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Research article

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Niphargus dancaui sp. nov. (Amphipoda, Niphargidae) – a new species thriving in sulfidic groundwaters in southeastern Romania

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Abstract. *Niphargus dancaui* sp. nov., previously referred to as *Niphargus* cf. *stygius*, was sampled from various groundwater sites in and near the town of Mangalia (SE Romania) and described with Movile Cave (a sulfidic, chemoautotrophically based ecosystem) as type locality. A short comparison with *Niphargus stygius* specimens from Slovenia was made, together with a morphological analysis of interpopulational variability. Males of *N. dancaui* sp. nov. were relatively large (17 mm), with long antennae, pereiopods and uropod III. Females were slightly smaller, with shorter antennae, pereiopods and uropod III. Interpopulational variability was noticed in the chaetotaxy of the telson lobes and uropod III. *N. dancaui* sp. nov. is morphologically very close to *N. lessiniensis* and *N. tridentinus*, two species present in northern Italy, but distinct genetically from them based on 28S rRNA sequences. Instead, the closest relative of *N. dancaui* sp. nov. sequenced so far for this marker is *N. montanarius*, which inhabits a sulfidic cave system in central Italy. The work presented here contributes to our knowledge of groundwater crustacean biodiversity in general and of the systematics of the genus *Niphargus* in particular.

Keywords. 28S phylogeny, hypogenic cave, interpopulational diversity, Movile Cave.

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Introduction

The amphipod genus *Niphargus* Schiödte, 1849 is widely distributed in the western Palearctic and represents the most speciose genus of freshwater amphipods in the world (Väinölä *et al.* 2008). This genus is taxonomically demanding and its species richness is far from being completely described. The morphology of niphargids is not a very good proxy for the phylogenetic relationships among these species (Fišer *et al.* 2008; Trontelj *et al.* 2012; Flot *et al.* 2014), and cryptic species (i.e., species that are nearly indistinguishable from a morphological point of view but possess distinctive DNA sequences) appear common in this group (Trontelj *et al.* 2009).

Most *Niphargus* species are confined to subterranean waters (Fišer 2012), where they represent the dominant group of macroinvertebrates. *Niphargus* thrives in all types of subterranean waters, from shallow hypotelminorheic ones (i.e., shallow seeps filled with groundwater; Fišer *et al.* 2010) to epikarst (Sket 1981), sinking streams and phreatic lakes (Trontelj *et al.* 2012). Niphargid amphipods have also been noted for their dominant presence in hypogenic caves, i.e., caves that result from limestone dissolution by hydrogen sulfide arising from deep reservoirs (Latella *et al.* 1999; Sarbu *et al.* 2000; Forti *et al.* 2002; Flot *et al.* 2010, 2014). These sulfidic caves are of particular interest from an ecological viewpoint: instead of sunlight (photoautotrophy) or allochthonous organic material (organoheterotrophy), they derive their energy from inorganic chemical compounds (chemoautotrophy; Sarbu *et al.* 1996, 2000). Although the fauna of these unique ecosystems should be highly protected, not all *Niphargus* species present in hypogenic caves have been described. As incomplete taxonomy is a major impediment to conservation biology, the present study aims to contribute to bridging this gap.

The two best-studied hypogenic cave ecosystems in Europe are the Frasassi cave system in Italy and Movile Cave in Romania. The niphargid fauna of Frasassi was recently studied molecularly, revealing the presence of four species (Flot *et al.* 2010; Karaman *et al.* 2010). A similar molecular study conducted in Romania revealed the presence of seven niphargid species in and around Movile Cave: *Niphargus* cf. *stygius* (Schiödte, 1847), *N. decui* Karaman & Sarbu, 1995, *N. dobrogicus* Dancău, 1964, *N. gallicus* Schellenberg, 1935, *N. hrabei* Karaman, 1932, *Pontoniphargus racovitzai* Dancău, 1970, and *P. ruffoi* Karaman & Sarbu, 1993 (Flot *et al.* 2014; Fišer *et al.* 2015). The sulfidic waters of Movile Cave are host to two of these species. One of them, *P. racovitzai*, was originally described as belonging to the distinct genus *Pontoniphargus* Dancău, 1970, although molecular analyses show it clearly nested within the genus *Niphargus* (Flot *et al.* 2014). The second species was called *Niphargus* cf. *stygius* by Dan Dancău in the 1980s (in an unpublished determination) as it presented morphological similarities with *Niphargus stygius* (Schiödte, 1847). Sarbu & Popa (1992) considered it as a putative new species, and a recent molecular analysis provided additional support for this hypothesis (Flot *et al.* 2014). However, this species was never named nor properly described.

Material and methods

Morphological analysis

Niphargus cf. *stygius* specimens were sampled from existing wells in the town of Mangalia (SE Romania), from Movile Cave and from a spring in Hagieni Forest (Table 2, Fig. 1). In Mangalia, we either descended in the wells and picked specimens directly from the walls; pulled and dragged a planktonic net through the mass of water in the well; or examined large amounts of water (typically 100-150 liters) brought up to the surface using a rope and a bucket; in Movile Cave, we picked specimens directly from the edges of the cave lake; in Hagieni Forest spring, we collected them under the rocks and in detritus. Niphargids were immediately transferred to 70% or 96% ethanol to allow for both morphological and genetic analyses. Morphological inspection, drawings and measurements were performed using an MBS-1 stereo microscope (Lytkarino, USSR) and a Carl Zeiss microscope (Jena, Germany). The terminology used for body parts and the choice of appendages taken into consideration for measurements were as in

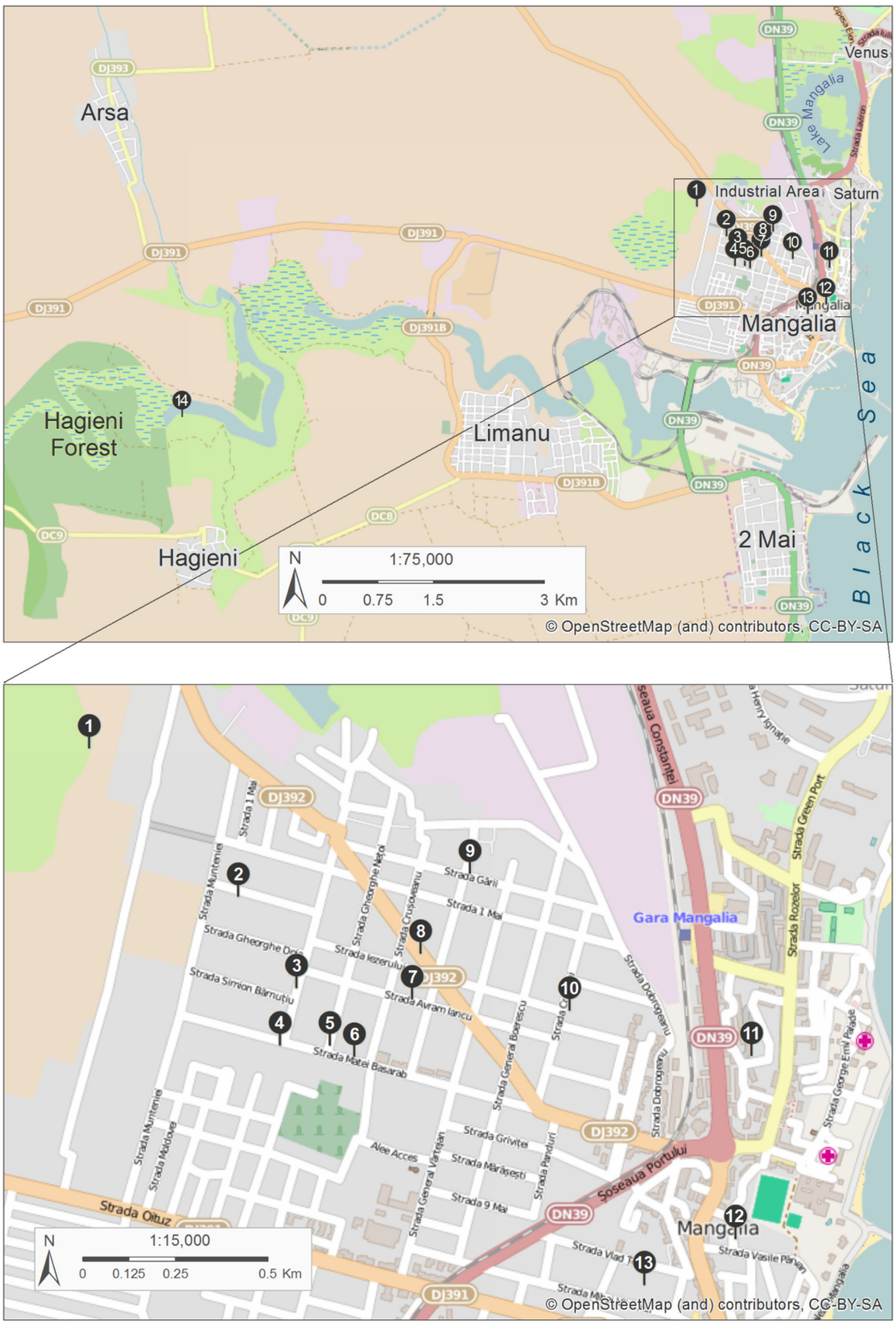

Fig. 1. Map of the research area with indication of sampling locations

Fišer *et al.* (2009). True spines, i.e., extrusions of cuticle, are not known in *Niphargus* (Fišer *et al.* 2009). Species of this genus have appendages armed with flexible thin setae, flexible plumose setae and stout spiniform setae. To simplify descriptions, we refer to the thin flexible setae as 'setae' and stout spiniform setae as 'spines' as in Švara *et al.* 2015.

Molecular analyses

To shed light on the relationships of *Niphargus* cf. *stygius* with other species of the genus *Niphargus*, all nuclear ribosomal 28S sequences of members of the Niphargidae available on 28 Jul. 2015 were downloaded from GenBank (Benson *et al.* 2015). This dataset of 494 sequences was then completed with unpublished 28S sequences of topotypes of *N. montellianus* Stoch, 1998 and *N. tridentinus* Stoch, 1998 (sequenced using the same protocol as in Flot *et al.* 2010; GenBank accession numbers KT878856 and KT878857) and pre-aligned in MAFFT using the FFT-NS-i mode to identify unsuitable sequences (originating from a different 28S region than our target region). After filtering out these sequences, the remaining dataset of 422 sequences from Lefébure *et al*. (2006, 2007), Fišer *et al*. (2008), Trontelj *et al*. (2009), Flot (2010), Flot *et al*. (2010, 2014), Hartke *et al*. (2011), Trontelj *et al*. (2012), Hekmatara *et al*. (2013), Fišer *et al*. (2013), McInerney *et al*. (2014), Altermatt *et al*. (2014), Ntakis *et al*. (2015) and Esmaeili-Rineh *et al*. (2015) were screened for perfect duplicates using FaBox (Villesen 2007), resulting in a dataset a 260 unique sequences, to which two outgroup sequences from the genera *Synurella* Wrzesniowski, 1877 and *Gammarus* Fabricius, 1775 were added as in Flot *et al*. (2014). The sequences were aligned in MAFFT version 7 (Katoh & Standley 2013) using the E-INS-i option and analyzed in FastTree 2 (Price *et al*. 2010) using the GTR model (Lanave *et al*. 1984; Tavaré 1986) with 1000 bootstrap replicates (Felsenstein 1985). The resulting Newick tree was turned into PDF using MEGA6 (Tamura *et al*. 2013), then beautified using Inkscape (Bah 2011).

The holotype and paratypes are deposited in the collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana.

Results

Order Amphipoda Latreille, 1816
Family Niphargidae Bousfield, 1977
Genus ***Niphargus*** Schiödte, 1849

Niphargus dancaui sp. nov.
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Figs 1–15

Niphargus cf. *stygius* – Sarbu & Popa 1992: 651.

Diagnosis

Mid to large-sized *Niphargus* of robust appearance, with acute to right postero-ventral angle of epimeral plates. Pleon with at most 4 setae along the posterior margin of each segment and a single tiny seta in postero-lateral position on urosomite I. Antenna I shorter than half of the total body length. The outer lobe of maxilla I has 7 spines with 1–3 teeth. The inner lobe of maxilliped has 9 spines. The propodus of both gnathopods are almost square-shaped, with 5 setae along the outer margins of the dactyli. The pereopods are shorter than half of the total body length, with one spine at the nail base. The uropods III are sexually dimorphic and elongated in males. The male telson bears 3 apical spines, 1 lateral and 1 dorsal spine on each lobe, plus 1 subapical spine per lobe for the female telson.

Etymology

The specific name is derived from the name of the late Dan Dancău (1933–1994), who first studied the amphipod fauna in the Dobrogea region and described some of its species, notably *Pontoniphargus racovitzai* from Doi Mai and Mangalia (Dancău 1970) and *Niphargus dobrogicus* from Doi Mai, Schitu and Vama Veche (Dancău 1964).

Material examined

Holotype

ROMANIA: ♂, Movile Cave, Mangalia (Fig. 1, Table 2). The holotype specimen collected from location 1 in Fig. 1 was not dissected, but was deposited intact in 75% ethanol.

Paratypes

ROMANIA: Mangalia, 1 ♂, 3 ♀♀, well 12, Aleea Cetăţii 1; 1 ♂, well 7, str. Avram Iancu 26; 1 ♀, well 10, str. Crinului 34; 1 ♀, well 3, str. General Dragalina; 1♂, 1 ♀, well 8, str. Horia, Cloşca şi Crişan 13.

Remark

The description was performed and species variability was examined on the basis of several paratypes collected from various hand-dug wells in the town of Mangalia (SE Romania) (Fig. 1, Table 2). The male and female described here were sampled from a well on Aleea Cetăţii 1. The other specimens were included in order to examine the variability.

Description (male)

The total male body length is 17.4 mm (Fig. 2A). A detailed chart containing the measurements of all diagnosis-relevant body appendages for both male and female is presented in Table 1.

Head

The head (Fig. 3A) represents 8.5 % of total body length, no rostrum was observed.

Antennae

Antenna I (Fig. 3B) almost half of total body length (Table 1), with a flagellum formed of 32 articles. Most flagellum articles bear one short aesthetasc (Fig. 3C). Length of peduncle slightly more than one third of the total length of antenna I. Accessory flagellum (Fig. 3D) biarticulated, the proximal article exceeds half of second article of main flagellum, distal article is approximately one fifth of total length of accessory flagellum. Antenna II (Fig. 3E) with flagellum formed of 13 articles, half as long as antenna I. Peduncle almost twice as long as flagellum.

Mouthparts

Labium (Fig. 4A) bilobate; length of inner lobes half length of outer lobes. Both inner and outer lobes with distally fine setae. Labium displayed in Fig. 4A from female sampled from str. Horia, Cloşca şi Crişan 13; identical to all inspected labia from other specimens, males and females.

Left mandible (Fig. 4B) with five teeth on incisor process, four teeth on lacinia mobilis and a row of eight serrate spines between lacinia mobilis and molar process (Fig. 4C).

Right mandible (Fig. 4D) with four teeth on incisor process, several small denticles on lacinia mobilis and a row of five denticulate setae between lacinia mobilis and molar process (Fig. 4E).

Two mandibular palps (Fig. 4B and Fig. 4D), highly similar and of same length. The three articles represent 21% (article 1), 37% (article 2) and 42% (article 3) of total palp length (Table 1). Proximal article without setae, article 2 with 8–11 ventral setae and article 3 with one group of 5–6 A setae, three groups of 3–4 B setae, approximately 36 D setae and 5 E setae (Fišer *et al.* 2009).

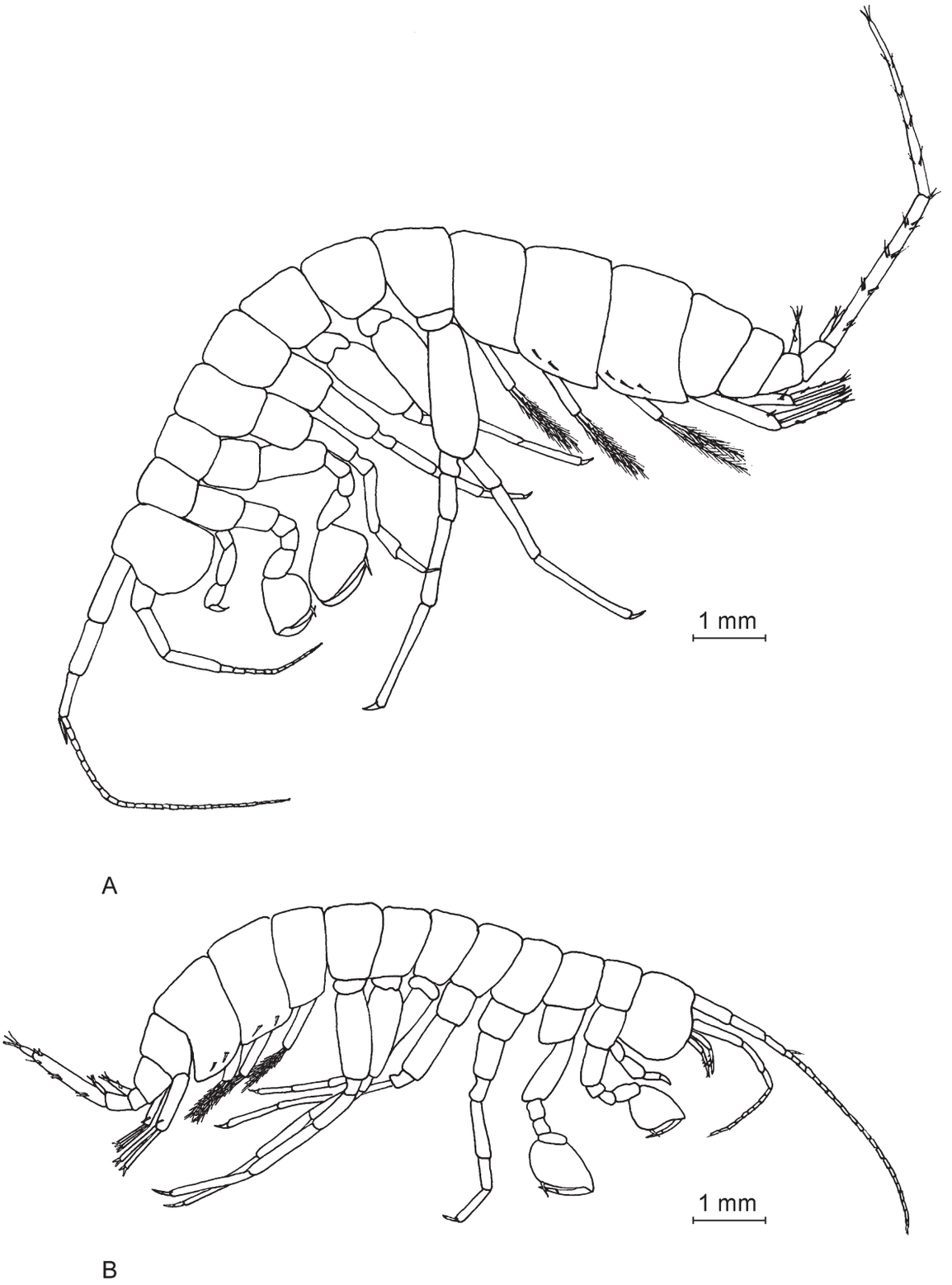


Fig. 2. General appearance of *Niphargus dancaui* sp. nov. **A**. ♂. **B**. ♀.

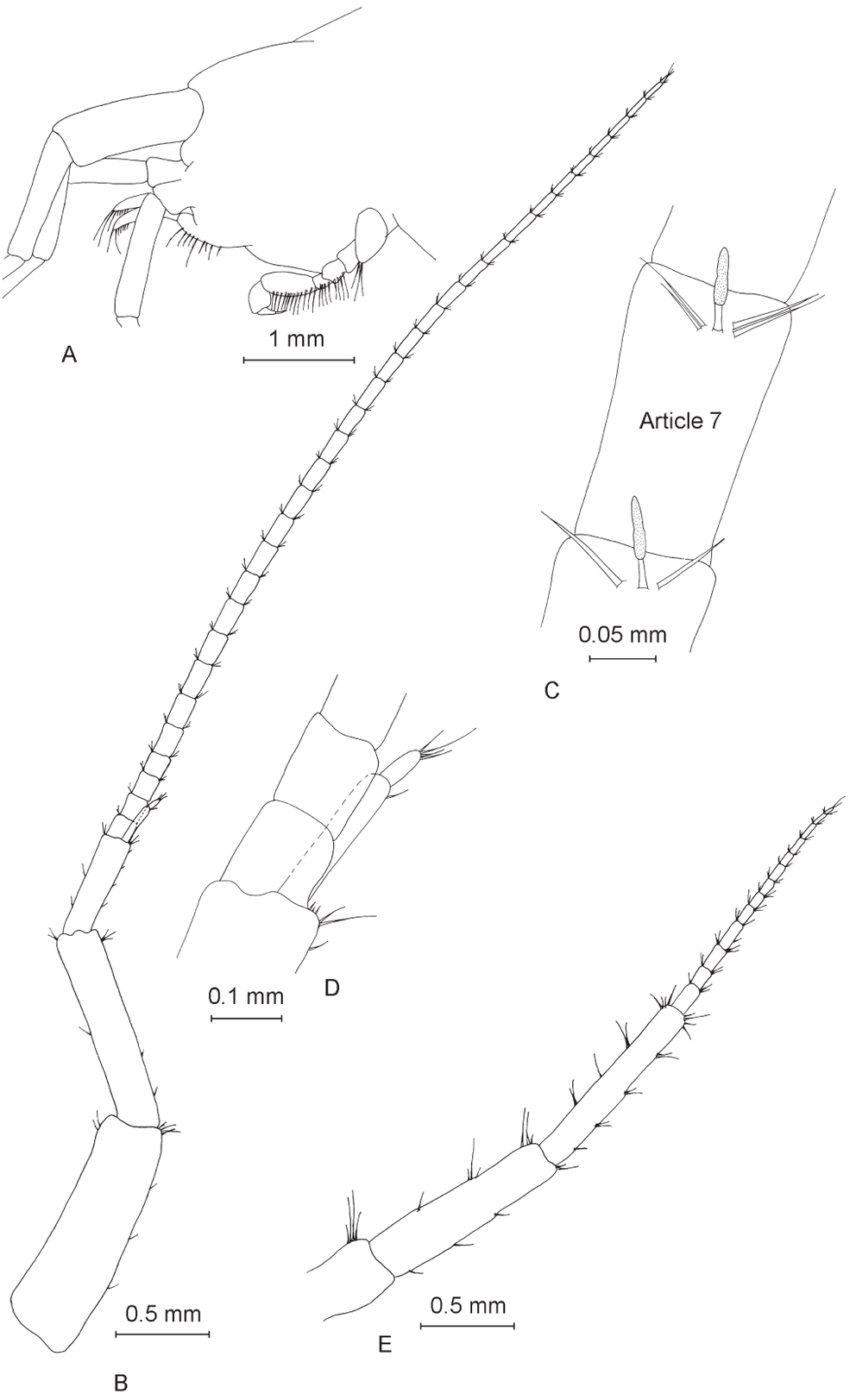


Fig. 3. ♂. **A**. Head. **B**. Antenna I. **C**. Aesthetascs of antenna I. **D**. Accessory flagellum of antenna I. **E**. Antenna II.

Table 1. Measurements in mm of the various appendages of male and female *Niphargus dancaui* sp. nov. sampled from a well located on Aleea Cetăţii 1 in the town of Mangalia (SE Romania).

Appendage		Male	Female	Appendage		Male	Female
Body length		17.4	14.3	PIII	Coxal plate depth	1.4	1.3
Head length		1.5	1.4		Coxal plate width	1.0	1.0
Antenna I	Peduncle length	3.1	2.3		Basis	2.0	1.7
	Flagellum length	5.2	3.4		Ischium	0.3	0.2
	Art 1 Fl. acc.	0.2	0.2		Merus	1.2	1.0
Antenna II	Peduncle length	2.6	2.0		Carpus	0.7	0.6
	Flagellum length	1.3	1.1		Propodus	1.0	0.7
Mandible palp	Art 1	0.3	0.2		Dactylus 1	0.2	0.2
	Art 2	0.5	0.4		Dactylus 2	0.2	0.1
	Art 3	0.6	0.4	PIV	Coxal plate depth	1.4	1.3
Gnathopod I	Propodus length	1.2	0.9		Coxal plate width	1.4	1.0
	Propodus width	1.0	0.8		Basis	2.0	1.7
	Propodus depth	0.9	0.8		Ischium	0.3	0.3
	Dactylus 1	0.9	0.6		Merus	1.0	0.8
	Dactylus 2	0.3	0.3		Carpus	0.8	0.6
	Carpus length	0.8	0.5		Propodus	0.9	0.7
	Basis width	0.6	0.5		Dactylus 1	0.2	0.2
	Basis length	1.4	1.1		Dactylus 2	0.2	0.1
	Coxal plate depth	1.3	1.1	PV	Basis length	1.7	1.5
	Coxal plate width	0.8	0.7		Basis width	1. 3	1.0
Gnathopod II	Propodus length	1.6	1.2		Ischium	0.3	0.3
	Propodus width	1.5	1.2		Merus	1.0	0.9
	Propodus depth	1.2	1.0		Carpus	1.2	1.0
	Dactylus 1	1.3	1.0		Propodus	1.3	1.0
	Dactylus 2	0.4	0.3		Dactylus 1	0.2	0.1
	Carpus length	1.0	0.8		Dactylus 2	0.1	0.1
	Basis width	0.6	0.5	PVI	Basis length	1.9	1.7
	Basis length	1.9	1.7		Basis width	1.3	1.1
	Coxal plate depth	1.3	1.0		Ischium	0.3	0.3
	Coxal plate width	1.1	0.9		Merus	1.3	1.1
Uropod I	Basis length	1.4	1.3		Carpus	1.7	1.5
	Endopodite length	1.1	1.0		Propodus	2.1	1.8
	Exopodite length	1.1	1.0		Dactylus 1	0.3	0.2
Uropod II	Basis length	0.8	0.6		Dactylus 2	0.2	0.1
	Endopodite length	0.7	0.7	PVII	Basis length	2.0	1.6
	Exopodite length	0.8	0.6		Basis width	1.3	1.1
Uropod III	Basis length	0.9	0.5		Ischium	0.3	0.4
	Endopodite length	0.5	0.3		Merus	1.2	1.0
	Exopodite art 1	3.4	2.1		Carpus	1.7	1.4
	Exopodite art 2	3.2	0.3		Propodus	2.4	2.0
Telson	Cleft length	0.5	0.4		Dactylus 1	0.4	0.2
	Telson width	0.6	0.5		Dactylus 2	0.2	0.1
	Telson length	0.8	0.7				
	Longest spine length	0.2	0.2				

Table 2. List of sampling locations with their geographic position and physico-chemical characteristics. The numerals in the first column relate to the location numbers in Fig. 1. The specimens dissected and analyzed for this study were selected from the locations highlighted in grey.

Nr.	Location	Latitude N	Longitude E	pH	T°C	EC µS cm^{-1}	H_2S mg/l
1	Movile Cave	43°49'36"	28°33'43"	7.4	21.2	1071	8.3
2	Mangalia, str. Dumitru Ana 13	43°49'23"	28°34'01"	7.3	19.1	1052	3.4
3	Mangalia, str. General Dragalina 10	43°49'15"	28°34'08"				0
4	Mangalia, str. Matei Basarab 74	43°49'10"	28°34'06"	7.4	19.5	1550	12.2
5	Mangalia, str. Gheorghe Netoi 1	43°49'10"	28°34'12"	7.4	18.6	1078	4.5
6	Mangalia, str. Matei Basarab 62	43°49'09"	28°34'15"				0
7	Mangalia, str. Avram Iancu 26	43°49'14"	28°34'22"	7.5	19.6	1540	6.4
8	Mangalia, str. Horia, Cloşca şi Crişan 13	43°49'18"	28°34'23"	7.7	18.7	1870	0
9	Mangalia, str. Ion Mecu 51	43°49'25"	28°34'29"	7.68	19.9	1135	2.2
10	Mangalia, str. Crinului 34	43°49'13"	28°34'41"	7.43	20.2	1490	0
11	Mangalia, str. Pictor Tonitza 1	43°49'09"	28°35'03"	7.27	19.0	1242	0
12	Mangalia, Aleea Cetăţii 1	43°48'53"	28°35'01"	7.42	19.3	1650	0
13	Mangalia, str. Mihai Viteazul 20	43°48'49"	28°34'50"	7.3	19.5	1770	0
14	Hagieni Spring	43°48'08"	28°28'29"	7.6	10.5	905	5.2

Maxilla I (Fig. 4F) with 7 apical setae on distal palp article. Outer lobe with 7 spines with 1–3 teeth, inner lobe with 3 apical setae.

Maxilla II (Fig. 4G) with inner lobe slightly shorter than outer lobe; both lobes with numerous apical setae.

Maxilliped (Fig. 4H) with palp formed of four articles. Article 2 with numerous setae in approximately 10 groups aligned along inner margin. Article 3 with three groups of 4–5 setae on inner margin, one group of 3 setae on dorsal margin and one apical group with 5 setae. Article 4 without setae. Outer lobe of maxilliped with 6 shorter, flattened spines and 7 longer, slightly hairy, apical spines. Inner lobe with 9 setae-like spines.

Gnathopod I

Gnathopod I (Fig. 5A) with relatively ovoid coxal plate with depth greater than its width (ratio depth:width 1.0:0.6). Basis length:width ratio 1.0:0.4. Ischium with one posteroventral group of 4 setae. Basis length:carpus length 1.0:0.6. Carpus with two groups of 8–10 setae on ventral margin, and one group of 4 setae located anterodorsally. Length:width ratio of propodus 1.0:0.8. Propodus with 7 groups of 3–4 setae on ventral margin, one anterodorsal group with 6 setae and one antero-apical group of 4 setae. Two groups with 2–3 setae on lateral surface of propodus close to its ventral side,and two groups of 4–5 setae closer to propodus dorsal margin. One group of 3 long setae present close to palmar spine. Strong palmar spine and 4 outer denticulate spines in palmar corner. Dactylus (Fig. 5B) strong, with claw representing one quarter of total dactylus length and with five setae along outer margin.

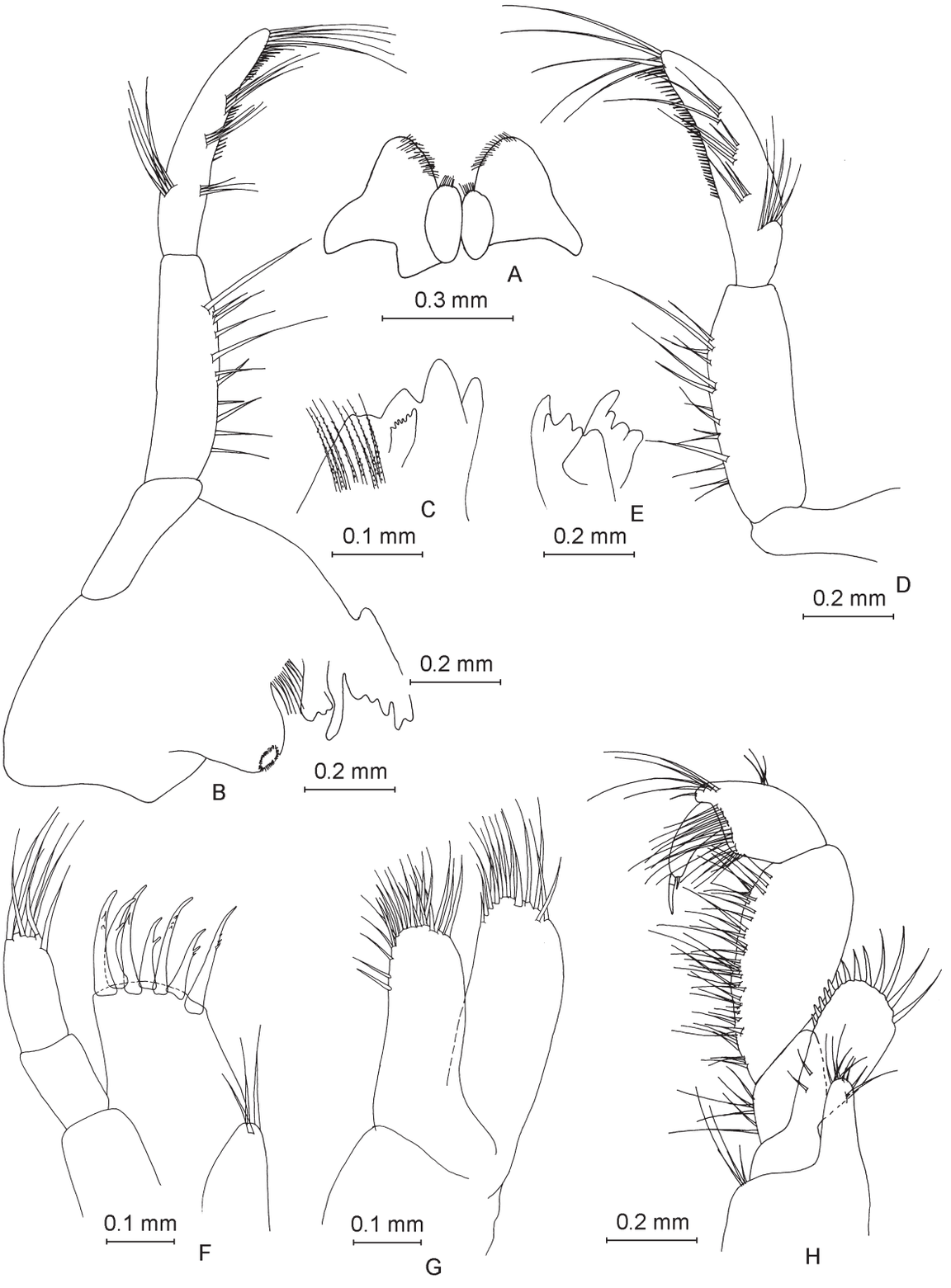


Fig. 4. ♂, mouthparts. **A**. Labium. **B**. Left mandible. **C**. Detail (the incisor process and lacinia mobilis) of left mandible. **D**. Right mandible. **E**. Detail (the incisor process and lacinia mobilis) of right mandible. **F**. Maxilla I. **G**. Maxilla II. **H**. Maxilliped.

Gnathopod II

Coxal plate (Fig. 5C) with rectangular shape, deeper than wide (ratio depth:width 1.0:0.8). Basis length:width ratio 1.0:0.3. Ischium with one anteroventral group of 2 setae. Basis length:carpus length 1.0:0.6. Carpus has with two groups of 8–10 setae on ventral margin and one group of 2 setae located anterodorsally. Propodus almost square-shaped, with length:width ratio of 1.0:0.96. Propodus with 7 groups of 2–4 setae on ventral margin, one anterodorsal group of 2 setae, and one apical group of 4 setae. Lateral surface of gnathopod II propodus without setae, with only two long setae close to palmar spine.

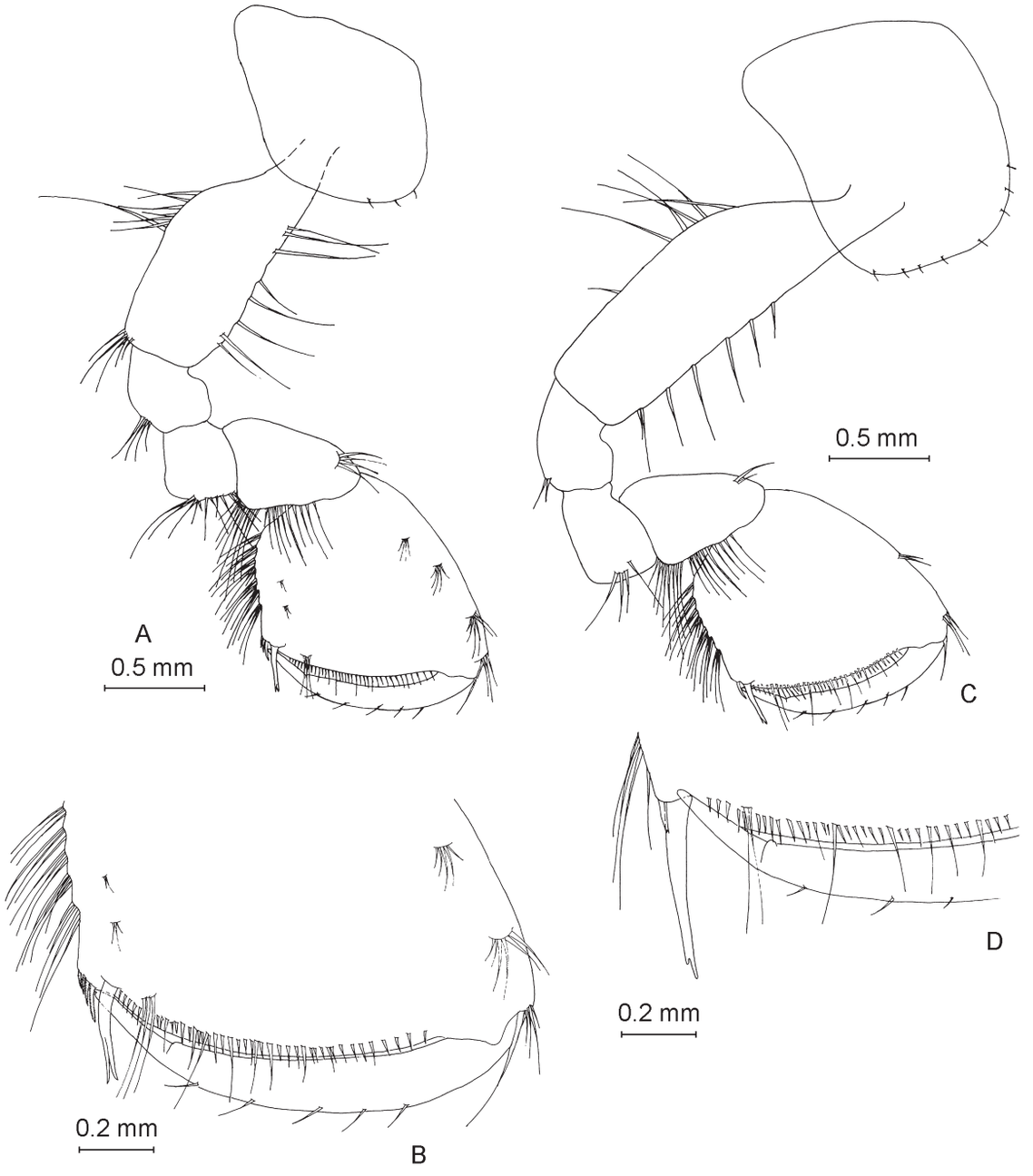


Fig. 5. ♂. **A**. Gnathopod I. **B**. Propodus of gnathopod I. **C**. Gnathopod II. **D**. Propodus of gnathopod II.

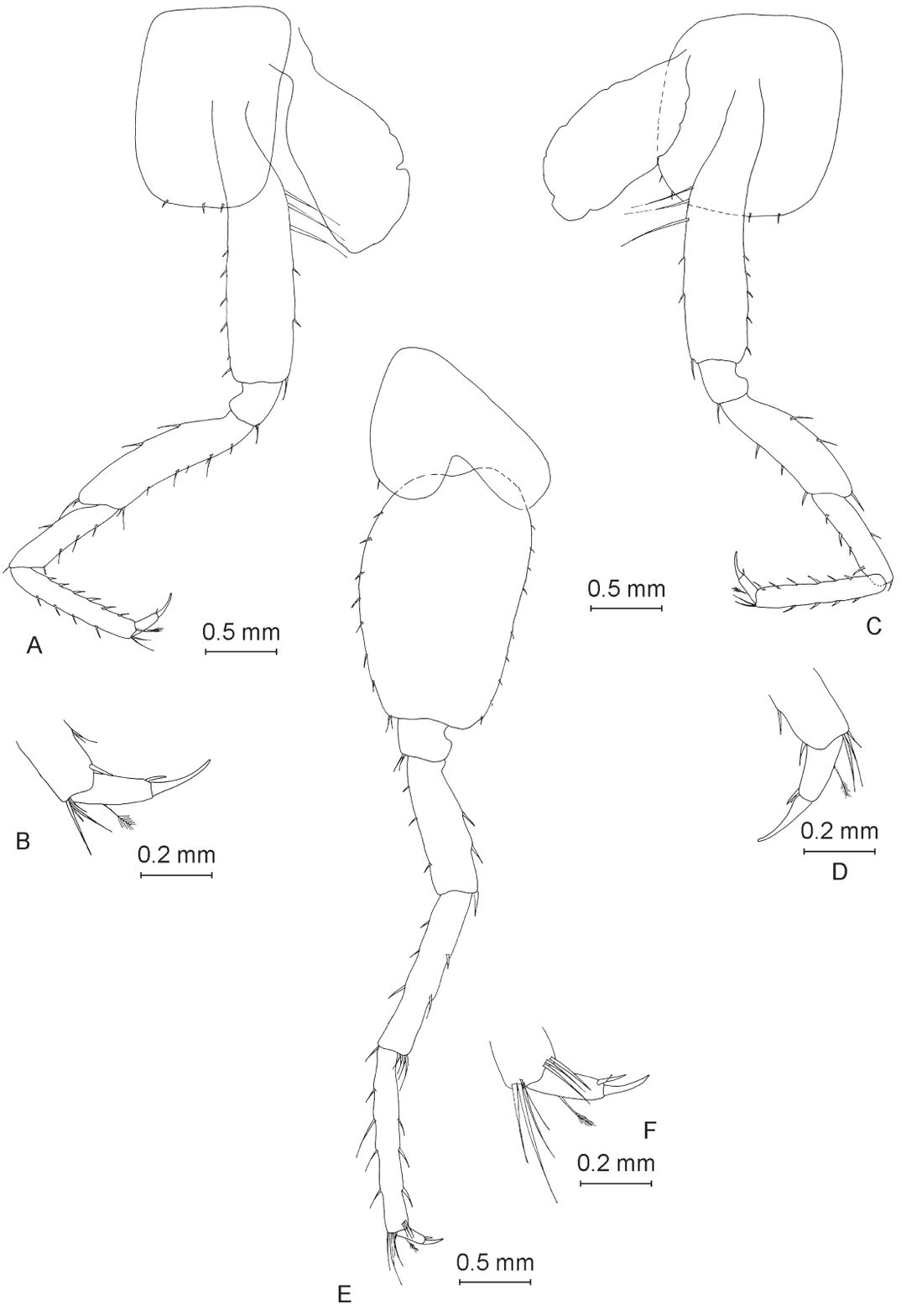


Fig. 6. ♂. **A**. Pereopod III. **B**. Dactylus of pereopod III. **C**. Pereopod IV. **D**. Dactylus of pereopod IV. **E**. Pereopod V. **F**. Dactylus of pereopod V.

One strong spine and one outer, smaller spine on palmar corner. Dactylus (Fig. 5D) strong, with claw representing one quarter of total dactylus length and with five setae along outer margin.

Pereopod III

Coxal plate of pereopod III (Fig. 6A) with rectangular shape, with depth:width ratio of 1.0:0.7. Posterior margin concave, with three setae. Gill irregularly ovoid. Dactylus (Fig. 6B) robust, with a nail measuring half of total dactylus length; with one dorsal seta with plumose tip and one spine at nail base. Propodus length:dactylus length ratio 1.0:0.34. Pereopod III nearly equal in length to pereopod IV (pereopod III length:pereopod IV length ratio 1.0:0.95).

Pereopod IV

Coxal plate of pereopod IV (Fig. 6C) almost square-like, depth:width ratio 1.0:0.96. Posterior margin concave, with four setae. Gill irregularly ovoid. Dactylus (Fig. 6D) robust, with nail slightly longer than half of total dactylus length; with one dorsal seta with plumose tip, one spine and one seta with plumose tip at nail base. Propodus length:dactylus length ratio 1.0:0.35.

Pereopod V

Coxal plate of pereopod V (Fig. 6E) shape of heart, with one small seta on anterior lobe. Basis with ovoid-trapezoidal shape, with length:width ratio of 1.0:0.68. Basis with 9 spine-like setae on anterior margin and 9 small setae on posterior margin. Dactylus (Fig. 6F) with one seta with plumose end on outer margin and one spine and one smaller seta with plumose end at base of nail. Nail represents 41% of total dactylus length.

Pereopod VI

Coxal plate of pereopod VI (Fig. 7A) highly similar to that of pereopod V. Basis with ovoid-trapezoidal shape, with length:width ratio of 1.0:0.68. Basis with 9 spine-like setae on anterior margin and 9 small setae on posterior margin. Dactylus (Fig. 7B) with one seta with plumose end on outer margin and one spine and one smaller seta with plumose end at base of nail. Nail represents 34% of total dactylus length.

Pereopod VII

The pereopod VII (Fig. 7C) is almost half of the total body length. The coxal plate pereopod VII is half-ovoid, with one small seta on its posterior margin. The basis has a ovoid-trapezoidal shape, with a ratio length:width of 1.0:0.66. The basis presents 6 spine-like setae on the anterior margin and 11 small setae on the posterior margin. The dactylus (Fig. 7D) has one seta with a plumose end on the outer margin, one spine and one smaller seta with a plumose end at the base of the nail. The nail represents 33% of the total dactylus length.

Pereopods V:VI:VII equal 1.0:1.35:1.40.

Pleopods

Pleopods I–III (Fig. 8A, Fig. 8B and Fig. 8C) highly similar, with rami of unequal length and 2 retinacles each.

Uropod I (Fig. 8D) with two dorsolateral spines onto peduncle. Length of endopodite equal to that of exopodite, segments with a low number of spines. One strong spine at base of uropod I.

Uropod II (Fig. 8E) with three dorsolateral spines onto peduncle. Exopodite slightly longer than endopodite, exopodite length:endopodite length ratio 1.0:0.88, both rami with a low number of spines.

Uropod III (Fig. 8F) long (43% of body length) and sexually differentiated. Protopodite with 4–5 small apical spines. Endopodite as long as protopodite with two apical setae. Proximal segment of exopodite

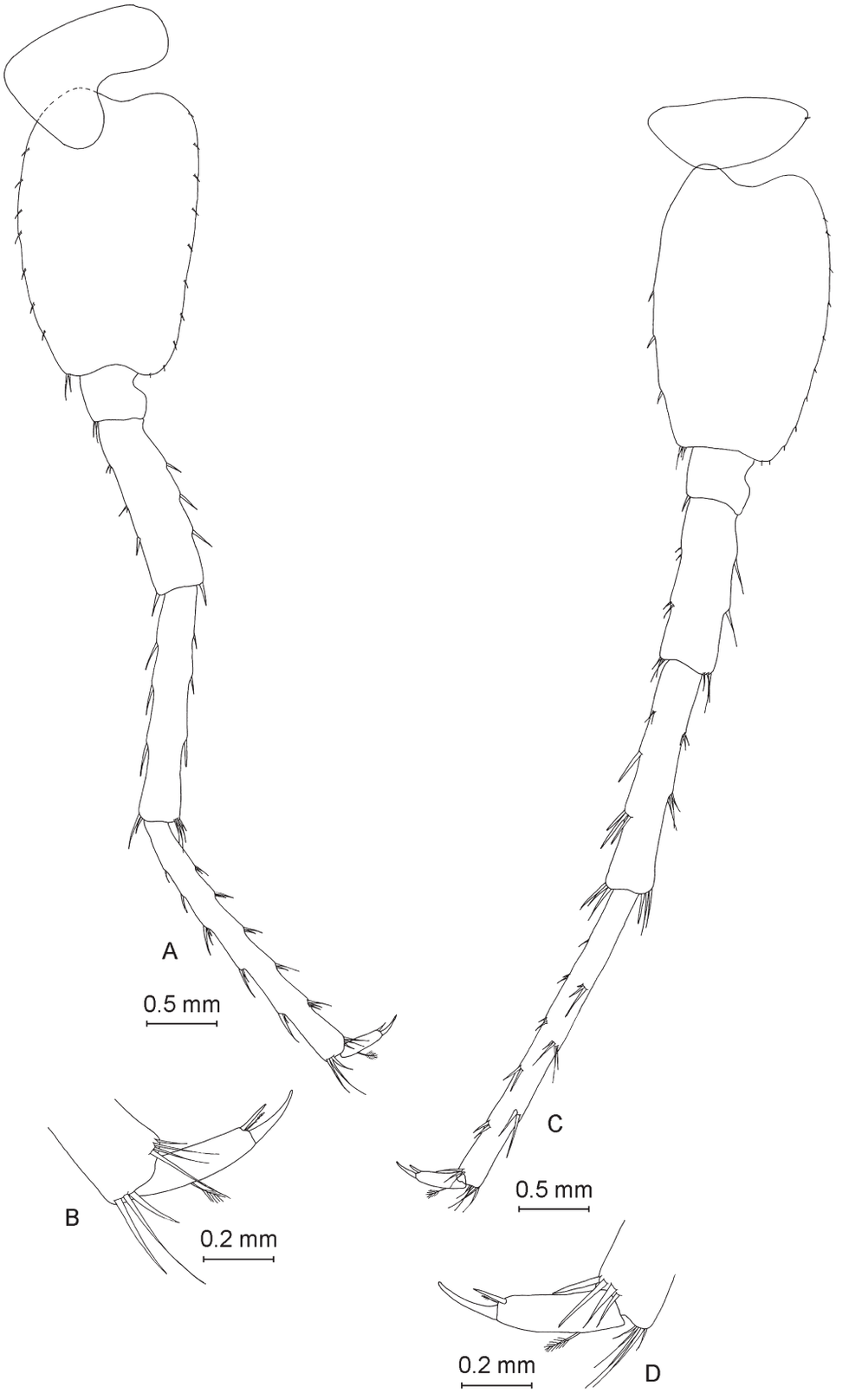


Fig. 7. ♂. **A**. Pereopod VI. **B**. Dactylus of pereopod VI. **C**. Pereopod VII. **D**. Dactylus of pereopod VII.

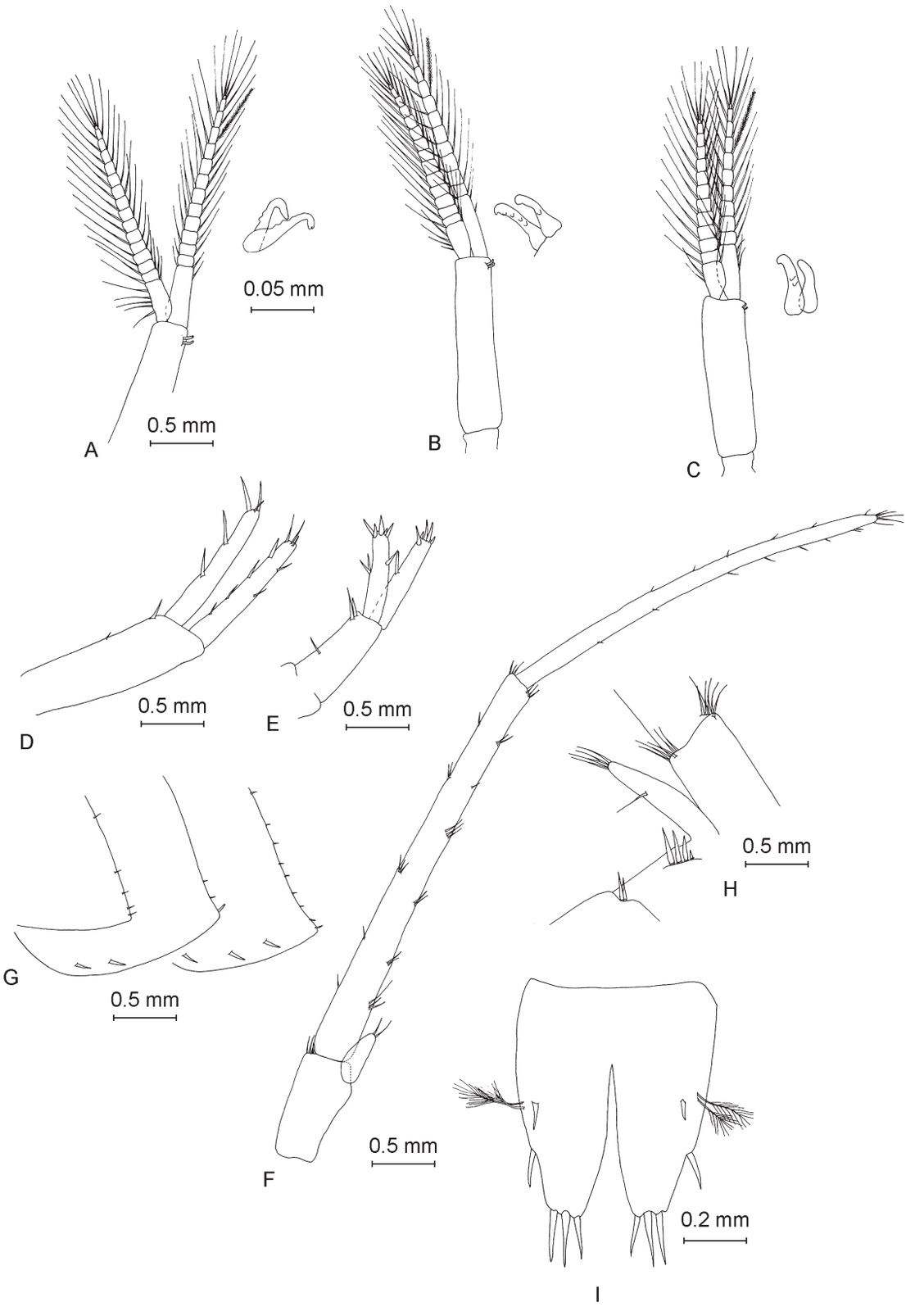


Fig. 8. ♂. — **A–C**. Pleopods with detail of retinacles **A**. Pleopod I. **B**. Pleopod II. **C**. Pleopod III. — **D**. Uropod I. **E**. Uropod II. **F**. Uropod III. **G**. Epimeral plates. **H**. Urosome of ♂ sampled from str. Horia, Cloşca şi Crişan13. **I**. Telson.

almost equal to distal segment (ratio 1.0:0.94). Proximal segment with seven groups of 1–3 spines on inner margin, and six groups with 1–3 spines on outer margin. Distal segment of exopodite with 8 spines on inner margin, 6 spines on outer margin and 5 apical setae.

Epimeral plates

Epimeral plate I (Fig. 8G) with acute postero-ventral angle, convex ventral margin with no spines and straight posterior margin with five setae.

Epimeral plate II (Fig. 8G) with right postero-ventral angle, straight posterior margin and convex ventral margin. Two spines present along ventral margin, one strong spine in postero-ventral angle and two shorter setae along posterior margin.

Epimeral plate III (Fig. 8G) slightly different compared to epimeral plate II; postero-ventral angle rather acute, posterior margin slightly concave, ventral margin convex. Three spines present along ventral margin and nine setae along posterior margin.

Urosomite I with two dorsolateral spines, whereas urosomite II with four dorsolateral spines of various lengths. Dorsal margin of urosomite III spineless (Fig. 8H). Urosome on Fig. 8H belonged to ♂ analyzed from str. Horia, Cloşca şi Crişan 13. Number of setae on urosomites identical on all inspected specimens.

Telson

Telson (Fig. 8I) slightly longer than wide (width:length ratio 1.0:1.2). Three apical spines relatively short, approximately one fifth of telson length. Telson with two fragile, plumose-ended setae along each side, as well as one lateral spine and one dorsal spine per lobe.

Sexual dimorphism

The female (Fig. 1B) is smaller (body length 14.3 mm) compared to the male. The female appendages (Figs 9–14) are highly similar to those of males, with a few exceptions. Antenna I, with 22 articles, reaches only one third of the total body length (Fig. 9B). Antenna II has just 9 articles (Fig. 9E). The female gnathopods I and II (Fig. 11) are similar to those of the male, except that the female propodus has a more inclined palmar margin, conferring it a rather trapezoidal shape in comparison to the more rectangular shape of the male propodus (Fig. 5). The female telson (Fig. 14H) is slightly different from the male one, with one sub-apical spine on each lobe. The uropod III (Fig. 14F) presents a distal segment of the exopodite shorter than that of the male. For the female, the proximal segment:distal segment ratio of the exopodite is 1.0:0.17, vs. 1.0:0.94 for the male.

Intraspecific variability

The intraspecific variability of *Niphargus dancaui* sp. nov. appears to be relatively low. The individuals sampled from various locations differed mainly in age-related size. The male and female described here were fully grown adults. These two specimens sampled from the well on Aleea Cetăţii 1 were larger and probably older than the other inspected specimens. However, their appendages, including their mouth parts, gnathopods, pereopods, pleopods and uropods, were largely similar with a few exceptions. The number of articles in the flagellum of antennae I and II appears variable, with numbers of articles ranging from 17 to 24 and from 9 to 11, respectively. Although the telson has always three apical spines and a pair of lateral spines its number of subapical and dorsal spines is variable (Fig. 14I–J). The shape of the epimeral plates is largely similar but the number of spines along their ventral margins ranges from 1 to 2 and from 1 to 3 in epimeral plates II and III, respectively.

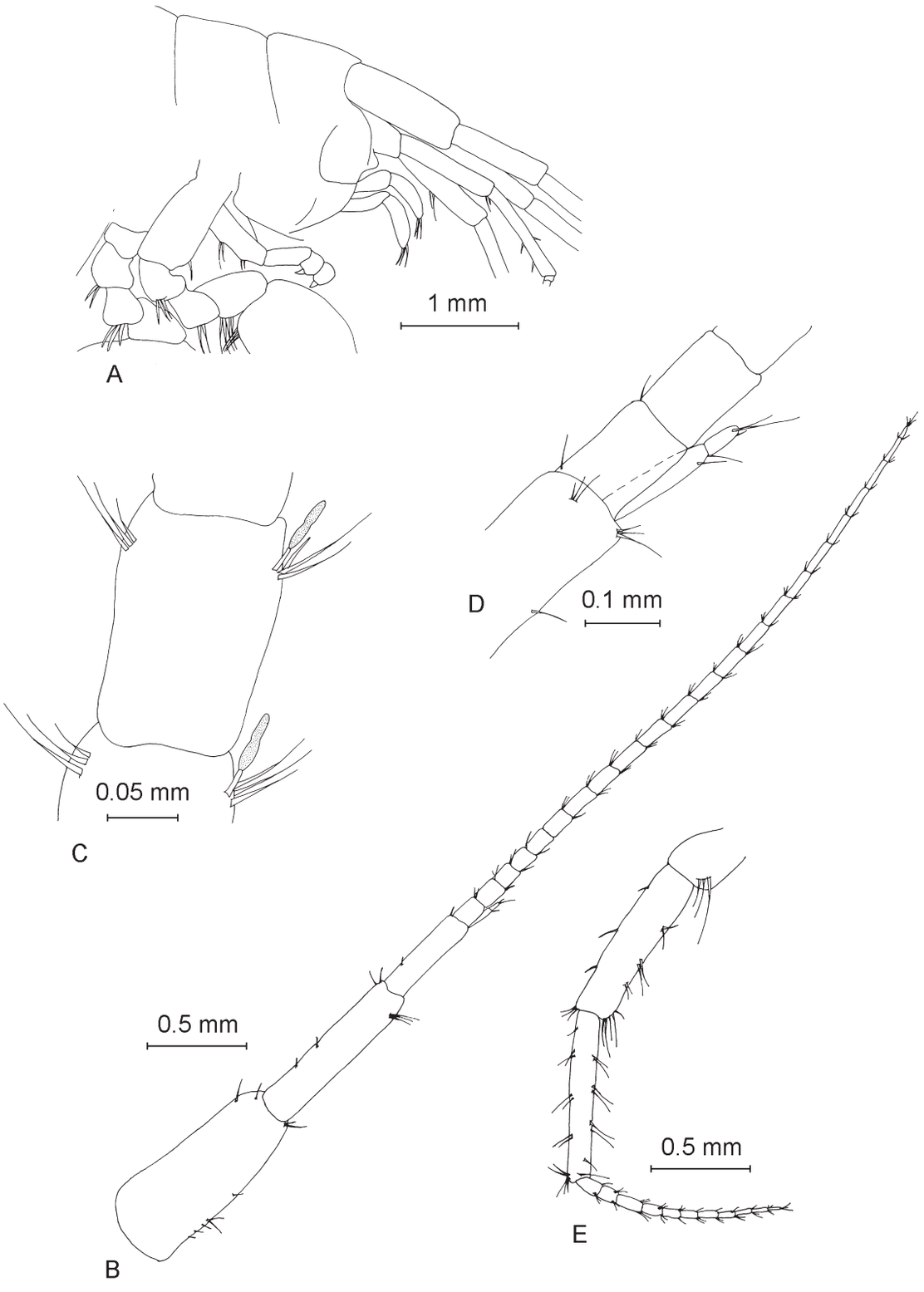


Fig. 9. ♀. **A**. Head. **B**. Antenna I. **C**. Aesthetascs of antenna I. **D**. Accessory flagellum of antenna I. **E**. Antenna II.

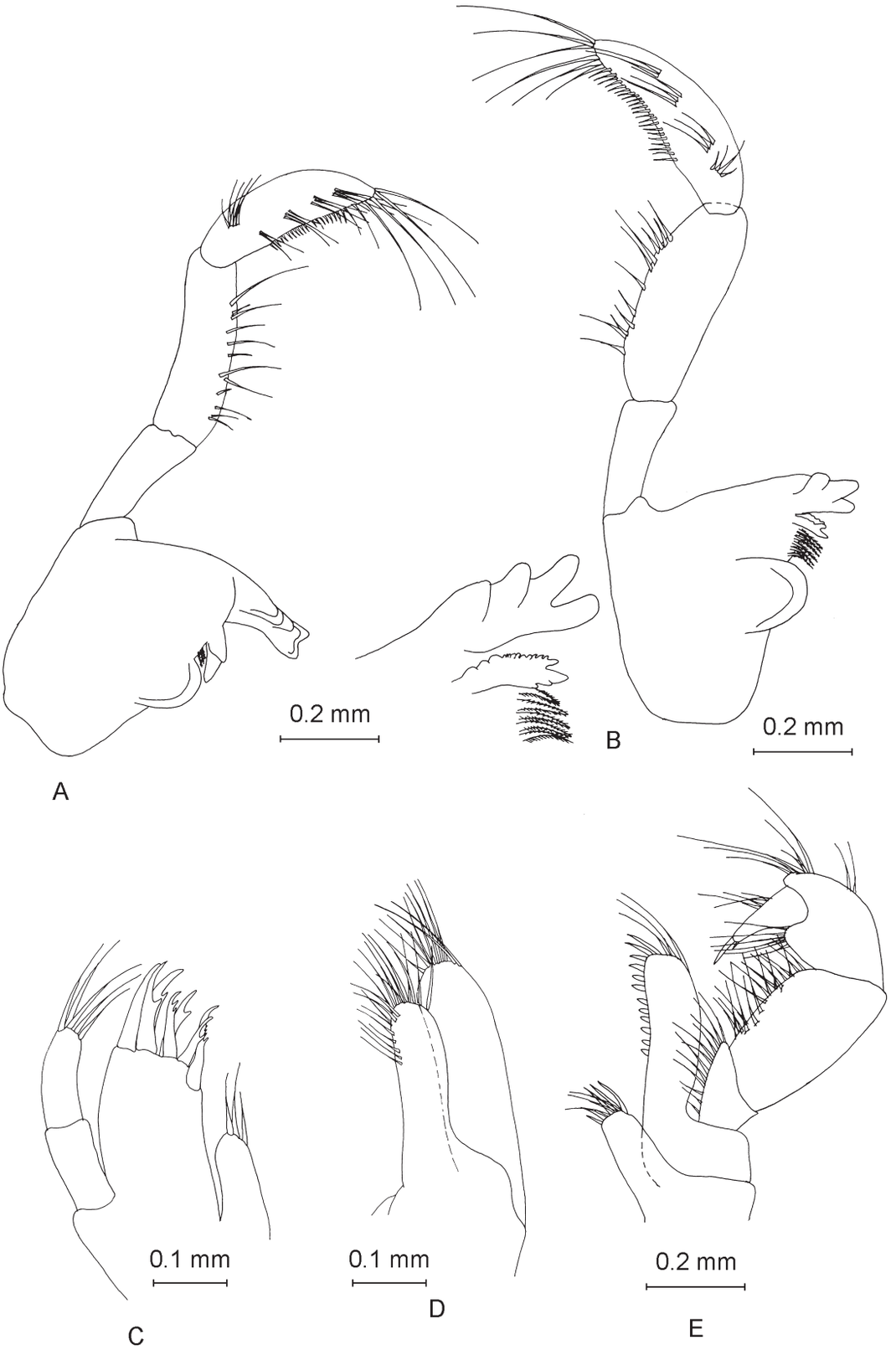


Fig. 10. Female mouthparts. **A**. Left mandible. **B**. Right mandible. **C**. Maxilla I. **D**. Maxilla II. **E**. Maxilliped.

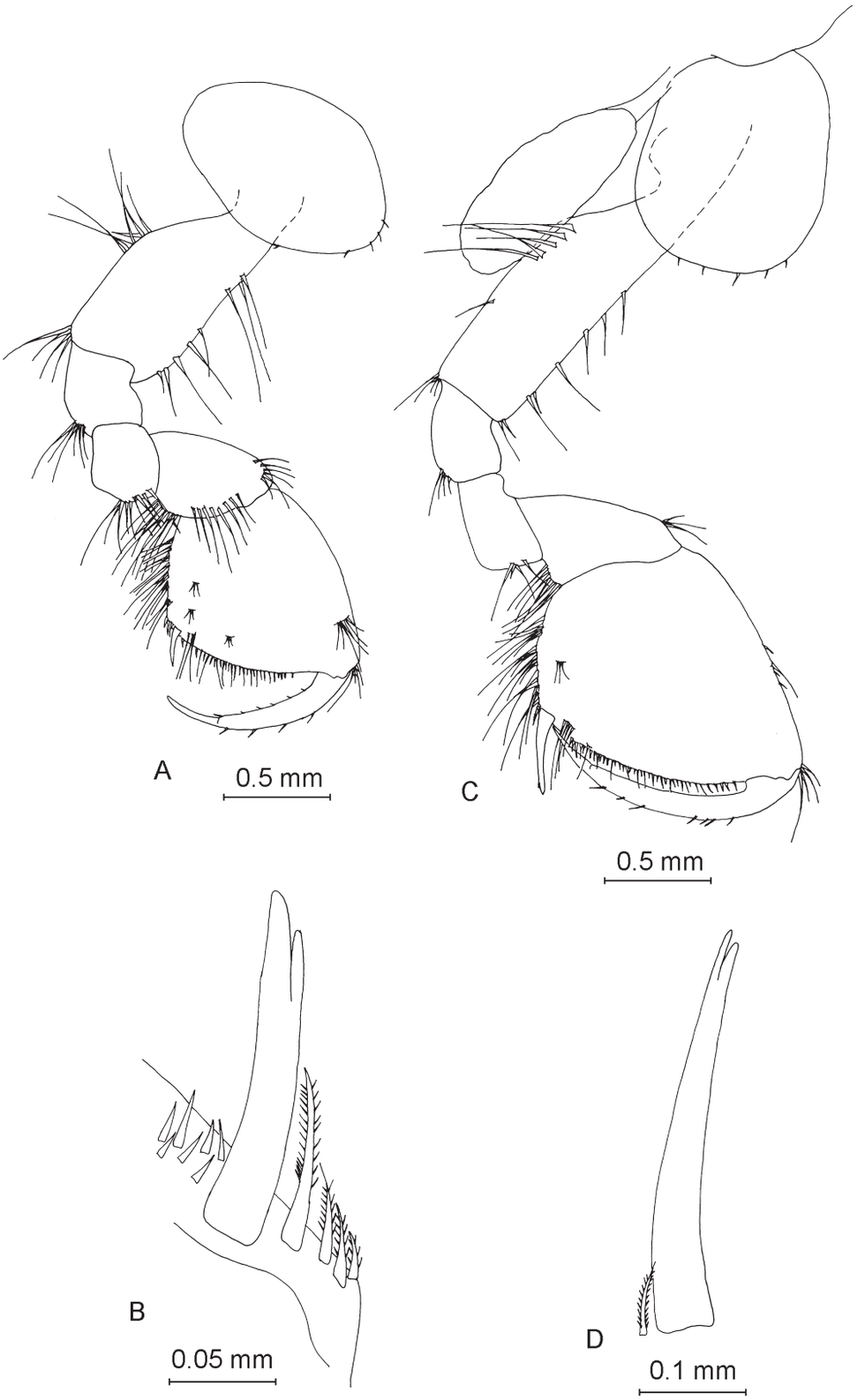


Fig. 11. ♀. **A**. Gnathopod I. **B**. Propodus teeth of gnathopod I. **C**. Gnathopod II. **D**. Propodus teeth of gnathopod II.

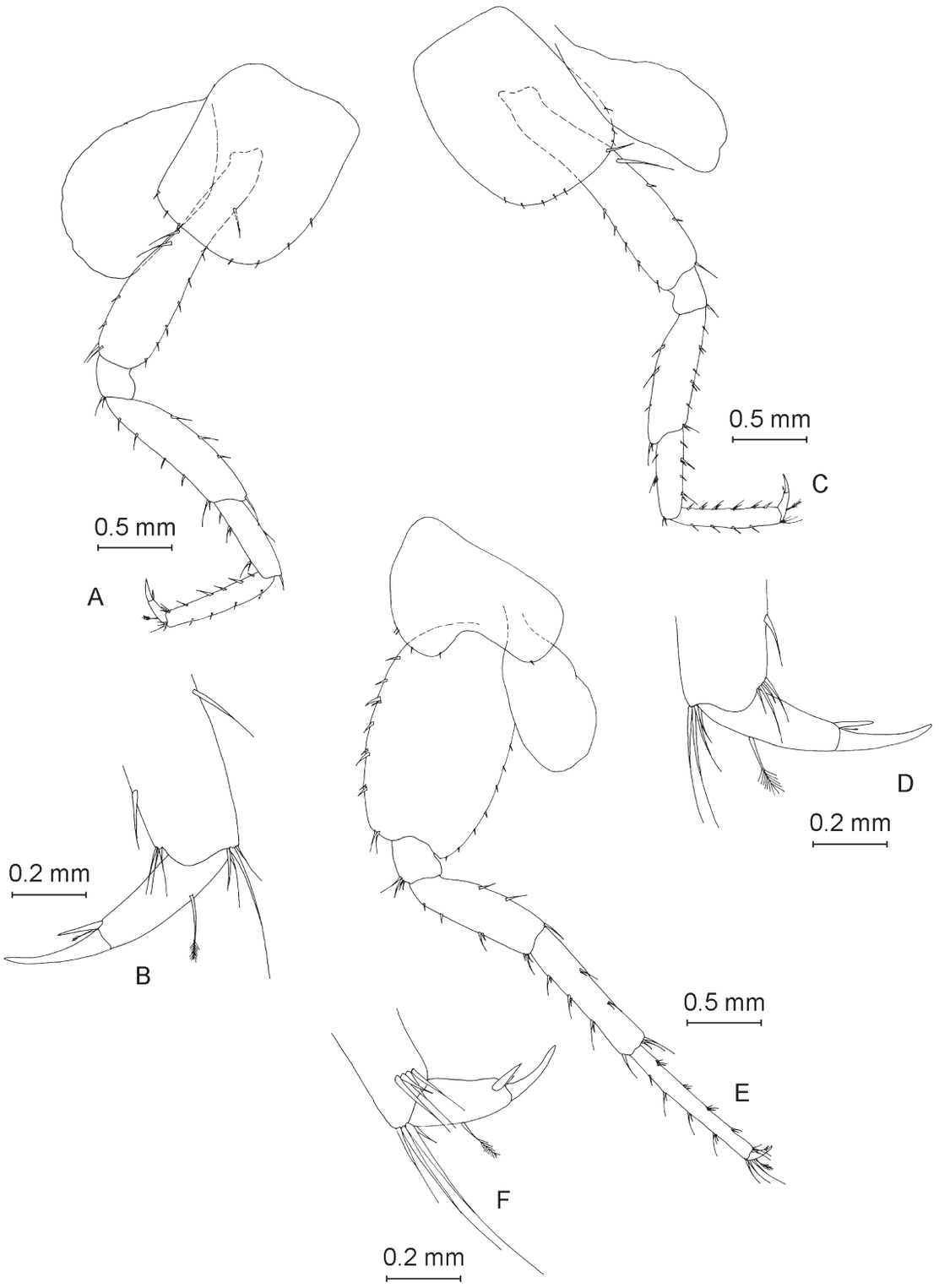


Fig. 12. ♀. **A**. Pereopod III. **B**. Dactylus of pereopod III. **C**. Pereopod IV. **D**. Dactylus of pereopod IV. **E**. Pereopod V. **F**. Dactylus of pereopod V.

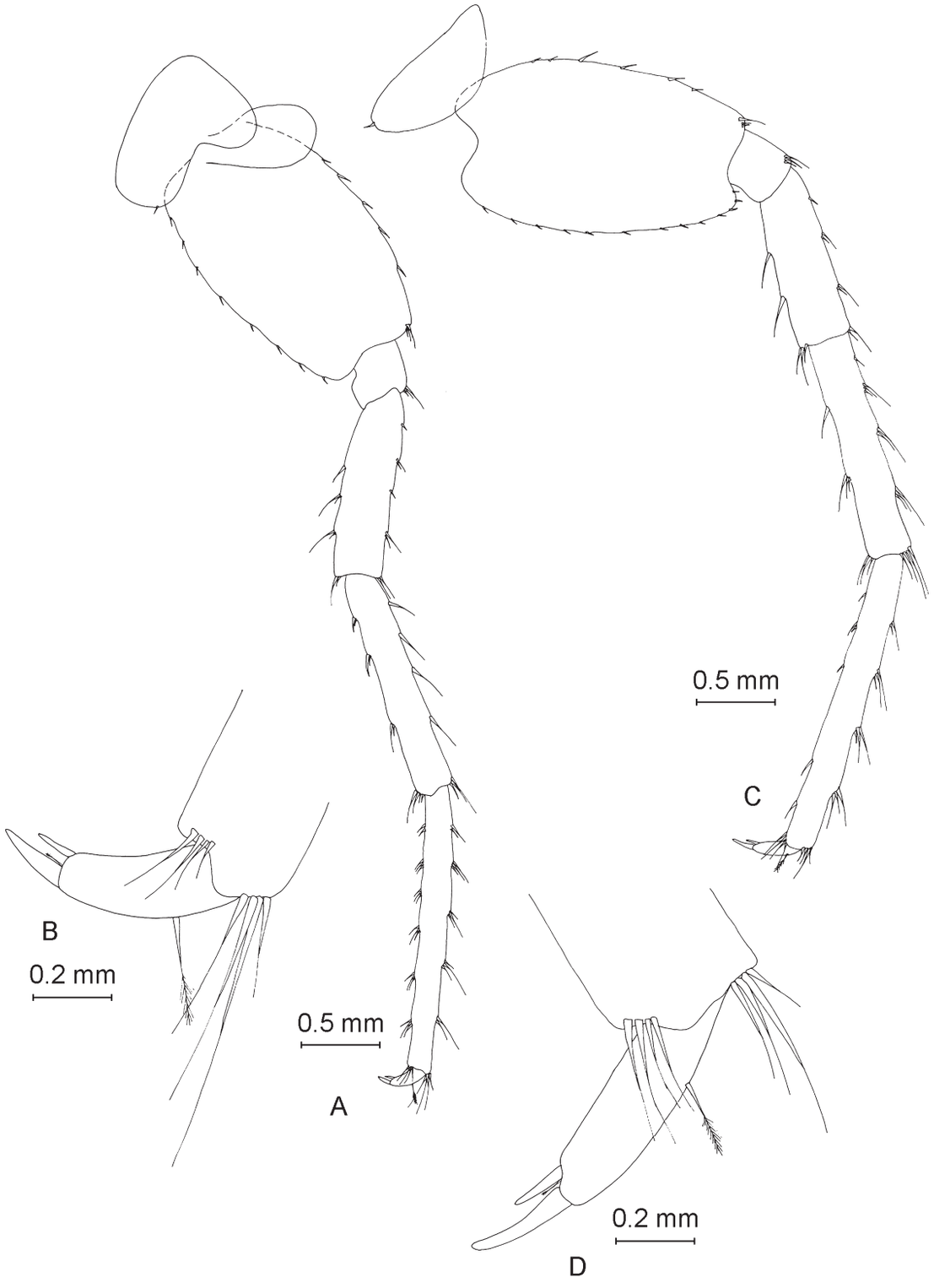


Fig. 13. ♀. **A**. Pereopod VI. **B**. Dactylus of pereopod VI. **C**. Pereopod VII. **D**. Dactylus of pereopod VII.

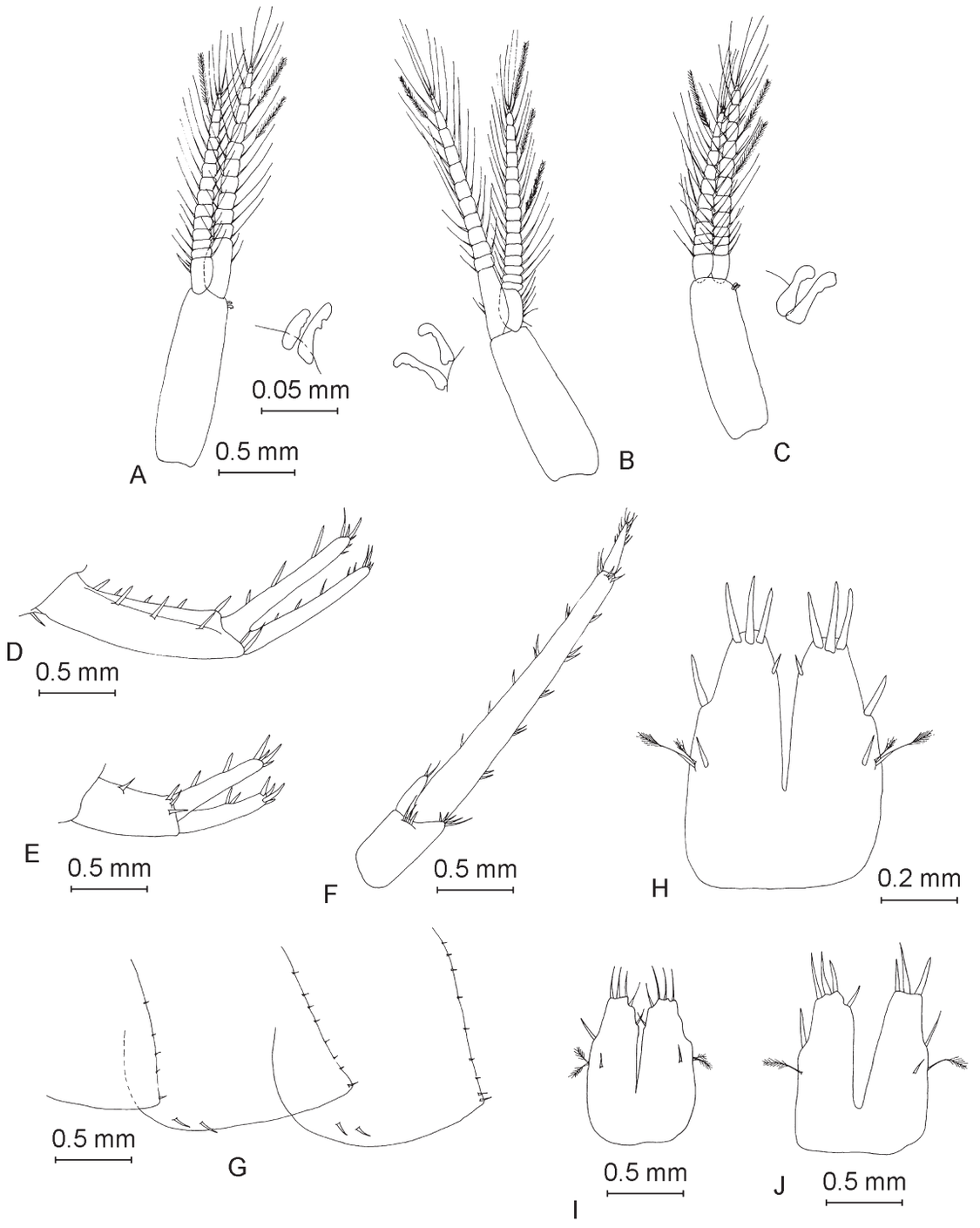


Fig. 14. — **A–H**. ♀. **A**. Pleopod I (with detail of retinacles). **B**. Pleopod II (with detail of retinacles). **C**. Pleopod III (with detail of retinacles). **D**. Uropod I. **E**. Uropod II. **F**. Uropod III. **G**. Epimeral plates. **H**. Telson. — **I**. Telson of ♂ sampled from str. Avram Iancu 26. **J**. Telson of ♀ sampled from str. Crinului 34.

Discussion

Morphological affinities of *Niphargus dancaui* sp. nov.

Niphargus dancaui sp. nov. shares several similarities with *N. stygius* (Sket 1974; personal observations). Both species are mid- to large-sized, robust and have similar lengths of appendages (e.g., pereopods, antennae) compared to their body lengths. Moreover, both species present similar mouthparts, gnathopods (notably the shape of the propods), spine patterns on pereopod dactyli, sexually non-dimorphic rami of uropod I, as well as similar sexually dimorphic traits such as elongated uropod III in males.

Yet, several details help distinguish *N. dancaui* sp. nov. from *N. stygius*. As pointed out by S. Karaman (1952), *N. stygius* and some related species (*N. novomestanus* Karaman, 1952, *N. likanus* Karaman, 1952, *N. podpecanus* Karaman, 1952, *N. kenki* Karaman, 1952, *N. karamani* Schellenberg, 1935) share four main traits: the outer margins of the dactyls of both gnathopods are armed with groups of setae, at most 4 setae are present along the posterior margin of the pleon segments, the first urosomite segment has a single tiny seta in a postero-lateral position, and the uropod III is sexually dimorphic. Some of these characters are also present in *N. dancaui* sp. nov. (namely, its sexually dimorphic uropod III and the fact that the dorso-posterior margin of its pleonites presents less than 4 setae). However, the setae along the gnathopod dactyls of *N. dancaui* sp. nov. are not in groups, and the urosomite I of this species has more than one seta, which sets *N. dancaui* sp. nov. apart from *N. stygius* and related species.

In this aspect, *N. dancaui* sp. nov. resembles some other species from the *N. costozzae* group living in northern Italy (*N. costozzae* Stoch, 1998, *N. lessiniensis* Stoch, 1998, *N. montellianus* and *N. tridentinus*; see Stoch 1998 for a revision) or the *N. sphagnicolus*-*N. plurispinosus* group from Slovenia and Slovakia (Rejic 1956; Hudec & Mock 2014). The latter group differs from *N. dancaui* sp. nov. by having more than three dorsal spines per telson lobe. *Niphargus costozzae* and *N. montellianus* differ from *N. dancaui* sp. nov. by having their setae along the gnathopod dactyli arranged in groups and by having more than one dorsal spine per telson lobe. *Niphargus lessiniensis* and *N. tridentinus*, however, show striking similarities to *N. dancaui* sp. nov. as described here. The only difference we found is the number of setal groups along the proximal exopodite article of uropod III in males: in both Italian species up to five setal groups can be found along the inner or outer margin of this article, whereas this number is slightly higher in *N. dancaui* sp. nov. (up to seven groups of setae).

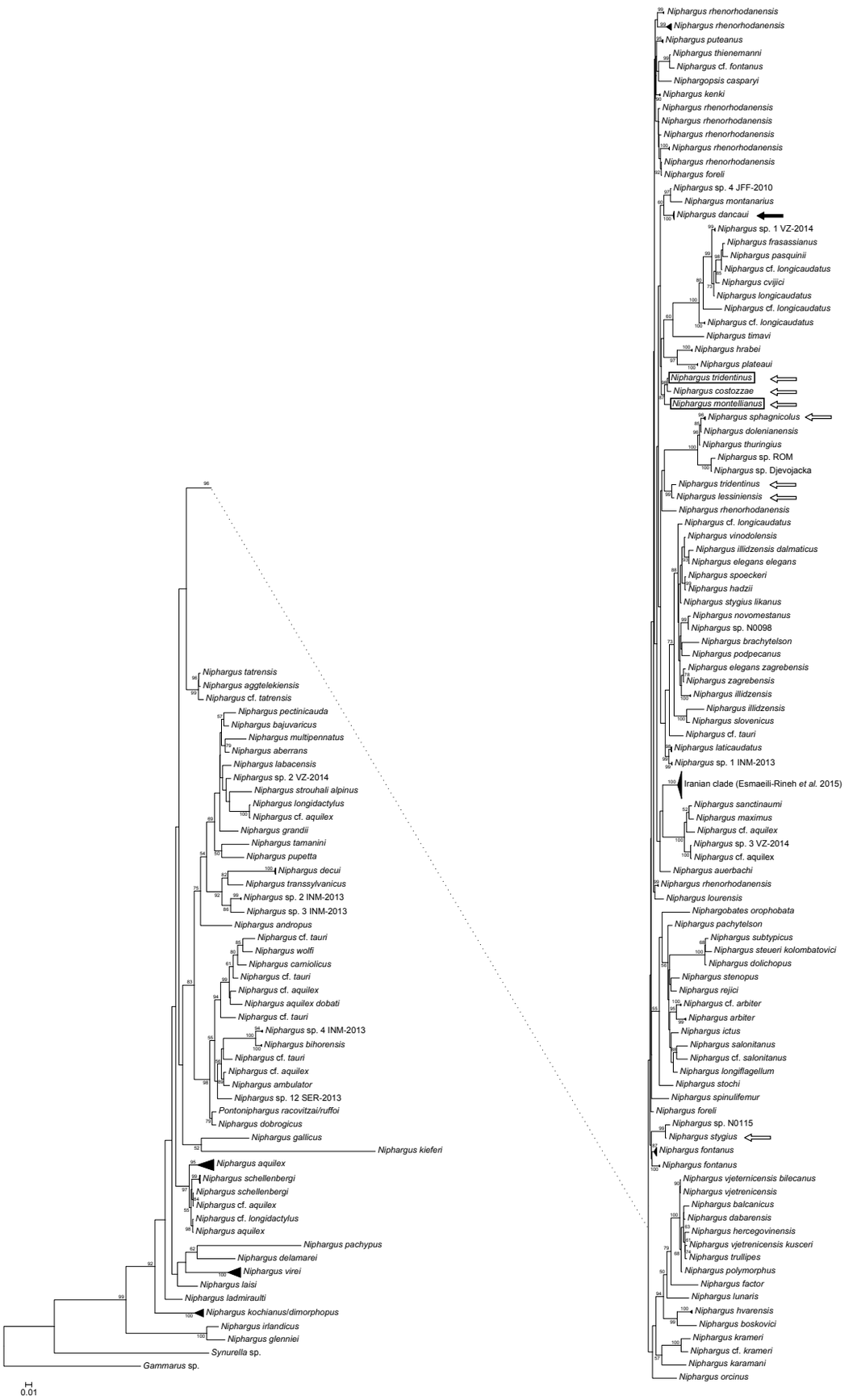
Position of *N. dancaui* sp. nov. in the niphargid tree of life

The updated 28S rRNA phylogeny of Niphargidae presented here (Fig. 15) is consistent with the ones in Flot *et al*. (2014) and McInerney *et al*. (2014): notably, the position of the two species *Niphargus glenniei* and *N. irlandicus* as a sister group to the rest of the genus is confirmed with a very strong bootstrap support. This phylogeny shows that *N. dancaui* sp. nov. (black arrow) is genetically very distinct from all the species to which it is morphologically similar (white arrows): instead, it may be related to *Niphargus montanarius* and *Niphargus* sp. 4 from the Frasassi Cave system (Flot *et al*. 2010), a relationship that only receives low bootstrap support.

Ecological data

Individuals of *N. dancaui* sp. nov. collected from various wells in the town of Mangalia, in Hagieni Spring and in Movile Cave were largely similar regardless of the concentration of hydrogen sulfide in the waters where they were collected (Table 2). These niphargids seem to be highly tolerant of the presence of hydrogen sulfide in water, but are not dependent on it for their survival.

Niphargus dancaui sp. nov. is probably more widely distributed in the area than our 14 sampled locations since it was found to occur in Hagieni Spring (Fig. 1), which is located at an aerial distance of 8 km from the town of Mangalia. However, *N. dancaui* sp. nov. is probably endemic to the area of Mangalia, given


Niphargus rhenorhodanensis
Niphargus rhenorhodanensis
Niphargus puteanus
Niphargus thienemanni
Niphargus cf. fontanus
Niphargopsis casparyi
Niphargus kenki
Niphargus rhenorhodanensis
Niphargus rhenorhodanensis
Niphargus rhenorhodanensis
Niphargus rhenorhodanensis
Niphargus rhenorhodanensis
Niphargus foreli
Niphargus sp. 4 JFF-2010
Niphargus montanarius
Niphargus dancaui
Niphargus sp. 1 VZ-2014
Niphargus frasassianus
Niphargus pasquinii
Niphargus cf. longicaudatus
Niphargus cvijici
Niphargus longicaudatus
Niphargus cf. longicaudatus
Niphargus cf. longicaudatus
Niphargus timavi
Niphargus hrabei
Niphargus plateaui
Niphargus tridentinus
Niphargus costozzae
Niphargus montellianus
Niphargus sphagnicolus
Niphargus dolenianensis
Niphargus thuringius
Niphargus sp. ROM
Niphargus sp. Djevojacka
Niphargus tridentinus
Niphargus lessiniensis
Niphargus rhenorhodanensis
Niphargus cf. longicaudatus
Niphargus vinodolensis
Niphargus illidzensis dalmaticus
Niphargus elegans elegans
Niphargus spoeckeri
Niphargus hadzii
Niphargus stygius likanus
Niphargus novomestanus
Niphargus sp. N0098
Niphargus brachytelson
Niphargus podpecanus
Niphargus elegans zagrebensis
Niphargus zagrebensis
Niphargus illidzensis
Niphargus illidzensis
Niphargus slovenicus
Niphargus cf. tauri
Niphargus laticaudatus
Niphargus sp. 1 INM-2013
Iranian clade (Esmaeili-Rineh et al. 2015)
Niphargus sanctinaumi
Niphargus maximus
Niphargus cf. aquilex
Niphargus sp. 3 VZ-2014
Niphargus cf. aquilex
Niphargus auerbachi
Niphargus rhenorhodanensis
Niphargus lourensis
Niphargobates orophobata
Niphargus pachytelson
Niphargus subtypicus
Niphargus steueri kolombatovici
Niphargus dolichopus
Niphargus stenopus
Niphargus rejici
Niphargus cf. arbiter
Niphargus arbiter
Niphargus ictus
Niphargus salonitanus
Niphargus cf. salonitanus
Niphargus longiflagellum
Niphargus stochi
Niphargus spinulifemur
Niphargus foreli
Niphargus sp. N0115
Niphargus stygius
Niphargus fontanus
Niphargus fontanus
Niphargus vjeternicensis bilecanus
Niphargus vjetrenicensis
Niphargus balcanicus
Niphargus dabarensis
Niphargus hercegovinensis
Niphargus vjetrenicensis kusceri
Niphargus trullipes
Niphargus polymorphus
Niphargus factor
Niphargus lunaris
Niphargus hvarensis
Niphargus boskovici
Niphargus krameri
Niphargus cf. krameri
Niphargus karamani
Niphargus orcinus
Niphargus tatrensis
Niphargus aggtelekiensis
Niphargus cf. tatrensis
Niphargus pectinicauda
Niphargus bajuvaricus
Niphargus multipennatus
Niphargus aberrans
Niphargus labacensis
Niphargus sp. 2 VZ-2014
Niphargus strouhali alpinus
Niphargus longidactylus
Niphargus cf. aquilex
Niphargus grandii
Niphargus tamanini
Niphargus pupetta
Niphargus decui
Niphargus transsylvanicus
Niphargus sp. 2 INM-2013
Niphargus sp. 3 INM-2013
Niphargus andropus
Niphargus cf. tauri
Niphargus wolfi
Niphargus camiolicus
Niphargus cf. tauri
Niphargus cf. aquilex
Niphargus aquilex dobati
Niphargus cf. tauri
Niphargus sp. 4 INM-2013
Niphargus bihorensis
Niphargus cf. tauri
Niphargus cf. aquilex
Niphargus ambulator
Niphargus sp. 12 SER-2013
Pontoniphargus racovitzai/ruffoi
Niphargus dobrogicus
Niphargus gallicus
Niphargus kieferi
Niphargus aquilex
Niphargus schellenbergi
Niphargus schellenbergi
Niphargus cf. aquilex
Niphargus cf. longidactylus
Niphargus aquilex
Niphargus pachypus
Niphargus delamarei
Niphargus virei
Niphargus laisi
Niphargus ladmiraulti
Niphargus kochianus/dimorphopus
Niphargus irlandicus
Niphargus glenniei
Synurella sp.
Gammarus sp.
0.01

that it was never found in the wells sampled in the neighboring villages (i.e., Limanu, Vama Veche, Doi Mai, Arsa, Albeşti, Vânători, Coroana, Pecineaga, Dulceşti, 23 August - data not shown).

Groundwater crustaceans are in general stenobiontic: they do not tolerate large fluctuations of the abiotic conditions in their environment (Gibert 2001). In places inhabited by humans, groundwater ecosystems are in general polluted with various anthropogenic wastes produced by industries, agricultural practices or household activities. *Niphargus dancaui* sp. nov. therefore appears vulnerable to extinction according to IUCN Red List categories and criteria. Apart from the occurrence of this species in Hagieni Spring, all other recorded sampling locations (Movile Cave and in 12 old hand-dug wells in the town of Mangalia) are spread over approximately 2 km^2. The wells were used in the past as drinking water sources but have now been replaced with a modern water supply system. As a result, most of these wells are abandoned or are even being used for dumping various wastes, with potentially severe consequences for groundwater crustaceans inhabiting the aquifer. It is our hope that the description of *N. dancaui* sp. nov. and its recognition as a species endemic to Mangalia and its vicinity will lead to conservation measures to protect the fauna of this unique sulfidic ecosystem.

Acknowledgements

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Fig. 15. (previous page) Maximum-likelihood phylogenetic tree of all published 28S niphargid sequences. This tree was obtained using FastTree2 under the GTR model; support values >50% (from 1000 bootstrap replicates) are indicated next to the nodes. The black arrow shows the location of *Niphargus dancaui* sp. nov. in the niphargid phylogeny, whereas white arrows point at niphargid species morphologically similar to *N. dancaui* sp. nov. The two black boxes highlight the positions of the topotypes that were newly sequenced for the present article.

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