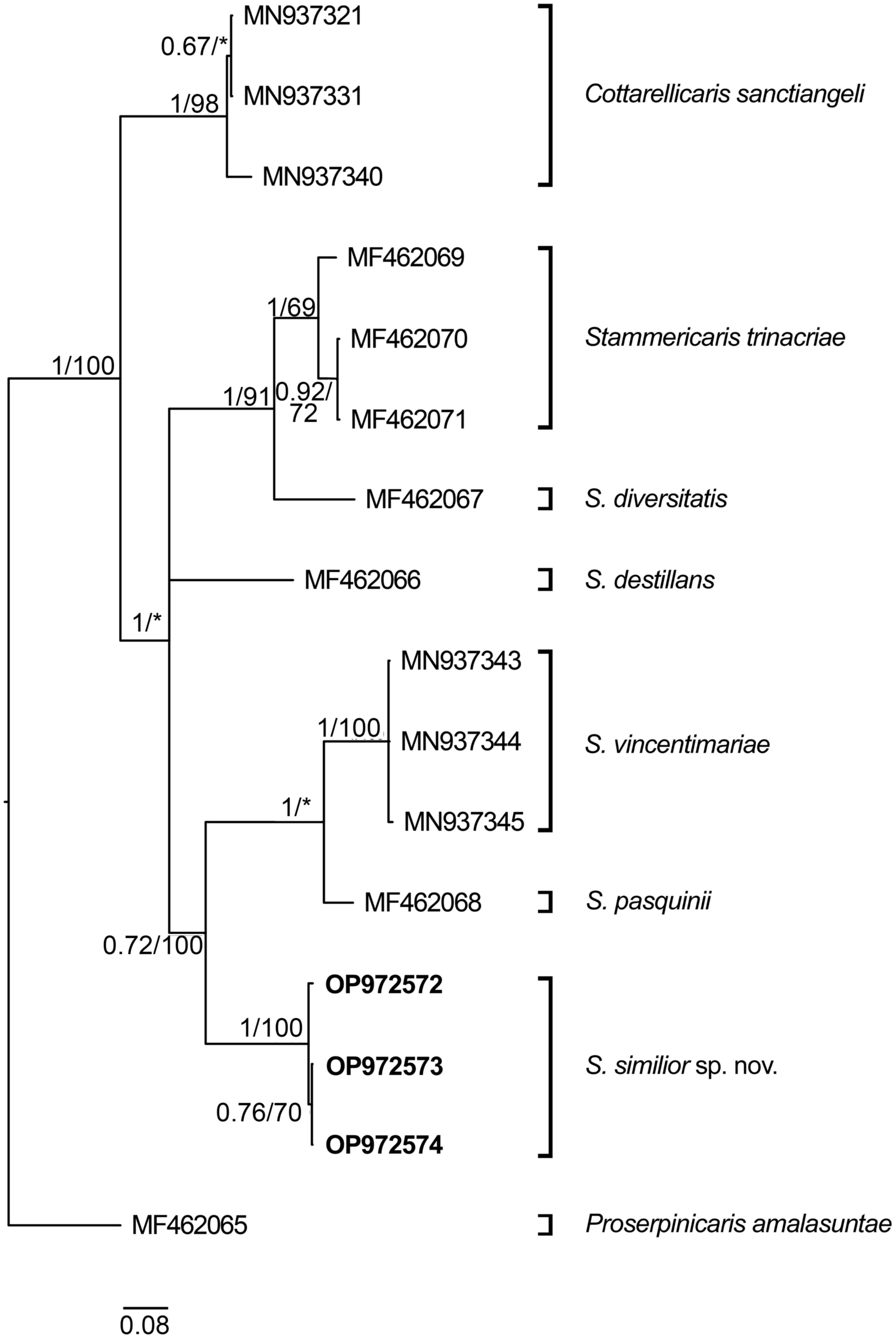

FIGURE 9. Bayesian phylogram (95% majority rule consensus tree) for *Stammericaris* spp. based on the 564 bp fragment of the mtDNA COI, listed by their Accession Number in GenBank. Samples of *Proserpinicaris amalasuntae* was used as outgroup to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI) / bootstrap values (Maximum Likelihood, ML). * Nodal statistical supports <0.50. Novel sequences are reported in bold.

Phylogenetic relationships

The phylogenetic relationships among the analysed Parastenocarididae species show, in agreement with what was previously stated by Bruno *et al.* (2020), a clear separation within the Parastenocaridinae clade for the genera *Cottarellicaris* and *Stammericaris*. The relationships among the members of the *Stammericaris* spp. clade are partially unresolved due to the polytomy of *Stammericaris* spp. sequences (see Figure 9). Nonetheless, the novel COI sequences of *Stammericaris similior* **sp. nov.** show a well-supported sister clade relationship to a subclade which includes the sequences belonging to *S. vincentimariae* and *S. pasquinii*. Such an arrangement is in contrast with the possible close relationship of *S. similior* **sp. nov.** and *S. destillans*, which is supported by their close morphological affinity. The absence of a strict phylogenetic relationship between *S. similior* **sp. nov.** *and S. destillans* suggested by the mtDNA COI sequences is in striking contrast with the available morphological evidence. Unfortunately, lacking nuclear sequences of *S. similior* **sp. nov.**, we do not have an independent line of evidence to support or reject the sister clade relationship proposed for these taxa. In fact, it is well-known that mitonuclear discordance phenomena often occur among crustaceans (e.g., Thielsch *et al.* 2017; Deng *et al.* 2022) and other taxa (i.e., Belaiba *et al.* 2019; Franco *et al.* 2015; Toews & Brelsford, 2012). Therefore, further analyses based on nuclear DNA are desirable, in order to better understand the phylogenetic relationships among Parastenocarididae species.

Ecology and distribution

The results of the research on Parastenocarididae we conducted in the last decade (Cottarelli & Bruno 2012, 2022; Bruno *et al.* 2017, 2020) suggest that caves, and particularly the cave epikarst, seem to represent an optimal habitat at least for the genus *Stammericaris*, even if the environmental conditions of the epikarst are harsh. In fact, well-structured populations exist even in fossil or semi-fossil cavities, and the network of small cavities and fractures of the epikarst, where water is retained by capillarity, seems to foster the survival of these microcrustaceans even during prolonged periods of drought, as it is frequently occurring in Sicily and possibly has been increasing in the last decades due to climate change. We also observed that the epikarstic species of *Stammericaris* and of the related genus *Cottarellicaris* are mainly and peculiarly represented by small species (compared to related taxa of, e.g., the fluvial hyporheos or the lacustrine psammon); the cuticle of the epikarstic taxa is very thin and often without the elliptical respiratory windows used for gas exchange. The small size and thinning of the exoskeleton and thus the increased flexibility may represent an advantage to move easily and colonize even the narrowest micro-crevices. The limited thickness of the cuticle could also account for the recorded frequent absence of respiratory windows, as gas exchange can occur throughout the whole body surface. In the case of *S. similior* **sp. nov.** and *S. destillans*, the degree of body miniaturization, which would improve the efficiency of this process, seems to be related to environmental harshness: *S. destillans* is the only crustacean found in a cave (Molara Cave, see Bruno *et al.* 2017 for details) located in an aera with a thinner epikarstic layer and much drier conditions than the one of Scrivilleri Cave. Notably, *S. destillans* is smaller than *S. similior* **sp. nov.**, perhaps this is an adaptation to move into the narrowest crevices and cracks where capillarity better preserves water. Hence, at least in the case of these two species, the higher hydrological stress might have selected for a stronger reduction of body size and resulting increased gas absorption efficiency. As previously stated (Bruno *et al.* 2017), “The lower diversity recorded in the Sicilian caves could be due to the stressful environmental conditions such as hydrological intermittency, high surface temperatures, and high ionic concentration (e.g., the very high sulfates concentration recorded in the drip pools of Entella Cave by GR, MTS, MCB, VC, unpubl. data), which only allow the survival of tolerant taxa such as some stygobiotic Parastenocarididae”. In synthesis, the selective environmental conditions characterizing some of the caves colonized by *Stammericaris* could represent the main factor determining the evolutionary history of these species.

In the whole Sicilian territory, all species of *Stammericaris* are present exclusively in caves, except for *S. trinacriae*, which lives also in phreatic water, i.e., two wells at 3-10 m depth (Pesce *et al.*, 1988). The extensive research conducted by Cottarelli, Grasso and Spena in the hyporheic of many Sicilian streams (Bruno *et al.* 2017; Cottarelli *et al.* 2012, and unpublished data), have not led to the discovery of *Stammericaris* or other Parastenocarididae, except for specimens of *Cottarellicaris* spp. collected almost exclusively in interstitial estuarine habitat. These data support the hypothesis that the epikarstic system represents the primary habitat of *Stammericaris* in Sicily.

Remarkably, during the biospeleological campaigns we have been conducting in Sicily (Cottarelli *et al.* 2012; Bruno *et al.* 2017), we have recorded four species of *Stammericaris* (i.e., *S. diversitatis*, *S. destillans*, *S. trinacriae*, and *S. similior* **sp. nov.**), all of them endemic or present only in one cave (i.e., Grotta Conza, Grotta Molara, Grotta di Entella, and Grotta Scrivilleri, respectively), even when other caves with comparable topographical development and with similar habitat conditions were present nearby which, although always carefully explored, did not host these new parastenocaridids. These *Stammericaris* are therefore stygobionts, characterized, as mentioned earlier, by a type of endemicity that could be defined as punctiform.

As regards the geonemy of the genus, the map in figure 10 shows a high concentration of species for Sicily (4 species): evidently, this datum has a faunistic interest but not a biogeographical one, due to the geographical imbalance of field research. It is however certain that the recent finding of *Stammericaris* in the USA (Cottarelli & Bruno 2021) has greatly expanded the distribution range of this genus, which may lead to the further discovery of new taxa.

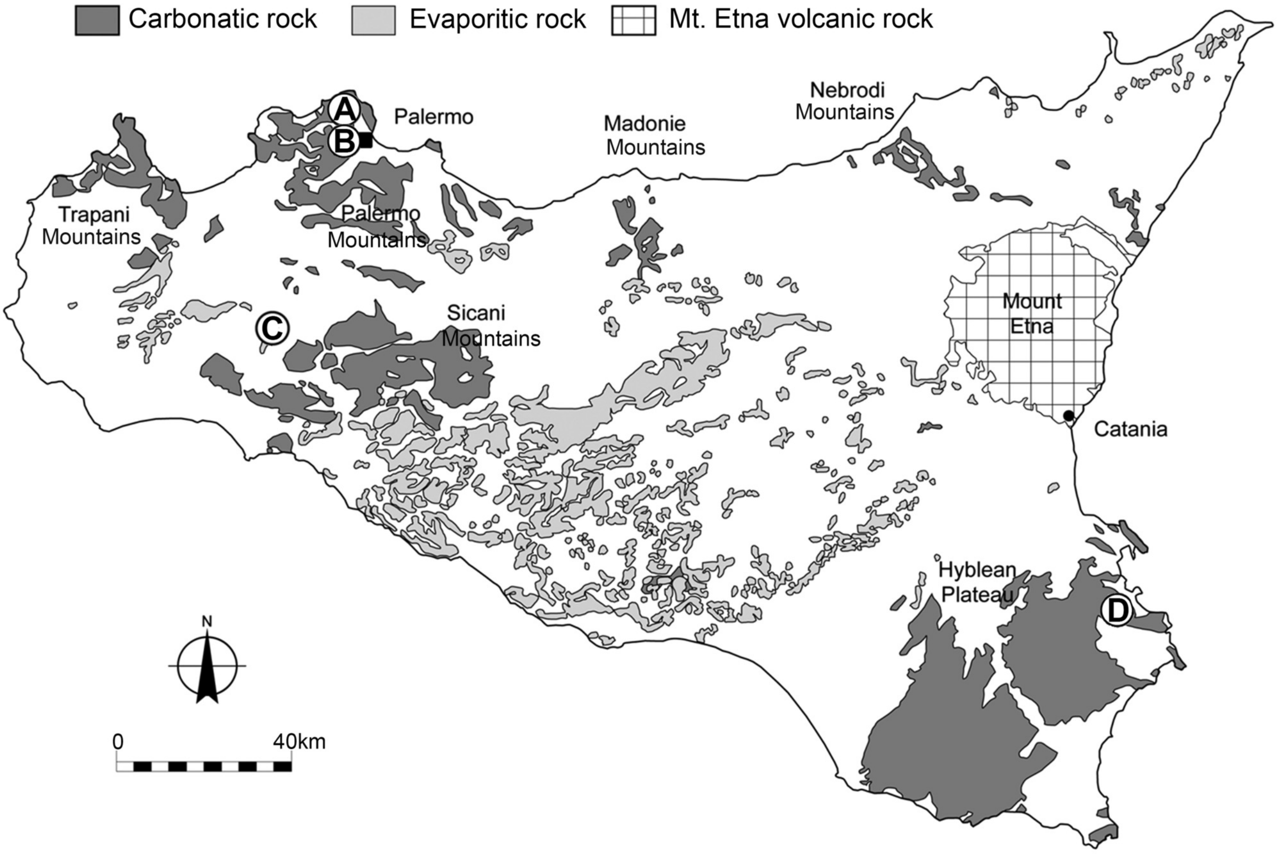

FIGURE 10. Map of Sicily showing the carbonatic, evaportitic and volcanic outcrops, and the position of the caves where endemic *Stammericaris* were collected: A) *Stammericaris diversitatis:* Conza Cave; B) *Stammericaris destillans:* Molara Cave; C) *Stammericars trinacriae:* Entella Cave*; D) *Stammericaris similior* **sp. nov.**: Scrivilleri Cave. * *S. trinacriae* was originally described from specimens collected from two wells in Trapani (Sicily, Italy) (Pesce & Galassi 1987; Pesce *et al.* 1988) and later redescribed based on specimens collected in Entella Cave (Bruno *et al.* 2017). Redrawn from Bruno *et al.* 2017.

Accompanying fauna

Elaphoidella elaphoides Chappuis 1923. A widely distributed species in Palearctic surface and ground water habitats, it was already known for Sicilian groundwater (Pesce & Galassi 1987) but not yet for caves. Petkovski (1959) already remarked that the taxon might actually be "a collection of morphologically similar taxa, presently grouped under a single name" (Pesce 1985). In Scrivilleri Cave, we collected specimens of both sexes from pools 1 and 2.

Metacyclops sp. An interesting stygobitic cyclopoid, characterized by several morphological characters that allow to consider it a new species of *Metacyclops*; however, this cyclopoid also possesses other characteristics not attributable to the above genus; the definition of this taxon will require further study. Remarkably, *Speocyclops* Kiefer 1938 was so far the only genus of cyclopoid recorded for Sicilian caves (with one species, *Speocyclops italicus* Kiefer 1938 reported in Cottarelli *et al.* 2012); the collection of *Metacyclops* sp. together with *Stammericaris similior* **sp. nov.** from pool 1, certainly represents an interesting faunistic and biogeographic novelty.

Diacyclops crassicaudis lagrecai Pesce & Galassi 1987. This subspecies was so far reported only from a well in the province of Palermo and described only with females (Pesce & Galassi 1987); we collected 31 specimens (from pool 11) which will allow redescribing the species.

CONCLUSIONS

Overall, albeit the great morphological similarity of *Stammericaris similior* **sp. nov.** and *S. destillans*, the new species can be characterized by the two apomorphies described for the male P4, which, coupled with the molecular analysis, allow to attribute to the population of Scrivilleri Cave the status of new species., which further increases the faunistic and biogeographical interest of the Sicilian stygobitic fauna.

In conclusion, we would like to reiterate that the significance of the present paper lies in the attempt to broaden and develop the knowledge needed for a better understanding of the biodiversity of the stiygobitic fauna of the epikarst and their habitat. These taxa, which are endemic and often with a very limited distribution range, are particularly sensitive and highly susceptible to disturbance events such as habitat alterations and/or ecological imbalances, occurring both on a large scale (e.g., the climate crisis heavily evident in Sicily), as well as on a smaller, but no less damaging, local scale. Specifically, since they are so threatened, these sensitive organisms should be carefully protected; in this regard, in agreement with Nitzu *et al.* (2018), endemic and rare stygobitic cave species can be used as bioindicators of habitat vulnerability, with the aim of developing protection and management plans for biotope prioritization. In this way these “insignificant” organisms might gain a new importance; although invisible to the public, they could contribute greatly to the better protection of the fascinating and complex cave habitat.

TAXONOMIC KEY FOR THE IDENTIFICATION OF MALES OF THE KNOW SPECIES OF *STAMMERICARIS*

(Note: the measurements of the P4 endopod length refer to the longest protrusion, which is usually the middle pinnate or lobate one, numbered 3 in Figures 7D, 7E). * species description and illustrations not consistent with the current morphological details requirements.

1 P4 endopod similar to the one in figure 7D (i.e., endopod represented by a cylindrical element distally enlarged in three pointed protrusions (marked 2, 3, 4 in figure 7D) 2
- P4 endopod similar to the one in figure 7E (i.e., P4 endopod represented by a basal plate distally enlarged into an inner pointed protrusion ending in a fused spinule (marked as 2 in Figure 7E); a second spinule is inserted at half of the outer margin (marked as 4 in Figure 7E); the endopod extends on the distal outer corner into a long bipinnate process (fused seta, marked as 3 in Figure 7E) 3
2 Caudal rami sub-cylindrical, slightly shorter than last abdominal somite. P1 basis with hook on inner margin; P3 exp-1 with longitudinal row of two spinules proximally on outer margin; P4 basis with two spiniform processes of different size on inner margin, the one closest to the endopod is the largest; P4 endopod represented by a small cylindrical segment, as long as ½ of the corresponding exp-1, with three small apical protrusions . . . . *S. destillans*
- Caudal rami sub-cylindrical, shorter than last abdominal somite. P1 basis with hook and spinule on inner margin; P3 exp-1 with longitudinal row of three spinules proximally on outer margin; P4 basis with two spiniform processes of different size on inner margin, the one closest to the endopod is the smallest; P4 endopod represented by a small cylindrical segment, apically bent at 90°, slightly longer than ½ of the corresponding exp-1, with three small apical protrusions . . . . *S. similior* **sp. nov.**
3 P3 exp-1 without longitudinal rows of spinules on outer margin . . . . *S. trinacriae*
- P3 exp-1 with longitudinal rows of spinules on outer margin 4
4 Caudal rami with pointed dorsal distal apophysis 5
- Caudal rami without pointed dorsal distal apophysis 8
5 Caudal rami sub-cylindrical, shorter than last abdominal somite. P1 basis with one spinule on inner margin . . . . . *S. lorenzae*
- Caudal rami sub-cylindrical, shorter than last abdominal somite. P1 basis with one spinule and one hook on inner margin . . .6

6 P3 exp-1 with proximal row of two stronger, and distal row of five thinner, longitudinal spinules on outer margin; enp P4 slightly shorter than the first two segments of the corresponding exp, the inwardly curved inner tip with lateral pinnate expansion . . . _S. pasquinii_

\- P3 exp-1 with proximal row of two and distal row of four longitudinal spinules on outer margin; enp P4 as long as or slightly longer than the corresponding exp-1, inwardly-curved inner tip apically bilobate . . . 7

7 P1 basis with spinule and longer hook on inner margin; P3 exp-1 with proximal row of two, distal row of four longitudinal spinules, all of them of similar size, on outer margin; P4 basis with three processes with blunt tip on inner margin, the medial-most much larger than the other ones . . . _S. diversitatis_

\- P1 basis with spinule and hook of similar length on inner margin; P3 exp-1 with proximal row of two spinules, and distal row of two larger and two smaller spinules (almost divided in two groups), on outer margin; P4 basis with four spinules on inner margin, the distalmost from the endopod is transversally inserted, the remaining ones decrease in size from inner to outer . . . _S. vincentimariae_

8 Caudal rami cylindrical and narrow, not tapering, longer than the last abdominal somite; enp P4 slightly longer than the corresponding exp-1, with only inner tip and apical pinnate outgrowth (proximal spiniform outgrowth missing) . . . . _S. orcina_

\- Caudal rami sub-cylindrical and narrow, tapering distally, shorter than the last abdominal somite; enp P4 of different shape . 9

9 P4 basis, inner margin with three spinules increasing in length from outer to inner one. . . . 10

\- P4 basis with only one spinule on the inner margin . . . 12

10 Exp-2 apophysis much longer than thumb . . . *_S. phreatica_

\- Exp-2 apophysis slightly longer than thumb . . . 11

11 P3 exp-1, outer margin with proximal longitudinal row of two spinules and distal longitudinal row of six spinules, all of same length; enp P4 longer than the corresponding exp-1 . . . *_S. stammeri_

\- P3 exp-1, outer margin with proximal and distal longitudinal row of two spinules all of same length; enp P4 as long as the corresponding exp-1 . . . _S. amyclaea_

12 P3 exp-1, outer margin with proximal row of three spinules and distal row of four spinules; P4 basis with one thin and straight spinule on the inner margin . . . *_S. acherusia_

\- P3 exp-1, outer margin with two longitudinal rows of spinules, both inserted in the proximal half of the outer margin; P4 basis with one large, inwardly-curved spinule on the inner margin . . . 13

13 Urosomites not pitted. P3 exp-1, outer margin with proximal and distal longitudinal row of two spinules; enp P4 represented by plate curved inwards in an almost L-shape, with strongly bifid tip; P4 exp-1 characteristically enlarged and strongly bent inwards . . . _S. remotaepatriae_

\- Urosomites pitted (character present only in this species); P3 exp-1, outer margin with proximal row of three or four spinules and distal row of four spinules; P4 endopod of different shape; P4 slightly enlarged at base and straight . . . . _S. palmerae_

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