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Gammarus sezgini sp. nov. (Arthropoda, Amphipoda, Gammaridae), a new amphipod species from the Eastern Black Sea region of Türkiye

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Abstract

A new amphipod species belonging to the genus *Gammarus* was identified in the rivers of the Eastern Black Sea Region of Türkiye: *G. sezgini* **sp. nov.** The authors described the new species using a taxonomic approach that combines morphological and molecular data. The newly identified species belongs to the *G. komareki* species complex because of the setation of antenna 2, pereopods 3 and 4, and the uropod 3. Some of its characteristic features are as follows: A medium-large species (holotype male, 9.8 mm). The body is yellowish; no dorsal keel or hump; eyes well developed, kidney-shaped; extremities not elongated; the second antenna bears numerous groups of long setae on the peduncle and flagellar segments; antennal gland cone long, not curved; the posterior margin of pereopod 3 is densely setose; the setae on the posterior edge of pereopod 4 are shorter and fewer in number; the anterior margins of pereopods 5 to 7 bear spines in the male; epimeral plates are not pointed. The newly identified species looks similar to *G. komareki* but differs from it by having a longer antennal gland cone, having fewer D-setae (33) in the third segment of the mandible palp, having shorter setae on the ventral part of the peduncular segment of the antenna 2, and having longer antenna 1, having fewer setae along the posterior margins of pereopods 3 and 4, and the absence of setae along the anterior margins of merus and carpus of pereopod 7. The new species is distinct from its relatives by high genetic distance (COI: 17.10% and 28S: 0.88%) and was resolved from them as an independent lineage with high support (ML: 78%, NJ: 70%, and BI: 1.0) in all phylogenetic results, based on the concatenated dataset (28S+COI). Additionally, species delimitation analyses (ASAP and PTP) based on the COI gene supported the conclusion that the new species constitutes an independent lineage. Detailed descriptions and drawings of the male holotype and the female allotype are given, and the morphology of the newly identified species is compared with that of its relatives.

Key Words

Introduction

The order Amphipoda Latreille, 1816, is comprised of six suborders represented by approximately 11,000 species. The suborder Senticaudata Lowry & Myers, 2013, which also includes the family Gammaridae Latreille, 1802, encompasses around 6,000 species, hosting nearly all freshwater species and numerous marine benthic species. The genus *Gammarus* Fabricius, 1775, with approximately 200 described species, exhibits a wide distribution in the Holarctic region. Previous studies suggest that *Gammarus* originated from ancient Tethys and then diversified due to plate tectonic activities between Eurasia and Africa/India (Hou et al. 2011; Horton et al. 2024).

Studies on *Gammarus* species in Türkiye began in the early 20th century and have continued until the present day (Vavra 1905; Coifman 1938; Bacescu 1954; Özbek and Ustaoğlu 1998, 2001, 2005a, 2005b; Sarı et al. 2001; Balık et al. 2004; Özbek et al. 2004, 2007; Akbulut et al. 2009; Albayrak and Özuluğ 2016; Özbek and Özkan 2017; Baytaşoğlu and Gözler 2018). Especially in recent years, newly recorded species from the inland waters

of Türkiye have been evaluated not only based on their morphological characteristics but also through molecular analysis (Rewicz et al. 2016; Özbek et al. 2023a, 2023b). So far, 55 species belonging to the genus *Gammarus* have been identified in Turkish inland waters (İpek and Özbek 2022; Özbek et al. 2023a, 2023b).

The Eastern Black Sea Basin covers Artvin, Rize, Trabzon, Gümüşhane, Giresun, and Ordu provinces. The rivers of the area are mainly fed by precipitation and have a regular regime. The flow rate is normal during the summer months, while the flow rate increases with melting snow. Some of the rivers flow directly into the Black Sea after a short flow, and some of them originate in central Anatolia and reach the Black Sea by crossing the North Anatolian Mountains (Selim 2011). In previous studies conducted in this basin, six species [*G. balcanicus* Schäferna 1922, *G. birsteini* Karaman & Pinkster, 1977, *G. kischineffensis* Schellenberg 1937, *G. komareki* Schäferna 1923, *G. pulex pulex* (Linnaeus 1758), and *G. fossarum* Koch 1835] were reported (Karaman 2003). However, no detailed distribution data for these species has been presented to date.

This study aimed to investigate the amphipod samples collected from streams (Balat-Yeşildere-Taşlı) in the Eastern Black Sea Basin (Rize) of Türkiye both morphologically and genetically. As a result of the study, a new amphipod species was described, *Gammarus sezgini* sp. nov., detailed descriptions and drawings of the extremities of the male holotype and female allotype were given, and the morphology of the newly described species was compared with its relatives.

Materials and methods

Study area

Samplings were conducted in Balat Stream, Taşlı Stream, and Yeşildere Stream within the borders of Rize province, the northeastern part of Türkiye. Balat Stream is a tributary of the Büyük Stream, which flows from Rize/Çayeli district to the Black Sea. There are trout farming facilities on the stream. Yeşildere Stream is a tributary of the Taşlı Stream, flowing from Rize/Andon Hot Springs to the Black Sea. In these locations, there are trout farming facilities and tea collection centers. The map of the stations where the species was identified is given in Fig. 1. A map was created using the QGIS v.3.8.3-Zanzibar software available at http://diva-gis.org (Fig. 1).

Data collection and analysis

Samplings were carried out at three stations in October 2019 and September 2020. A 30×30 cm sized hand net (D-Frame net) with a 250 μ mesh size was used to collect the specimens. The collected samples were placed in plastic sample containers, and the labels on which the date, the name of the station, the coordinate, the altitude, and the name of the city where they are located are written both inside the container and on the outside. The first fixation of the samples was made with 96% alcohol in the field. The samples brought to the laboratory were cleared of their sludge under tap water with the help of sieves with a mesh size of 4 mm–63 μm. Each individual was examined under a Leica MC 170 HD brand stereomicroscope.

Morphological identification

One adult male and one female individual from the samples were selected as holotype and allotype individuals, respectively. Both selected individuals were kept in a lactic acid and 10% NaOH solution for 2 hours. The holotype male individual was photographed under a stereomicroscope before being dissected. After holotype and allotype individuals were dissected in a glycerin alcohol solution, permanent slides were prepared with a CMCP-10 high-viscosity mount. Detailed photographs of the extremities
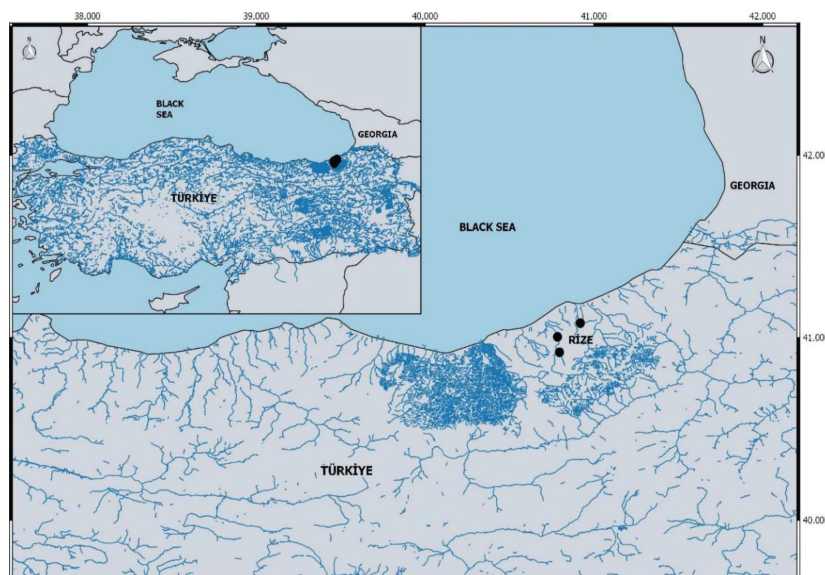
Figure 1. Map of the sampling area and the localities.

were taken with a 5-megapixel resolution digital camera mounted on an Olympus CKX-41 model binocular microscope. For detailed drawings of the extremities, a digitizer board (Wacom PTH-451) and a standard pen connected to the computer were used. Image processing programs were used in the drawings of the extremities, and the drawing techniques specified by Coleman (2003) were followed.

Molecular identification

Total DNA isolation, PCR amplification, and sequencing

The DNA of *Gammarus* specimens was extracted on the Qiacube Automated DNA Isolation Device (Qiagen, Valencia, CA) according to the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) protocol. The mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified with the primers UCOIF (5'-TAWACTTCDGGRT-GRCCRAAAAAYCA-3') and UCOIR (5'-ACWAAY-CAYAAAGAYATYGG-3') as described by Costa et al. (2009), and cycle conditions were as follows: 3 min. first denaturation at 95 °C, followed by 35 cycles of denaturing for 30 sec. at 95 °C, annealing for 30 sec. at 47 °C, extension for 45 sec. at 72 °C, and final extension for 7 min. at 72 °C. The nuclear large subunit ribosomal RNA gene (28S) was amplified with the primers 28F (5'-TTAGTAGGGG-

CGACCGAACAGGGAT-3') and 28R (5'-GTCTTCGC-CCCTATGCCCAACTGA-3') as described by Hou et al. (2007), and cycle conditions were as follows: 3 min. first denaturation at 95 °C, followed by 35 cycles of denaturing for 35 sec. at 95 °C, annealing for 30 sec. at 62 °C, extension for 1.15 min. at 72 °C, and final extension for 7 min. at 72 °C. The QIAquick PCR Purification Kit (Qiagen) was utilized to purify the amplified PCR products. Two-directional sequencing of PCR products was performed with an ABI PRISM 3730×1 Genetic Analyser using a BigDye Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystems) at Macrogen Europe.

Molecular data analysis

We carried out analyses to genetically compare the potential new species with its congeners and to generate its first molecular records. We sequenced the COI and 28S genes of a total of five specimens from three populations (Balat, Yeşildere, and Taşlı streams) of the new species (see "Genetic material" section). In addition, we downloaded the COI and 28S sequences of valid *Gammarus* species from GenBank. Detailed information on these species is available in Table 1.

The raw COI and 28S sequences of the new species were corrected by checking their chromatograms in the Bioedit 7.2.5 program (Hall 1999). All sequences were

Table 1. Information on sequences used in molecular analyses.

Species	Locality	28S	COI	References
G. sezgini sp. nov. (T)	Balat stream, Rize, Türkiye	PP456724	PP457381	This study
G. sezgini sp. nov. (T)	Balat stream, Rize, Türkiye	PP456725	PP457382	This study
G. sezgini sp. nov. (T)	Yeşildere stream, Rize, Türkiye	PP456726	PP457383	This study
G. sezgini sp. nov. (T)	Yeşildere stream, Rize, Türkiye	PP456727	PP457384	This study
G. sezgini sp. nov. (T)	Taşlı stream, Rize, Türkiye	PP456728	PP457385	This study
G. kunti (T)	Fakıllı Cave, Türkiye	OP650556	OP642558	Özbek et al. (2023a)
G. tumaf (T)	Gökgöl Cave, Türkiye	ON751931	ON749780	Özbek et al. (2023b)
G. baysali (T)	Cumayanı Cave, Türkiye	ON751932	ON749781	Özbek et al. (2023b)
G. kesslerianus (T)	Simferopol, Crimea, Ukraine	JF965721	JF965909	Hou et al. (2011)
G. komareki (T)	ca 200 km SE Sofia, Bulgaria	JF965725	JF965913	Hou et al. (2011)
G. komareki	Mazandaran, Iran	JF965723	JF965911	Hou et al. (2011)
G. rambouseki (T)	Bitola, Macedonia	JF965770	JF965946	Hou et al. (2011)
G. roeselii	Netherlands	JF965771	JF965947	Hou et al. (2011)
G. fossarum (T)	Regensburg, Germany	JF965696	JF965886	Hou et al. (2011)
G. plaitisi	Tinos, Komi, Greece	MT999102	MT999049	Hupało et al. (2020)
G. uludagi	Evia, Greece	JF965817	JF965986	Hou et al. (2011)
G. monspeliensis (T)	Montpellier, France	JF965738	JF965923	Hou et al. (2011)
G. ibericus	Lascaux, France	JF965713	JF965901	Hou et al. (2011)
G. pulex (T)	Slovenia	JF965767	JF965943	Hou et al. (2011)
G. lacustris	Bled, Slovenia	JF965728	JF965915	Hou et al. (2011)
G. italicus	Rieti, Lazio, Italy	JF965716	JF965904	Hou et al. (2011)
G. varsoviensis (T)	Secymin, Poland	JF965818	JF965987	Hou et al. (2011)
G. kischineffensis (T)	Targu Bujor, Romania	MG987529	MG987571	Copilaş-Ciocianu et al. (2018)
G. spelaeus (T)	Simferopol, Crimea, Ukraine	JF965801	JF965971	Hou et al. (2011)
G. balcanicus (T)	Kolašin, Montenegro	JF965640	JF965834	Hou et al. (2011)
G. bosniacus (T)	Sarajevo, Bosnia and Herzegovina	JF965680	JF965872	Hou et al. (2011)
G. leopoliensis (T)	Vistula, Poland	JF965734	JF965919	Hou et al. (2011)
G. stojicevici (T)	Bela Palanka, Serbia	JF965808	JF965978	Hou et al. (2011)
G. halilicae (T)	Lazaropole, Macedonia	JF965711	JF965900	Hou et al. (2011)
G. pljakici	Galicica planina, Macedonia	JF965758	JF965936	Hou et al. (2011)
G. stankokaramani (T)	Ohrid, Macedonia	JF965806	JF965976	Hou et al. (2011)
G. salemaai	Gradište, Macedonia	JF965780	JF965955	Hou et al. (2011)
Pontogammarus robustoides	Delta Volgi, Russia	JF965822	JF965990	Hou et al. (2011)

Note: (T) Topotype samples of nominal taxa.

then aligned with the Clustal W method (Thompson et al. 1994). The pairwise genetic distances were calculated for the COI and 28S genes according to the uncorrected *p*-distance in the MEGA X software (Kumar et al. 2018).

To reconstruct the phylogeny of the genus *Gammarus*, the COI and 28S sequences of all species were added end-to-end, resulting in a concatenated data set (28S+COI) for each species. Phylogeny was estimated by using Neighbour-Joining (NJ; Saitou and Nei 1987), Maximum Likelihood (ML; Felsenstein 1981) methods in MEGA X software, and Bayesian inference (BI) in MrBayes v3.2.1 (Ronquist et al. 2012). The appropriate nucleotide substitution models were selected according to the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) in jModeltest 0.1.1 (Posada 2008). The NJ tree was constructed using the *p*-distance node support, which was calculated (Felsenstein 1985) using 1000 bootstrap pseudo-replicates. The ML tree was constructed using the Tamura-Nei model (TN93; Tamura and Nei 1993) with gamma-correction and invariant sites (G+I), and node support was calculated with 500 bootstrap pseudo-replicates. The BI tree was constructed using the TN93+G+I model. The analysis was run for 5 million generations with Metropolis-coupled Monte Carlo Markov Chains (MCMC) sampled every 1000 generations. As burn-in, the first 25% of generations were discarded. The convergence of the runs was confirmed using Tracer v1.7.1 (Rambaut et al. 2018). The iTOL (Interactive Tree of Life; https://itol.embl.de/), a web-based program, was used to visualize the BI tree. In all phylogenies, *Pontogammarus robustoides* (Sars, 1894) (Table 1) was chosen as the outgroup.

We applied one distance-based method, Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2020), and one tree-based method, Poisson Tree Processes (PTP; Zhang et al. 2013), to identify the Molecular Operational Taxonomic Units (MOTUs) based on the COI dataset. To implement the ASAP method, we used the Kimura 2-parameter (K2P) distances and transition/transversion ratio (R:1.4) settings at the web address https://bioinfo.mnhn.fr/abi/public/asap/. The transition/transversion ratio (R) for the COI data was calculated in MEGA X software. PTP with a maximum likelihood solution was implemented via a web server (http://mptp.h-its.org/#/tree) (accessed on February 2, 2024).

Results

Gammarus sezgini sp. nov.

https://zoobank.org/BE51BA07-80D8-4832-96F3-594D0CD087FF
Figs 2–7

Material examined. *Holotype*: Türkiy • Male; 9.8 mm; Rize Province, Yeşildere stream/Balat stream/Taşlı Stream; coordinates: 40.9493°N, 40.5394°E / 41.0227°N, 40.7130°E / 40.8701°N, 40.5859°E. Specimens collected by Hazel BAYTAŞOĞLU; 16 October 2019 and 1 September 2020. Holotypes with paratypes are stored under catalog number RTEÜ-FFR200001;

(GenBank accession numbers: PP457383, PP457384 for COI, and PP456726, PP456727 for 28S; PP457381, PP457382 for COI, and PP456724, PP456725 for 28S; PP457385 for COI and PP456728 for 28S).

Paratypes: 38 males and 34 females, same data as the holotype.

Genetic material. RTEÜ-FFR-DNA K2, K4, Yeşildere stream, Rize Province, Türkiye, 40.9493°N, 40.5394°E (GenBank accession numbers: PP457383, PP457384 for COI, and PP456726, PP456727 for 28S) - RTEÜ-FFR-DNA K5, K8, Balat stream, Rize Province, Türkiye, 41.0227°N, 40.7130°E (GenBank accession numbers: PP457381, PP457382 for COI, and PP456724, PP456725 for 28S) - RTEÜ-FFR-DNA K9, Taşlı stream, Rize Province, Türkiye, 40.8701°N, 40.5859°E (GenBank accession numbers: PP457385 for COI and PP456728 for 28S).

Diagnosis. A medium-large species. The body is yellowish; no dorsal keel or hump; the eyes are well developed; kidney-shaped; the extremities are not elongated; the second antenna bears numerous groups of long setae on the peduncle and flagellar segments; the antennal gland cone is straight and reaches to the distal end of the third peduncular segment; posterior margin of pereopod 3 densely setose; the setae on the posterior edge of pereopod 4 are shorter and fewer in number; the anterior margins of pereopods 5 to 7 bear spines in the male, while they bear long setae along with the spines in females; epimeral plates are pointed; the inner ramus of uropod 3 is slightly longer than 0.8 of the outer one; each telson lobe bears a pair of spines distally and setae longer than the spines.

Description of male holotype. Head: Rostrum absent, inferior antennal sinus deep, rounded. Eyes kidney-shaped; length is slightly shorter than the diameter of the first peduncular segment of antenna 1 (Fig. 2).

Antennae: Antenna 1 (Fig. 4A) is half as long as the body length; the length ratio of the peduncular segments is 1:0.75:0.38; peduncle segments bear a few groups of minute setae; the length of the setae is much shorter than the segment where they are implanted; the main flagellum with 32 segments; each segment bears a few short setae in distal side; aesthetasc absent; accessory flagel-

Figure 2. Habitus of the holotype male of *Gammarus sezgini* sp. nov.
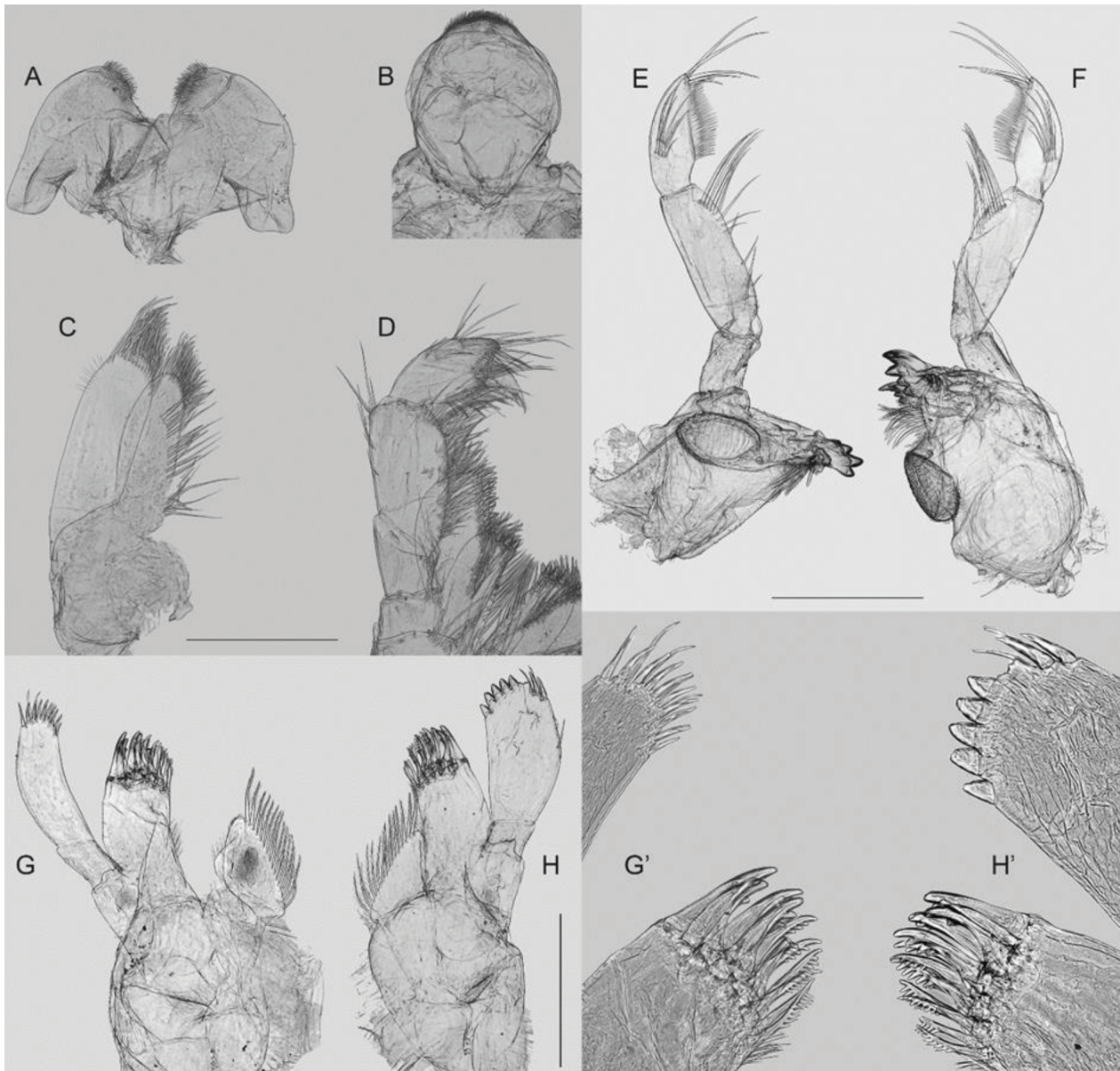
Figure 3. Mouthparts of the holotype male of *Gammarus sezgini* sp. nov. **A.** Lower lip; **B.** Upper lip; **C.** Maxilla 2; **D.** Maxilliped; **E.** Right mandible; **F.** Left mandible; **G.** Left maxilla 1; **H.** Right maxilla 1; **G', H'.** Detail of the left and right maxilla 1.

lum with five segments. Antenna 2 (Fig. 4B) is shorter than antenna 1 (ratio 1:0.7); the antennal gland cone is straight and reaches the distal end of the third peduncular segment; setation is rich both on peduncular and flagellar segments; peduncular segments 4 and 5 bear many groups of setae; the setae on the ventral part of the peduncle segments are shorter than the diameter of the segment but longer than those on the dorsal part; flagellum consists of 12 segments; flagellar segments are setose and swollen; each segment bears many long setae groups on both dorsal and ventral sides; calceoli absent.

Mouthparts: Upper lip (Fig. 3B) with numerous minute setules in the distal part.

Left mandible (Fig. 3F) with 4-toothed incisor, lacinia mobilis with 4 dentitions, molar triturative. The first article of palp without setae; the second one bears 14 setae; the setae become shorter from distal to proximal. The

third segment has 33 D-setae, 4–5 E-setae, one group of A-setae, and one group of B-setae. C-setae absent.

Right mandible (Fig. 3E) has a 4-toothed incisor and bifurcate lacinia mobilis.

Right maxilla 1 (Fig. 3H, H') is asymmetric to the left; it has 16 plumose setae along the inner margin of the inner lobe. The outer lobe bears 11 distal stout serrate spines and some tiny setules on the inner margin. Palp of the outer lobe with no setae in the first segment and six stout spines (one of them lost) and three setae (two of them robust) on the distal part of the second segment, in addition to a marginal seta along the outer margin. The second article of left palp is elongated and bears 10 spines, five simple setae on its distal part, and no setae along the outer margin (Fig. 3G, G').

Lower lip (Fig. 3A) has no inner lobe and bears numerous small simple setae along the distal margins of both lobes.
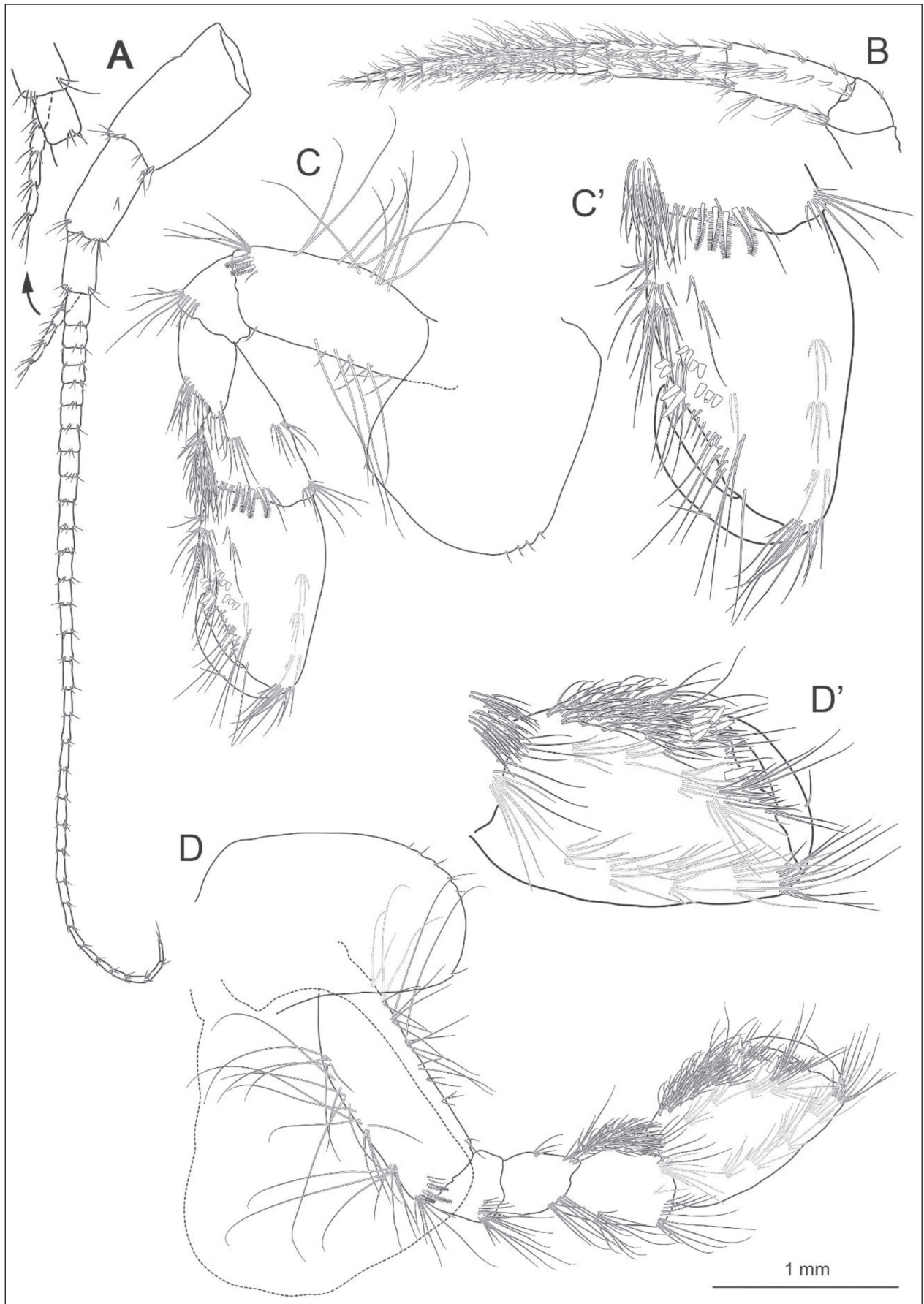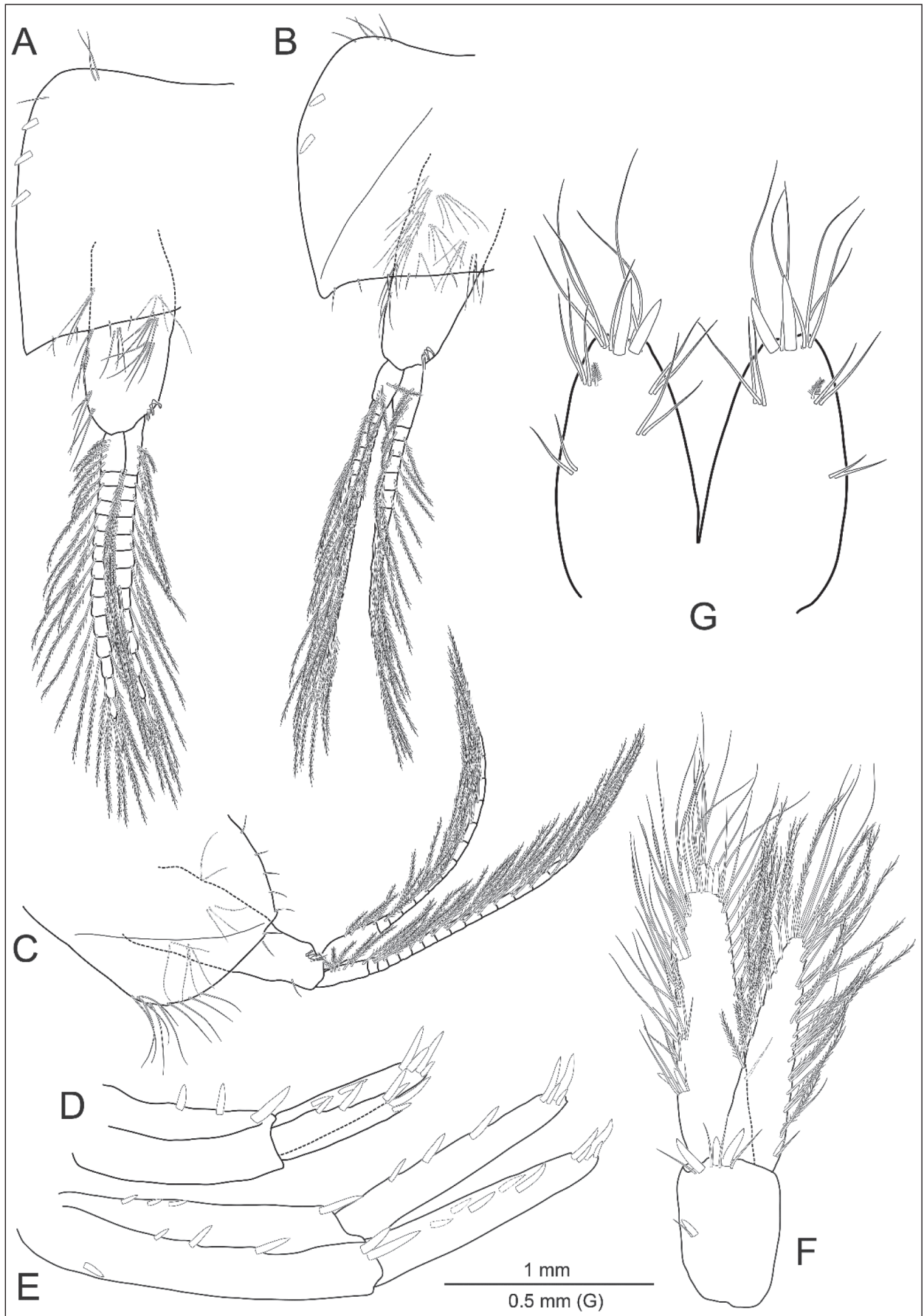
Figure 4. Extremities of the holotype male of *Gammarus sezgini* sp. nov. **A.** Antenna 1; **B.** Antenna 2; **C.** Gnathopod 1; **C'.** Palm of Gnathopod 1; **D.** Gnathopod 2; **D'.** Gnathopod 2.
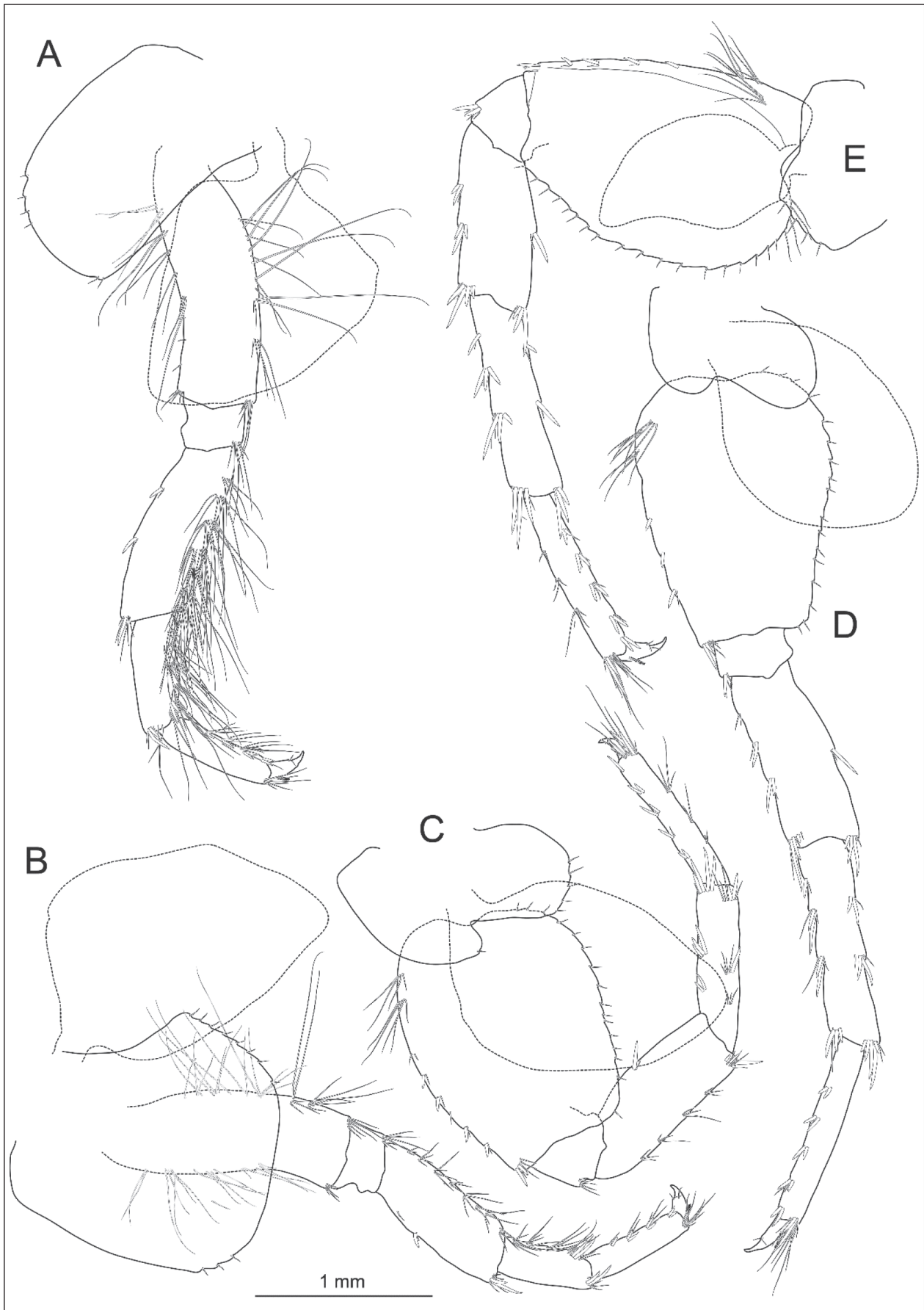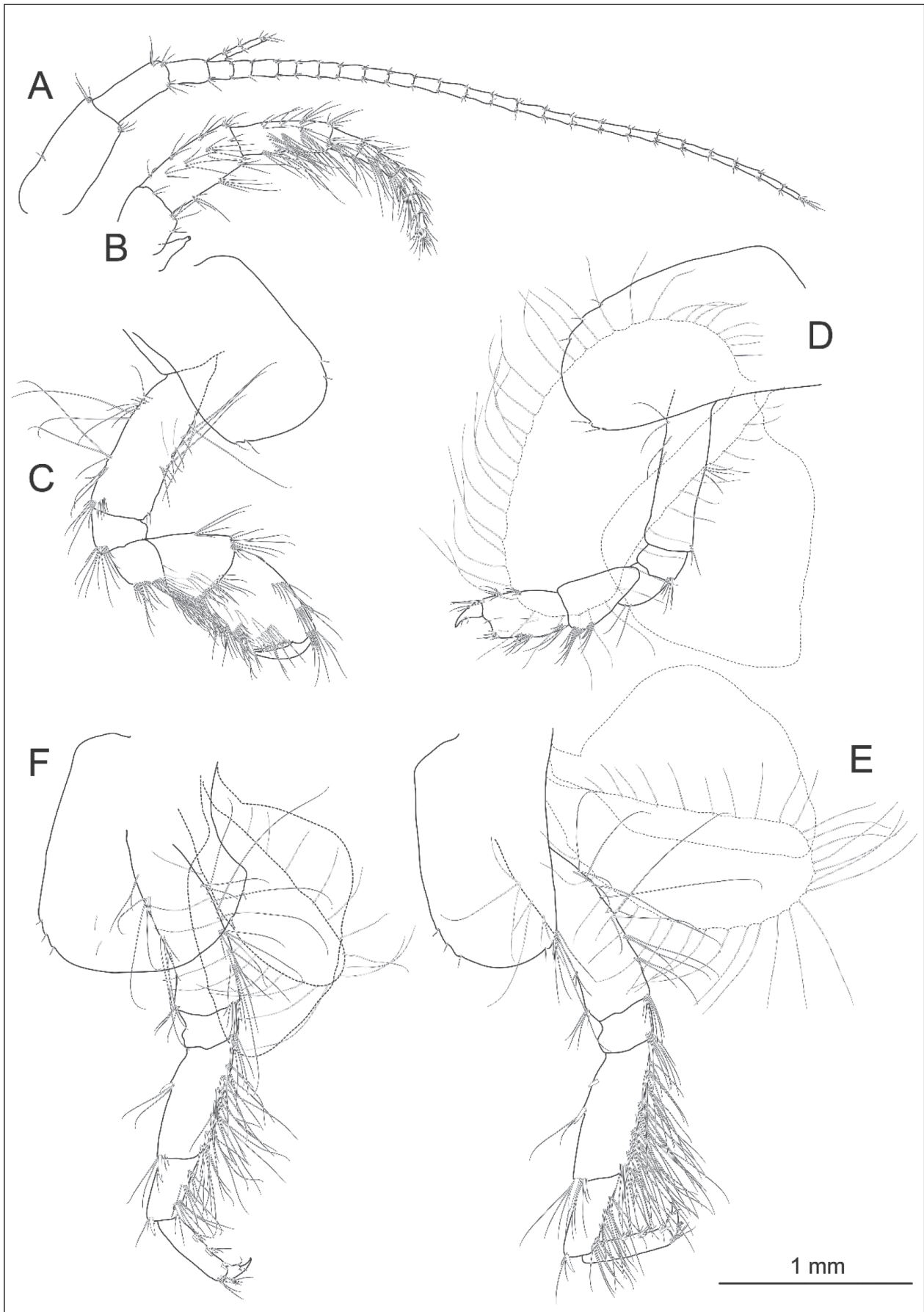
Figure 5. Pereopods of the holotype male of *Gammarus sezgini* sp. nov. **A.** Pereopod 3; **B.** Pereopod 4; **C.** Pereopod 5; **D.** Pereopod 6; **E.** Pereopod 7.

Figure 6. Extremities of the holotype male of *Gammarus sezgini* sp. nov. **A.** Pleopod 3 and Epimeral plate 3; **B.** Pleopod 2 and Epimeral plate 2; **C.** Pleopod 1 and Epimeral plate 1; **D.** Uropod 2; **E.** Uropod 1; **F.** Uropod 3; **G.** Telson.

Figure 7. Appendages of the allotype female of *Gammarus sezgini* sp. nov. **A.** Antenna 1; **B.** Antenna 2; **C.** Gnathopod 1; **D.** Gnathopod 2; **E.** Pereopod 3; **F.** Pereopod 4.

Maxilla 2 (Fig. 3C) has 20–30 simple setae in the distal part of the outer lobe and a few tiny hairs along the outer margin. The inner lobe also has 10–15 simple setae in the distal part in addition to 14–15 (two of them lost) plumose setae located in a diagonal row along the inner margin. There are also a few tiny hairs on the proximal part of the inner margin of the lobe.

Maxilliped (Fig. 3D) inner plate has three tooth-like spines and a spine in the distal part and the distal corner, respectively. Additionally, there are 10 plumose setae along the inner margin of the lobe. Outer plate armed with 5–6 serrate stout setae in the distal part and 12 spines along its inner margin.

Coxal plates: Coxal plate 1 (Fig. 4C) is rectangular, the distal part slightly widened, the ventral margin slightly convex, and bears four antero-distal setae and two postero-distal setae. Coxal plate 2 (Fig. 4D) is in the shape of an elongated rectangle; distal part narrower than the proximal; the ventral margin is highly convex; anterodistal part with five setae; and postero-distal part with one seta. Coxal plate 3 (Fig. 5A) is similar to coxal plate 2 in shape, with four and two setae in the antero- and postero-distal ends, respectively. The ventral edge of the coxal plate 4 (Fig. 5B) is slightly convex and bears three and eight setae along the anteroventral and posterior margins, respectively. Coxal plate 5 (Fig. 5C) bilobate and has one and five setae in the anterior and posterior lobes, respectively. Coxal plate 6 (Fig. 5D) bilobate and has one seta in the posterior lobe. Coxal plate 7 (Fig. 5E) is characterized by the presence of four setae on the postero-ventral margin.

Gnathopods: Basal segment of gnathopod 1 (Fig. 4C, C') bears many long setae along both margins; the length of the setae can be longer than twice the diameter of the segment. Ischium bears a group of setae in postero-ventral corner. Merus is in diamond shape and bears some setae along its disto-posterior margin. Carpus is triangular and bears two groups of setae along the anterior margin, in addition to many setae groups on both ventral and posterior sides. Propodus pyriform, the length/width ratio is 1:0.60; anterior margin with four groups of setae; medial palmar spine is present; posterodistal corner armed with two strong spines in addition to some small spines; posterior margin bears 4–5 groups of setae. Dactylus reaches the postero-distal corner and bears a simple seta along the outer margin in addition to a small setule around the distal part of the inner margin.

The basis and ischium of gnathopod 2 (Fig. 4D, D') are similar to those of gnathopod 1 but have denser setae. Merus and carpus are more setose than those of gnathopod 1. Carpus is triangular, densely setose along the posterior margin, in addition to two groups of setae along the anterior margin. Propodus is densely setose and has a sub-rectangular shape; the length/width ratio is 1: 0.6; anterior margin bears six groups of setae; posterior margin with many groups of setae; medial palmar spine is present; the postero-distal corner is armed with six strong spines. Dactylus reaches the postero-distal corner and bears a simple seta along the outer margin in addition to a small setule around the distal part of the inner margin.

Pereopods: Anterior and posterior margins of the basal segment of pereopod 3 bear long setae; the setae along the posterior margin are longer than those in the anterior margin; posterior margins of the merus, carpus, and propodus bear long setae; the setae can be more than three times the diameter of the segment where they are implanted. Dactylus slim, a minute plumose seta occurs on the outer margin; the inner margin with two small setules (Fig. 5A).

The basal segment of pereopod 4 (Fig. 5B) has a similar setation to that of pereopod 3. Ischium, merus, carpus, and propodus have groups of setae along their posterior margins, but they are much shorter and less than those in pereopod 3. The length of the setae can be as long as (or slightly longer) than the diameter of the segment where they are implanted. Dactylus slim, a minute plumose seta, occurs on the outer margin; the inner margin with two small setules.

Posterior margins of the basal segments of pereopods 5 to 7 (Fig. 5C–E) are more or less convex and bear many short setae, anterior margins with 3–7 small spines, and no setae present on the inner surfaces of the basal segments; no spine exists in the postero-ventral corner of the basal segment of pereopod 7. Pereopod 7 bears no setae along the anterior margins of merus and carpus, while pereopods 5 and 6 have a few setae accompanying spines along with the mentioned segments. Propodus of pereopods 5 to 7 with 2–3 groups of long setae groups along their outer margins in addition to 5–6 groups of small spines along their inner margins. Dactylus slim, a minute plumose seta, occurs on the outer margin; the inner margin with two small setules.

Epimeral plates: They are slightly pointed. Epimeral plate 1 (Fig. 6C) bears 10 long setae along the antero-ventral margin, and the postero-ventral corner is angular. Epimeral plate 2 (Fig. 6B) bears five setae in the anteroventral corner; the ventral margin is armed with two spines; the posterior margin with 4–5 setules; the postero-ventral corner is pointed. Epimeral plate 3 (Fig. 6A) is pointed; the anteroventral corner bears two setae; the ventral margin is armed with three spines in addition to a seta; the posterior margin bears 5–6 setules.

Urosomites: Not elevated (Fig. 2). Each segment bears a median and two dorsolateral groups of armaments; each of them consists of 1–2 spines and 3–4 accompanying setae.

Uropods: Uropod 1 (Fig. 6E) has a spine in the outer margin of the base; inner margins bear 4+5 spines; the peduncle is longer than rami; the length ratio is about 1:0.7. Peduncle with a spine in the outer margin of the proximal part in addition to three spines along the inner margin and three spines in the distal part. The inner ramus is slightly longer than the outer ramus and bears three spines along their inferior margin in addition to 4–5 distal spines. The outer ramus has two and three spines along the inferior and outer margins in addition to 4–5 distal spines, respectively.

Uropod 2 (Fig. 6D) is smaller than the first one; the length ratio is about 1:0.6; the peduncle segment is slightly longer than the rami and bears 2+1 spines along the inner margin and the distal part, respectively. The outer margin is bare. The length and armaments of both rami are similar to each other; they bear two spines along their

inner margins in addition to 4–5 longer spines on their distal tips.

Uropod 3 (Fig. 6F) is setose and bears both simple and plumose setae. The peduncle segment is much shorter than the outer ramus, and the length ratio is about 1:0.48. The outer ramus is two articulated and densely setose along both margins; the outer margin bears three groups of spines accompanied by groups of long simple setae; the inner margin with plumose setae; the second article is well developed and longer than the surrounding distal spines. The inner ramus is about 0.78 times the length of the outer ramus. It bears one spine in the proximal part of the outer margin in addition to groups of simple and plumose setae; the inner margin bears both simple and plumose setae.

Telson: Telson (Fig. 6G) lobes cleft; each lobe bears two spines and 5–6 simple setae in their distal parts. The setae are twice as long as the spines. There are 3–4 groups of short setae on the dorsal surface of the lobes in addition to two plumose setules. The length/width ratio of each lobe is about 1:0.5.

Description of females. Smaller than males. Except for the sexual dimorphism indicated for the genus *Gammarus*, females do not show obvious differences from males. At first glance, the morphological differences between the female allotype and the male holotype can be listed as follows: More setose antenna 2, less setose and small gnathopod 2, more setose pereopod 4; more setose anterior margins of pereopods 5 to 7 (Figs 7, 8).

Variability. Some of the paratypes are immature. The eyes are kidney-shaped, or elongated, and oval. The number of flagellar segments in antenna 1 varies between 26 and 29. Similarly, there are 10–11 flagellar segments in antenna 2.

Etymology. The species epithet is derived from the name of our dear friend Prof. Dr. Murat Sezgin (R.I.P.), who made valuable contributions to the marine amphipod species in Türkiye.

Results of molecular data analyses

We tested the new species with molecular methods as well as morphological characters. For this, firstly, the COI (573 bp.) and 28S (911 bp.) genes of the new species from type and paratypes were amplified and sequenced. The obtained sequences were deposited in Genbank with the corresponding accession numbers: PP457381–PP457385 for COI and PP456724–PP456728 for 28S. For molecular comparison, sequences of topotype samples of valid congeners of the new species or otherwise correct sequences of valid species were downloaded from GenBank (see Table 1) and used in all analyses.

The pairwise genetic distance amongst *Gammarus* species based on the COI was calculated to range from 5.24% (*G. stankokaramani* G. Karaman, 1976 - *G. salemaai* G. Karaman, 1985) to 28.97% (*G. sezgini* sp. nov. - *G. roeselii* Gervais, 1835). The species most closely related to *G. sezgini sp. nov.* is *G. tumaf* Özbek, Aksu & Baytaşoğlu, 2023, with 17.10%, approximately three times larger than

the minimum genetic distance. The pairwise genetic distance amongst *Gammarus* species based on the 28S was calculated to range from 0.11% (*G. halilicae* G. Karaman, 1969 - *G. pljakici* G. Karaman, 1964) to 7.73% (*G. rambouseki* G. Karaman, 1931 - *G. stojicevici* (S. Karaman, 1929)). The species most closely related to *G. sezgini* sp. nov. is *G. kesslerianus* Martynov, 1931, with 0.88%, eight times larger than the minimum genetic distance. All pairwise genetic distance values calculated with the *p*-distance model based on COI and 28S genes amongst *Gammarus* species are given in Suppl. material 1.

Phylogenetic trees constructed with ML, NJ, and BI methods based on the concatenated dataset (28S+COI) showed similar topologies with a few exceptions and had high bootstrap (ML and NJ BP≥70%; Fig. 9) and posterior probability (BI PP≥0.7; Fig. 9) support for a large number of nodes. In the phylogenies constructed according to all three methods, the newly identified species, *G. sezgini* sp. nov., formed the sister clade of the *G. kunti* Özbek, Baytaşoğlu & Aksu, 2023, *G. tumaf*, and *G. baysali* Özbek, Yurga & Külköylüoğlu, 2013 (*G. sezgini* sp. nov., (*G. kunti*, (*G. tumaf* + *G. baysali*))) and was resolved from it with strong support (ML BP: 78%, NJ BP: 70%, and BI PP: 1.0; Fig. 9).

The species delimitation analysis we performed according to the ASAP method identified 27 MOTUs for 27 morphologically valid species (Fig. 9). The best ASAP score was 3.0 (p = 0.01) at a threshold distance of 0.079053. The analysis identified the species *G. stankokaramani* and *G. salemaai* as a single MOTU, while the Bulgarian and Iranian samples of *G. komareki* were identified as separate MOTUs. The PTP method identified 28 MOTUs for 27 species (Fig. 9). p=0.001, null-model score: 84.937039, best score for single coalescent rate: 95.647569. Similar to ASAP, Bulgarian and Iranian samples of *G. komareki* formed separate MOTUs, while unlike ASAP, *G. stankokaramani* and *G. salemaai* species also formed separate MOTUs. *Gammarus sezgini* sp. nov. formed a single MOTU independently of other species according to both methods (Fig. 9).

Discussion

The consensus of morphological and molecular findings has shown that the Balat, Taşlı, and Yeşildere streams at Rize province populations of *Gammarus* are distinct from their congeners and should be recognized as a separate species. *Gammarus sezgini* sp. nov. belongs to the *G. komareki*-group due to the characteristic setation of the posterior part of pereopod 3 and 4, the setation of antenna 2 and uropod 3 (Karaman and Pinkster 1977).

At first glance, the newly identified species looks similar to *G. komareki* by the setation of the antenna 2, pereopod 3, and uropod 3, by the presence and the shape of the eyes; but *G. sezgini* sp. nov. differs from *G. komareki* by having a longer antennal gland cone, having fewer D-setae (33) in the third segment of the mandible palp,
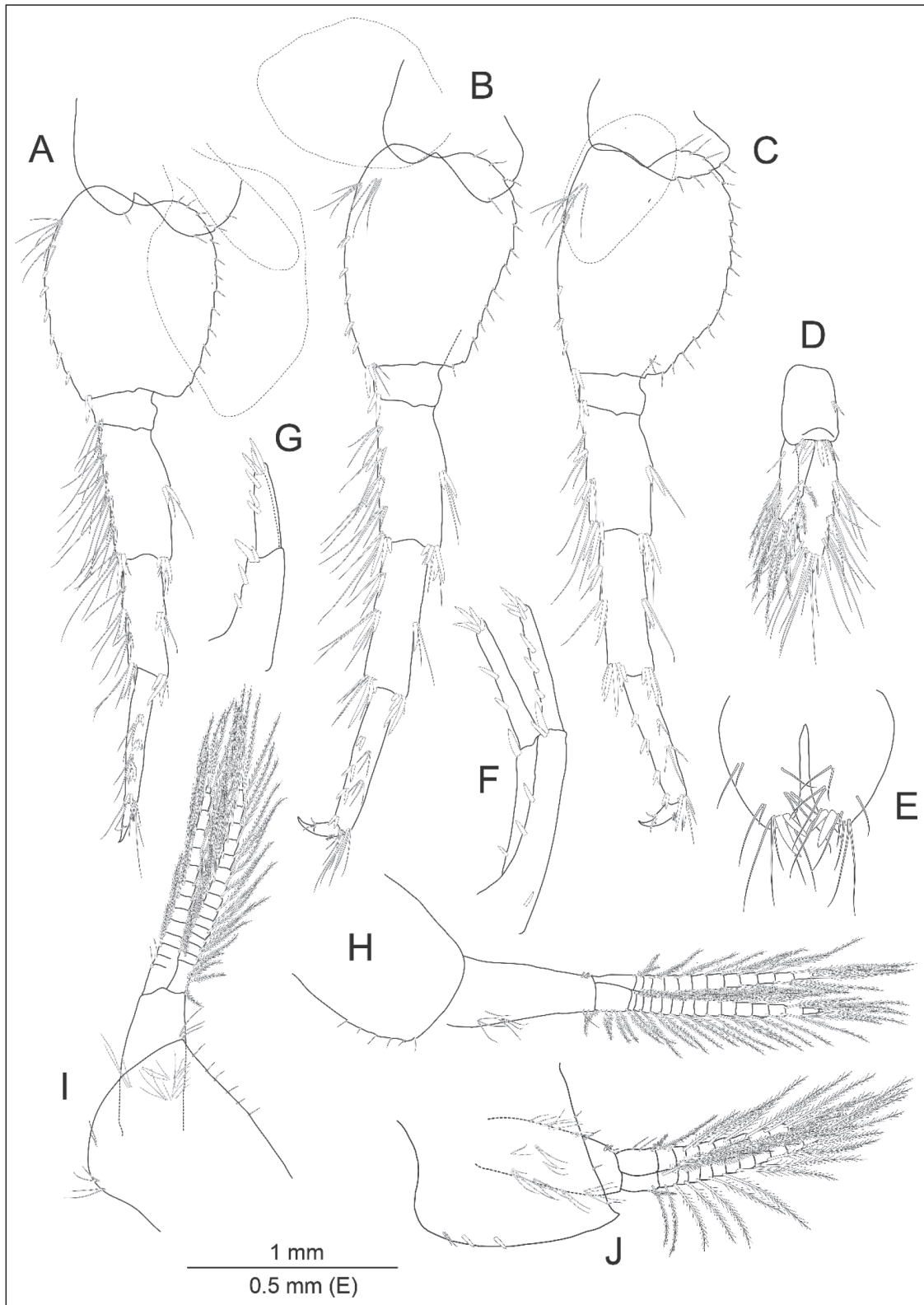
Figure 8. Appendages of the allotype female of *Gammarus sezgini* sp. nov. **A.** Pereopod 5; **B.** Pereopod 6; **C.** Pereopod 7; **D.** Uropod 3; **E.** Telson; **F.** Uropod 1; **G.** Uropod 2; **H.** Pleopod 1 and Epimeral Plate 1; **I.** Pleopod 2 and Epimeral Plate 2; **J.** Pleopod 3 and Epimeral Plate 3.

having shorter setae on the ventral part of the peduncular segment of the antenna 2, and having a longer antenna 1, having fewer setae along the posterior margins of pereopod 3 and 4, by the absence of setae along the anterior margins of merus and carpus of pereopod 7.

Gammarus komareki has been recorded from the Black Sea coasts, Eastern Europe, the Balkans, and Iran in previous studies (Copilaş-Ciocianu et al. 2014; Grabowski and Pešic 2007; Zamanpoore et al. 2011). The species was reported from the Zonguldak, Trabzon, Sinop, and
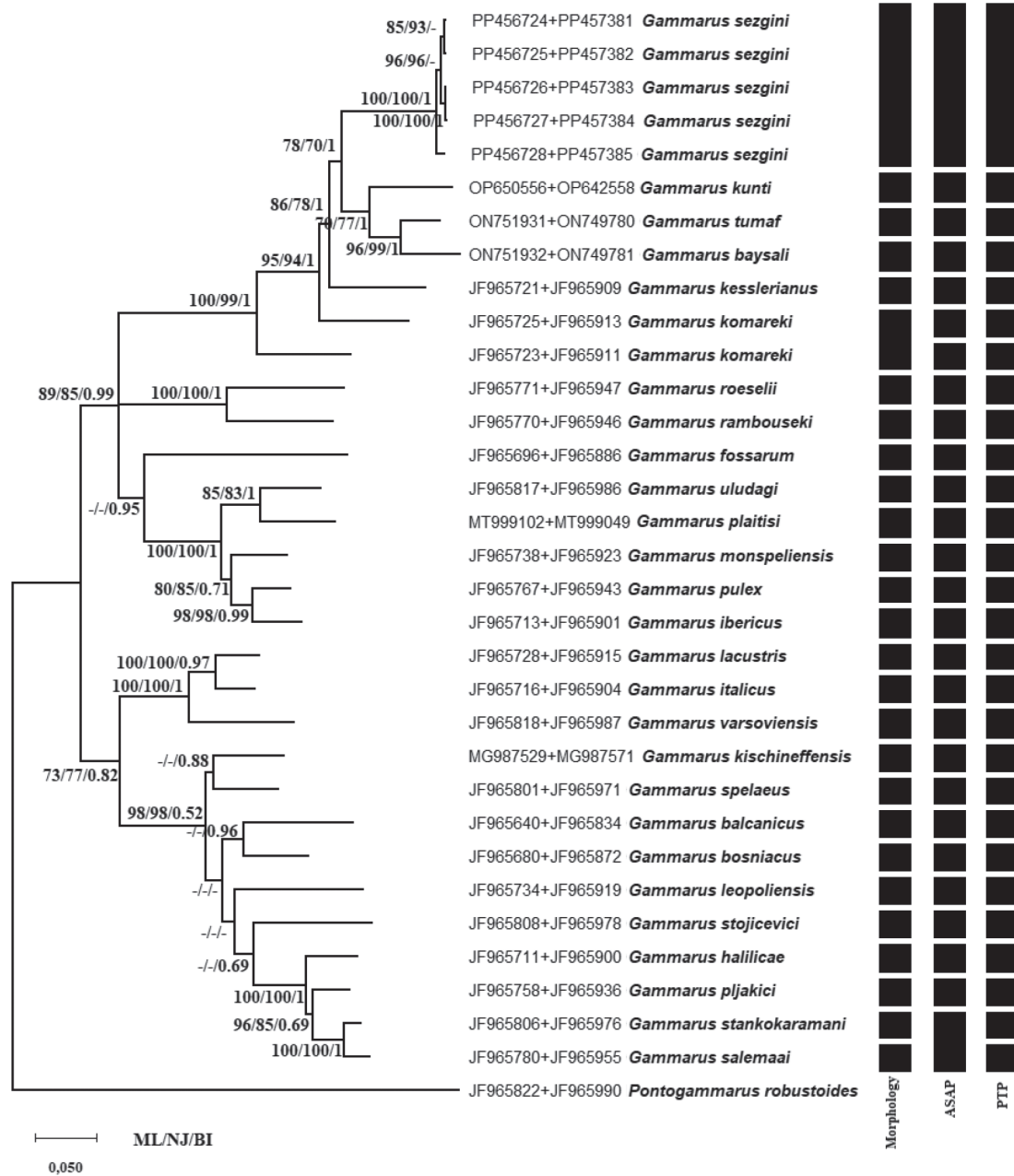
Figure 9. Phylogenetic relationships of *Gammarus* species reconstructed with the ML method based on the concatenated data set (28S+COI). Since the ML, NJ, and BI methods generally yield similar topologies, only the ML phylogeny is shown. The nodes (ML, NJ, and BI) show the Bayesian posterior probabilities and the bootstrap percentage. For the support values of the nodes, ML ≥ 70%, NJ ≥ 70%, and BI ≥ 0.70 are shown. Black bars indicate OTUs. The first column shows morphology-based results, the second column shows ASAP results, and the third column shows PTP results.

Rize provinces previously (Karaman 2003; Özbek 2011). Additionally, it was reported from the inland waters of Gökçeada Island (Özbek and Özkan 2017), from the lakes of the Western Black Sea Region and the Sakarya River Basin (Özbek 2008), from the inland waters of Sinop and Samsun provinces (Akbulut et al. 2009), and from the inland waters of Ordu (Ekinci and Miroğlu 2016).

Due to the lack of detailed sampling in the rivers in the Eastern Black Sea Basin, it is likely that the species *G. sezgini* sp. nov. has been diagnosed as *G. komareki* or has not been reached. The present study reveals the morphological and molecular differences between the two species in detail.

Gammarus obruki Özbek, 2012, *G. baysali*, *G. tumaf*, and *G. kunti* have been recently identified from four different caves (Inderesi Cave/Bartın province, Cumayanı Cave/Zonguldak province, Gökgöl Cave/Zonguldak province, and Fakıllı Cave/Düzce province, respectively) within the Western Black Sea Basin (Özbek 2012; Özbek et al. 2013; Özbek et al. 2023a, 2023b). They are the closest relatives of the newly described species due to their presence in geographically close localities and their morphological similarities. The four species mentioned above, including *Gammarus sezgini* sp. nov., are morphologically very similar to *G. komareki*, especially due to the dense setation on the flagellum and peduncle

segments of antenna 2. As a result of the study, morphological and genetic similarities and differences were defined in detail (Table 2, Fig. 9).

Gammarus sezgini sp. nov. is similar to *G. obruki* in that it has a yellowish body color, kidney-shaped eyes, and densely setose flagellum and peduncle segments of antenna 2. But the newly described species is almost half the size of *G. obruki*, has a much shorter antenna 1 (52 vs. 32 segments), and no elongated extremities. Additionally, the inner ramus of the uropod 3 is shorter in the newly identified species (Table 2).

Although the newly described species is similar to *G. baysali* in having setose antenna 2, it is quite different from it in terms of both morphological characters and habitat. *G. baysali*, like *G. obruki*, is a large species and is approximately 2 times larger than the newly described species. Additionally, *G. baysali* is a hypogean eyeless species and has elongated extremities. *Gammarus sezgini* sp. nov. is an epigean species with well-developed kidney-shaped eyes and does not have elongated extremities. Although there are spines and setae on the anterior margins of pereopods 6 and 7 in *G. baysali*, the newly described species has no setae along the mentioned margins. While the inner lobe/outer lobe length ratio is 0.9 in the third uropod of *G. baysali*, this ratio is 0.78 in *G. sezgini* sp. nov. (Table 2).

The newly described species is similar to *G. kunti* in having kidney-shaped eyes and setose antennae 1 and 2, but differs from it in the following features: Its body has a yellowish rather than whitish color; it bears one seta instead of two along the anterior margin of the inner lobe of the right maxilla 1; the inner lobe of the right maxilla bears more than 14 plumose setae. In addition, the 2nd and 3rd epimeral plates are more pointed, and the telson lobes bear more and longer setae on the dorsal surface and the distal part (Table 2).

Gammarus sezgini sp. nov. differs from *G. tumaf* by having kidney-shaped eyes, a less setose inner lobe of right maxilla 1 (16–17 vs. 20), the armaments of the palp of maxilla 1, having fewer plumose setae on maxilla 2 (14–15 vs. 20), and having more setose telson. Additionally, the newly identified species has 33 D-setae, while the number is 28 in *G. tumaf* (Table 2).

Although the newly described species is similar to *G. kesslerianus* in having a setose second antenna 2, *Gammarus sezgini* sp. nov. differs from it in having almost half a smaller body length (20 mm vs. 9.8 mm), a shorter flagellum of antenna 2 (13 vs. 17 segments), and a shorter inner lobe of uropod 3.

Anatolia is a peninsula very rich in biodiversity, as it is located at the intersection of three different biodiversity hot spots. The presence of several unique habitats is an important factor in increasing the number of endemic species on the peninsula. Türkiye's Black Sea region hosts fast-flowing streams that are generally fed by snow water. The authors believe that *G. komareki*, which is a typical species of these types of habitats, still contains many cryptic and undefined species and should be ex-

Table 2. Some morphological features of *Gammarus sezgini* sp. nov. and its sister species (*G. baysali*, *G. tumaf*, *G. kunti*) and *G. obruki* (reproduced from Özbek et al. 2023a).

Characters	*Gammarus sezgini* sp. nov.	*G. obruki*	*G. baysali*	*G. tumaf*	*G. kunti*	*G. komareki*
Body length	9.8 mm	21.0 mm	18.1 mm	12.6 mm	11.5 mm	15 mm
Eyes	kidney-shaped	kidney-shaped	eyeless	minute	kidney-shaped	reniform
Body color	yellowish	yellowish	colorless, whitish	whitish	whitish	
Antenna 1	32+5 flagellar segments	52+6 flagellar segments	41+6 flagellar segments	30+5 flagellar segments	32+6 flagellar segments	39+5 flagellar segments
Antenna 2	peduncular and flagellar segments densely setose; flagellum 12 segmented	fifth peduncular and flagellar segments densely setose; flagellum 17 segmented	peduncular and flagellar segments setose; flagellum 20 segmented	peduncular and flagellar segments densely setose; flagellum 13 segmented	peduncular and flagellar segments densely setose; flagellum 15 segmented	peduncular and flagellar segments densely setose; flagellum 13 segmented
Antennal gland cone	straight, reaches to the distal end of the third peduncular segment	straight, not reaches to the distal end of the third peduncular segment	straight, reaches to the distal end of the third peduncular segment	straight, reaches to the distal end of the third peduncular segment	straight, reaches to the distal end of the third peduncular segment	Short, about half as long as the third peduncle segment
Inner lobe of right maxilla 1	with 16 (17) plumose setae	with 18 plumose setae	with 19 plumose setae	with 20 plumose setae	with 14 plumose setae	No data in original description
Palp of right maxilla 1	6 stout spines, 1 seta along the anterior margin	6 stout spines, 3 setae along the anterior margin	6 stout spines, 4 setae along the anterior margin	5 stout spines, 2 setae along the anterior margin	6 stout spines, 2 setae along the anterior margin	No data in original description
Maxilla 2	inner lobe with 14–15 plumose setae	inner lobe with 21 plumose setae	inner lobe with 21 plumose setae	inner lobe with 20 plumose setae	inner lobe with 15 plumose setae	No data in original description
Number of D-setae	33	37	34	28	28	40
Pereopods	not elongated	slightly elongated	elongated	not elongated	not elongated	No data in original description
Pereopods 6–7	anterior margins without setae	anterior margins without setae	anterior margins with setae	anterior margins without setae	anterior margins without setae	anterior margins without setae
Uropod 3	setose, inner/outer lobe ratio: 0.78	setose, inner/outer lobe ratio: 0.9	setose, inner/outer lobe ratio: 0.9	setose, inner/outer lobe ratio: 0.75	setose, inner/outer lobe ratio: 0.77	setose, inner/outer lobe ratio: 0.75
Telson (each lobe)	with 2 distal spines and 5–6 longer setae; l/w ratio 1:0.5	with 1–2 distal spines and 3–4 longer setae; l/w ratio 1:0.5	with 2 distal spines and 4–5 longer setae; l/w ratio 1:0.5	with 2 distal spines and 3–4 longer setae; l/w ratio 1:0.5	with 2 distal spines and 3–4 longer setae; l/w ratio 1:0.5	with 2 distal spines and 3–4 longer setae; l/w ratio 1:0.5

amined in detail from both molecular and morphological perspectives. Such an integrative approach would help highlight the true biodiversity of gammarids in Anatolia.

Acknowledgments

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Supplementary material 1

The pairwise genetic distance values amongst the *Gammarus* species, based on the COI datasetand 28S dataset

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