

Original Article

Deep molecular divergence and shallow morphological differentiation: three new pseudocryptic *Gammarus* (Crustacea: Amphipoda) species from the Carpathian region

Luiz F. Andrade^{1,*}, Denis Copilaş-Ciocianu², Tomasz Mamos¹, Michał Grabowski¹

¹Department of Invertebrate Zoology and Hydrobiology, Faculty of Biology and Environmental Protection, Łódź 90-237, Poland

²Laboratory of Evolutionary Ecology of Hydrobionts, State Scientific Research Institute Nature Research Centre, Vilnius LT-08412, Lithuania

*Corresponding author. Department of Invertebrate Zoology and Hydrobiology, Faculty of Biology and Environmental Protection, Banacha 12/16, Łódź 90-237, Poland.
E-mail: luiz.felipe.de.andrade@biol.uni.lodz.pl

ABSTRACT

Freshwater amphipods often include highly divergent molecular lineages with limited morphological variation, resulting in many reported cryptic species. However, most of these cases rely solely on mitochondrial markers, lacking nuclear and morphological characters. This study integrates multilocus phylogenetics and morphological examination to describe three new species in the *Gammarus balcanicus* complex from Romania. *Gammarus balcanicus* consists of narrowly endemic lineages spanning south-eastern Europe to Central Asia, many of which remain undescribed due to morphological stasis. The newly described species, *Gammarus carausui* sp. nov., *G. dobrogicus* sp. nov., and *G. pinkfloydorom* sp. nov., show significant divergence at mitochondrial and nuclear levels, along with consistent, yet subtle morphological differences compared to each other and to topotypical *G. balcanicus* from Montenegro. These species diverged in the Late Miocene, pre-dating Quaternary glaciations. Despite extensive sampling, two are known only from their type localities in the Carpathians, while *G. dobrogicus* is endemic to the Dobrogea plateau. Considering global threats to freshwater habitats, these species may already be endangered. This work highlights the role of molecular tools in resolving cryptic species' taxonomy and highlights the Carpathians as a crucial refugial region for relict pre-Quaternary fauna.

Keywords: integrative taxonomy, phylogeography, cryptic diversity, biodiversity hotspot

INTRODUCTION

The geological history of Europe has left an impact on its freshwater biota. Its dynamic past shaped by the formation of the continent and the regression of the Tethys Ocean and Paratethys Sea influenced the composition and diversification of its freshwater taxa (Banarescu 1992, Popov *et al.* 2004, Hou *et al.* 2011, McInerney *et al.* 2014). Given this, it is important to consider the impact of local sea-level fluctuations and the emergence of new land masses, as well as the flooding of certain areas, which have played significant roles in speciation processes (Banarescu 1992, McInerney *et al.* 2014). The Pleistocene glaciations wielded a vast influence on Europe's biodiversity, leading to the refuge and diversification of numerous taxa in the southern European refugia (Hewitt 1999, 2004).

The Romanian territory is crossed by the predominant extension of one of the major mountain ranges in Europe, the Carpathians. The region is recognized as a diversity hotspot and by a

substantial role in the formation and Pleistocene glaciation survival of several taxa (Schmitt 2007, Bálint *et al.* 2011). Amphipods encompass a diverse epigeal freshwater fauna in the country, including *Synurella ambulans* (F.Müller, 1846), *Niphargus hrabei* S.Karaman, 1932, *N. valachicus* Dobrea and Manolache, 1933, and 10 species of the genus *Gammarus* Fabricius, 1775: *G. arduus* G.Karaman, 1975, *G. balcanicus* Schäferna, 1922, *G. dacicus* Dobrea and Manolache 1942, *G. fossarum* Koch in Panzer, 1836, *G. hamaticornis* Copilaş-Ciocianu *et al.*, 2018, *G. kischinefensis* Schellenberg, 1937, *G. komareki* Schäferna, 1922, *G. leopoliensis* Jazdzewski and Konopacka, 1989, *G. pulex* Linnaeus, 1758, and *G. roeselii* Gervais, 1835 (Copilaş-Ciocianu *et al.* 2014, 2018).

Gammaridae Leach (1814) is a diverse family of amphipods inhabiting springs, rivers, streams, lakes, lagoons, and marine littoral zones (Hou and Sket 2016). It encompasses 42 genera, with the genus *Gammarus*, exhibiting the highest level of species'

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richness and diversity. Widely distributed in the Holarctic, *Gammarus* comprises at least 284 species, and, although predominantly concentrated in Europe, its distribution extends to other regions such as North America in the west and South Korea in the east (Horton *et al.* 2025, Väinölä *et al.* 2008). Despite the high number of species and broad distribution, most of its diversity was described relying only on morphological traits, resulting in several taxonomic inaccuracies that have persisted for decades.

In an extensive taxonomic review of the genus from European freshwaters, Karaman and Pinkster (1977a, b, 1987) established three artificial groups of *Gammarus*, i.e. *balcanicus*-, *pulex*-, and *roselii*-group. This division was based mainly on morphological characters such as the presence/absence of dorsal carina and setal density on pereopods 3–4 and uropod 3. Although it was an essential step for the systematics of the genus, many previously described species were synonymized, assuming that some phenotypic differences were intraspecific variations.

According to Rudolph *et al.* (2018), it is necessary to conduct a comprehensive reassessment of morphological traits and their variability in *Gammarus* to redefine diagnostic characteristics for species' identification. Additionally, it is crucial to revisit existing literature and interpret these observations using modern molecular techniques. Recent studies using integrative approaches have successfully demonstrated the presence of cryptic diversity within *Gammarus* (Grabowski *et al.* 2017, Copilaş-Ciocianu *et al.* 2018, Hupalo *et al.* 2018, Mamos *et al.* 2021). Such an integrative approach to disentangle *Gammarus* taxonomy is crucial, provided the genus is commonly used in biomonitoring and ecotoxicological essays (e.g. Kunz *et al.* 2010). It has already been shown that presumed cryptic *Gammarus* species occupy different ecological niches (Kabus *et al.* 2023, Bystřický *et al.* 2025), and that different evolutionary histories may significantly determine cryptic species' ecology and sensitivity to pollutants, which impacts the outcomes of environmental risk assessments if the studied lineages are not correctly identified (Jourdan *et al.* 2023). Mamos *et al.* (2016) provided evidence indicating that divergence within the *G. balcanicus* group occurred during the Early Miocene (c. 19 Mya) in the Central Balkans, when the Paratethys Sea covered the region. It is assumed that habitat shifts during this period may have resulted in the separation and diversification of the *G. balcanicus* clade from other *Gammarus* lineages. Previous molecular investigations have revealed numerous cryptic lineages within this complex, particularly in the Carpathians (Mamos *et al.* 2016, Copilaş-Ciocianu and Petrusek 2017). Furthermore, one of the Carpathian lineages was resurrected and redescribed as *G. tatrensis* (S.Karaman, 1931), and another one was recently described as a new species as *G. stasiuki* Jażdżewski *et al.*, 2021 (Mamos *et al.* 2021).

Given that the Carpathian Arc is recognized as a significant European biodiversity and endemism hotspot and a crucial extra-Mediterranean glacial refugium for a plethora of both terrestrial and aquatic animal and plant species (Mráz and Ronikier 2016), it is reasonable to assume that its biodiversity is far from being fully documented. Consequently, further discoveries of new species can be expected in the area. Considering the phenomenon of morphological stasis commonly observed in *Gammarus*, Mamos *et al.* (2021) hypothesized that several highly divergent lineages of *G. balcanicus* in the Carpathians may represent cryptic or pseudo-cryptic species. In the light of this hypothesis, our paper undertakes an integrative, morphological, and molecular multimarker,

taxonomic re-examination of three cryptic, genetically divergent lineages of the *G. balcanicus* complex. Restricted to the Carpathian Arc and the adjacent Dobrogea Plateau, these lineages represent potentially undescribed species, which we formally delineate and name herein, as well as reveal their spatio-temporal onset and phylogenetic relationships with other lineages, contributing to a finer-scale understanding of biodiversity and evolutionary history in this eminent European biodiversity hotspot.

MATERIALS AND METHODS

Sample collection and data acquisition

The sampling sites of the *loci typici* of the newly described species are shown in Figure 1. The material was collected between 2005 and 2019, during several scientific expeditions organized by Michał



Figure 1. *Loci typici* of the newly described species: A, *Gammarus carausui* sp. nov., stream close to Vărciorog, Romania, 15 March 2011; *Gammarus pinkfloydorum* sp. nov., Raul stream, Prăpăstiile Zărneștilor, Zărnești, Piatra Craiului, Romania, 29 August 2005; *Gammarus dobrogeicus* sp. nov., cheshma in Șipotetele, Romania, 6 September 2007. Photos by Tomasz Mamos (A), Michał Grabowski (B, C).

Grabowski and Tomasz Mamos from the Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Poland, and, between 2011 and 2019, by Denis Copilaş-Ciocianu from the Nature Research Center, Lithuania (Fig. 2A). A list of all specimens and sampling points is given in the BOLD dataset DS-ROGAMBAL (<https://dx.doi.org/10.5883/DS-ROGAMBAL>). The material was gathered from a variety of fluvial habitats using a benthic hand-net and preserved in 96% ethanol at room temperature. The majority of data used in this paper comes from already published studies done by Copilaş-Ciocianu *et al.* (2017) and Mamos *et al.*

(2016, 2021), the list of all sequences with respective GenBank codes is given in DS-ROGAMBAL (<https://dx.doi.org/10.5883/DS-ROGAMBAL>).

Morphological analysis

For the taxonomic study, appendages and mouthparts of selected individuals were dissected under a Nikon SMZ1270 stereo-microscope and mounted on glass slides with Euparal. The slides were photographed on a Nikon Eclipse Ci microscope coupled with a camera DLT Cam Pro SMP using the software Delta

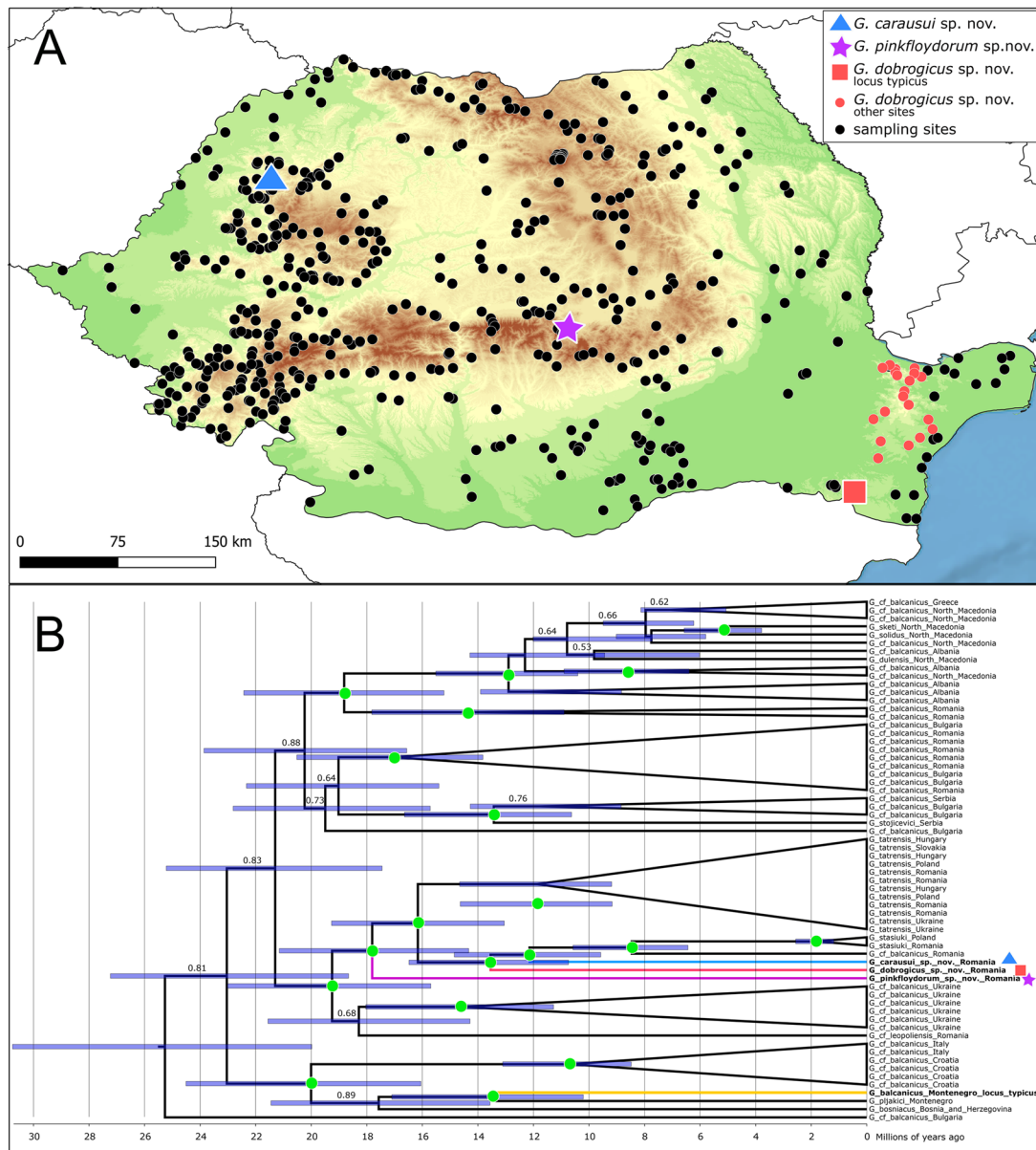


Figure 2. A, map of Romania indicating the distribution of the newly described species. Blue triangle—*Gammarus carausui* sp. nov.; purple star—*Gammarus pinkfloydorum* sp. nov.; red square—*Gammarus dobrogicus* sp. nov.. The red dots indicate other sites where *G. dobrogicus* sp. nov. was found. Black dots indicate sampling localities where other epigeic freshwater amphipods were sampled (mainly *Gammarus* spp.; data from: Copilaş-Ciocianu *et al.* 2014, Mamos *et al.* 2016, this study). B, time-calibrated phylogeny based on COI, 16S, and 28S. Green dots at nodes indicate strong support (PP ≥ 0.95), numbers indicate moderately supported clades (PP = 0.94–0.5), while unannotated nodes are weakly supported. Blue bars indicate estimated age credibility intervals. The newly described species are highlighted with symbols and colours corresponding to the map. The type locality lineage of *G. balcanicus* (occurring in Montenegro) is highlighted in yellow. Non-focal clades were collapsed for simplicity.

Optical DLT-Cam, and digitally prepared with CorelDRAW Graphics Suite 2018. Habitus photographs of the specimens were captured with a Leica M205 C stereo-microscope using the software LAS X. The examined type materials are stored in the Museum and Institute of Zoology Polish Academy of Sciences (MIIZ PAN). All the materials other than holotypes and paratypes (if not indicated otherwise) are deposited in the collection of the Department of Invertebrate Zoology and Hydrobiology, University of Lodz. Morphological intraspecific variability is found among individuals of the newly described species, which may reflect different maturity stages (Karaman and Pinkster 1977a, b, 1987). These differences can be observed mainly in the number of articles in the flagella of antennae 1–2, setal arrangements in gnathopods, pereopods, and uropods, and length ratio between outer and inner rami of uropod 3.

DNA extraction, polymerase chain reaction amplification, and sequencing

Except for previously published data we have used sequences from newly collected specimens. The DNA was extracted from a total of 14 individuals using the Chelex non-boiling method (Casquet et al. 2012) or the Quick-DNA Miniprep Plus Kit (Zymo Research). Two mitochondrial markers, including fragments of the cytochrome *c* oxidase subunit I (*COI*, c. 650 bp) and 16S ribosomal RNA (c. 350 bp), as well as one nuclear marker: fragment of 28S ribosomal RNA (c. 900 bp), were chosen for amplification, the polymerase chain reaction (PCR) procedure, chemistry, and primers used follow the procedures from Mamos et al. (2016). Sequencing of the PCR products was performed using BigDye terminator technology by Macrogen Inc., Korea. The nuclear marker was sequenced in both directions.

Molecular data analysis, phylogenetic reconstruction, and molecular clock dating

All newly generated data were combined with those previously published, representing the same phylogenetic clade according to Mamos et al. (2016). The sequences together with metadata are publicly available in the portal Barcode of Life Datasystem (BOLD, <https://boldsystems.org/>) dataset: DS-ROGAMBAL (<https://dx.doi.org/10.5883/DS-ROGAMBAL>). The molecular distances and barcode index numbers (BINs), equivalents of molecular species (Ratnasingham and Hebert 2013), were calculated and assigned within BOLD.

To ensure that the new species represent separate evolutionary lineages, we reconstructed maximum likelihood phylogenetic trees for each marker using RAxML (Stamatakis 2014). The substitution models were selected through ModelTestNG (Darriba et al. 2020). A thorough bootstrap with an auto MRE algorithm was used, to visualize the possible conflicts, and trees were plotted against each other using the *cophylo* function in R package APE 5.7-1 (Paradis and Schliep 2019) (Supporting Information, Fig. S1).

To visualize phylogenetic relationships through time, we reconstructed phylogeny, using all three markers set as separate partitions in BEAST 2.7.7 (Bouckaert et al. 2019) employing molecular clock dating. The substitution models for each marker were set with bModelTest (Bouckaert and Drummond 2017), and the birth–death process was used as a tree prior. The molecular clock was calibrated using the *COI* rate of 0.0166 substitutions/site Ma^{−1} (SD: 0.0022). The value is an average of rates from previous studies

focused on the *G. balcanicus* group, which employed different calibration schemes: 0.0167 Ma – 1 SD: 0.0026 (Mamos et al. 2016) and 0.0165 Ma – 1, SD: 0.0018 (Copilaş-Ciocianu et al. 2018). For *COI*, a strict clock based on the evolutionary rate mentioned above, for 28S the log-normal relaxed clock, were used. Three instances of Markov chain Monte Carlo (MCMC), each with 60 000 000 generations sampled every 1000 generations, were applied. All runs and parameters were examined to ensure an effective sample size (ESS) >200. The tree files were combined and resampled at a lower frequency (3000). A burn-in of 10% was discarded using LogCombiner. The final maximum clade credibility tree was computed and annotated using TreeAnnotator (Bouckaert et al. 2019).

Phylogeographic analyses

We investigated in detail the phylogeographic patterns within *G. dobrogicus* sp. nov., since this species has a larger range than the other two newly described species and occurs in an area that is a known glacial refugium (Copilaş-Ciocianu et al. 2019a). Specifically, we wanted to assess if its phylogeography has been shaped by post-glacial dispersal.

We reconstructed a *COI* haplotype network to examine the mitochondrial genetic structure. The network was constructed in HAPLOVIEWER (Salzburger et al. 2011) using a maximum-likelihood tree obtained with MEGA 7 (Kumar et al. 2016).

A time-calibrated phylogenetic tree was constructed in BEAST 1.8.2 (Drummond et al. 2012) based on a dataset consisting of unique *COI* haplotypes, and corresponding 16S and 28S sequences. The HKY evolutionary model was assigned to the 16S and 28S partitions, while the HKY+G model was assigned to *COI*, which was further split into two partitions (positions 1 + 2, position 3). Model selection was based on the Bayesian information criterion (BIC) in MEGA 7. Due to the intraspecific nature of the divergence, each marker was assigned a strict molecular clock. Estimation of divergence times was based on a *COI* substitution rate ranging from 0.0177 substitutions Ma^{−1} (typical amphipod interspecific rates; Copilaş-Ciocianu et al. 2019b) to 0.033 substitutions Ma^{−1} (faster intraspecific crustacean rates; Crandall et al. 2012). Thus, a uniform prior ranging between 0.0177 and 0.033 was employed, with a starting value of 0.02. Speciation was modelled using the Yule process. The analysis was run for 10 000 000 generations with a sampling frequency of 1000 and 10% burn-in removal.

To investigate demographic patterns through time, we applied an extended Bayesian Skyline Plot (eBSP) in BEAST 1.8.2. The analysis had the same parameters and settings as the preceding one, except for the eBSP tree prior. Furthermore, three of the most powerful statistical tests for detecting population size changes were implemented: R_s , Fu's F_s , and Tajima's D . These analyses were run in DnaSP 6 (Rozas et al. 2017).

Data collection

BOLD dataset: DS-ROGAMBAL (<https://dx.doi.org/10.5883/DS-ROGAMBAL>).

RESULTS

Molecular diversity and phylogeny

Each of the newly described species represents a separate phylogenetic lineage in a large clade grouping cryptic lineages of *G. balcanicus* and other species (e.g. *G. leopoliensis*, *G. tatrensis*, and *G.*

stasiuki) inhabiting a vast area of the Carpathian Arc. This clade is deeply divergent (more than 20 Mya) from the Western Balkan clade, including *G. balcanicus* from its typical locality in Montenegro. In the Carpathian clade, *G. pinkfloydorum* inhabiting the Piatra Craiului Mountains in the Southern Carpathians is sister to a subclade grouping *G. tatrensis* and *G. stasiuki*, inhabiting the Western and Eastern Carpathians, as well as the newly described *G. carausui* from the Apuseni Mountains in the Western Romanian Carpathians, and *G. dobrogicus* inhabiting the Dobrogea Plateau. The divergence of *G. pinkfloydorum* started already in Early Miocene, at c. 18 Mya. In turn, *G. dobrogicus* diverged from the

subclade composed of *G. carausui*, *G. stasiuki*, and a '*G. balcanicus*' cryptic lineage in the Middle Miocene, at c. 13.5 Mya. Finally, *G. carausui* diverged from the rest of the subclade at c. 12 Mya, also in the Middle Miocene (Supporting Information, Fig. S1, 2B).

Each of the new species belongs to a separate single Barcode Index Number in BOLD: *G. carausui* sp. nov.: ADD2200; *G. dobrogicus* sp. nov.: ACQ6316; and *G. pinkfloydorum* sp. nov.: AGV6335.

Phylogeography

Phylogeographic analyses indicate that *G. dobrogicus* sp. nov. comprises three well-supported lineages (northern, central, and

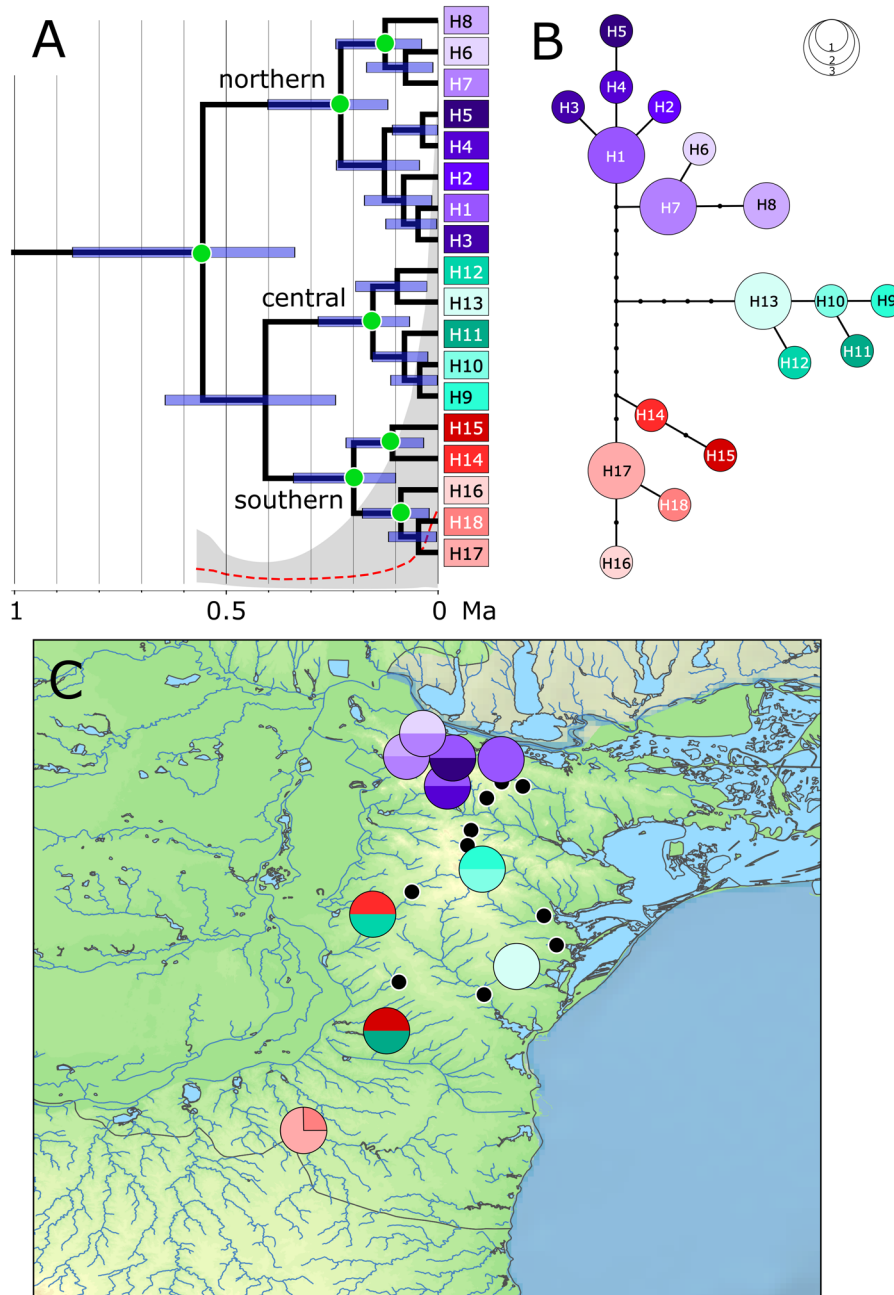


Figure 3. Phylogeography of *Gammarus dobrogicus* sp. nov. A, BEAST chronogram based on COI, 16S, and 28S markers. Green dots indicate strongly supported nodes (PP ≥ 0.95) and blue bars indicate 95% confidence intervals for node age. Terminals represent unique haplotypes. In the background, an eBSP is shown with the dotted red line indicating mean population size through time (95% confidence intervals in gray). B, COI haplotype network. Each circle represents a unique haplotype and its size reflects sample size (see legend on the upper right). Each line segment between two black dots indicates one substitution. C, geographical distribution of haplotypes. Pie-charts at each site indicate the proportion of haplotypes. Black dots indicate additional sites where the species was identified based on morphology.



Figure 4. *Gammarus carausui* sp. nov., male paratype. Habitus. Scale bar: 3 mm.

southern) that diverged between 0.55 and 0.40 Mya (Fig. 3A). The relationships among these lineages are not strongly supported, though the northern lineage might be a sister to the other two. Each lineage has a relatively localized distribution and comprises populations harbouring unique *COI* haplotypes (Fig. 3B, C). The eBSP analysis indicates demographic growth of the populations during the last 30 Ka. However, the demographic analyses with Tajima's *D* and *R_s* tests indicate no sign of population growth (Tajima's *D* = 0.469, *P* > .1; *R_s* = 0.137, *P* > .1), though Fu's *F_s* indicates population expansion just above statistical significance (Fu's *F_s* = -4.205, *P* = .057).

TAXONOMY

Suborder Senticaudata Lowry and Myers, 2013

Family Gammaridae Latreille, 1802

Genus *Gammarus* Fabricius, 1775

Gammarus carausui sp. nov.

(Figs 4–8)

Material examined: Holotype, male, 12.8 mm, stream close to Vărciorog, Romania (Lat. 46.950017 Long. 22.288617), 325 alt. (m a.s.l.), T. Mamos and H. Bogdan coll., 15 March 2011 (MIZ PAN CRU 001316). Allotype, female, 11 mm, same data as holotype (MIZ PAN CRU 001317). Paratypes: five males, same collection data as for holotype (MIZ PAN CRU 001318–001322); five females, same data as holotype (MIZ PAN CRU 001323–001327); one male, used for habitus photography; 83 males, same data as holotype; 64 females, same collection data as for holotype; seven intersex, same collection data as for holotype.

BOLD BIN number: doi.org/10.5883/BOLD: ADD2200.

Etymology: The specific epithet is dedicated to the eminent Moldavian/Romanian carcinologist and ichthyologist Sergiu Cărașușu (1907–1997) for his pivotal contributions to understanding the taxonomy and biogeography of amphipods in Romania.

Description of male (based on holotype, 12.8 mm)

Head: Eyes ovoid, about twice as long as wide. *Lateral cephalic lobe* slightly truncate. *Antenna 1* weakly setose; primary flagellum 27-articulate; accessory flagellum 4-articulate. *Antenna 2* gland cone reaching about half of peduncle article 3; article 4 with two

groups of posteromarginal setae; article 5 with sparse posteromarginal setae; flagellum 11-articulate, calceoli present. *Upper lip* and *Lower lip* apically setose. *Mandibles* incisor with six (left) and four (right) teeth, lacinia mobilis with three teeth (left) and bifid and apically cuspidate (right), molar ridged, left accessory setal row with nine multicuspidate setae; palp articles 1–3 ratios: 1 : 2.7 : 1.9, article 2 with 3 proximal, one medial and seven distal setae, article 3 with 23 D-setae, five E-setae, one group of A-setae and bearing one B-seta. *Maxilla 1* inner plate with 14 plumose setae; outer plate with nine robust serrate/multicuspidate setae; palp 2-articulate, right article 2 apex with six robust short setae and one lateral longer seta, left article 2 with eight spines with few slender setae; *Maxilla 2* inner plate slightly shorter than outer, with facial row of plumose setae and marginally covered with slender setae; outer plate apex with two rows of slender setae. *Maxilliped* inner plate with three apical spines and plumose setae; outer plate mesial margin with spines increasing in size apically, apex with cuspidate and plumose setae; palp article 2 mesial margin setose, article 3 lateral apex slightly produced, bearing seven setae, article 4 about 0.4 times the length of article 3, bearing a long unguis.

Pereon: *Coxae 1–3* subrectangular, with an anteroventral notch with one setule and three anterodorsal notches with one setule each. *Coxa 4* with antero- and posteroventral notches bearing setules, posterodorsal margin weakly excavated. *Coxae 5–6* bilobed. *Coxa 7* subrectangular. *Gnathopods 1–2* basis bearing anterior and posterior long setae; ischium, merus and carpus with dense posterodistal row of setae; propodus 1.3 times longer than carpus. *Gnathopod 1* palm margin slightly crenulate, bearing one medial spine, palmar corner with two spines, 2.6 times longer than the posterior margin of propodus; dactylus curved, shorter than palm, with unguis. *Gnathopod 2* palm margin with a shallow excavation, bearing one medial spine, palmar corner with two spines; dactylus slightly curved, reaching the palmar corner. *Pereopods 3–4* basis anterior and posterior margins with long setae; merus posterior margin with five groups of setae, anterior margin with one distal spine and slender setae, reaching about one-third the length of carpus; carpus and propodus subequal in length, bearing few posterior spines; dactylus short, with unguis. *Pereopods 5–7* basis posterior margin serrate, about 1.4–1.6 times as long as wide. *Pereopod 7* basis inner margin with four setae and a short spine on the posteroventral corner; ischium, merus, carpus and propodus bearing sparse short and long spines; dactylus short, with unguis.

Pleon: *Epimeron 1* posteroventral corner bluntly produced. *Epimera 2–3* with one to two ventral spines, posteroventral corner moderately pointed. *Urosomites 1–2* with a pair of spines dorsally and on each lateral side, accompanied by slender setae. *Urosomite 3* with a pair of dorsal slender setae and two spines on each lateral side, accompanied by slender setae. *Uropod 1* peduncle longer than rami; rami subequal, with four apical spines; outer ramus with three marginal spines; inner ramus with two marginal spines. *Uropod 2* peduncle longer than rami; rami with marginal spines and four apical spines; outer ramus 0.8 times the length of inner. *Uropod 3* peduncle with four distoventral setae; inner ramus 0.6 times the length of outer, bearing plumose setae laterally and one spine and slender setae apically; outer ramus article

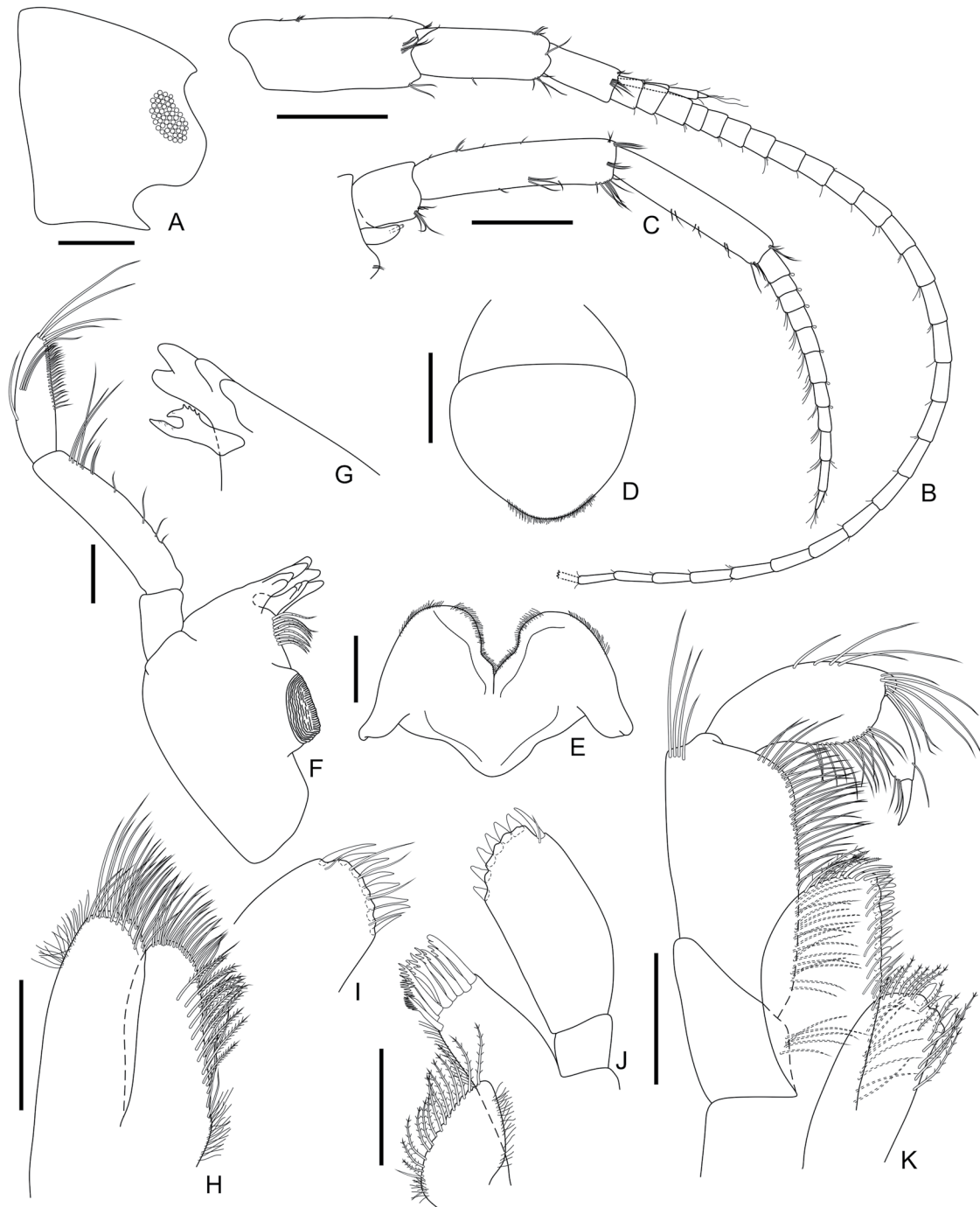


Figure 5. *Gammarus carausui* sp. nov., male holotype. A, head; B, antenna 1; C antenna 2; D, upper lip; E, lower lip; F, left mandible; G, incisor of right mandible; H, maxilla 2; I, palp of left maxilla 1; J, right maxilla 1; K, maxilliped. Scale bars: 0.5 mm for A–C, 0.2 mm for the remaining.

1 with few slender setae and three pairs of spines, article 2 with three short setae. *Telson* deeply cleft, each lobe with dorsoproximal pair of a robust and a plumose seta, dorso-medially with one long plumose and one short seta, apically with up to two spines, inner margin of lobes with two plumose setae (right lobe) and one robust and one plumose (left lobe).

Description of female (based on allotype, 11 mm)

Head: Antenna 2 peduncle articles 4–5 with longer setae; flagellum 8-articulate.

Pereon: *Gnathopod* 1 palm more acute than in male, without medial spine, corner with three spines. *Gnathopod* 2 palm less excavated than in male, without medial spine, corner with two

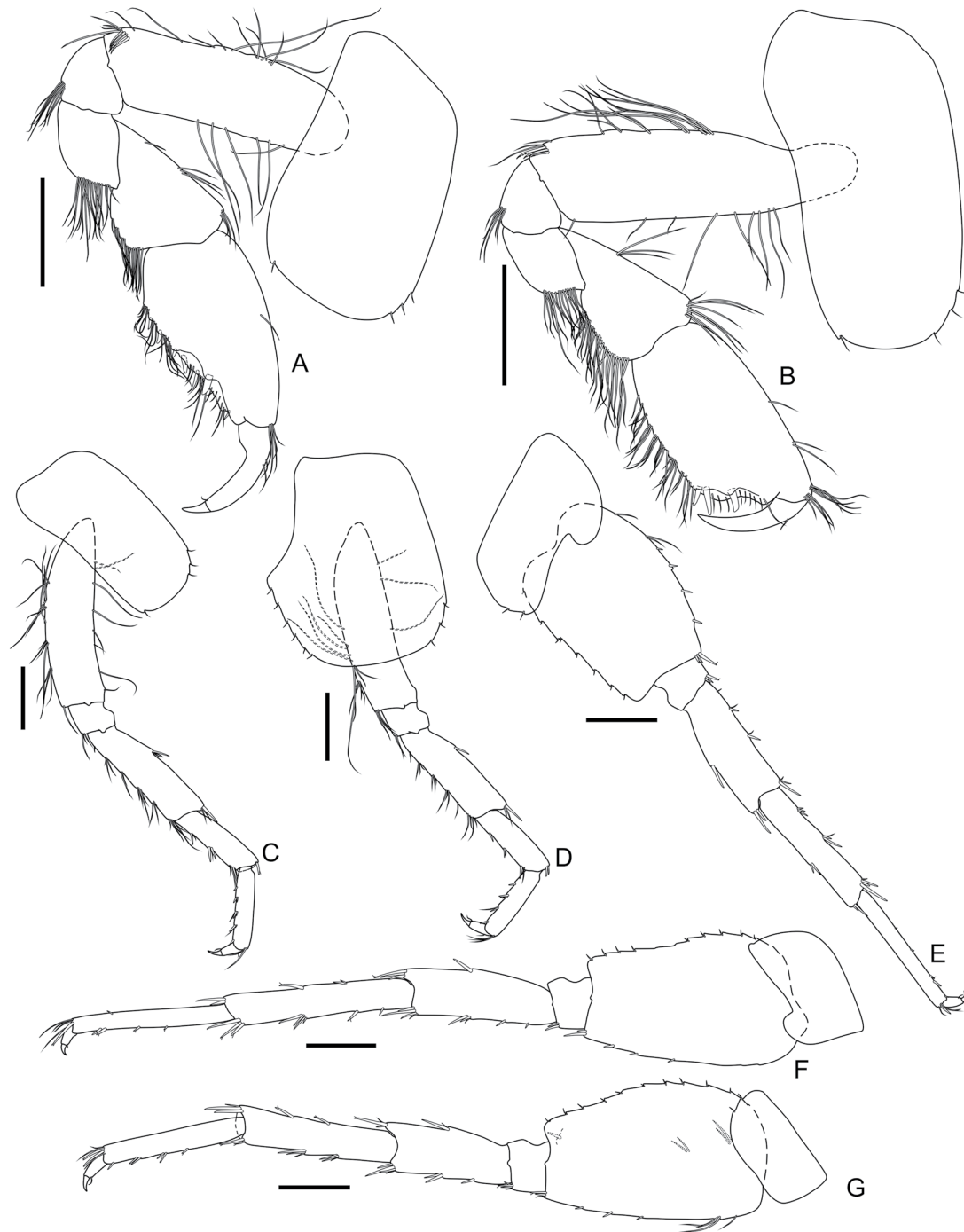


Figure 6. *Gammarus carausui* sp. nov., male holotype. A, gnathopod 1; B, gnathopod 2; C, pereopod 3; D, pereopod 4; E, pereopod 5; F, pereopod 6; G, pereopod 7. Scale bars: 0.5 mm.

spines. *Pereopods* 3–4 merus posterior margin with much longer setae. *Pereopods* 5–7 basis less elongated. *Pereopod* 7 basis inner margin with four setae.

Pleon: *Uropod* 3 rami without plumose setae. *Telson* presenting the same pattern, but without the short plumose setae.

Differential diagnosis: The new species differs from the topotypic material of *G. balcanicus* described by Karaman and Pinkster (1987) by the following: antenna 2 gland cone

reaching the middle of article 3 (vs. exceeding article 3), article 4 with two groups of posteromarginal setae (vs. three groups), article 5 with pairs of posteromarginal setae (vs. tufts of setae); pereopod 3 merus anterodistal setae reaching one-third the length of carpus (vs. reaching halfway); coxa 4 about 1.2 times as long as wide (vs. as long as wide); urosomite 1 with a pair of spines dorsally (vs. group of slender setae); urosomite 3 with a pair of dorsal slender setae and two spines on each lateral side (vs. one dorsal and a

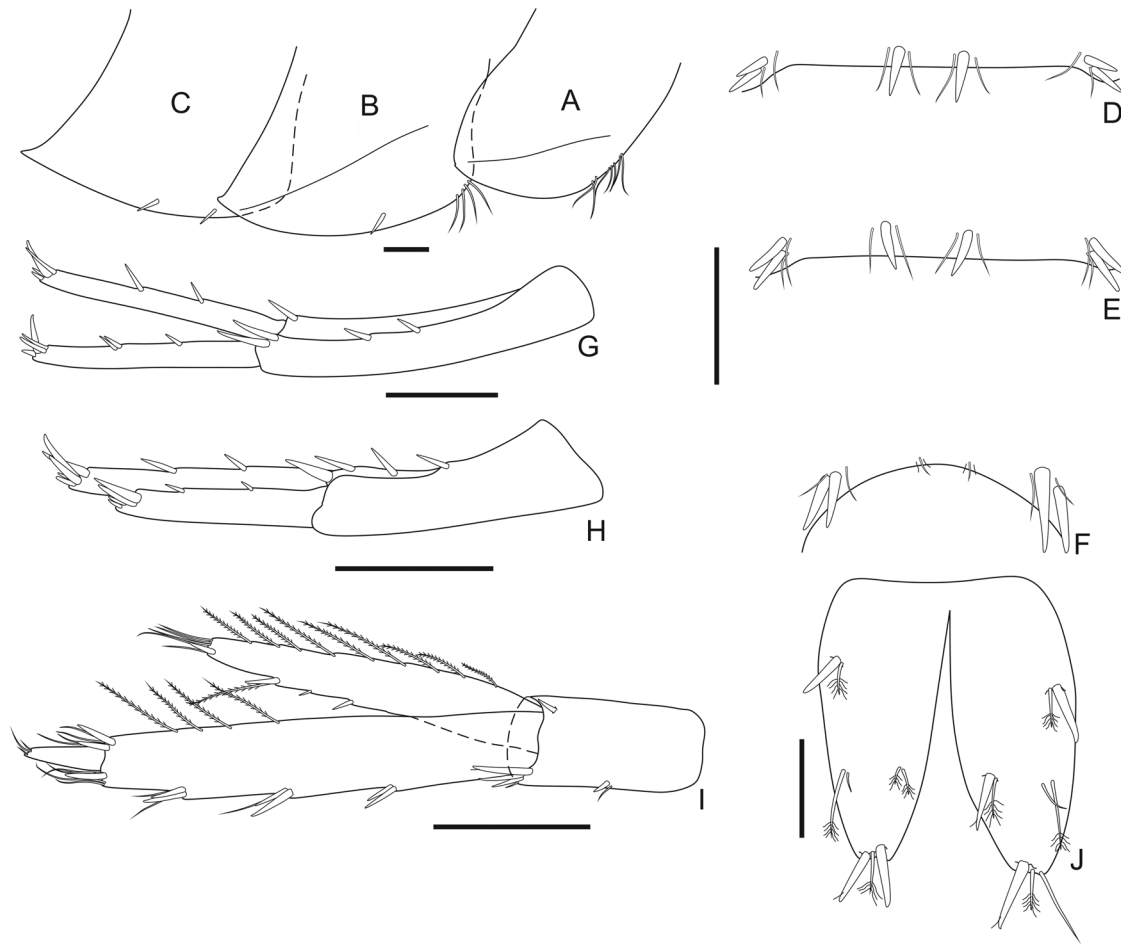


Figure 7. *Gammarus carausui* sp. nov., male holotype. A, epimeron 1; B, epimeron 2; C, epimeron 3; D, urosomite 1; E, urosomite 2; F, urosomite 3; G, uropod 1; H, uropod 2; I, uropod 3; J, telson. Scale bars: 0.2 mm for A–F, 0.5 mm for the remaining.

lateral group of spines); telson with a dorsoproximal spine (vs. without).

Gammarus carausui sp. nov. can in addition be distinguished from *G. dobrogicus* sp. nov. by characters such as: antenna 2 gland cone medium (vs. short); mandibular incisor with six teeth and lacinia mobilis with three (vs. four on both); maxilla 1 palp apex with regular short spines on right side and slightly longer spines on left (vs. thicker spines on right side and longer ones on left); gnathopod 1 with slightly crenulated palm (vs. smooth), corner with two spines (vs. one long and five short spines); epimeron 1 with posteroventral corner bluntly produced (vs. rounded); epimeron 3 posteroventral corner more pointed (vs. less pointed); urosomite 3 with slender dorsal setae (vs. spines).

Distribution and ecological data: Known from the type locality only.

***Gammarus pinkfloydorum* sp. nov.**
(Figs 9–12)

Material examined: Holotype, male, 10.2 mm, Raul stream, Prăpăștiile Zărneștilor, Zărnești, Piatra Craiului, Romania (Lat. 45.54205, Long. 25.29596667), 734 alt. (m a.s.l.), M. Grabowski and K. Bączela-Spychalska coll., 29 August 2005 (MIZ PAN CRU 001328).

Allotype, female, 9.7 mm, same collection data as for holotype (MIZ PAN CRU 001329). Paratypes: two males, same data as holotype (MIZ PAN CRU 001330–001331); three females, same collection data as for holotype (MIZ PAN CRU 001332–001334); one male, used for habitus photography; three males, same data as holotype; 15 females, same collection data as for holotype.

BOLD BIN number: doi.org/10.5883/BOLD: AGV6335.

Etymology: The species is named in honour of the iconic, psychedelic, and progressive rock band Pink Floyd, intended to express the long-standing passion of the last author of this paper and collector of the species to the music of the band. Just as Pink Floyd is renowned for their innovative, complex, and eclectic music, often complemented by sophisticated visual elements in their album art and concerts, the genus *Gammarus* exhibits remarkably intricate patterns and subtle differences in morphological features among its hundreds of species. The specific epithet was chosen to capture the complexity and remarkable nature of the new species' morphology, drawing a parallel to the multifaceted artistry of Pink Floyd's music. The name is a genitive plural, referring collectively to the members of the band.

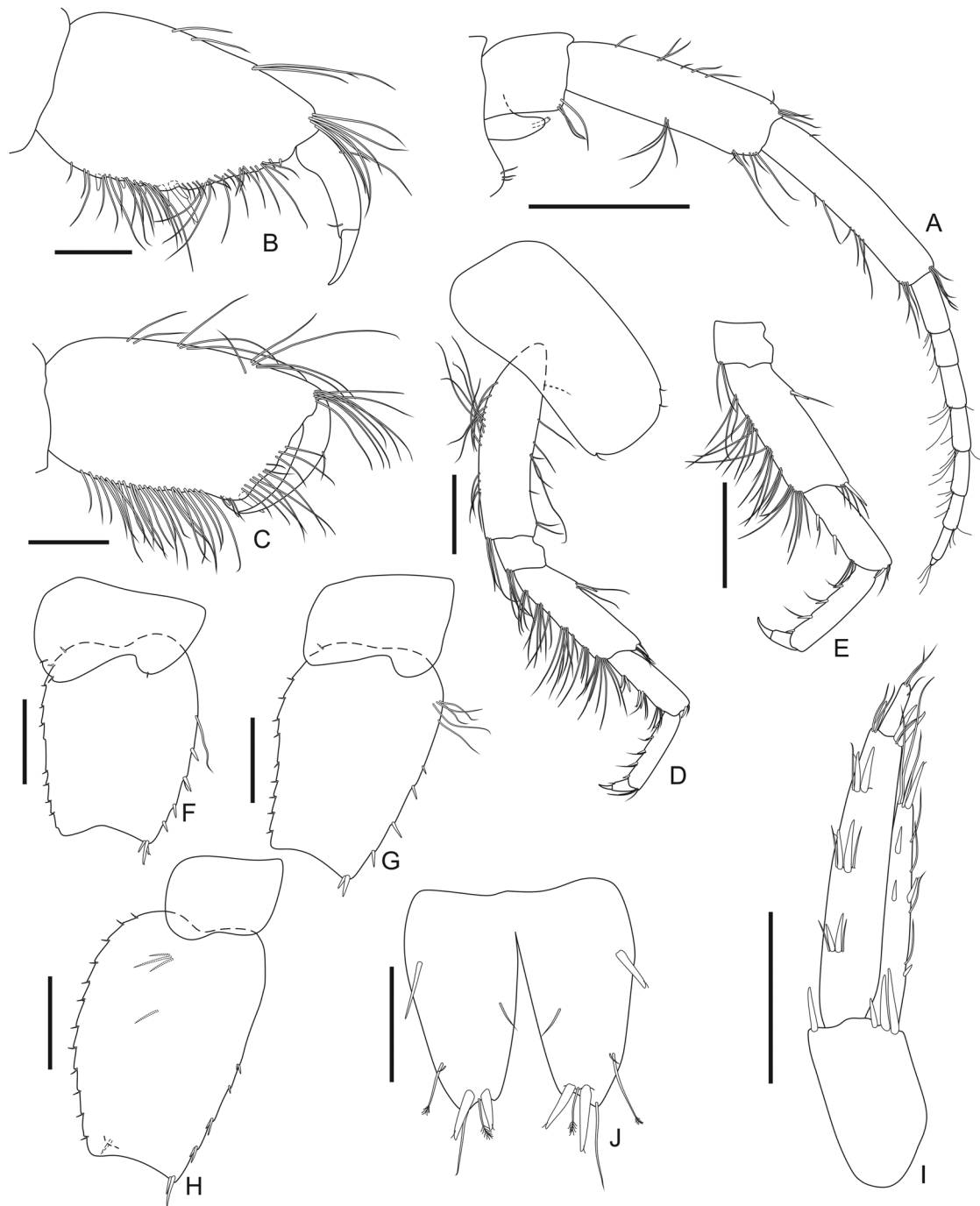


Figure 8. *Gammarus carausui* sp. nov., female allotype. A, antenna 2; B, gnathopod 1 propodus; C, gnathopod 2 propodus; D, pereopod 3; E, pereopod 4 (partial); F, pereopod 5 basis; G, pereopod 6 basis; H, pereopod 7 basis; I, uropod 3; J, telson. Scale bars: 0.5 mm for A–I, 0.2 mm for J.

Description of male (based on holotype, 10.2 mm)

Head: Eyes ovoid, about twice as long as wide. **Lateral cephalic lobe** slightly truncate. **Antenna 1** weakly setose; primary flagellum 21-articulate; accessory flagellum 3-articulate. **Antenna 2** gland cone almost reaching the apex of peduncle article 3; articles 4–5 with six groups of very long posteromarginal setae; flagellum 10-articulate, calceoli absent. **Left mandible** incisor with three

teeth, lacinia mobilis with four teeth, molar ridged, accessory setal row with eight multicuspidate setae; palp articles 1–3 ratios: 1 : 3.0 : 1.8, article 2 with lateral setae increasing in length to apex, article 3 with 32 D-setae, four E-setae, two groups of A-setae and two B-setae. Other mouthparts nearly identical to *Gammarus carausui* sp. nov., differing only on some few setal arrangements.



Figure 9. *Gammarus pinkfloydorum* sp. nov. male paratype. Habitus. Scale bar: 3 mm.

Pereon: *Coxae* 1–2 subrectangular with an anteroventral notch with one setule and three anterodorsal notches with one setule each. *Coxa* 4 with antero- and posteroventral notches bearing setules, posterodorsal margin excavated. *Coxae* 5–6 bilobated. *Coxa* 7 subrectangular. *Gnathopod* 1 palm with one medial spine, corner with three spines, slightly shorter than the posterior margin of propodus; dactylus curved, reaching the palmar corner, with unguis. *Gnathopod* 2 palm margin with one medial spine, palmar corner with one spine; dactylus slightly curved, longer than palm, with unguis. *Pereopods* 3–4 merus posterior margin with five groups of long setae (pereopod 3) and short setae (pereopod 4), anterior margin with distal spines and slender setae, reaching about one-third the length of carpus; carpus longer than propodus (pereopod 3) and slightly shorter (pereopod 4); propodus with posterior spines; dactylus short, with unguis. *Pereopods* 5–7 basis posterior margin serrate, about 1.3–1.5 times as long as wide. *Pereopod* 7 basis slightly expanded posteriorly, inner margin with three setae and a short spine on the posteroventral corner.

Pleon: *Epimeron* 1 posteroventral corner rounded. *Epimeron* 2 with one lateral and two ventral spines, posteroventral corner weakly pointed. *Epimeron* 3 with three ventral spines, weakly pointed. *Urosomite* 1 with simple dorsal setae and one spine on each lateral side. *Urosomite* 2 with a pair of spines dorsally and on each lateral side, accompanied by slender setae. *Urosomite* 3 with a pair of short spines and three spines on each lateral side. *Uropod* 1 peduncle longer than rami; rami subequal, with marginal spines and three to four apical spines. *Uropod* 2 peduncle longer than rami; rami with marginal spines and five apical spines; outer ramus slightly shorter than inner. *Uropod* 3 peduncle with three distoventral setae; inner ramus 0.4 times the length of outer, bearing few simple setae and spines laterally, apex with two spines; outer ramus article 1 with few slender setae and two spines, article 2 with three short setae. *Telson* deeply cleft, each lobe with one lateral setae, apically with two to three spines and few slender setae.

Description of female (based on allotype, 9.7 mm)

Head: *Antenna* 2 peduncle articles 4–5 more densely setose.

Pereon: *Gnathopod* 1 propodus more setose; palm less acute than in male, without medial spine, palmar corner with three spines. *Gnathopod* 2 propodus more setose; palm less acute than in male, without medial spine, corner with four spines. *Pereopods* 3–4 merus more setose. *Pereopods* 5–7 basis less elongated. *Pereopod* 7 basis inner margin posterior margin more expanded.

Pleon: *Uropod* 3 rami reduced. *Telson* with more and longer slender setae.

Differential diagnosis: The new species can be easily distinguished from the topotypic material of *G. balcanicus* described by Karaman and Pinkster (1987), and from *G. carausui* sp. nov. and *G. dobrogicus* sp. nov. by the following: antenna 2 peduncle articles 4–5 with groups of very long posteromarginal setae (longer than the width of the underlying segment), and flagellum without calceoli; gnathopod 2 dactylus longer than palm; pereopods 3–4 merus more setose; pereopod 7 basis posterior margin slightly expanded, inner margin with three setae; epimeron 2 posteroventral corner weakly pointed.

Distribution and ecological data: Known only from the type locality.

***Gammarus dobrogicus* sp. nov.**
(Figs. 13–16)

Material examined: Holotype, male, 11.8 mm, cheshma (Romanian *cișmea*—drinking fountain) in Șipotele, Romania (Lat. 44.042067, Long. 27.962733), 50 alt. (m a.s.l.), M. Grabowski and K. Băcela-Spychalska coll., 6 September 2007 (MIZ PAN CRU 001335). Allotype, female, 10.6 mm, same collection data as for holotype (MIZ PAN CRU 001336). Paratypes: five males, same collection data as for holotype (MIZ PAN CRU 001337–001341; five females, same collection data as for holotype (MIZ PAN CRU 001342–001346); one male, used for habitus photography; 10 males, same data as holotype; 27 females, same collection data as for holotype; 24 males, Stream on the road near Luncavița, Romania, (Lat. 45.22803333, Long. 28.31116667), 9 alt. (m a.s.l.), M. Grabowski and K. Băcela-Spychalska coll., 2 September 2005.

BOLD BIN number: doi.org/10.5883/BOLD: ACQ6316.

Etymology: The specific epithet *dobrogicus* is a latinized adjective meaning ‘of Dobrogea’, referring to the Dobrogea (Dobruddzha in Bulgarian) region that straddles the Black Sea in SE Romania and NE Bulgaria. The range of this species overlaps remarkably well with this region.

Description of male (based on holotype, 11.8 mm)

Head: Eyes ovoid, about twice as long as wide. *Lateral cephalic lobe* truncate. *Antenna* 1 weakly setose; primary flagellum 24-articulate; accessory flagellum 4-articulate. *Antenna* 2 gland cone short, reaching about one-third the length of the peduncle article 3; articles 4–5 with three groups of posteromarginal setae; flagellum 13-articulate, calceoli present. *Left mandible* incisor and lacinia mobilis with four teeth, molar ridged, accessory setal row with nine multicuspidate setae; palp articles 1–3 ratios: 1 : 2.5 : 1.7, article 2 with sparse setae, distally with four long setae, article 3. *Maxilla* 1 palp right article 2 apex with five spines and two lateral slender setae, left article 2 with eight long spines and three slender setae. Other mouthparts nearly identical to

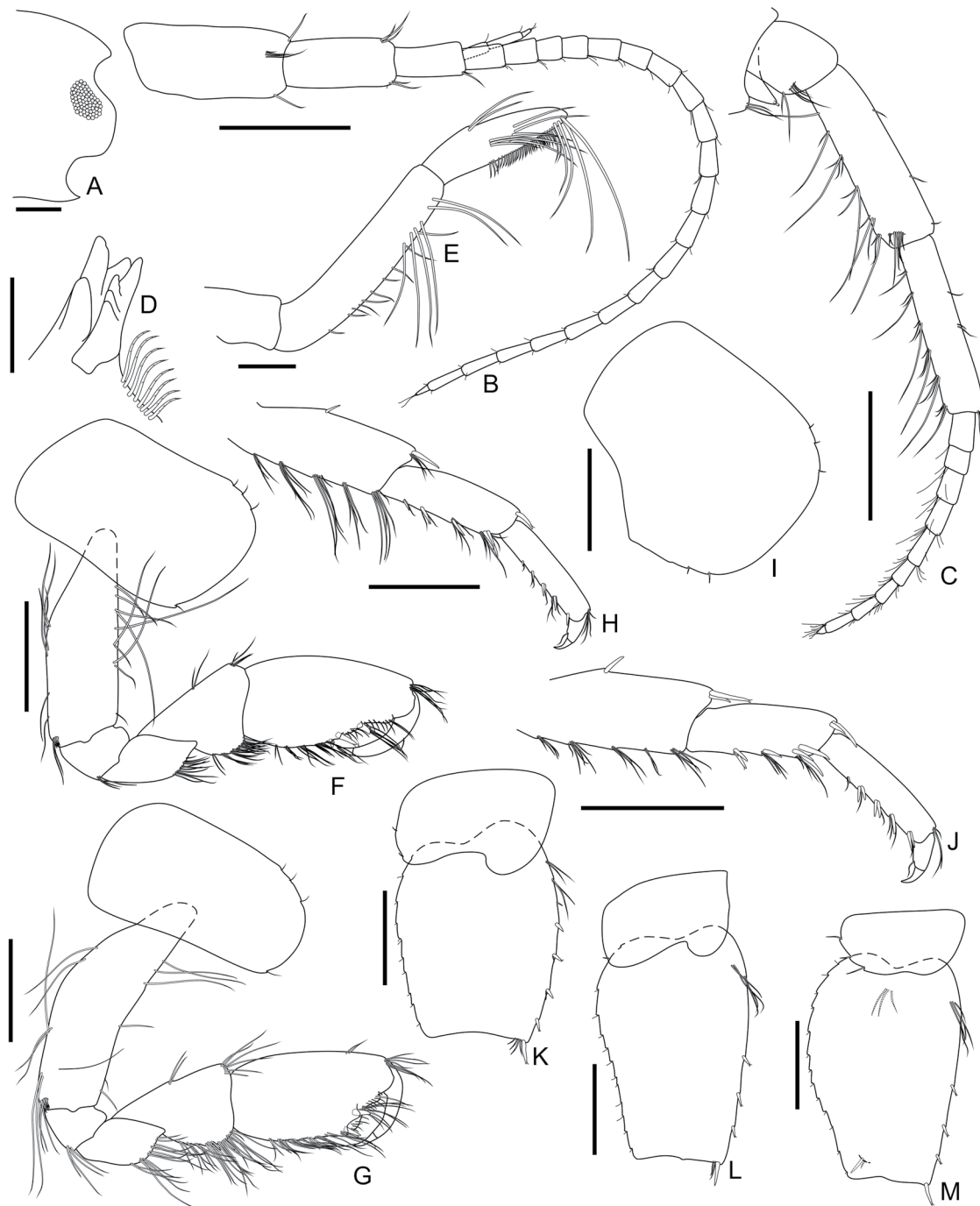


Figure 10. *Gammarus pinkfloydorum* sp. nov., male holotype. A, head; B, antenna 1; C, antenna 2; D, left mandible incisor; E, left mandible palp; F, gnathopod 1; G, gnathopod 2; H, pereopod 3 (partial); I, coxa 4; J, pereopod 4 (partial); K, pereopod 5 basis; L, pereopod 6 basis; M, pereopod 7 basis. Scale bars: 0.3 mm for A, 0.1 mm for D and E; 0.5 mm for the remaining.

Gammarus carausui sp. nov., differing only in some few setal arrangements.

Pereon: *Coxae* 1–3 with an anterodorsal notch with one setule and two anterodorsal notches with one setule each. *Coxa* 4 with antero- and posteroventral notches bearing setules, posterodorsal margin excavated. *Coxae* 5–6 bilobated. *Coxa* 7 subrectangular. *Gnathopod* 1 palm with one medial spine, corner with one long

robust and five short spines; dactylus curved, reaching the palmar corner. *Gnathopod* 2 palm margin with one medial spine, palmar corner with three spines; dactylus curved, slightly longer than palm. *Pereopods* 3–4 merus posterior margin with long setae (pereopod 3) and short setae (pereopod 4), anterior margin with distal spines reaching up to one-third the length of carpus; carpus and propodus subequal; propodus with posterior spines; dactylus

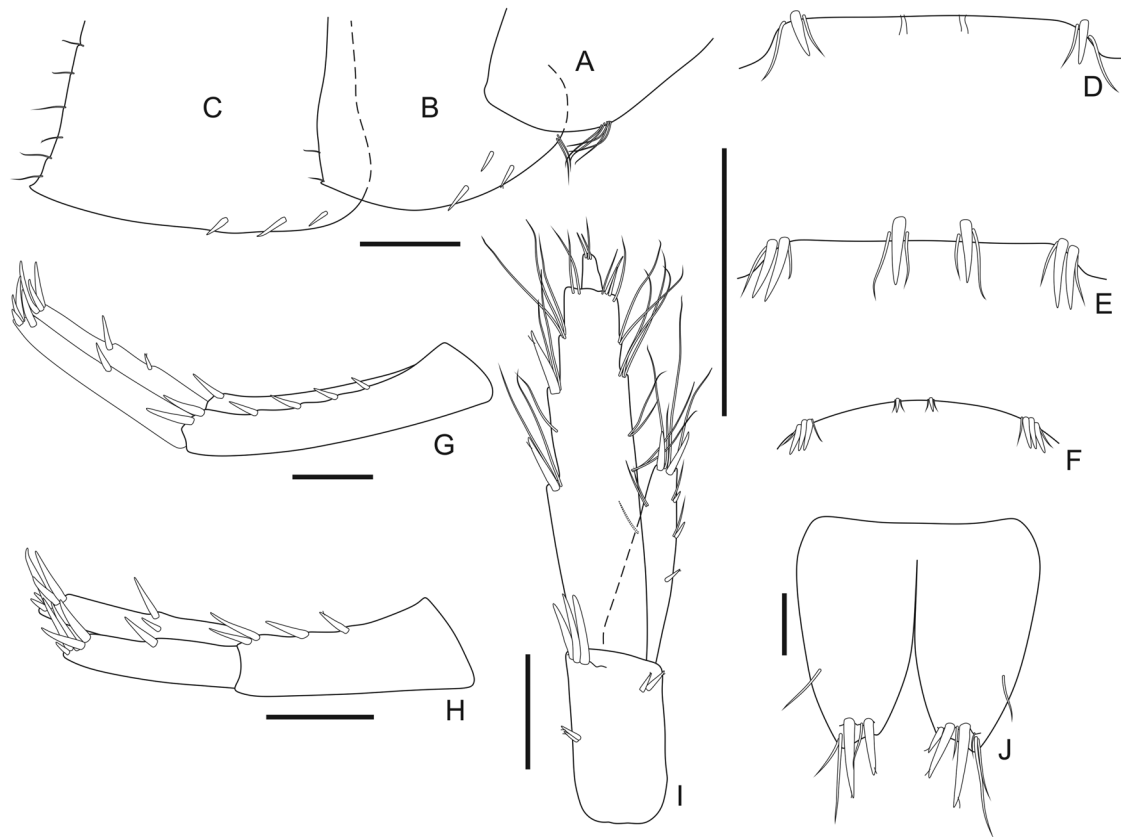


Figure 11. *Gammarus pinkfloydorum* sp. nov., male holotype. A, epimeron 1; B, epimeron 2; C, epimeron 3; D, urosomite 1; E, urosomite 2; F, urosomite 3; G, uropod 1; H, uropod 2; I, uropod 3; J, telson. Scale bars: 0.1 mm for J, 0.3 mm for the remaining.

short, with unguis. *Pereopods* 5–7 basis posterior margin serrate, about 1.3–1.5 times as long as wide. *Pereopod* 7 basis inner margin with two setae.

Pleon: *Epimeron* 1 posteroventral corner rounded. *Epimeron* 2 with one lateral and two ventral spines, posteroventral corner moderately pointed. *Epimeron* 3 with three ventral spines, moderately pointed. *Urosomite* 1 with a pair of dorsal spines and three spines on each lateral side. *Urosomites* 2–3 with a pair of dorsal and lateral spines. *Uropod* 1 peduncle longer than rami; rami subequal, with marginal spines and four to five apical spines. *Uropod* 2 peduncle longer than rami; rami with marginal spines and five apical spines; outer ramus 0.7 times the length of inner. *Uropod* 3 peduncle with three distoventral setae; inner ramus 0.6 times the length of outer, bearing plumose setae laterally and two robust and slender setae apically; outer ramus article 1 with few plumose and six spines, article 2 with two short setae. *Telson* deeply cleft, each lobe with a dorsoproximal spine and a dorsomedial seta, apically with two spines, slender and plumose setae.

Description of female (based on allotype, 10.6 mm)

Head: *Antenna* 2 peduncle articles 4–5 with longer setae; flagellum 9-articulate.

Pereon: *Gnathopod* 1 palm less acute than in male, without medial spine, palmar corner with two spines. *Gnathopod* 2 palm less acute

than in male. *Pereopods* 3–4 merus posterior margin with much longer setae. *Pereopods* 5–7 basis less elongated. *Pereopod* 7 basis inner margin with two setae.

Pleon: *Uropod* 3 with longer plumose setae. *Telson* inner margin of lobes with one to two setae.

Differential diagnosis: The new species differs from the topotypic material of *G. balcanicus* described by Karaman and Pinkster (1987) by the following characters: antenna 2 gland cone short, reaching about one-third the length of the peduncle article 3 (vs. long, exceeding the apex of article 3); maxilla 1 palp apex with thicker spines on right side and longer ones on left (vs. regular short spines on right side and slightly longer spines on left); maxilliped inner plate with three apical spines (vs. four); pereopods 3–4 carpus posterior margin with setae length never longer than the width of the underlying segment (vs. longer); urosomite 1 with a pair of dorsal spines (vs. slender setae); telson with a dorsoproximal spine (vs. without); setae on uropod 3 rami are always shorter than the width of underlying segment (vs. longer); setae on inner margin of uropod 3 outer ramus are plumose (vs. simple).

Distribution and ecological data

This species is endemic to the Dobrogea (Dobrudzha) region of Romania and is the most widespread *Gammarus* species in

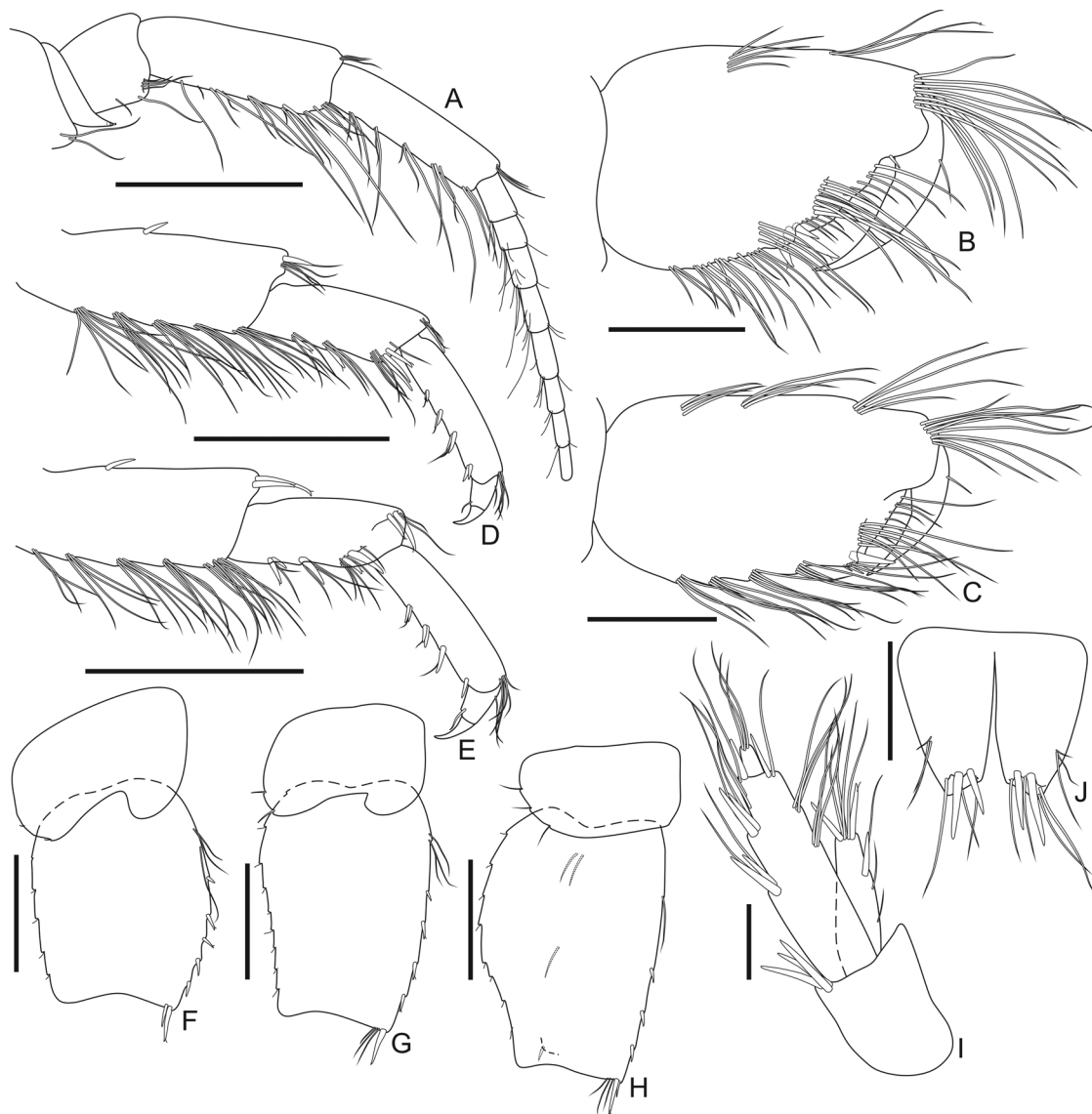


Figure 12. *Gammarus pinkfloydorum* sp. nov., female allotype. A, antenna 2; B, gnathopod 1 propodus; C, gnathopod 2 propodus; D, pereopod 3; E, pereopod 4 (partial); F, pereopod 5 basis; G, pereopod 6 basis; H, pereopod 7 basis; I, uropod 3; J, telson. Scale bars: 0.2 mm for B, C, I, and J, 0.5 mm for the remaining.



Figure 13. *Gammarus dobrogicus* sp. nov., male paratype. Habitus. Scale bar: 3 mm.

this region. It lives in small- to medium-sized streams and springs, especially with rocky substrates and moderate flow. At the type locality, cheshma in Șipotele in Romania, the species was found in a spring used as a water source for cattle, in the middle of a vast pasture. The spring was characterized by slightly acidic (pH 6.18) water, high temperature (18.6°C) and very high conductivity ($K\ 1,5928\text{mS}\cdot\text{cm}^{-1}$), making it quite an unusual habitat for a member of the strictly freshwater and, generally coldwater, *Gammarus* species group. Throughout the northern half of its range, the species often co-occurs with *G. hamaticornis*, another regional endemic (Copilaș-Ciocianu et al. 2019). In the southern half of its range, it is gradually replaced by more typically Balkan elements such as *G. arduus* (Kazanavičiūtė and Copilaș-Ciocianu 2024), *G. komareki* (Copilaș-Ciocianu 2013), and *G. pulex* (Copilaș-Ciocianu et al. 2014), which prefer streams with a slower flow and more vegetation. The species probably occurs in the north-eastern part of Bulgaria as well.

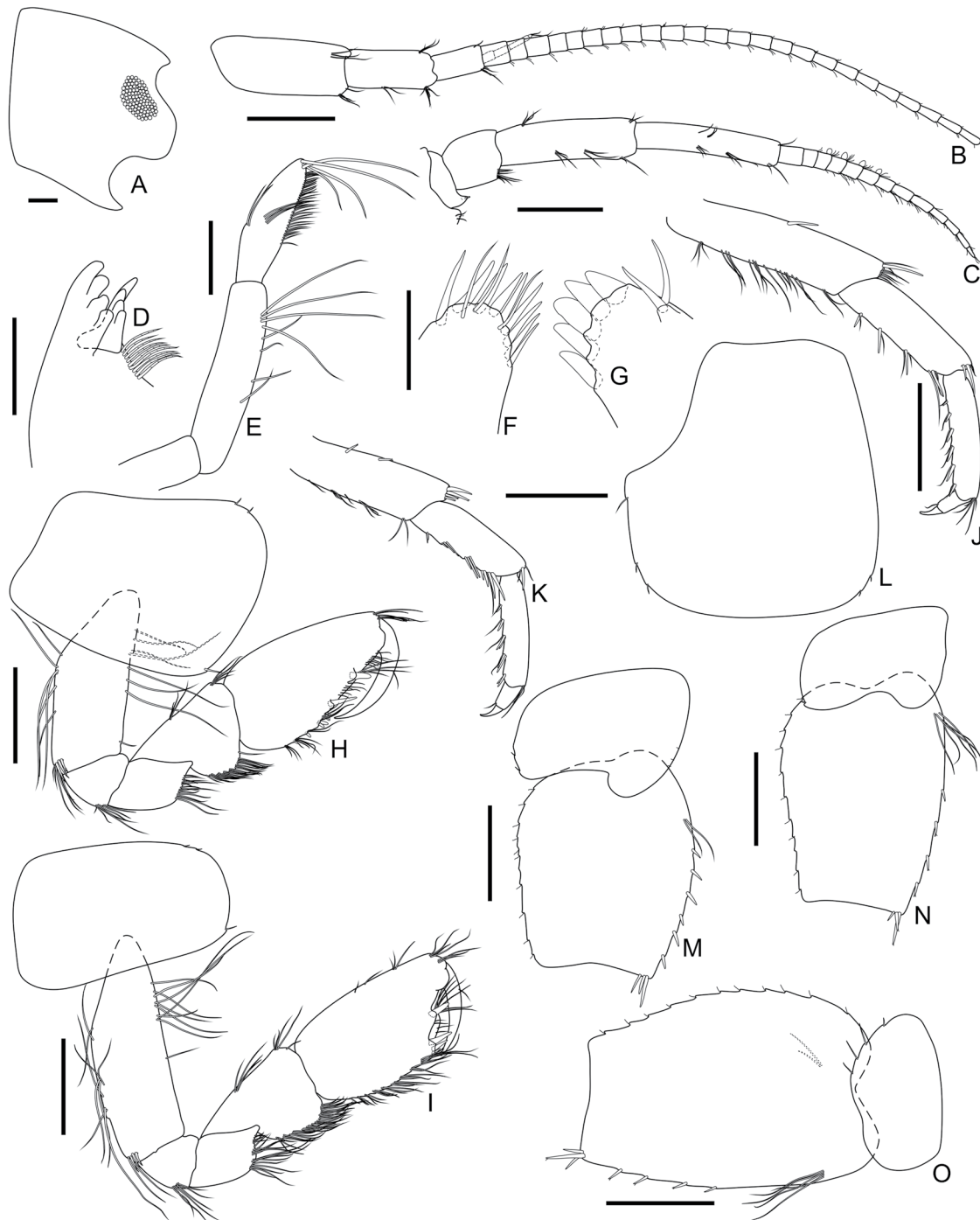


Figure 14. *Gammarus dobrogicus* sp. nov., male holotype. A, head; B, antenna 1; C, antenna 2; D, left mandible incisor; E, left mandible palp; F, palp of left maxilla 1; G, palp of right maxilla 1; H, gnathopod 1; I, gnathopod 2; J, pereopod 3 (partial); K, pereopod 4 (partial); L, coxa 4; M, pereopod 5 basis; N, pereopod 6 basis; O, pereopod 7 basis. Scale bars: 0.2 mm for A, D and E, 0.1 mm for F and G, 0.5 mm for the remaining.

DISCUSSION

Taxonomy

Our study, employing an integrative approach combining multilocus phylogenetics and detailed morphological examination, revealed the presence of three previously unrecognized pseudocryptic species within the *Gammarus balcanicus* species complex: *Gammarus carausui* sp. nov. and *Gammarus pinkfloydorum* sp. nov. in the Carpathians, as well as *Gammarus dobrogicus* sp. nov. on the Dobrogea Plateau, south-east of the Carpathians.

These findings corroborate our earlier molecular investigations that indicated the existence of cryptic lineages within this complex, particularly in the Carpathian region (Mamos *et al.* 2016, Copilaş-Ciocianu and Petrusek 2017). While initial assessments, based primarily on the mitochondrial *COI* marker, might suggest cryptic, our comprehensive analysis, including two mitochondrial and one nuclear markers as well as morphological traits showing subtle yet consistent differences, supports their delineation as distinct species. Our results align with recent

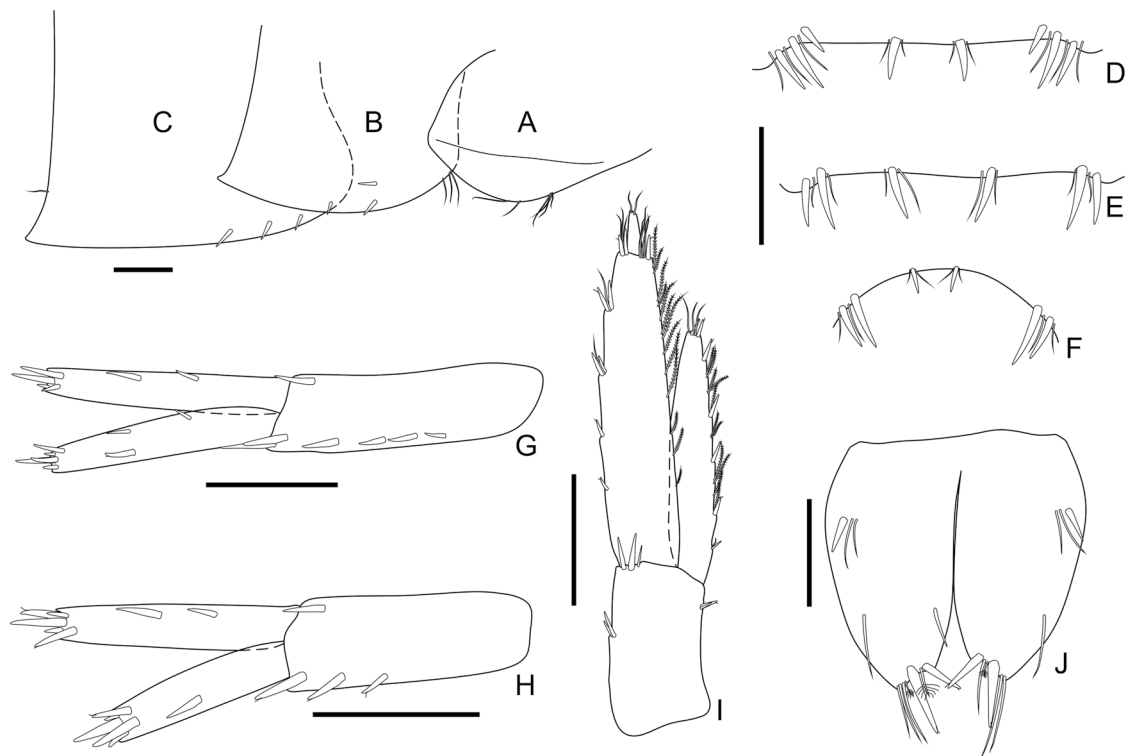


Figure 15. *Gammarus dobrogicus* sp. nov., male holotype. A, epimeron 1; B, epimeron 2; C, epimeron 3; D, urosomite 1; E, urosomite 2; F, urosomite 3; G, uropod 1; H, uropod 2; I, uropod 3; J, telson. Scale bars: 0.2 mm for A–F, 0.5 mm for the remaining.

studies, using the integrative approach combining morphological and molecular traits, targeting other *Gammarus balcanicus* lineages that were also found to exhibit minor but consistent morphological characters differentiating species, and suggesting that many putative cryptic lineages might, in fact, be pseudocryptic. This led, for example, to the identification and description of a new species (*G. stasiuki*) and the resurrection of another species (*G. tatrensis*) (Mamos et al. 2021). Overall, our findings suggest that a significant part of the uncovered cryptic lineages of the *G. balcanicus* species complex might be pseudocryptic species, i.e. found to exhibit slight phenotypic divergence perceived only after a detailed study based on initial molecular species' delimitation. This further underscores the critical importance of DNA barcoding, best if integrated with nuclear markers, in delimiting and ultimately describing new species in taxonomic groups that exhibit morphological stasis or in which the phenotypic diagnostic features are not well established.

Phylogeny and phylogeography

The deep molecular divergence observed among the newly described species, dating back to the Middle Miocene (10–15 Mya), significantly predates the onset of the Quaternary glaciations. As shown in our previous broader-scale studies, the evolutionary history of *G. balcanicus* species complex is powerfully shaped by and, in turn, reflects the palaeogeographic dynamism of south-eastern Europe, including the regression of the Tethys Ocean and Paratethys Sea and the associated emergence of new landmasses and hydrological networks during the Mid-Late

Miocene (Mamos et al. 2016, Copilaş-Ciocianu and Petrusek 2017). In particular, the distribution and age of *Gammarus dobrogicus* sp. nov. are remarkably congruent with the geological history of the Dobrogea region, which existed as an island or peninsula within the Paratethys Sea during the time of divergence (Popov et al. 2004). Furthermore, the presence of three narrowly endemic sub-lineages within *G. dobrogicus* sp. nov., diverging in the Late Pleistocene, provides compelling evidence for their survival in three distinct local refugia during at least one glaciation cycle, followed by localized dispersal in the Holocene. This pattern mirrors that observed in another endemic Dobrogean gammarid, *G. hamaticornis* (Copilaş-Ciocianu et al. 2018), collectively emphasizing the under-appreciated role of the little-studied Dobrogea region as a crucial local glacial refugium for both aquatic and terrestrial fauna (Giurginca and Ćurčić 2003). The other newly described species herein, *G. carausui* sp. nov. and *G. pinkfloydorum* sp. nov., currently known only from their type localities in the Carpathians, also exhibit similarly old divergences, further highlighting the long-term persistence of ancient lineages in this region. Our findings reinforce the already recognized importance of the Carpathian Arc as a major European biodiversity and endemism hotspot and a vital extra-Mediterranean glacial refugium that has facilitated the survival and diversification of numerous taxa through Pleistocene climatic oscillations. The discovery of anciently divergent *Gammarus* species with restricted ranges underscores the high level of undocumented biodiversity probably present in this area and the potential for further discoveries.

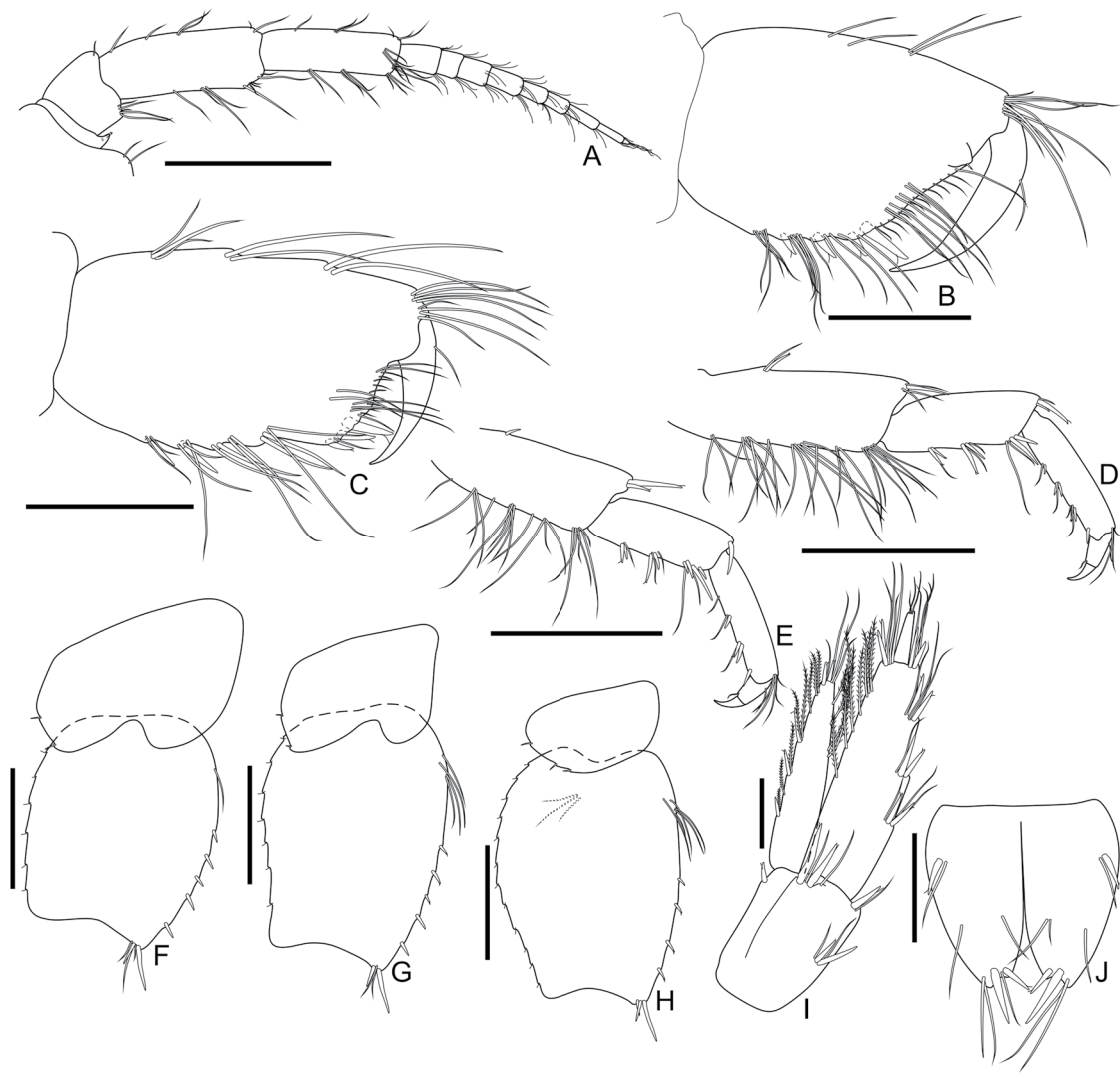


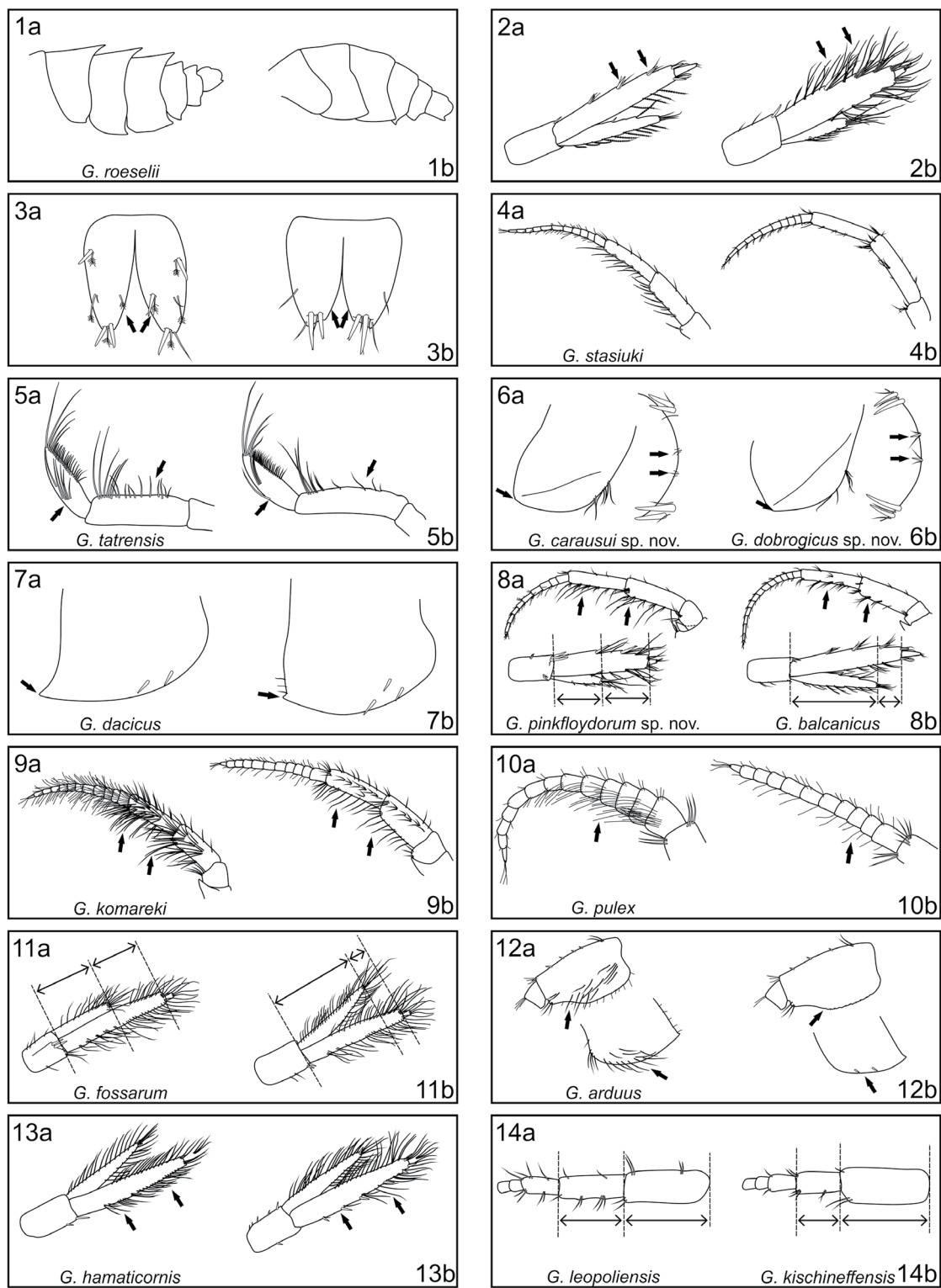
Figure 16. *Gammarus dobrogicus* sp. nov., female allotype. A, antenna 2; B, gnathopod 1 propodus; C, gnathopod 2 propodus; D, pereopod 3; E, pereopod 4 (partial); F, pereopod 5 basis; G, pereopod 6 basis; H, pereopod 7 basis; I, uropod 3; J, telson. Scale bars: 0.2 mm for B, C, I and J, 0.5 mm for the remaining.

CONCLUSION

The genus *Gammarus*, with its wide distribution and high species richness, serves as a valuable model organism for studying the evolutionary history of freshwater biodiversity (Väinölä *et al.* 2008, Hou *et al.* 2011). The patterns of deep divergence and limited morphological change observed in the *G. balcanicus* species complex exemplify how historical geological and climatic events have shaped the diversification and distribution of freshwater fauna.

Beyond their significance in evolutionary and biogeographic studies, *Gammarus* species play a crucial role in aquatic ecosystems (e.g. MacNeil *et al.* 1997) and are widely utilized in biomonitoring and ecotoxicological assessments (Kunz *et al.* 2010). Recognizing cryptic and pseudocryptic diversity within the genus has essential implications for these applications. Different cryptic

lineages can occupy distinct ecological niches and exhibit varying sensitivities to pollutants, which can influence the outcomes of environmental risk assessments if species are not correctly identified (Jourdan *et al.* 2023). Such isolated and highly divergent lineages should also be treated as separate conservation units (Jabłońska *et al.* 2020), which is fundamental for the adequate and effective protection of biodiversity, particularly in highly fragmented freshwater habitats, and should be in focus of protection and conservation programs (Hughes *et al.* 2008, Van Dyke 2008). Therefore, accurate taxonomic resolution, as provided by integrative approaches, is essential for effective biomonitoring programs and for informing conservation strategies aimed at protecting the genetic and ecological diversity of European aquatic habitats and their associated fauna, including the often overlooked but ancient lineages of *Gammarus*.



Pictorial identification key to the *Gammarus* species of Romania (A1, antenna 1; A2, antenna 2; E1, epimeron 1; E2, epimeron 2; P7, pereopod 7; U3, uropod 3)

1a. Pleonites produced with large dorsal spines	<i>G. roeslii</i>
1b. Pleonites not produced, without dorsal spines	2
2a. Setation of external margin of U3 outer ramus is scarce and as long as or shorter than the width of underlying segment	3
2b. Setation of external margin of U3 outer ramus is dense and longer than the width of the underlying segment	9
3a. Inner margins of telson lobes with seta/spine	4
3b. Inner margins of telson lobes without seta/spine	7
4a. A2 moderately setose	<i>G. stasiuki</i>
4b. A2 weakly setose	5
5a. Mandible palp article 2 with a weak inner row of setae medio-proximally, article 3 without B-setae	<i>G. tatrensis</i>
5b. Mandible palp article 2 with sparse inner setae medio-proximally, article 3 with B-setae	6
6a. Posteroventral corner of E1 bluntly produced, urosomite 3 with dorsal slender setae	<i>G. carausui</i> sp. nov.
6b. Posteroventral corner of E1 rounded, urosomite 3 with dorsal spines	<i>G. dobrogicus</i> sp. nov.
7a. Posteroventral corner of E2 strongly pointed	<i>G. dacicus</i>
7b. Posteroventral corner of E2 slightly/moderately pointed	8
8a. Setation of A2 peduncle articles 4–5 very long, inner ramus of U3 is half length of the outer ramus	<i>G. pinkfloydorom</i> sp. nov.
8b. Setation of A2 peduncle articles 4–5 short, inner ramus of U3 is longer than half length of the outer ramus	<i>G. balcanicus</i>
9a. Setation of A2 peduncle dense and ca. 2x longer than the width of the underlying segment	<i>G. komareki</i>
9b. Setation of A2 peduncle scarce and shorter than or slightly exceeding the width of the underlying segment	10
10a. Flagellum of A2 swollen and bearing flag-like brush of setae as long as or exceeding the width of the underlying segment	<i>G. pulex</i>
10b. Flagellum of A2 slender	11
11a. Inner ramus of U3 reaches at most half length of the outer ramus	<i>G. fossarum</i>
11b. Inner ramus of U3 is longer than half length of the outer ramus	12
12a. Posteroinferior surface of P7 basis with setae, distal margin of E2 setose	<i>G. arduus</i>
12b. Posteroinferior surface of P7 basis without setae, distal margin of E2 not setose	13
13a. More than half of external margin of U3 outer ramus setose	<i>G. hamaticornis</i>
13b. Upper half of external margin of U3 outer ramus setose	14
14a. First and 2nd peduncle segments of A1 subequal in length	<i>G. leopoliensis</i>
14b. First peduncle segment of A1 much longer than the 2nd one,	<i>G. kischineffensis</i>

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SUPPLEMENTARY DATA

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

CONFLICT OF INTEREST

None declared.

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DATA AVAILABILITY

All newly generated sequences together with metadata (collection data, GPS codes, taxonomy) and NCBI GenBank codes are publicly available in the BOLD dataset DS-ROGAMBAL (<https://dx.doi.org/10.5883/DS-ROGAMBAL>).

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