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Research Article

Integrative taxonomy of the cave-dwelling mysids of the genus *Hemimysis*

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The genus *Hemimysis* (Malacostraca: Mysida: Mysidae) encompasses near-bottom, demersal and cave-dwelling mysids living in the marine, brackish and freshwater habitats around the European coast, from the Caspian Sea to the Scandinavian Peninsula. We conducted cladistic analysis of 52 morphological characters of all nine species and three subspecies of the genus *Hemimysis.* We also completed a molecular analysis based on three molecular markers of *Hemimysis lamornae* (Couch, 1856) found in the English Channel, the Mediterranean Sea, and the Black Sea. Both analyses did not support monophyly of *Hemimysis lamornae*. We thus consider the former subspecies *H. lamornae pontica* (Czerniavsky, 1882) and *H. lamornae mediterranea* Bacescu, 1936 as valid species. Analysis of mitochondrial cytochrome oxidase subunit I (COI) sequences of *H. pontica* shows no significant divergence between mysids living in the marine caves of Crimea and Bulgaria. Morphological trends in *Hemimysis* are discussed, *H. pontica* Czerniavsky, 1882 is redescribed, and a new key to all 11 species of the genus is given.

Key words: Black Sea, cave, *Hemimysis*, integrative taxonomy, mysid, phylogeny

Introduction

Marine caves are inhabited by various animals belonging to diverse taxa, which are often adapted to live in this environment (Gerovasileiou et al., 2016). Living in cave conditions can lead to niche partitioning and the evolution of closely related species living in neighbouring caves or even different parts of the same cave (Lejeusne & Chevaldonné, 2006; Neiber, Hansen, Iliffe, Gonzalez, & Koenemann, 2012; Rastorgueff, Harmelin-Vivien, Richard, & Chevaldonné, 2011). The study of fauna living in the marine caves of the Black Sea may clarify the species composition of poorly studied underwater cave communities as well as the evolution of species adapted to low salinity (Ereskovsky et al., 2018; Ereskovsky, Kovtun, & Pronin, 2016). One of the most interesting targets of these studies is the cave-dwelling mysids representing genus *Hemimysis* G.O. Sars, 1869 (Chevaldonné, Rastorgueff, Arslan, & Lejeusne, 2015; Lejeusne & Chevaldonné, 2006; Rastorgueff et al., 2011; Rastorgueff, Chevaldonne, Arslan, Verna, & Lejeusne, 2014).

The genus *Hemimysis* currently includes nine species inhabiting marine and brackish waters along the European coast from the Caspian Sea to the Scandinavian Peninsula (Mees & Meland, 2012). The Ponto-Caspian freshwater mysid *Hemimysis anomala* has widely dispersed during the last 60 years into

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Europe and was found in the Great Lakes of North America (Kestrup & Ricciardi, 2008). Six of the nine species (*H. lamornae*, *H. maderensis*, *H. margalefi*, *H. sophiae*, *H. speluncola*, *H. spinifera*) were found in the marine caves of the Mediterranean Sea (Ledoyer, 1989; Rastorgueff & Bianchimani, 2016). Observations of the microdistribution of co-occurring mysid species in caves near Marseilles showed an unusual change in the thermocline depth, which has led to a replacement of the cold-living *H. speluncola* by the eurythermic *H. margalefi* (see Chevaldonné & Lejeusne, 2003). Studies of the molecular markers of *H. margalefi* from the marine caves of the Mediterranean Sea highlighted a number of cryptic species, each occurring in a distinct geographic area, as well as the possibility of the existence of their ancestor during the time of the Tethys Sea (Rastorgueff et al., 2014). The cave mysids were not known to be from the Black Sea until recent studies of the marine caves on the western coast of Crimea revealed swarms of *H. lamornae pontica* and rare *H. serrata* (see Petrjachev & Kovtun, 2011). The latter species was found earlier in crevices of the eastern coast of Crimea (the Azov Sea) and studied in detail by Reznichenko (1959). However, this or other species of *Hemimysis* could not be found on the coast of the Azov Sea during our intensive sampling in 2016 (unpublished observation). A revision of the taxonomy and phylogenetic relations of the Black Sea representatives of *Hemimysis lamornae* may clarify the taxonomic status of the geographic forms of this species, as well as verify the presence of endemic species of mysids in the Black Sea (Czerniavsky, 1882).

According to the latest studies, the genus *Hemimysis* includes nine species: *Hemimysis abyssicola* G.O. Sars, 1869, *H. anomala* G.O. Sars, 1907, *H. lamornae* (Couch, 1856), *H. maderensis* Ledoyer, 1989, *H. margalefi* Alcaraz, Riera & Gili, 1986, *H. serrata* Bacescu, 1938, *H. sophiae* Ledoyer, 1989, *H. speluncola* Ledoyer, 1963, *H. spinifera* Ledoyer, 1989 and three subspecies: *H. lamornae lamornae* Couch, 1856, *H. lamornae mediterranea* Bacescu, 1936, and *H. lamornae pontica* Czerniavsky, 1882.

The genus was established after the discovery of the type species *Hemimysis abyssicola* Sars, 1869. In 1882, Czerniavsky described *H. pontica* based on one specimen collected at the eastern coast of the Black Sea (in the vicinity of Sukhum) on a dark night. Norman studied specimens of *Mysis lamornae* from Naples, the Norwegian coast, West Sweden and Denmark; he moved the species into *Hemimysis* and indicated that *H. lamornae pontica* is a juvenile of *H. lamornae* (Norman, 1892). Bacesco (1936) named specimens of *H. lamornae* from the North Sea, Naples and the Romanian coast as *H. lamornae typica*, *H. lamornae mediterranea*, and *H. lamornae reducta*, respectively. Later, *H. lamornae reducta* Bacescu, 1936 was synonymized with *H. lamornae pontica*, and *H. lamornae typica* Bacescu, 1936 with *H. lamornae lamornae* (see Bacescu, 1954).

The aim of this paper was to analyse the morphological characters of all nine species and three subspecies of the genus *Hemimysis* and couple these results with the molecular analysis in order to test the taxonomic status and phylogenetic relationships of three subspecies of *H. lamornae*.

Materials and methods

Specimen collection

Hemimysis lamornae pontica was collected in the Black Sea caves of Crimea (Fig. 1, points 1 and 2, Table S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2019.1596175) and Bulgaria (Fig. 1, points 3–6) (see Ereskovsky et al., 2016, 2018). *Hemimysis l. lamornae* was collected in the English Channel in a darkened niche of breakwaters (Table S1, see supplemental material online). The mysids were collected at a depth of 1–4 m by nets (mesh size 0.1 mm, during scuba and snorkelling) and fixed in 95% ethanol.

Morphological examinations

Morphological studies were completed using light microscopes Olympus CX41RF and Olympus BX 51. Specimens were dissected under the microscope Olympus BX 51 and mounted on slides in a drop of glycerine. Measurements were taken using eyepiece micrometres. Drawings were made using a camera lucida.

Character matrix

Analysis of the morphology of all of the species within the genus was made using both original and literature data. All 11 recognized species and subspecies of *Hemimysis* were included as terminals. Two morphological analyses were performed with two outgroups, *Gastrosaccus wittmanni* Deprez, Wooldridge, & Mees, 2000 and *Mysis relicta* Lovén, 1862. These species belong to the different subfamilies and different molecular clades within the family Mysidae. *M. relicta* is more closely related to *Hemimysis* and belongs to the same subfamilies and to the same molecular clade (Remerie *et al.*, 2004).

Twelve species were included in the analysis; 52 morphological characters were identified (Table S2, see

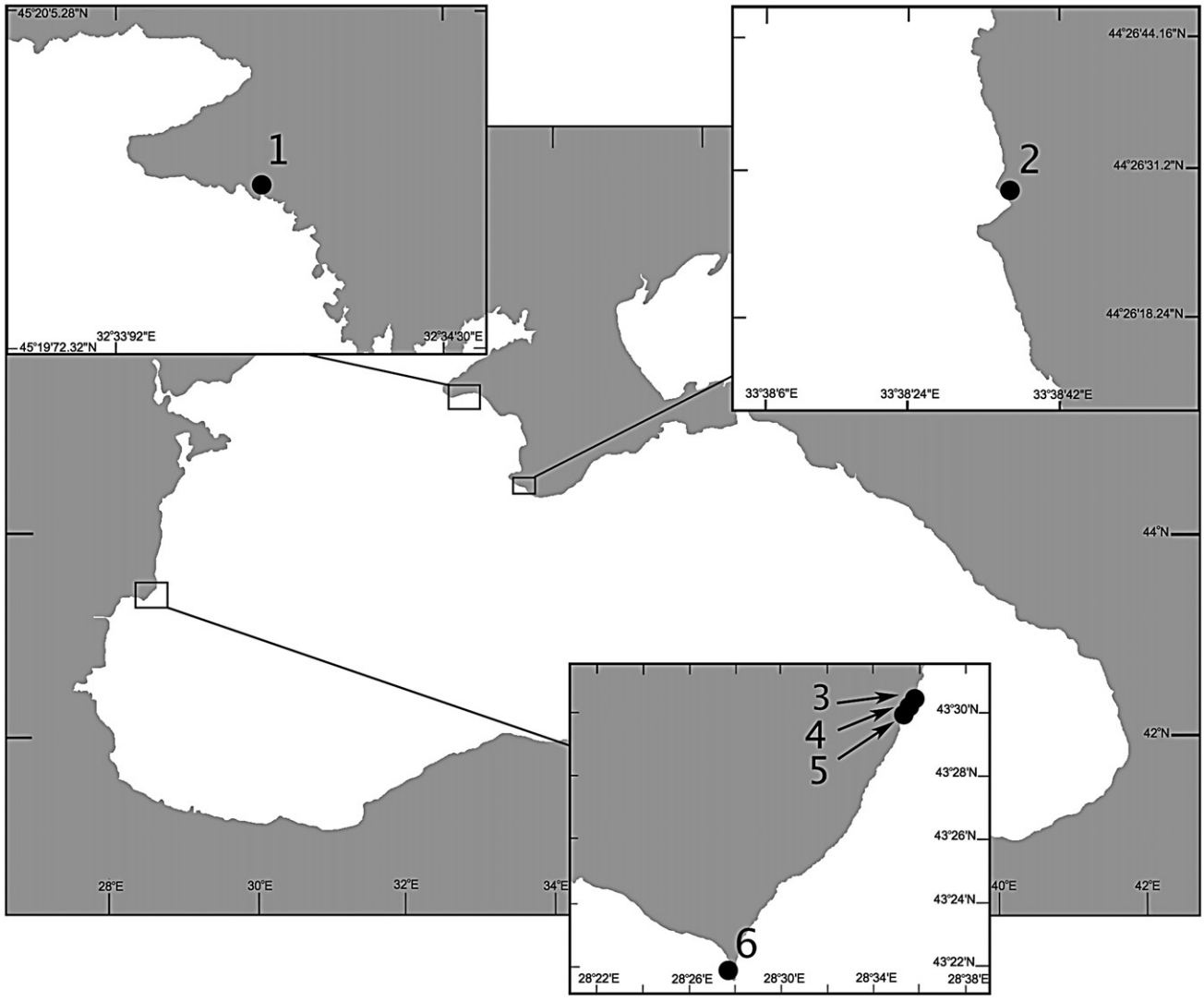

Fig. 1. The sampling localities of *Hemimysis* in the Black Sea (Bulgaria – 3–6, Crimea – 1 and 2). See Table S1 (see supplemental material online).

supplemental material online). The characters used in this study were divided into six groups: 12 characters related to the telson, eight characters related to the antennae, four characters related to the maxilla II, four characters related to the thoracopods, 18 characters related to the pleopods and five characters related to the uropods. The telson shows much variation within the genus. Differences were observed in the depth of the sinus, in the number of the lateral and posterior teeth and in the shape of the distal lobes (Fig. 3.9). The antennal scale varies significantly in shape and armature. The scale may be subrectangular or lanceolate and may have a different number of articulate movable spines on the outer margin (Fig. 2.3). The palp of the maxilla II bears a varied number of modified setae, and the distal segment of palp may be oval or subtriangle (Fig. 2.5). The thoracopods III–VIII vary in the number of their carpo-propodal segments (from three to six – Figs 2.9 and 3.1). The female pleopods are rudimentary and not included in the analysis. The first two male pleopods are rudimentary, as were the females' but larger: the pleopods III–V are variable within the genus; the exopod and endopod may be rudimentary, one- or multisegmented (Figs 3.5 and 3.6). The uropodal endopod may be armed with a variable number of spines, either arranged in one or two rows or not arranged. The data matrix is presented in Table S3 (see supplemental material online).

Cladistics analysis of morphological data

The data were analysed using a combination of programs by maximum parsimony: Winclada/Nona and

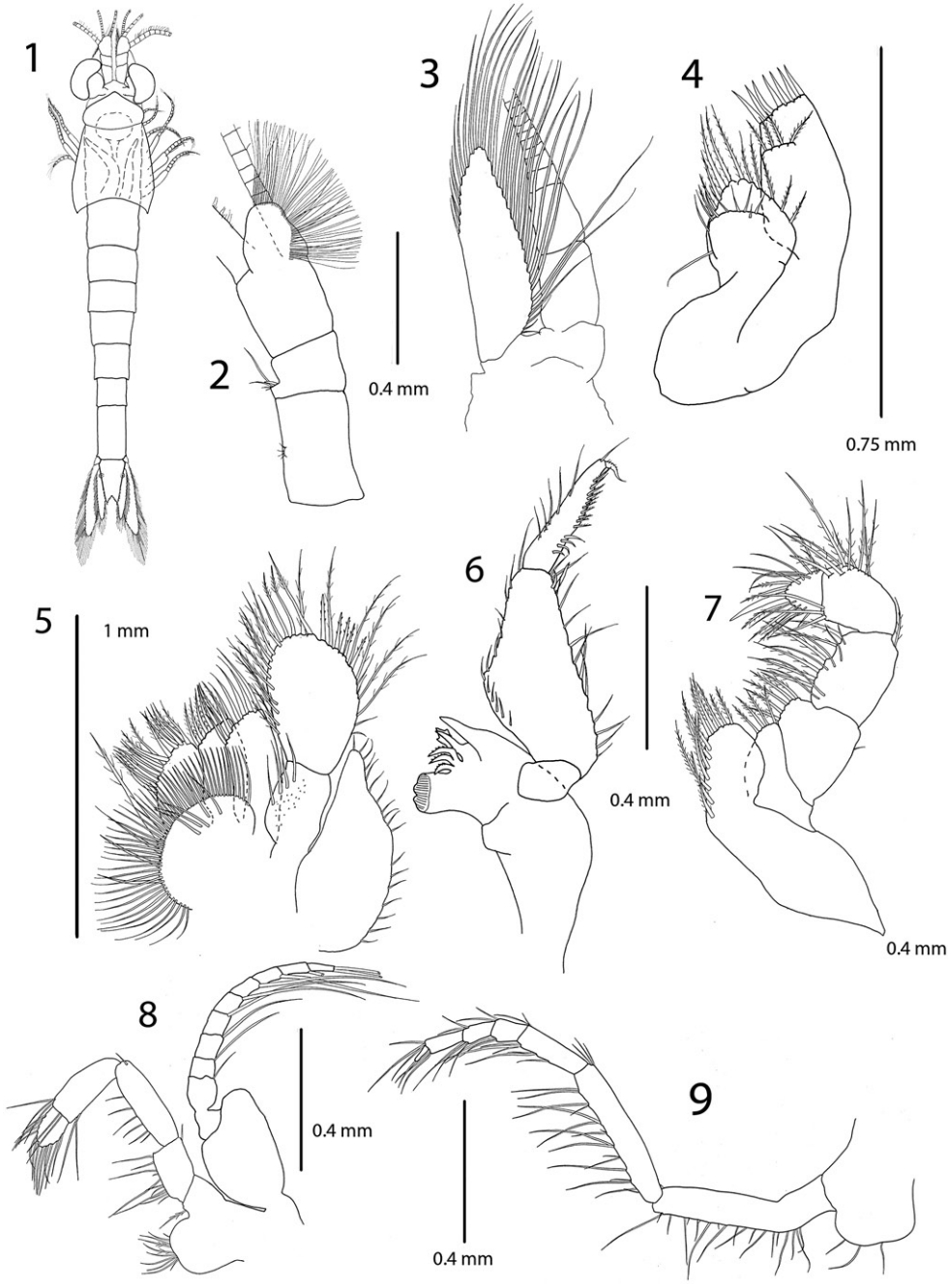

Fig. 2. *Hemimysis pontica* Czerniavsky, 1882. Female: 1 – body, body length from tip of rostrum to distal margin of telson 9 mm; 2 – antenna I; 3 – antenna II; 4 – maxilla I; 5 – maxilla II; 6 – mandible; 7 – thoracopod I; 8 – thoracopod II; 9 – thoracopod III.

TNT (Goloboff, Farris, & Nixon, 2000; Nixon, 1999). Winclada was used for the coding of characters; TNT was used for the generation of trees and for performing bootstrap and Bremer support analyses. All characters were unordered (non-additive) and equally weighted; missing (unavailable) data were scored as unknown. Characters were unordered, so the score given for each state (i.e., 0, l) implies nothing about the order in a transformation series. Trees were generated in TNT under the 'implicit enumeration' algorithm (Goloboff et al., 2000). The relative stability of clades was assessed by standard bootstrapping (sample with replacement) with 10,000 pseudoreplicates (Goloboff et al., 2000). We considered the clades robust if they received bootstrap support $\geq$75 and/or Bremer support $\geq$3.

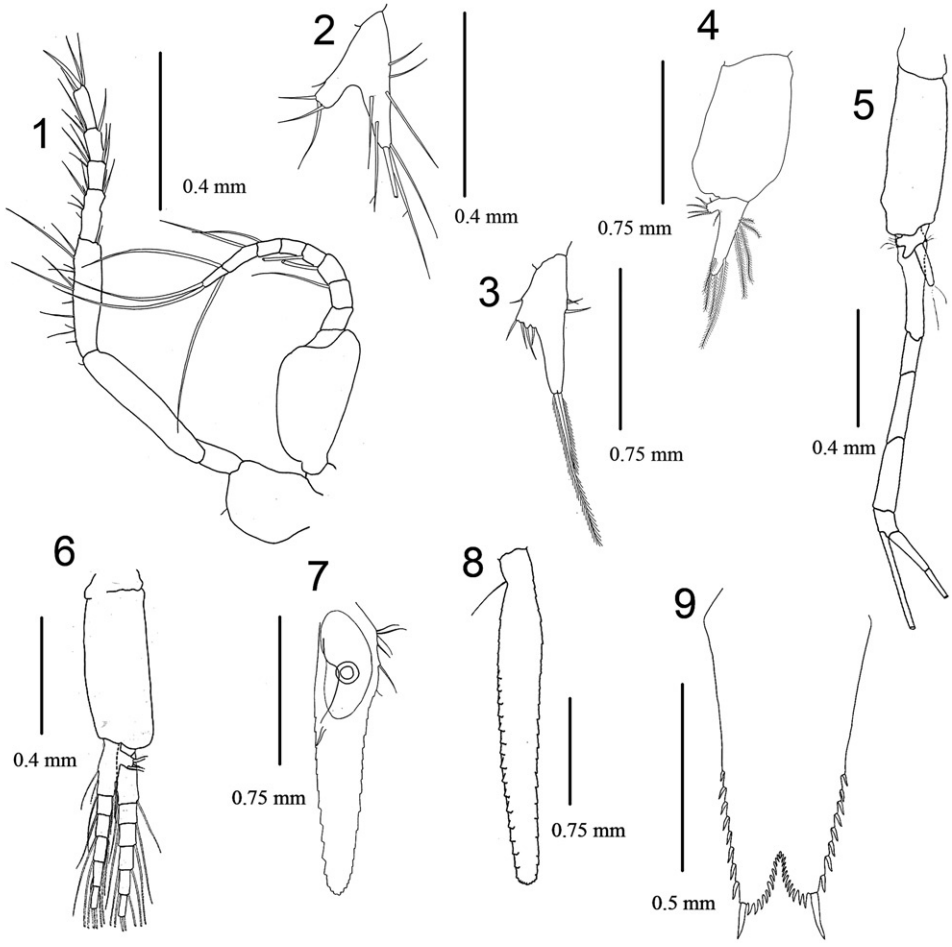

Fig. 3. *Hemimysis pontica* Czerniavsky, 1882. Female (A–G, I) and male (H). 1 – thoracopod VII; 2 – pleopod I; 3 – pleopod II; 4 – pleopod III; 5 – pleopod IV; 6 – pleopod V; 7 – uropod endopodite; 8 – uropod exopodite; 9 – telson.

DNA extraction and amplification

Eleven specimens of *Hemimysis lamornae lamornae* from the English Channel and 22 specimens of *H. lamornae pontica* from the Black Sea were selected for DNA analysis (Table S1, see supplemental material online). An eye or one thoracopod of each specimen was digested in 50 µl of lysis mix (20 mM Tris-HCl, 20 mM EDTA, 1% SDS, 0.0001 g/mL proteinase K) at 37 °C for 1 hour, and DNA was extracted from the lysis mix using a Diatom DNA Prep 100 Kit (Isogene, Moscow, Russia), following the manufacturer's protocol.

Three molecular markers were chosen for analysis: 18S nuclear ribosomal DNA, nuclear protein-coding gene glutamyl-prolyl-tRNA Synthetase (EPRS) and mitochondrial cytochrome oxidase subunit I (COI). Both 18S and COI are widely used in molecular phylogenetics, and large datasets of these genes are available for most animal taxa. The EPRS gene was shown to perform better then 18S and COI specifically within Mysidae (Audzijonyte, Daneliya, Mugue, & Väinölä, 2008). DNA fragments were amplified using an Encyclo Plus PCR kit (Evrogen, Moscow, Russia) and specific primers (18S: Medlin, Elwood, Stickel, & Sogin, 1988; EPRS: Audzijonyte et al., 2008; COI: Geller, Meyer, Parker, & Hawk, 2013). The PCR conditions are 3 min at 95 °C, 37 cycles of 94 °C for 20 s, followed by annealing at temperatures for 30 s, 72 °C for 1 min 30 s and then a final elongation at 72 °C for 5 min. Annealing temperatures were 56 °C for 18S rDNA gene and 48 °C for COI gene. Nested PCR was performed for the EPRS gene as in Audzijonyte et al., 2008. PCR products were visualized with electrophoresis in 1% agarose gel. The amplified DNA was purified with exonuclease I and shrimp alkaline phosphatase (1 unit of each per PCR tube, 1 hour at 37 °C and 10 min at 90 °C). Purified PCR products were sequenced in the sequencing facility of Evrogen JSC (Moscow) on an ABI 3730 capillary sequencer in both directions.

The sequences were assembled and edited using Geneious 7.1.2, deposited in GenBank (the accession numbers are in Table 1) and compared with other

Table 1. GenBank accession numbers for COI, EPRS, and 18S sequences of species used for phylogenetic analyses.

Species name	DNR marker			Sequences used for concatenation
	18S	EPRS	COI	
Hemimysis anomala	AJ566104.1		EU029162.1	EU029162.1
			EU029163.1	
			EU029164.1	
			EU029165.1	
			EU029166.1	
			EU029167.1	
			EU029168.1	
			EU029169.1	
			EU029170.1	
Hemimysis speluncola	HE613050.2		HE980623.1	
			AM114270.1	
			AM114271.1	
			AM114272.1	
Hemimysis margalefi	HE980453.2	HG797087.1	AM114242.1	AM114237.1, HE614239.1
	HE980454.1	HG797082.1	AM114247.1	
			AM114250.1	
			AM114252.1	
			HE614218.1	
			HE614246.1	
			HE614263.1	
			HE614278.1	
			HE614279.1	
Hemimysis lamornae lamornae	MK429928	MK439440, MK439441	MK439443 — MK439453	MK439444
Hemimysis lamornae pontica	MK429927	MK439442	MK439454 — MK439475	MK439455
Hemimysis lamornae mediterranea	LN717264.1	–	–	
Mysis oculata	AM422510.1		EF609270.1	
Mysis relicta	DQ189130.1		AY529027.1	
Mysis sergestralei	DQ189132.1	EU233591.1	DQ524909.1	

sequences using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (NCBI BLAST) (Altschul, Gish, Miller, Myers, & Lipman, 1990). Protein-coding sequences (EPRS and COI) were checked for reading frame and absence of stop codons.

We sequenced the COI gene fragment (658 bp) for 33 specimens (11 specimens of *H. lamornae lamornae* from the English Channel and 22 of *H. lamornae pontica* from Crimea and Bulgaria). Five COI haplotypes were found for *H. l. lamornae* and 15 for *H. l. pontica*. Sequences of 18S rDNA (1560 bp) are identical among all of the analysed specimens of *H. lamornae* subspecies. The EPRS gene fragment (400 bp) is identical among *H. l. pontica*; two haplotypes are present in *H. l. lamornae*. Alignments of 9 sequences for 18S rDNA (823 bp), 9 sequences for EPRS (436 bp) and 47 sequences for COI (644 bp) are generated using sequences of the *Hemimysis* species available in GenBank (Table 1).

Statistical phylogenetic analyses of molecular data

COI, 18S, and EPRS sequences of *Hemimysis* and the outgroup taxa (*Mysis oculata* for 18S and COI trees, *M. segerstralei* for EPRS tree, both species in combined dataset) were found in GenBank. Sequences obtained in this study were aligned using the MUSCLE algorithm integrated in MEGA 7.0 with default settings (Kumar et al., 2016).

Models of nucleotide evolution were estimated using ModelGenerator (Keane et al., 2006). The GTR + G + I model, as the best choice for all datasets by LnL criterion, was used for phylogenetic calculations. Maximum likelihood phylogenetic trees were built in RAxML 8 (Stamatakis, 2014) and with Bayesian analysis using MrBayes 3.2.6 (Ronquist et al., 2012). In Bayesian analysis, 1,000,000 generations were requested. Convergence was monitored with Tracer (Rambaut, Drummond, Xie, Baele, & Suchard, 2018) and was reached in all MrBayes runs (avg S.D. of split frequencies < 0.01, effective sample size > 200). All saved trees except for the first 20% (relburnin = yes burninfrac = 0.2) were used for consensus calculation.

Hypothesis testing

To statistically assess the paraphyly of *H. lamornae* we performed Kishino-Hasegawa (KH), Shimodairo-

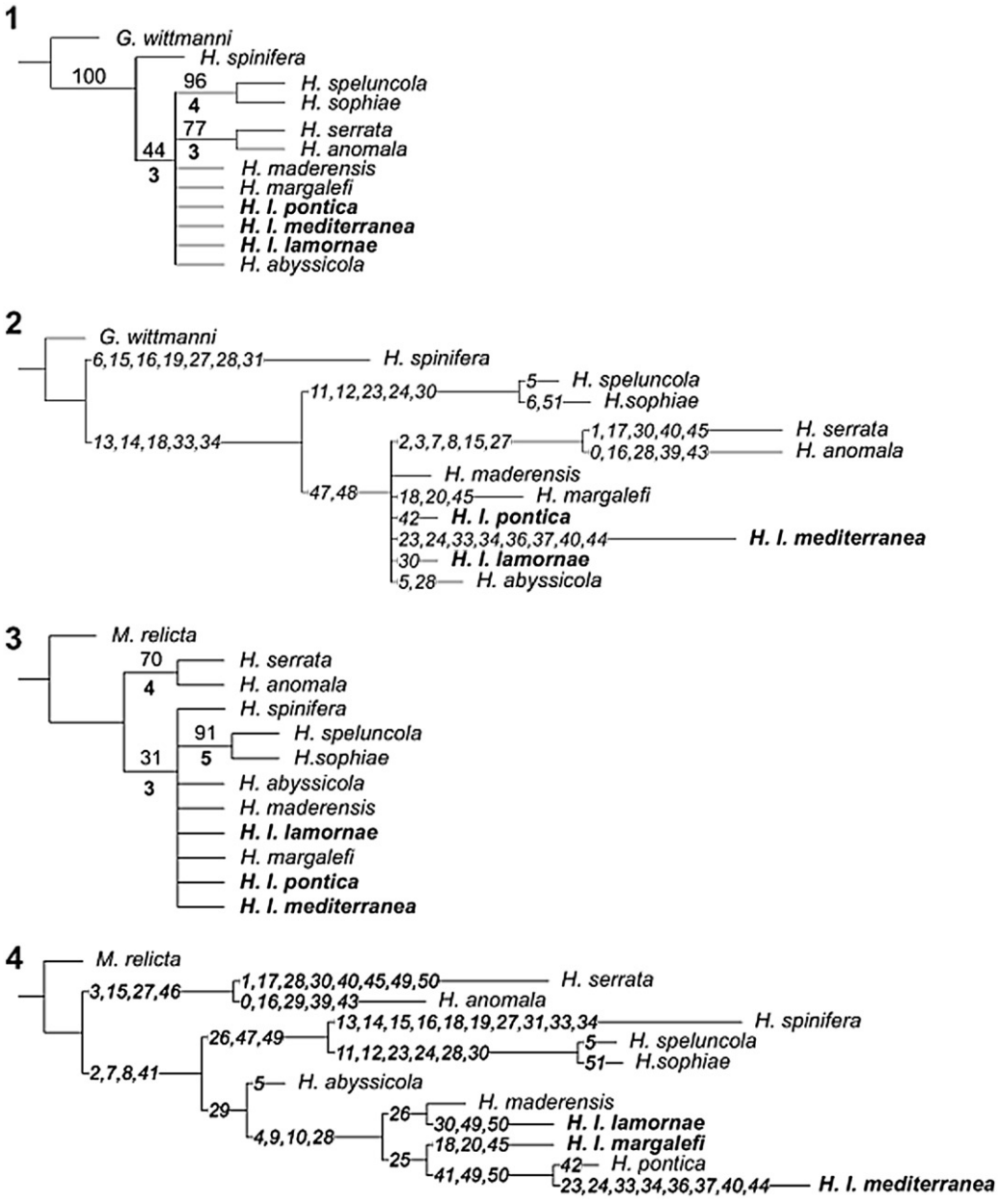

Fig. 4. Phylogenetic trees of the genus *Hemimysis* based on analyses of morphological characters. 1 & 3 – strict consensus trees with Bootstrap support >75 (numbers above the lines) and Bremer support ≥3 (numbers under the lines); 2 & 4 the same morphological trees with synapomorphies. Morphological characters and their states are in Table S2 (see supplemental material online). The subspecies of *Hemimysis lamornae* are in **bold**.

Hasegawa (SH), and unbiased (AU) tests of alternative tree topologies (Kishino & Hasegawa, 1989; Shimodaira, 2002; Shimodaira & Hasegawa, 1999). The tests were implemented in IQ-TREE Web (Trifinopoulos, Nguyen, von Haeseler, & Minh, 2016). Alternative tree topologies were drawn manually in MEGA 7.0 based on the Bayesian trees for each of the analysed datasets (18S, COI, EPRS, and concatenated) by moving either *H. l. lamornae* branch to the base of *H. l. pontica* or vice versa (tested topologies are in File S1, see supplemental material online).

To substantiate the species status of *H. l. pontica* we applied species delimitation analysis using ABGD (Automatic Barcoding Gap Detection) (Puillandre, Lambert, Brouillet, & Achaz, 2012) and bPTP (Bayesian Poisson Tree Process) (Zhang, Kapli, Pavlidis, & Stamatakis, 2013).

For analysis of the population structure of *H. l. pontica*, we built the haplotype network in PopArt

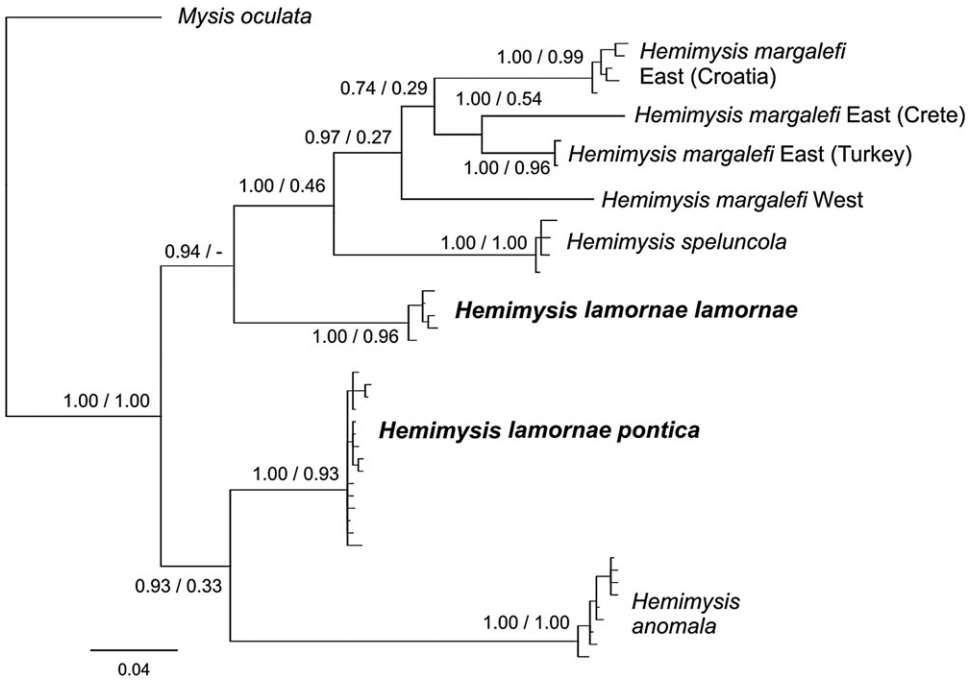

Fig. 5. Phylogenetic tree of the genus *Hemimysis* based on mitochondrial COI gene fragment. 50% majority consensus of 800 Bayesian trees is shown. ML tree on the same dataset have identical topology except the position of the *H. l. lamornae* as sister group of *H. lamornae pontica* + *H. anomala.* Fractions at nodes show Bayesian posterior probabilities (BPP) in numerator and bootstrap support in denominator. BPP and bootstrap support values for intra-specific nodes are not shown. Subspecies of *Hemimysis lamornae* are in **bold**.

using the median-joining method (Leigh & Bryant, 2015). Tajima's *D* statistics were calculated in PopArt (Tajima, 1989).

Results

Morphological data

Phylogeny of *Hemimysis*. Morphological analysis 1 with *G. wittmanni* as the outgroup retrieves seven minimal length trees of length 92 (Figs 4.1 and 4.2). *Hemimysis spinifera* is at the base of the tree and followed by two sister clades (*H. speluncula* + *H. sophiae*) and the rest of the genus. Within the rest, only two clades are statistically robust: (1) *H. speluncula* + *H. sophiae* and (2) *H. serrata* + *H. anomala* (Fig. 4.1).

Morphological analysis 2 with *M. relicta* as the outgroup retrieves one minimal length tree of length 90 (Figs 4.3 and 4.4). As in analysis 1, only two clades are statistically robust: (1) *H. speluncula* + *H. sophiae* and (2) *H. serrata* + *H. anomala.*

Morphological trends in *Hemimysis*. In both morphological analyses *Hemimysis spinifera* presents three groups of autapomorphic characters: a serration of the telson (character or char. 6), spination of the antennal scale (char. 15, 16, 19), and segmentation of the thoracopodal carpo-propodi (char. 27, 28) and of pleopodal endopodite (char. 31) (Table S2, see supplemental material online).

The main clade of *Hemimysis* (except *H. spinifera*) is supported by two groups of synapomorphies: lanceolate shape of antennal scale, naked posterior half of outer ridge of the scale (char. 13, 14, 18) and non-segmented endopodite of pleopod IV (char. 31).

H. speluncula + *H. sophiae* is a statistically supported species group in both analyses and is supported by the rounded distal lobes of the telson (char. 11, 12), the triangle shape of the distal segment of the maxillar palp (char. 23, 24) and the rudimentary exopodite of the third pleopod (char. 30).

The species group *H. serrata* + *H. anomala* is supported in both analyses by such synapomorphies as the serration of telson (less than 20 teeth on the posterior margin) (char. 3), an absence of the articulate spines on the outer margin of the antennal scale (char. 15) and 5-segmented carpo-propodi of the III–VIII thoracopods (char. 27).

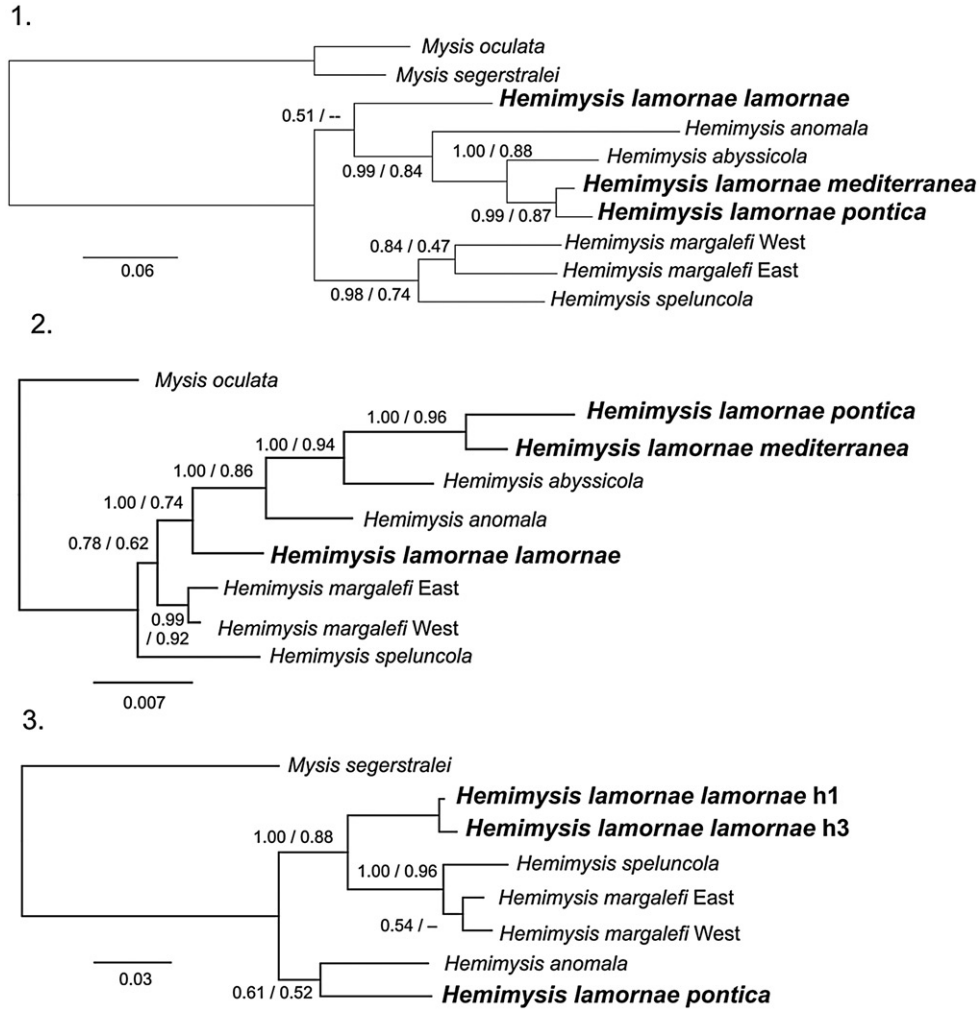

Fig. 6. Phylogenetic trees (MrBayes and RAxML) of the genus *Hemimysis* based on concatenated alignments of 18S, EPRS and COI (1), 18S (2) and EPRS (3). 1–50% majority consensus of 800 Bayesian trees built using concatenated alignments of 18S, EPRS, and COI of the genus *Hemimysis.* ML trees on the same dataset have identical topology except the position of the *H. l. lamornae* as sister group of *H. margalefi* + *H. speluncola.* 2–50% majority consensus of 800 Bayesian trees of the genus *Hemimysis* 18S rDNA genes. ML trees on the same dataset have identical topology. 3–50% majority consensus of 800 Bayesian trees of *Hemimysis* EPRS genes. ML trees on the same dataset have identical topology except the unresolved trichotomy of the *H. speluncola* and two subclades of *H. margalefi.* Fractions at nodes show Bayesian posterior probabilities (BPP) in numerator and bootstrap support in denominator. Subspecies of *Hemimysis lamornae* are in **bold**.

Molecular data

Molecular phylogeny of *Hemimysis*. Bayesian analysis of the combined three-gene dataset (Fig. 6.2) recovers two well-supported (Bayesian posterior probability (BPP)>0.8) clades *H. speluncola* + *H. margalefi* and *H. anomala* + *H. abyssicola* + *H. l. pontica* + *H. l. mediterranea. H. l. lamornae* is recovered as a sister to the latter clade, albeit with weak support (BPP = 0.49). In contrast, ML analysis recovers *H. l. lamornae* as sister to the *H. speluncola* + *H.margalefi* clade, albeit with little support (35% bootstrap).

In both Bayesian and ML trees, *H. anomala* and *H. abyssicola* are branching between *H. l. lamornae* and *H. l. pontica* with high support inconsistent with the monophyly of *H. lamornae*.

Single gene trees (Figs 5, 6.2, and 6.3) recover slightly different topologies from each other and the concatenated dataset. However, all trees show affinity of *H. l. pontica* with *H. anomala* and *H. abyssicola*, but not with *H. l. lamornae*. The position of *H. l. lamornae* is unstable. It is either sister to the clade *H. speluncola* + *H. margalefi* (ML concatenated dataset, Bayesian COI tree, both EPRS trees), or sister to the clade combining *H. anomala*, *H. abyssicola*, and *H. l. pontica* (Bayesian concatenated dataset, ML COI tree, and both 18S trees).

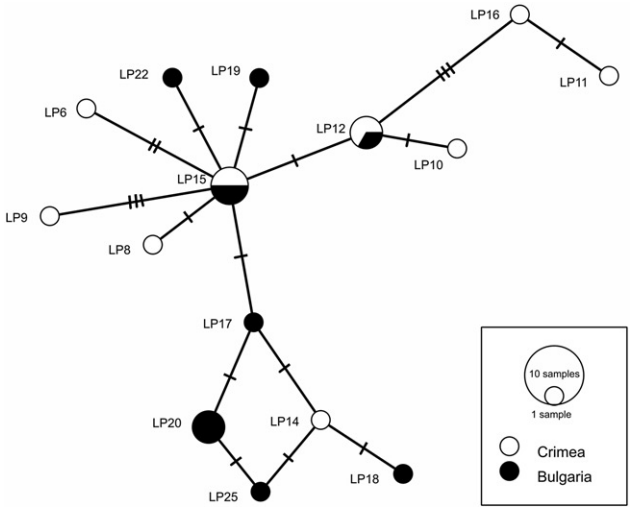

Fig. 7. Median-joining network of COI haplotypes for *Hemimysis pontica* Czerniavsky, 1882. Circle size depicts haplotype frequency, notches on lines – number of substitutions between haplotypes. Black indicates specimens from Bulgaria, white from Crimea.

Only one sequence of 18S rDNA is available for *H. l. mediterranea* placed as the sister group of *H. l. pontica* (Fig. 6.2).

The alternative tree topology tests assuming monophyly of *H. lamornae* rejected monophyly of *H. lamornae*, $P < 0.1$ for 18S, $P < 0.05$ for EPRS and combined three-gene dataset. COI data show no significant rejection of any of the tested alternative topologies.

Molecular taxonomy of *Hemimysis lamornae*. ABGD and PTP methods of molecular species delimitation used for analysis of COI sequences show significant distinction between *H. l. lamornae* and *H. l. pontica* as well as between these two subspecies and other species of the genus *Hemimysis*. The interspecies DNA barcoding gap in ABGD is 15%. The partition support values in PTP analysis are 0.94 and 0.98 for *H. l. lamornae* and *H. l. pontica*, respectively.

Only one 18S rDNA sequence is available for *H. l. mediterranea.* It is 1.0% different from 18S rDNA of *H. l. pontica*. This difference is within the range of interspecific distances of the genus *Hemimysis* (0.5–2.9%). The intraspecific diversity of 18S rDNA in the analysed sequences is low, not more than 0.12% (one substitution per analysed 823 bp fragment). See sequence divergences (Tables S4–S6, see supplemental material online).

Intraspecific diversity in *Hemimysis lamornae pontica*. Twenty-two specimens from the Black Sea have 15 haplotypes of the COI fragment. Three haplotypes are in more than one animal; two of these groups are present in both Bulgaria and Crimea (1 – LP12, LP13, LP23, 2 – LP7, LP15, LP21, LP26). The median-joining haplotype network shows no sign of genetic isolation between mysids living in marine caves of Crimea and Bulgaria (at an approximate distance of 370–400 km) (Fig. 7).

Tajima's *D* statistic ($D = -1.80331$, $P = 0.974784$) is below zero and indicates an absence of mutational-drift equilibrium (Tajima, 1989).

Combined results of molecular and morphological analyses

Molecular analyses result in paraphyletic split of *Hemimysis lamornae* into the two clades: (1) *H. l. lamornae* and (2) *H. l. pontica* + *H. l. mediterranea.* Few molecular data and morphological data on *H. l. mediterranea* suggest that *H. l. mediterranea* and *H. l. pontica* are sister taxa. Significant differences in COI sequences of *H. l. lamornae* from the English Channel, *H. l. pontica* from the Black Sea and other species of *Hemimysis* indicate that both subspecies are distinct species. The 0.1% difference between *H. l. pontica* and its sister *H. l. mediterranea* based on fragments of 18S is within the range of interspecific distances of the genus *Hemimysis.* The results of both molecular and morphological analyses suggest the validity of *Hemimysis pontica* Czerniavsky, 1882 and change of the subspecies status of *H. l. mediterranea* to the species one: *Hemimysis mediterranea* Bacescu, 1936 stat. nov.

Classification

Hemimysis G.O. Sars, 1869

Synonymized names: *Mysis aurantia* G.O. Sars, 1864 (synonym); *Mysis lamornae* Couch, 1856 (basionym).

Diagnosis

Carapace with very short triangular rostrum; posterior margin emarginate. Telson small and narrow, either with a cleft which is armed with a close row of teeth or truncate, with or without a slight median emargination. Antennal scale rather small, broadly lanceolate; proximal portion of the outer margin naked, the unarmed portion not ending in a tooth; remaining part of inner margin and outer margin armed with plumose setae. Distal segment of maxillar palp broad and flattened. Endopods of the thoracic limbs moderately long with combined carpopropodus divided into four to five subsegment; dactylus with a strong nail. Female pleopods rudimentary, male pleopods reduced; third pair with large sympod, simple unsegmented or two-segmented endopod and the exopod, if present, extremely small and knob-like; fourth pair with a two-segmented

sympod; endopod small, two-segmented; exopod long, five- or six segmented with two long modified setae, on the distal segments; fifth pair well developed biramous, multi-articulate and natatory. Uropod long and narrow; inner margin of endopod armed with a row of spines. Marsupium consisting of a pair of large brood lamellae on each of the seventh and eighth thoracic segments and a very small anterior pair on the sixth segment.

Type species. *Hemimysis abyssicola* G.O. Sars, 1869

Species included. *Hemimysis abyssicola* G.O. Sars, 1869; *Hemimysis anomala* G.O. Sars, 1907; *Hemimysis lamornae* (Couch, 1856); *Hemimysis maderensis* Ledoyer, 1989; *Hemimysis margalefi* Alcaraz, Riera & Gili, 1986; *Hemimysis serrata* Bacescu, 1938; *Hemimysis sophiae* Ledoyer, 1989; *Hemimysis speluncola* Ledoyer, 1963; *Hemimysis spinifera* Ledoyer, 1989; *Hemimysis pontica* Czerniavsky, 1882; *Hemimysis mediterranea* Bacescu, 1936 stat. nov.

Key to species of the genus *Hemimysis*

1. Telson with less than 20 teeth on posterior margin, lateral spines along the entire length of telson (Fig. 3.9) 2
– Telson with 20 or more teeth on posterior margin, lateral spines only in distal part of telson 3
2. Sinus on telson absent ..... *H. anomala* Sars, 1907
– Sinus on telson present ..... *H. serrata* Bacescu, 1938
3. Distal lobes of telson rounded 4
– Distal lobes of telson angular (Fig. 3.9) 5
4. Telson with 38–56 teeth on posterior margin; uropodal endopod with 8–14 spines. ..... *H. speluncula* (Krøyer, 1859)
– Telson with more than 70 teeth on posterior margin; uropodal endopod with 15–22 spines ..... *H. sophiae* Ledoyer, 1989
5. Telson with 60 or more teeth on posterior margin; antennal scale subrectangular, with articulate spines on outer ridge; uropodal endopod with continuous row of spines ..... *H. spinifera* Ledoyer, 1989
– Telson with less than 60 teeth on posterior margin; antennal scale lanceolate, without articulate spines on outer ridge; uropodal endopod with two separate groups of spines 6
6. Telson with 40 or more teeth on posterior margin and 12 or more lateral spines on each side … *H. abyssicola* Sars, 1869
– Telson with less than 40 teeth on posterior margin and less than 12 lateral spines on each side 7
7. Antennal scale not setose along about half length of the outer ridge (Fig. 2.3); uropodal endopod with 8–14 spines 8
– Antennal scale not setose along about 0.8 or 0.2 of the outer ridge; uropodal endopod with 0–7 spines …. 10
8. Maxillar palp with distal segment subcirculate/subtriangular; fourth pleopod with non-segmented endopod and 5-segmented exopod; fifth pleopod with 4-segmented endopod and 4-segmented exopod …… *H. mediterranea* Bacescu, 1936 stat. nov.
– Maxillar palp with distal segment enlarged and oval; fourth pleopod with two-segmented endopod (Fig. 3.5) and 6-segmented exopod (Fig. 3.5); fifth pleopod with 5- or 6-segmented endopod and 6-segmented exopod (Fig. 3.5) 9
9. Maxillar palp with distal segment bearing 16 or more modified setae; fifth pleopod with 5-segmented endopod ..... *H. lamornae* (Couch, 1856) (Figs 8.1 and 8.2)
– Maxillar palp with distal segment bearing 3–4 or less modified setae (Fig. 2E); fifth pleopod with 6-segmented endopod …… *H. pontica* Czerniavsky, 1882 (Figs 8.3 and 8.4)
10. Antennal scale not setose along about 0.2 of the outer ridge; maxillar palp with distal segment bearing less than 8 modified setae; thoracopods III–VIII with 3-segmented carpo-propodus; fifth pleopod with 5-segmented exopod ..... *H. margalefi* Alcaraz *et al*., 1986
– Antennal scale not setose along about half of the outer ridge; maxillar palp with distal segment bearing 14–17 modified setae; thoracopods III–VIII with 4-segmented carpo-propodus; fifth pleopod with 6-segmented exopod ..... *H. maderensis* Ledoyer, 1989

Redescription of *Hemimysis pontica* Czerniavsky, 1882

Class Malacostraca
Order Mysida
Family Mysidae Haworth, 1825
Hemimysis G.O. Sars, 1869
Hemimysis pontica Czerniavsky, 1882

Synonymy: *Hemimysis lamornae* Norman, 1892: 247; Hemimysis *lamornae reducta* Bacescu, 1936: 71; *Hemimysis lamornae pontica* Bacescu, 1949: 13.

Material examined: 65 specimens from Crimea and Bulgaria (Table S1, see supplemental material online).

Body length of mature individuals 9–10 mm from the anterior margin of the carapace to the end of the telson (Figs 8.3 and 8.4). Carapace short, thoracic segments VI and VII exposed dorsally. Anterior margin of the carapace forms a broad angle rostrum reaching the base of the eye stalks (Fig. 2.1). Cervical groove well defined.

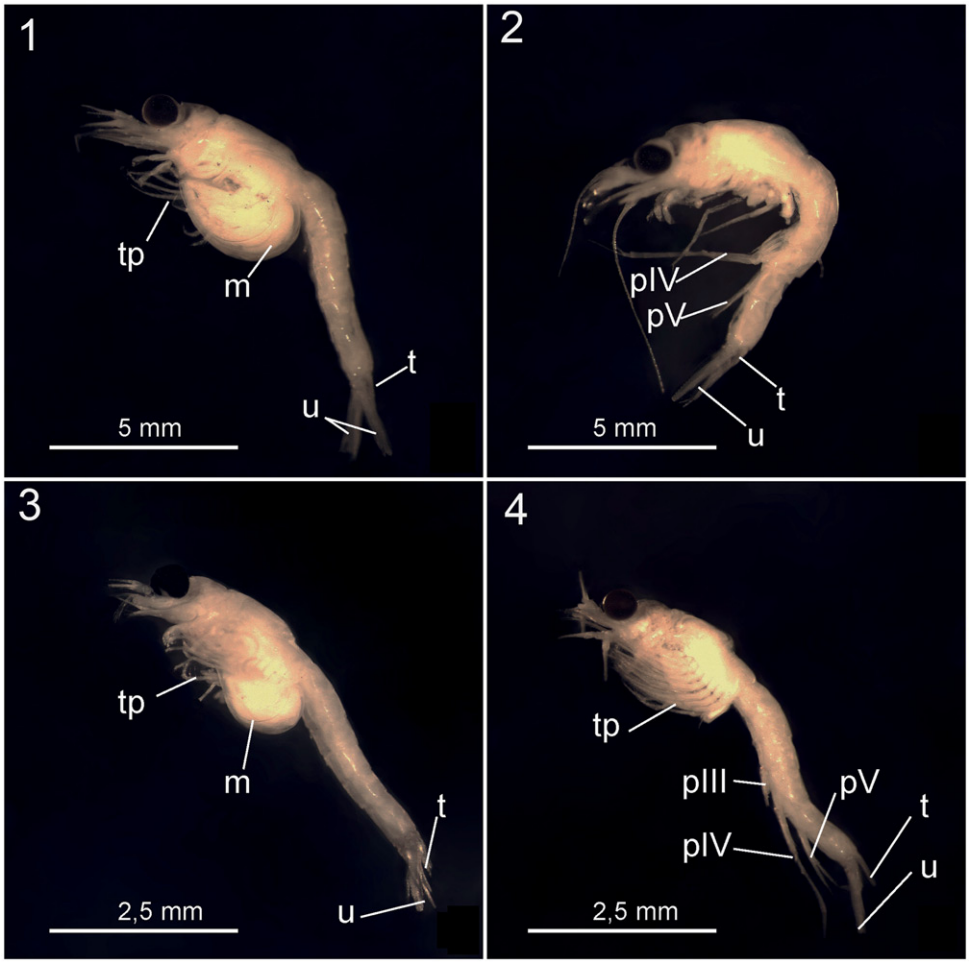

Fig. 8. Lateral view of female and male of *Hemimysis lamornae* (1, 2) and *Hemimysis pontica* Czerniavsky, 1882 (3, 4). 1, 3 – females, 2, 4 – males. Abbreviations: m – marsupium; pIII–V – pleopods III–V, t – telson; tp – thoracopod; u – uropode.

Abdominal somites I–V approximately equal; fourth segment two times as long as fifth one. Telson 1.7–2 times as long as wide; width of the distal part two times lesser than the broadest part. The distal half of the lateral margin armed with 5–10 lateral spines. Cleft of telson well defined, its depth is 1/10 the length of the telson, armed with 16–24 small teeth; each distal corner of telson bearing one apical spine.

Eyes large and bean-shaped in dorsal view, cornea pigment black. The third segment of antennula bearing an appendage with a row of long thin setae. Antennal scale lanceolate, consists of two segments; about 3.5 times as long as broad in the broadest part (Fig. 2.2). The outer margin straight, rounded in the distal third and armed with setae of different length. The inner margin of the scale fringed with setae over its entire length. Distal segment is short and armed with five setae.

Mandible is similar to those of the other species of the genus. The first segment of the palp small, second and third segments armed with short hard setae. Maxillule with endopod bearing seven terminal spines and four subterminal plumose setae (Fig. 2.3). Maxilla with terminal article of the endopod oval, widened distally and armed with setae and 5–6 spines alternating with setae (Fig. 2.4).

First thoracopod consists of six segments. Inner margin of the endopod is densely covered with setae. Endopod of second thoracopod bearing less setae than first thoracic limb. Third thoracic limb with 3-segmented carpo-propodus. Male pleopods I–II rudimentary and have the form of simple undifferentiated plates armed with setae. Pleopods III two-segmented, uniramous, without exopod. Pleopods IV with two-segmented endopod; small 6-segmented exopod many times longer than endopod. Pleopods V biramous, exopod and endopod consist of six segments. Pleopods of females small, rudimentary and have form of simple unsegmented plates, similar to the male pleopods of

the first two pairs. Exopod of uropod long, 4.5 times as long as wide in the broadest part. Endopod shorter than exopod, its length is equal to 4/5 of the exopod length. Endopod armed with long setae on the outer and inner margins from the statocyst to distal end (Fig. 2.5). A single spine at the inner margin near the statocyst present.

Intraspecific variation appears in varying number of lateral spines and cleft teeth. Number of lateral spines ranging from 5–9 in females and from 5–10 in males. Number of cleft teeth is 16–24 in females and 21–24 in males. Length of apical spines 3–3.5 times as long as lateral spines. Number of lateral spines and cleft teeth may be unequal at the right and left sides of the telson.

Discussion

The results of both molecular and morphological analyses suggest the validity of the *Hemimysis pontica* Czerniavsky, 1882 and change of the subspecies status of *H. l. mediterranea* to the species one: *Hemimysis mediterranea* Bacescu, 1936 stat. nov.

DNA analysis of *Hemimysis pontica* did not reveal genetic isolation of the mysids collected in caves and grottoes of Crimea and Bulgaria, as it was recently shown for the Mediterranean mysid *H. margalefi*, representing a group of geographically isolated cryptic species (Rastorgueff et al., 2014; Rastorgueff & Bianchimani, 2016). The genetic homogeneity of *H. pontica* can be explained by the features of the Black Sea coastline as well as the currents providing active gene exchange between the mysids living in caves of the analysed areas (Korotaev, Oguz, Nikiforov, & Koblinsky, 2003; Stanev, He, Staneva, & Yakushev, 2014; Zatsepin et al., 2003; Stanev et al., 2014). The genetic homogeneity may also result from population bottleneck effects that could have occurred during the expansion of *H. pontica* in the Black Sea (Allendorf, England, Luikart, Ritchie, & Ryman, 2008). The data on genetic homogeneity are based on the analysis of a relatively small amount of animals due to the lack of information for other areas of the Black and Azov Seas, where the marine grottos and caves remain unknown and unexplored.

Morphological analysis of species and subspecies of *Hemimysis* did not show statistically supported clades, including two or three subspecies of *H. lamornae*. We conclude that morphological data provide no basis for transferring the two species, *H. lamornae* and *H. pontica*, into *H. lamornae*, as well as description of *H. l. mediterranea* as a subspecies of *H. lamornae* (Bacescu, 1936).

Some of the auto- and synapomorphies of *Hemimysis* may be adaptive to habitats. Such characters as shape, serration, and spination of the telson and antennal scale may provide various types of defence. The shape of the distal segment of the maxillary palp may fit the size and type of the food particles. Finally, segmentation of the thoracopodal carpo-propodi and of the pleopodal exopod may provide a fine adjustment to movement within the caves.

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Disclosure statement

The authors declare that they have no conflict of interest.

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Supplemental data

Supplemental data can be accessed here: http://dx.doi.org/10.1080/14772000.2019.1596175.

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