

A new freshwater amphipod (Amphipoda, Gammaridae) from the Fakıllı Cave, Düzce Türkiye: *Gammarus kunti* sp. nov.

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Abstract

Aquatic species (such as fish, amphipods, isopods, hirudineans etc.) adapted to environmental conditions can live in caves connected to groundwater. The species of *Niphargus* and *Gammarus* are the most commonly encountered amphipods in caves. Türkiye is very rich in terms of karst areas and is home to more than 2000 known caves. Fakıllı Cave, located in Düzce Province in the Western Anatolian Region, has a length of 1071 m. A new amphipod species belonging to the *Gammarus* genus has been identified from the cave and named as *Gammarus kunti* sp. nov. Some of the characteristic features of the newly-identified species can be listed as “Medium-large size; smooth body, well-developed and reniform eyes; non-prolonged extremities; antennal gland cone is straight and long; second antenna with setose peduncular and flagellar segments; medial palmar spine present; posterior margin of pereopod 3 densely setose; anterior margins of pereopods 6 and 7 armed with spines only; epimeral plates not pointed”. Although the mentioned features are generally seen in epigean species, the members of this species were sampled from the dark zone of the Fakıllı Cave. The partial sequences of the COI (573 bp) and 28S (914 bp) genes of the newly-described species, *Gammarus kunti* sp. nov., were generated. The pairwise genetic distances between the new species, *Gammarus kunti* sp. nov. and other species ranged from a minimum of 16.23% (*G. tumaf*) to a maximum of 28.27% (*G. roeselii*) for the COI gene and a minimum of 0.88% (*G. tumaf*) to a maximum of 6.81% (*G. balcanicus*) for the 28S gene. Phylogenies generated by the NJ and ML methods, based on the combined data, assigned the new species as an independent lineage with high support values. In addition, the ASAP method identified the new species as a single MOTU independent of other species. *G. tumaf* and *G. baysali* are the sister taxa of *G. kunti* sp. nov. Detailed descriptions and drawings of the extremities of the male holotype and the female allotype are given and the morphology of the newly-identified species is compared with its relatives.

Key Words

benthos, cave, identification key, invertebrate, molecular identification, new species

Introduction

Caves, mid-ocean islands, deep seas, remote lakes and extremely cold and/or hot habitats are typical examples of extreme environments. Extreme conditions can lead to more effective functioning of organisms’ adaptation and evolution mechanisms resulting in morphological changes that can be associated not only with the absence of light in caves, but also with the presence of different microhabitats. In addition, morphological changes may

be niche-based and related to the presence of various micro-habitats (Trontelj et al. 2012).

Gammarus, the most widely distributed epigean freshwater genus of the Amphipoda order, has spread from the Western Palearctic to China and North America (Vainola et al. 2008). The representatives of the genus generally live in epigean habitats, but are also distributed in hypogean habitats, such as caves and wells (Karaman and Pinkster 1977). Reduced or vestigial eyes, elongated antennae and extremities and a non-pigmented body are

some of the morphological features frequently encountered in *Gammarus* species adapted to living in hypogean habitats (Pinkster and Karaman 1978; Fišer 2009; Özbek et al. 2013).

Türkiye is located between the Eurasian, African and Arabian plates and is situated on the Alpine-Himalayan Mountain Belt. As a result, it is a karst-rich country with more than 2000 known caves (Nazik et al. 2019; Yamaç et al. 2021). Studies on the amphipod fauna of Türkiye's inland waters, which started with the identification of *Gammarus argaeus* from Mount Erciyes (Vávra 1905), have increased over time and with a total of 51 *Gammarus* species reported. A total of 20 amphipod species belonging to the genus *Niphargus* Schiödt, 1849; *Gammarus* Fabricius, 1775; *Parhadzia* Vigna Taglianti, 1988 and *Bogidiella* Hertzog, 1933 have been reported from the caves and wells of Türkiye (İpek and Özbek 2022). In a recent study, this number increased by one more and *Gammarus tumaf* was identified from Gökgöl Cave, Zonguldak Province (Özbek et al. 2023).

The study aims to examine the individuals collected from Fakıllı Cave, Düzce Province, Türkiye, in terms of morphological and molecular features. Detailed descriptions and drawings of the extremities of the male holotype and female allotype are given and the morphology of the newly-identified species is compared with its relatives.

Materials and methods

Sampling area

Fakıllı Cave is located in Fakıllı Village, 8 km south-east of Akçakoca Town, Düzce Province, NW Türkiye. The total length of the cave is 1071 m and 350 m from the cave entrance is open for visitors. The entrance of the cave, which is 100 ms above sea level, has a width of 5–10 m and a ceiling height of 5–6 m. From the entrance of the cave, the sections are passed through long narrow corridors. There are many natural features including galleries, stalactites and stalagmites going in various directions inside the cave, which was registered as a first-degree protected area by the Ministry of Culture and Tourism's Regional Board of Protection of Cultural and Natural Assets (Zengin and Eker 2020).

Morphological identification

Individuals were collected with a hand aspirator from the dark zone of the cave, fixed in 70% ethanol in the field and transported to the laboratory for taxonomic identification. Specimens were dissected under a stereomicroscope, straightened with forceps and body length was measured from the base of the first antennae to the base of the telson. Permanent slides of the male holotype individual were prepared using the high-viscosity mount, CMCP-

10. Photographs of the extremities were taken with a digital camera connected to an Olympus CX41. A digitiser board (Wacom PTH-451) and a standard pen connected to a PC were used for detailed drawings of the extremities. Scaled drawings of the extremities were made on the photographs (Coleman 2003). The geographical location of the cave is shown in Fig. 1. The collected samples are kept in the Museum of the Faculty of Fisheries, Ege University (ESFM).

Molecular identification

DNA extraction, PCR amplification and Sequencing

Total DNA was extracted on the Automated DNA isolation device (QIAcube Qiagen, Germany) according to the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) protocol. Mitochondrial cytochrome c oxidase subunit I gene (COI) and the nuclear large subunit ribosomal RNA gene (28S) were amplified from the extracted DNA. Amplification of the COI marker was performed with the primers UCOIF (5'- TAWACTTCDGGRT-GRCCRAAAAAYCA-3') and UCOIR (5'- ACWAAY-CAYAAAGAYATYGG-3') according to the PCR protocol of Costa et al. (2009). Amplification of the 28S marker was performed with the primers 28F (5'- TTAGTAGGG-GCGACCGAACAGGGAT-3') and 28R (5'- GTCTTC-GCCCCTATGCCCAACTGA-3') according to the PCR protocol of Hou et al. (2007).

PCR products of the COI and 28S genes were purified by using the QIAquick PCR Purification Kit (Qiagen). Bidirectional sequencing of both PCR products was performed with an ABI PRISM 3730x1 Genetic Analyser using a BigDye Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystem) according to the Sanger method at MacroGen Europe.

Molecular data analyses

We sequenced the partial sequences of the COI and 28S genes from one individual to perform molecular analyses and generate the genetic record of the new species. In addition, we downloaded a total of 27 reference sequences (COI and 28S sequences for each species) from the GenBank (NCBI: National Centre for Biotechnology Information) for use in molecular analyses. Detailed information on the sequences used in molecular analyses is given in Table 1.

The raw COI and 28S sequences of the new species were corrected by checking their chromatograms in Bioedit 7.2.5 programme (Hall 1999). All sequences were then aligned with the Clustal W method (Thompson et al. 1994), trimmed at the ends and converted to a FASTA format file. The pairwise genetic distances were calculated separately for both genes according to the uncorrected *p*-distance in MEGA X software (Kumar et al. 2018).



Figure 1. The habitus of the male holotype (up) and the type locality of *Gammarus kunti* sp. nov. (down).

To perform the phylogenetic analyses, the COI and 28S sequences, both newly-generated and downloaded from GenBank, were added end-to-end to obtain a concatenated dataset (28S+COI) for each species. Phylogeny of *Gammarus* species was estimated by using Neighbour-Joining (NJ) and Maximum Likelihood (ML) methods in *MEGA X* software. The NJ tree was generated according to the *p*-distance parameter. The ML tree was generated according to the General Reversible Time (GTR) with gamma-distributed invariant sites (G+I) model (Tavaré 1986) and the best-fit substitution model was selected with the lowest Akaike Information Criterion (AIC) score in jModelTest 0.1.1 (Posada 2008). The

nodal support of the NJ and ML analyses was computed with the bootstrap test (Felsenstein 1985) using 1000 pseudoreplicates. To root the *Gammarus* phylogeny, *Pontogammarus robustoides* (also see Table 1) was used as an outgroup in the analyses.

The species delimitation analysis was carried out using the ASAP (Assemble Species by Automatic Partitioning) method, based on COI data. 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.

Table 1. Information of sequences used in molecular analyses.

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

Note: (T) Topotype samples of nominal taxa.

Results

Gammarus kunti sp. nov.

<https://zoobank.org/25D58B26-4577-460C-AF00-0D20B1D04397>

Figs 1–7

Type material. Holotype. Male, 11.5 mm (ESFM-MA-LI/20–15), Akçakoca District, Düzce Province, Türkiye (41°3'7.01"N, 31°10'38.70"E), 16.xiii.2020; collected by M. Elverici.

Paratypes. 3 males and 5 females, (ESFM-MA-LI/20–16), same data as holotype.

Diagnosis. A medium-large species. Body smooth, pigmentation weak; eyes well-developed, ovoid; extremities not prolonged; second antenna with setose peduncular and flagellar segments; antennal gland cone long; medial palmar spine present; posterior margin of pereopod 3 densely setose; anterior margins of pereopods 5 to 7 armed with spines and a few short setae; epimeral plates not pointed; inner ramus of uropod 3 longer than 0.75 of the outer one; telson weakly armed.

Description of male holotype. Head: Rostrum absent, inferior antennal sinus deep, rounded. Eyes kidney-shaped; shorter than the diameter of the first peduncular segment of antenna 1 (Figs 1, 5G).

Antennae: Antenna 1 is as long as half of the body length; the length ratio of the peduncular segments is 1:0.67:0.4; 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 flagellum 6 segmented (Fig. 3A). Antenna 2 is shorter than antenna 1 (ratio 1:0.67); the antennal gland cone is straight, reaches to 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 longer than the dorsal ones and can be up to 1.5 times longer than the diameter of the segment; flagellum consists of 15 segments; flagellar segments are setose and swollen; each segment bears many long setae on both dorsal and ventral sides; calceoli absent (Fig. 3B).

Mouthparts: Left mandible (Fig. 2A) with 5-toothed incisor, lacinia mobilis with 3 dentitions, molar triturative. The first article of palp without setae, the second one bears 12 setae; the setae become shorter from distal to proximal. The third segment has 28 D-setae, 4–5 E-setae, one group of A-setae and one group of B-setae. C-setae absent.



Figure 2. *Gammarus kunti* sp. nov., (male holotype). **A.** Left mandible; **B.** Right mandible; **C.** Maxilla 2; **D.** Lower lip 1; **E.** Maxilliped; **F.** Left maxilla 1; **G.** Right maxilla 1; **H.** Upper lip.

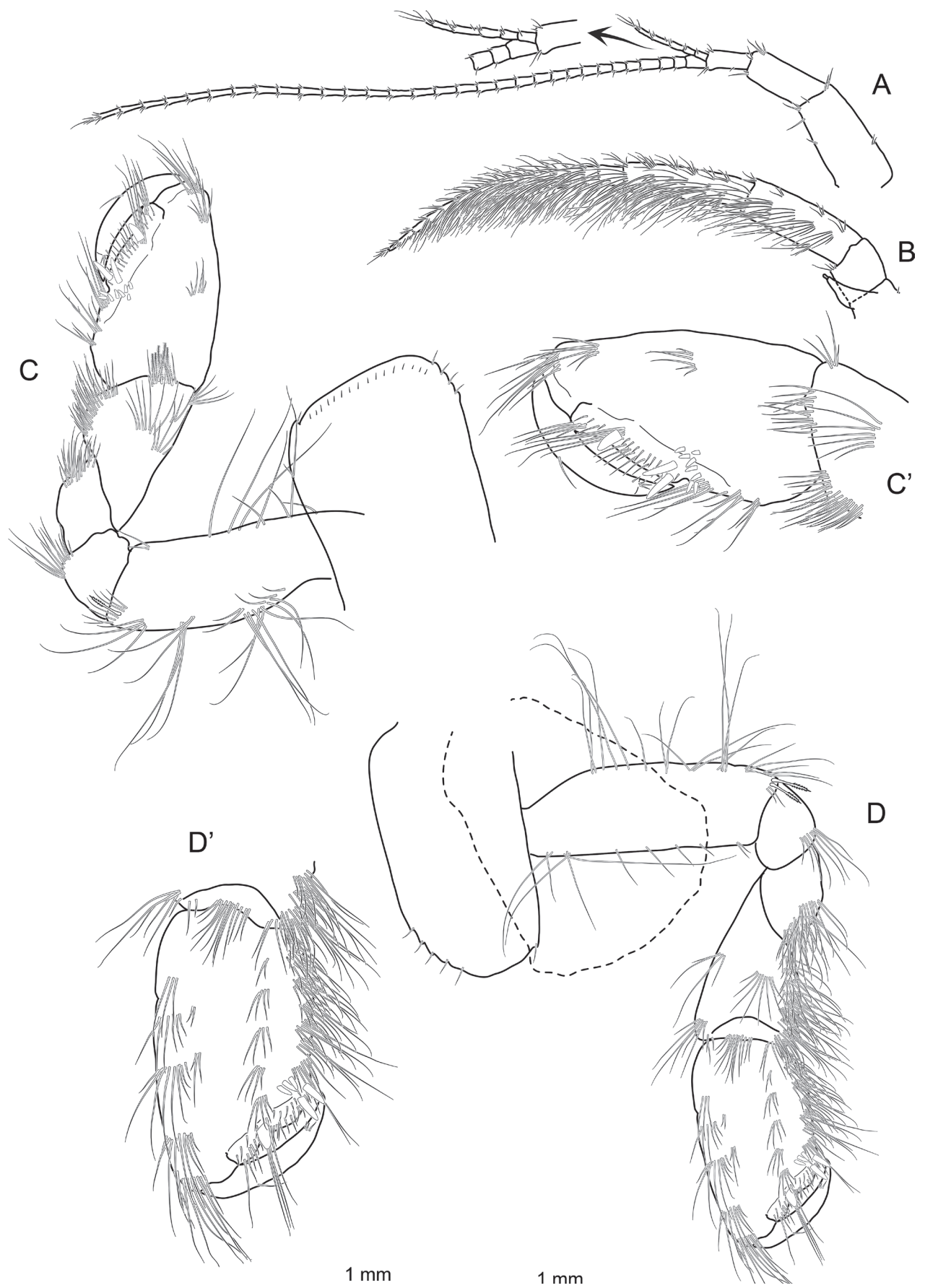


Figure 3. *Gammarus kunti* sp. nov., (male holotype). A. Antenna 1; B. Antenna 2; C. Gnathopod 1; C'. Palm of gnathopod 1; D. Gnathopod 2; D'. Palm of gnathopod 2.

Right mandible (Fig. 2B) has a 4-toothed incisor and bifurcate lacinia mobilis.

Right maxilla I (Fig. 2G) is asymmetric to the left, it has 14 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 and two simple setae on the distal part of the second segment, in addition to two marginal setae along the outer margin. The second article of left palp elongated and bears 8 spines and 3 simple setae on its distal part and no setae along the outer margin (Fig. 2F).

Lower lip (Fig. 2D) has no inner lobe and bears numerous small simple setae along the distal margins of both lobes.

Upper lip (Fig. 2H) with numerous minute setules in the distal part.

Maxilla II (Fig. 2C) has 20–25 simple setae in the distal part of the outer lobe and a few tiny hairs along the outer margin. The inner lobe also has 8–10 simple setae in the distal part in addition to 15 plumose setae located in a diagonal row along the inner margin. There are also a few tiny hairs in the proximal part of the inner margin of the lobe.

Maxilliped (Fig. 2E) inner plate has 3 tooth-like spines and a spine in the distal part and the distal corner, respectively. Additionally, there are 7 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 is rectangular, the distal part slightly widened, the ventral margin slightly convex and bears 4 antero-distal setae and one postero-distal seta in addition to some tiny setules along the ventral margin (Fig. 3C). Coxal plate 2 is in the shape of an elongated rectangle, distal part narrower than the proximal, the ventral margin is highly convex, antero-distal part with 5 setae and postero-distal part with one seta (Fig. 3D). Coxal plate 3 is similar to coxal plate 2 in shape, with 3 and 1 setae in the antero- and postero-distal ends, respectively (Fig. 4A). The ventral edge of the fourth coxal plate is slightly convex and bears 3 and 6 setae along the anteroventral and posterior margins, respectively (Fig. 4B). Coxal plate 5 (Fig. 5A) and Coxal plate 6 exhibit a bilobate structure (Fig. 5B), each having one seta in the anterior lobes and four and one setae in the posterior lobes, respectively. Coxal plate 7 is characterised by the presence of five setae on the postero-ventral margin (Fig. 5C).

Gnathopods: Basal segment of gnathopod 1 bears many long setae along both margins, the length of the setae can be as long as twice the diameter of the segment. Ischium bears a group of setae in the postero-ventral corner. Carpus is triangular and bears four 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: 0.57, anterior margin with two groups of setae, medial palmar spine is present, postero-distal 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 (Fig. 3C, C').

Basis and ischium of gnathopod 2 have a similar setation to that of gnathopod 1. Merus and carpus are more setose than those of gnathopod 1. Carpus 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: 0.53, anterior margin bears 6 groups of setae, posterior margin with many groups of setae, medial palmar spine is present, the postero-distal corner is armed with two strong spines in addition to some small 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 (Fig. 3D, D').

Pereopods: Anterior and posterior margins of the basal segment of pereopod 3 bear long setae, the setae along the posterior margin are much 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. 4A).

The basal segment of pereopod 4 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 (Fig. 4B).

Posterior margins of the basal segments of pereopods 5 to 7 are more or less convex and bear many short setae, anterior margins with 5–7 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. Pereopods 6 and 7 bear no setae along the anterior margins of ischium, merus and carpus, while pereopod 5 has a few setae longer than the 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 (Fig. 5A–C).

Epimeral plates: They are neither curved nor sharply pointed. Epimeral plate 1 bears 2 long setae in addition to 4–5 setules along the anterior margin, the postero-ventral corner is angular (Fig. 5D). Epimeral plate 2 bears 5–6 setae in the antero-ventral corner, the ventral margin is armed with 1 spine and two short setae, the posterior margin with 4–5 setules, the postero-ventral corner is angular (Fig. 5E). Epimeral plate 3 is slightly pointed; the antero-ventral corner bears 3–4 setae; the ventral margin is armed with 3 spines; the posterior margin bears 6–7 setules (Fig. 5F).

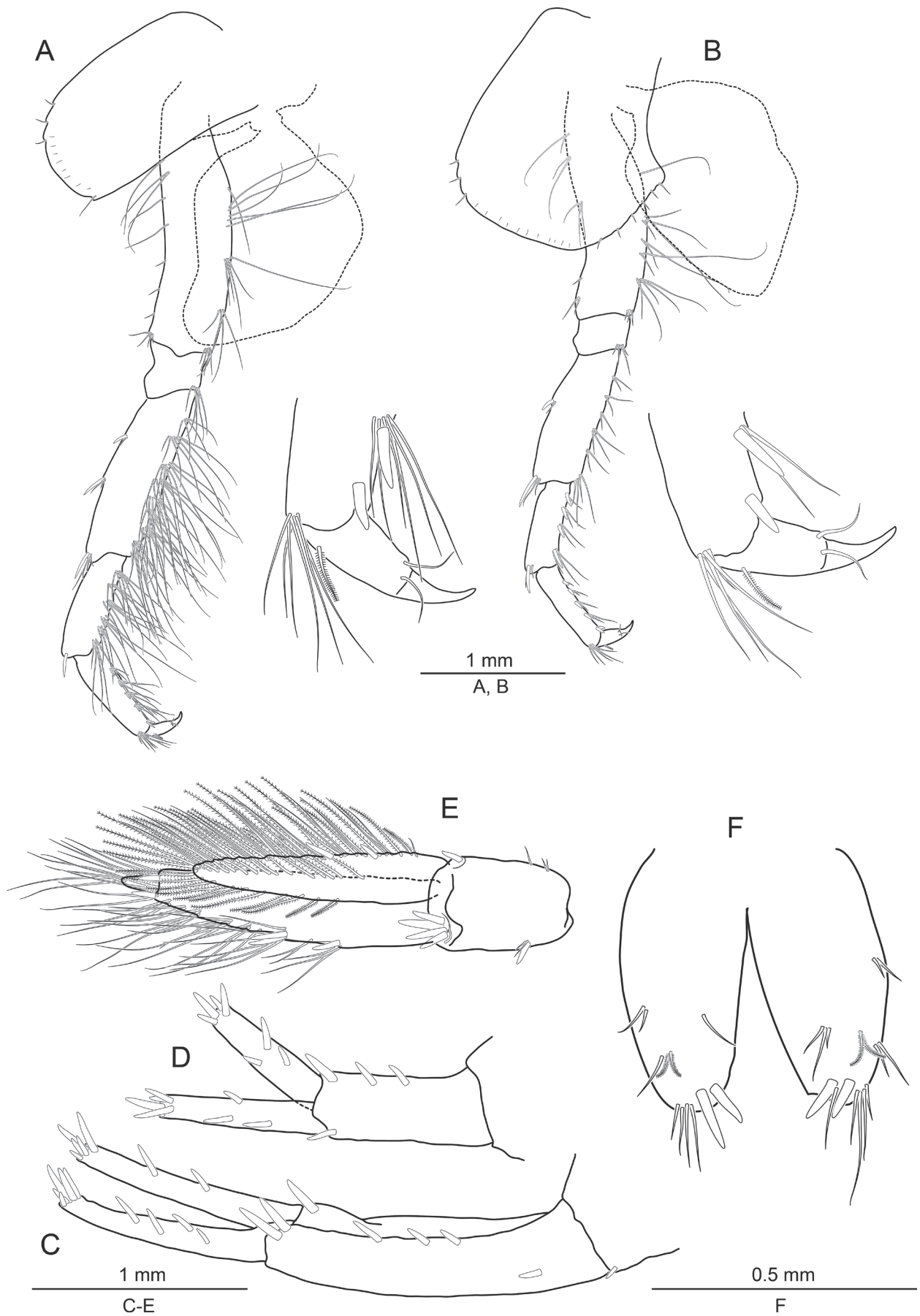


Figure 4. *Gammarus kunti* sp. nov., (male holotype). **A.** Pereopod 3; **B.** Pereopod 4; **C.** Uropod 1, **D.** Uropod 2; **E.** Uropod 3; **F.** Telson.

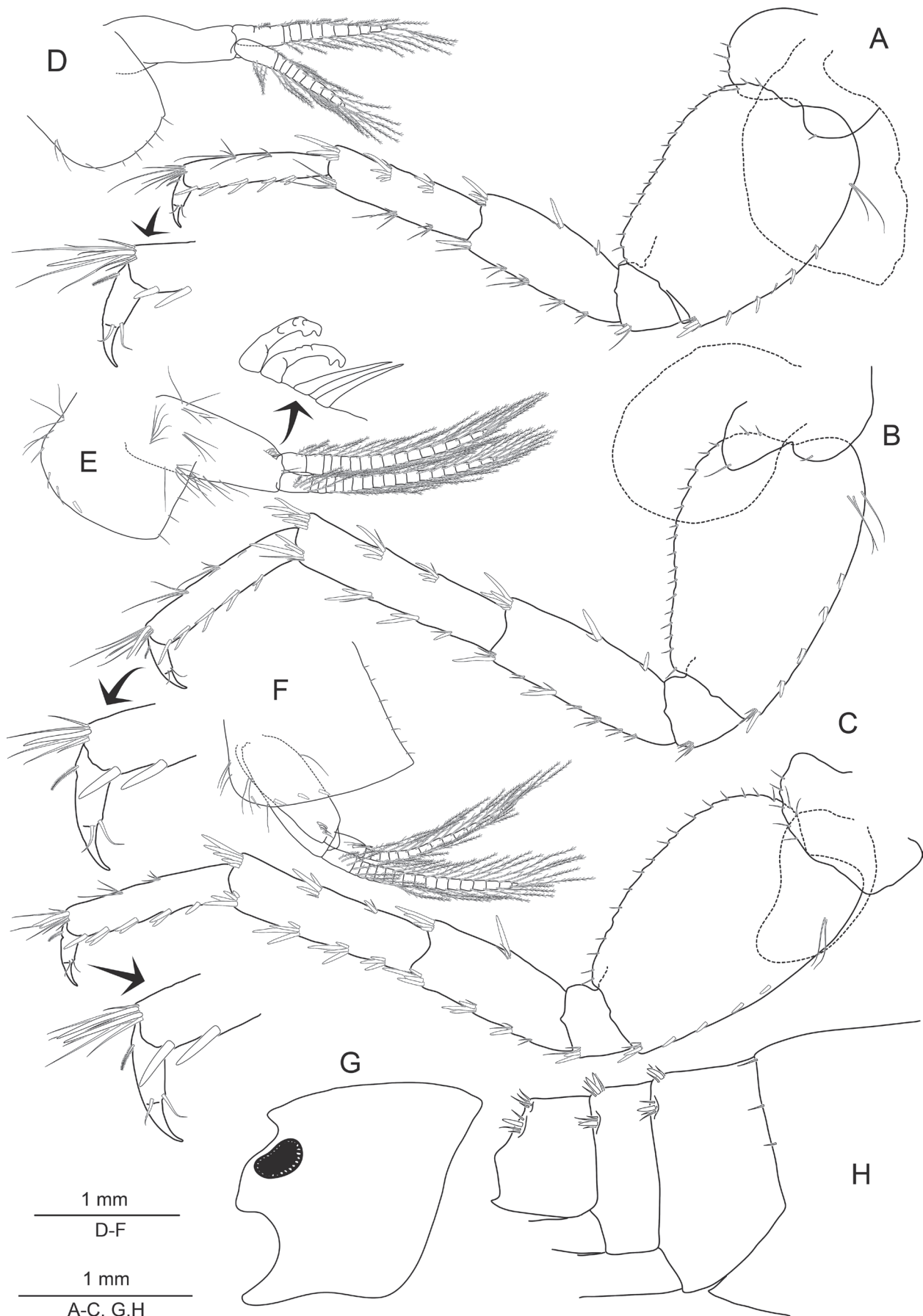


Figure 5. *Gammarus kunti* sp. nov., (male holotype). **A.** Pereopod 5; **B.** Pereopod 6; **C.** Pereopod 7; **D.** Pleopod 1; **E.** Pleopod 2; **F.** Pleopod 3; **G.** Head; **H.** Urosomites.

Urosomites: Not elevated. Each segment bears a median and two dorsolateral groups of armaments; each of them consists of 1–2 spines and 3–4 accompanying setae (Fig. 5H).

Uropods: Uropod 1 has a spine in the disto-ventral corner of the base; the peduncle is longer than the rami; the length ratio is about 1:0.7. Peduncle with a spine in the outer margin of the proximal part in addition to 3 spines along the inner margin and 3 spines in the distal part. The inner ramus is slightly longer than the outer ramus and bears 3–4 spines along their inferior margin in addition to 4–5 distal spines. The outer ramus with 2 spines along the inferior margin in addition to 4–5 distal spines (Fig. 4C).

Uropod 2 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+2 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, bearing 2–3 spines along their inner and outer margins in addition to 4–5 longer spines on their distal tips (Fig. 4D).

Uropod 3 is setose and bears simple and plumose setae. The peduncle segment is much shorter than the outer ramus and the length ratio is about 1:0.41. The outer ramus is two articulated and densely setose along both margins; the outer margin bears 2 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 0.77× the length of the outer ramus. It bears two spines along the outer margin in addition to groups of simple and plumose setae; the inner margin bears both simple and plumose setae (Fig. 4E).

Telson: Telson lobes cleft, each lobe bears 2 spines and 2–3 simple setae in their distal parts. The setae are longer than the spines. There are 2–3 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 (Fig. 4F).

Etymology. The species epithet is derived from the name of scientist Dr. Kadir Boğaç Kunt, who has valuable contributions to the Arachnida species of Türkiye and sent the materials for this study.

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: less setose antenna 2, not swollen flagellar segments of antenna 2, less setose gnathopod 2 and more setose pereopods 4–7 (Figs 6, 7).

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

Molecular data analyses

We generated the partial sequences of the COI (573 bp) and 28S (914 bp) genes of the newly-described species, *Gammarus kunti*. After all sequences were aligned, the total length is 1489 bp including gaps. While no stop codon, insertion, deletion and a gap was detected in the protein-coding mtDNA COI gene, there were insertions and deletions in the nuclear 28S gene. Additionally, newly-generated sequences are deposited in GenBank accession numbers, for COI; [OP642558](#) and 28S; [OP650556](#). Thus, the first genetic record of the newly-described species was created. We performed the genetic comparison of the new species with the reference sequences of the topotype samples of the nominal taxa in GenBank. In the absence of sequences of topotype samples, correct sequences considered representative of the species were preferred (Table 1).

For the COI gene, the pairwise genetic distance amongst the species ranged from a minimum of 5.24% (*G. stankokaramani* - *G. salemaai*) to a maximum of 28.62% (*G. kesslerianus* - *G. plaitisi*). The pairwise genetic distances between the new species *Gammarus kunti* sp. nov. and the other species ranged from a minimum of 16.23% (*G. tumaf*) to a maximum of 28.27% (*G. roeselii*). For the 28S gene, the pairwise genetic distance amongst the species ranged from a minimum of 0.11% (*G. halili-cae* - *G. pljakici*) to a maximum of 7.84% (*G. rambouseki* - *G. stojicevici*).

The pairwise genetic distances between the new species *Gammarus kunti* sp. nov. and the other species ranged from a minimum of 0.88% (*G. tumaf*) to a maximum of 6.81% (*G. balcanicus*). The genetic distance of the new species to the nearest species is approximately three times greater for the COI gene and eight times greater for the 28S gene than the minimum genetic distance between valid *Gammarus* species. This indicates that the new species is well differentiated genetically. All pairwise genetic distance values amongst *Gammarus* species are given in Suppl. material 1.

Phylogenies generated by the NJ and ML methods, based on the concatenated data, yielded fully compatible trees. Except for a few branches (ML: 16–67%; NJ: 25–69%), the other branches (ML: 82–100%; NJ: 83–100%) in the phylogenies were generally resolved and supported with high bootstrap values. *G. tumaf* Özbek et al., 2023 and *G. baysali* Özbek et al., 2013 are the sister taxa of *G. kunti* sp. nov. The phylogenetic position of the new species, *Gammarus kunti* sp. nov., indicates an independent lineage supported by high bootstrapping values (for NJ: 95%, for ML: 91%; Fig. 8).

The species delimitation analysis we implemented according to the ASAP method, based on COI data, identified 26 MOTUs (molecular operational taxonomic units) for 27 *Gammarus* species. The best ASAP score had 1.5 ($p=0.01$) at a threshold distance of 0.079053. The analysis identified species *G. stankokaramani* and *G. salemaai* as a single MOTU. The new species formed a single MOTU independent of other species.

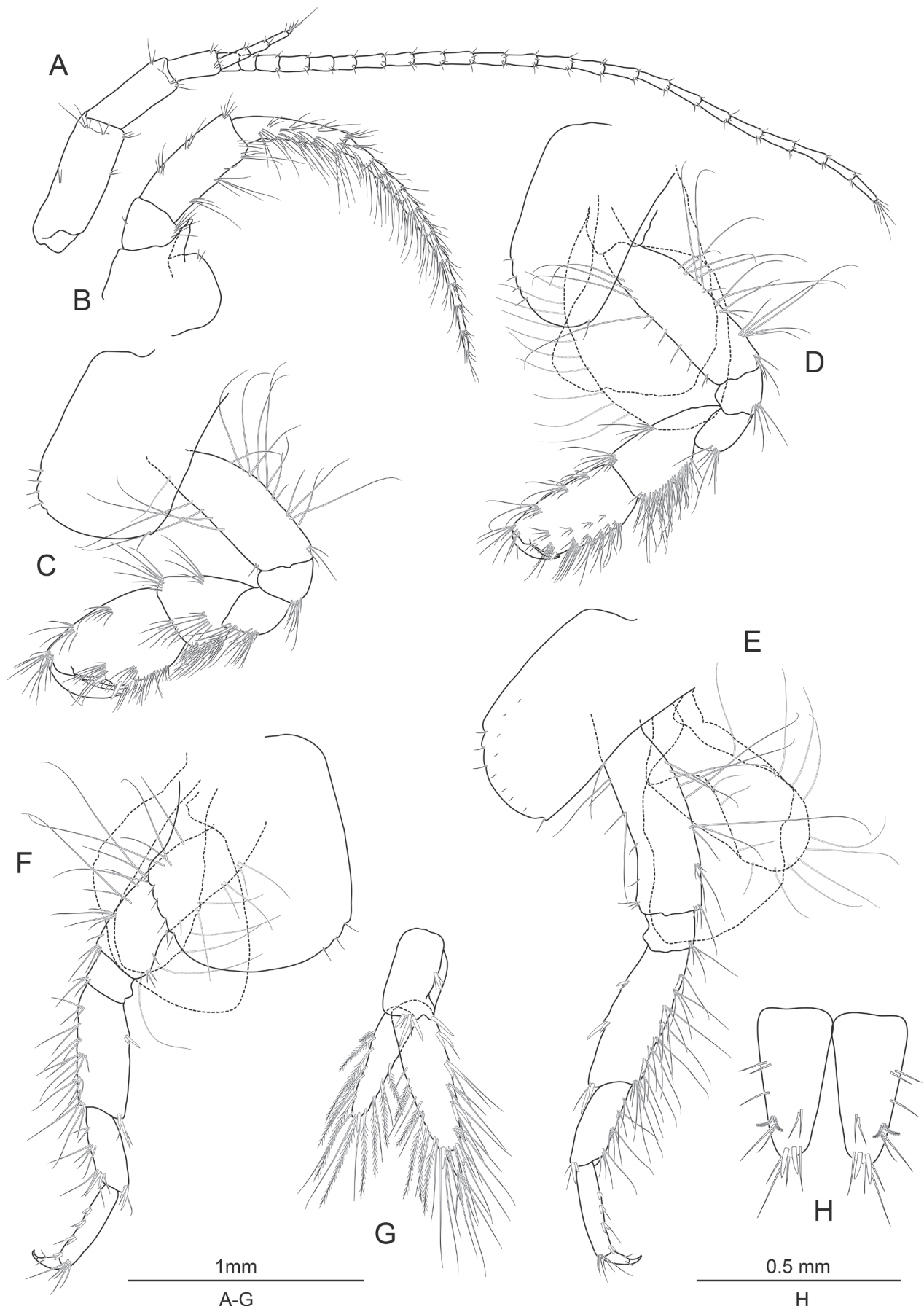


Figure 6. *Gammarus kunti* sp. nov., (female allotype). **A.** Antenna 1; **B.** Antenna 2; **C.** Gnathopod 1; **D.** Gnathopod 2; **E.** Pereopod 3; **F.** Pereopod 4; **G.** Uropod 3; **H.** Telson.

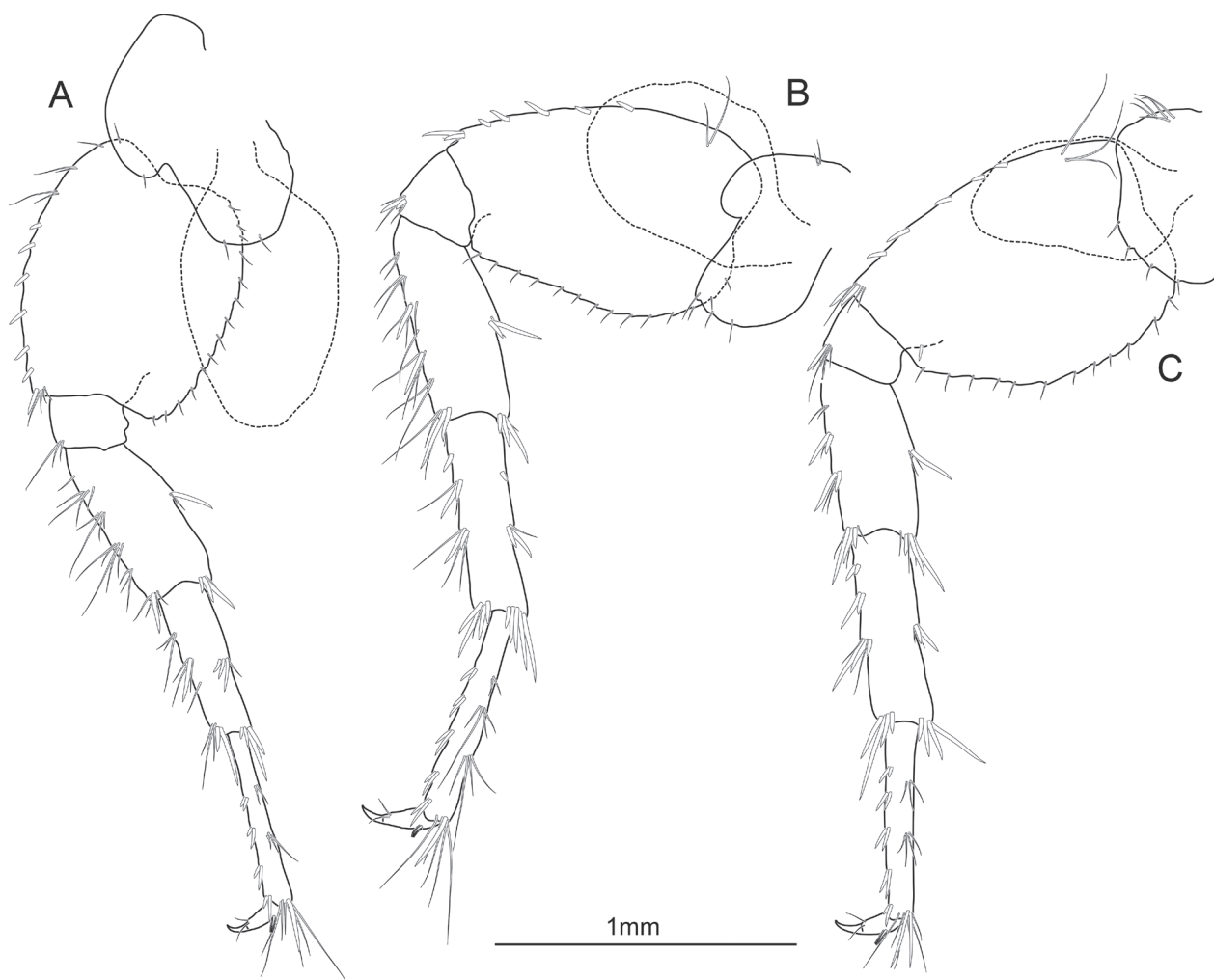


Figure 7. *Gammarus kunti* sp. nov., (female allotype). **A.** Pereopod 5; **B.** Pereopod 6; **C.** Pereopod 7.

Discussion

Gammarus kunti sp. nov. is a species belonging to the *Gammarus pulex*-group due to the setation of the posterior part of pereopods 3 and 4 and the setation of uropod 3 (Karaman and Pinkster 1977). *Gammarus kunti* sp. nov. shows close proximity to *G. baysali* and *G. tumaf*, considering the genetic analysis results (Fig. 8). In addition, the newly-described species show some similarities with *G. kesslerianus* and *G. komareki* (Schäferna, 1922). *G. kesslerianus* has not been recorded from Türkiye, while *G. komareki* has been reported from the entire Black Sea Region of Turkey, including the Thrace Region (İpek and Özbek 2022).

Although *Gammarus kunti* sp. nov. is genetically and morphologically close to *G. baysali*, it differs from *G. baysali* in having several morphological features. The newly-identified species is smaller than *G. baysali*. Additionally, having well-developed eyes, shorter antenna 1, more setose antenna 2, not elongated extremities and not setose anterior margins of pereopods 5–7 are some of the discriminant characteristics of *G. kunti* (Table 2).

G. kunti sp. nov. also resembles *G. tumaf* which is reported from the Gökgöl Cave, Zonguldak Province by

the same authors of the present study in 2023. The newly-identified species differs from it by having reniform eyes, while eyes are minute in *G. tumaf*. Inner lobe of right maxilla 1 bears 14 and 20 plumose setae in *G. kunti* and *G. tumaf*, respectively. *G. kunti* has six stout spines in the palp of right maxilla 1, while the number of the stout spines is five in *G. tumaf*. In addition, the newly-identified species has 15 plumose setae in the inner lobe of maxilla 2, while *G. tumaf* has 20 plumose setae (Table 2).

The new species is also similar to *Gammarus obruki* Özbek, 2012 by having kidney-shaped eyes, setose antenna 2 and armaments and setation of pereopods 5–6, but differs from it by being smaller and having much shorter antenna 1 and shorter inner/outer lobe ratio of uropod 3. In addition, *G. kunti* has 14 plumose setae in the inner lobe of right maxilla 1, while it has 18 in *G. obruki*. Similarly, the new species has two setae along the outer margin of the palp of the right maxilla 1, while *G. obruki* has three setae in the mentioned part of maxilla 1. Inner lobe of maxilla 2 bears 15 plumose setae in the newly-identified species and the number is 21 in *G. obruki*. Similarly, the number of D-setae in the palp of the mandible in *G. kunti* and *G. obruki* differs from each other (28 vs. 37, respectively) (Table 2).

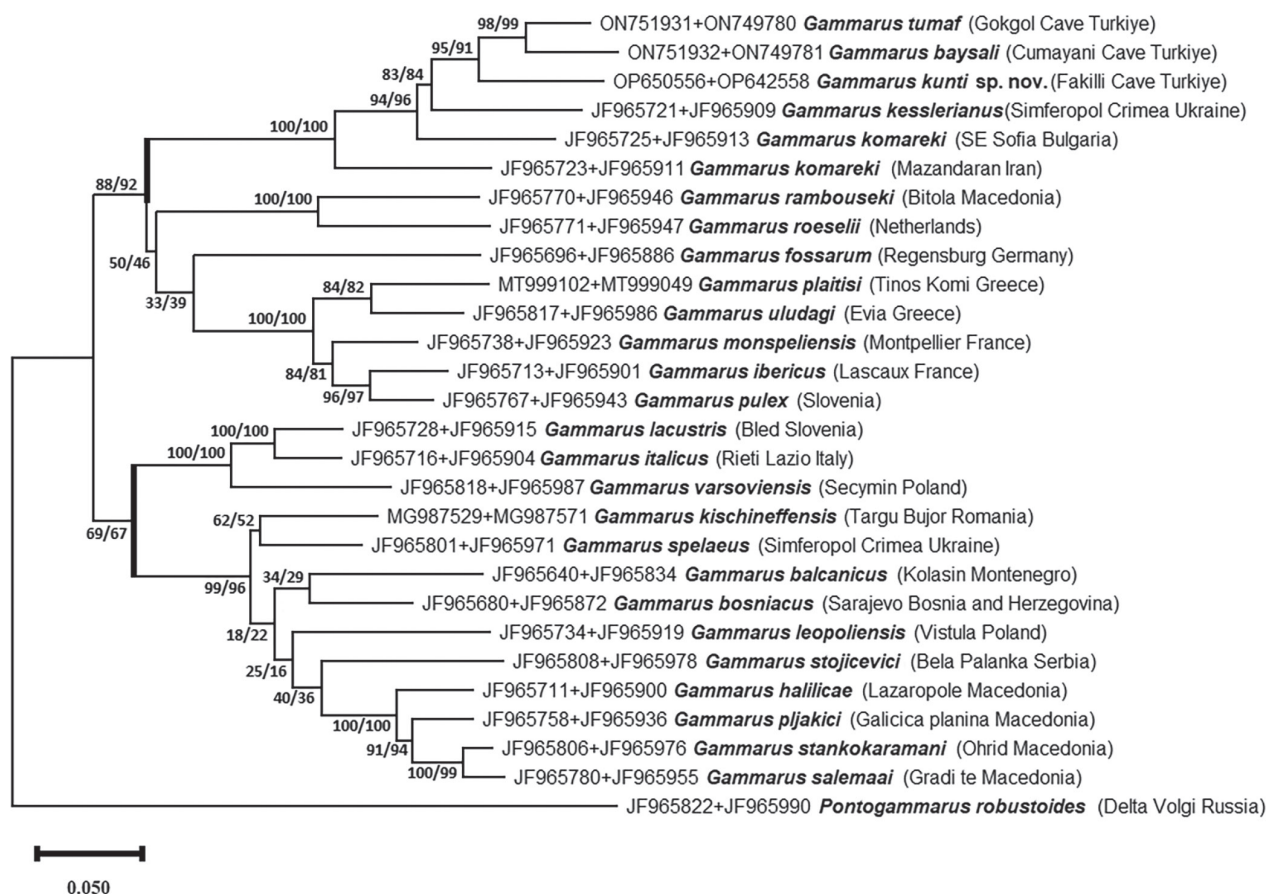


Figure 8. Maximum Likelihood (ML) phylogenetic tree generated, based on the concatenated dataset (28S+COI). ML and NJ methods yielded the same topologies and, therefore, only the ML tree is shown. The bootstrap values of NJ and ML are shown on nodes (NJ/ML).

Table 2. Some of the morphological features of *Gammarus kunti* sp. nov., *G. baysali*, *G. tumaf* and *G. obruki*.

Characters	<i>Gammarus kunti</i> sp. nov.	<i>G. baysali</i>	<i>G. tumaf</i>	<i>G. obruki</i>
Body length	11.5 mm	18.1 mm	12.6 mm	21.0 mm
Eyes	Kidney-shaped	eyeless	minute	Kidney-shaped
Body colour	whitish	colourless, whitish	whitish	yellowish
Antenna 1	32+6 flagellar segments	41+6 flagellar segments	30+5 flagellar segments	52+6 flagellar segments
Antenna 2	peduncular and flagellar segments densely setose	peduncular and flagellar segments setose	peduncular and flagellar segments densely setose	fifth peduncular and flagellar segments densely setose
Antennal gland cone	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	straight, not reaching to the distal end of the third peduncular segment
Inner lobe of right maxilla 1	with 14 plumose setae	with 19 plumose setae	with 20 plumose setae	with 18 plumose setae
Palp of right maxilla 1	6 stout spines, 2 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, 3 setae along the anterior margin
Maxilla 2	inner lobe with 15 plumose setae	inner lobe with 21 plumose setae	inner lobe with 20 plumose setae	inner lobe with 21 plumose setae
Number of D-setae	28	34	28	37
Pereopods	not elongated	elongated	not elongated	slightly elongated
Pereopods 6–7	anterior margins without setae	anterior margins with setae	anterior margins without setae	anterior margins without setae
Uropod 3	setose, inner/outer lobe ratio: 0.77	setose, inner/outer lobe ratio: 0.9	setose, inner/outer lobe ratio: 0.75	setose, inner/outer lobe ratio: 0.9

At first glance, the newly-identified species looks similar to *G. komareki* by the setation of the antennae, by the presence and the shape of the eyes, uropod 3 and telson, but the following characters are different. In *G. kunti* sp. nov., the antennal gland cone reaches to the distal end of the third peduncular segment, but it is shorter (roughly halfway) in *G. komareki*. Similarly, *G. kunti* sp. nov. has less D-setae in the third segment of the mandible palp (28 in *G. kunti*; 40 in *G. komareki*), less setose pereopod 4 and not setose anterior margins of pereopods 6–7. Another important differentiation is that, in males, the setation on the carpus and merus posterior margin of the 4th pereopod is significantly shorter in *G. kunti* (subequal to the diameter of underlying segment) than in *G. komareki* (longer than the diameter of underlying segment).

G. kunti sp. nov. also resembles *Gammarus komareki aznavensis* Özbek & Rasouli, 2014 in terms of setation of antenna 2, pereopods 3 and 4, but the newly-identified species differs from *G. komareki aznavensis* by its larger size, by having smaller eyes, longer antenna 1, by absence setae along the anterior margins of pereopod 6 and by the shorter setation of the telson (Özbek and Rasouli 2014).

The newly-identified species differs from *G. kesslerianus* by the body length (smaller), having fewer flagellar segments in antenna 2 and shorter endopod of uropod 3.

Gammarus kesslerianus wernerii S. Karaman 1934 was identified from Iznik Lake, NW Anatolia. After S. Karaman's record, the subspecies has been never re-described and collected again until G.S. Karaman's re-description (Karaman 2018). He elevated the subspecies to the specific rank as *Gammarus wernerii* and transferred it into the *Gammarus balcanicus*-group. So, *Gammarus kunti* sp. nov. distinctly differs from *G. wernerii* because the newly-identified species belong to the *Gammarus pulex*-group.

Gammarus kunti sp. nov. differs from *Gammarus ramboseki* (S. Karaman, 1931) by having reniform eyes, by the absence of long setae on the peduncular segments of antenna 1, by the absence of long setae along the anterior margins of pereopod 5 to 7 and by the presence of plumose setae on uropod 3. Additionally, *G. ramboseki* has less setose antenna 2 and more setose urosomites and telson (Karaman and Pinkster 1977).

Gammarus kunti sp. nov. is similar to *Gammarus fossarum* Koch, 1836 by having reniform eyes, a setose posterior margin of pereopod 3 and the armaments of pereopods 5 to 7. However, the newly-identified species differ from *G. fossarum* by having much more setose antenna 2 and by having a more elongated inner lobe of the uropod 3.

Studies conducted in recent years suggest that the western Black Sea Region of Türkiye is quite rich in terms of freshwater amphipods. Many new and endemic species have been identified from the caves and water bodies in the region (Andreev and Kenderov 2012; Karaman 2012; Özbek 2012; Özbek et al. 2013, 2023). To reveal the biodiversity of Turkish inland waters, studies supported by molecular analyses should be increased.

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Supplementary material 1

The pairwise genetic distance values amongst the *Gammarus* species, based on the COI dataset (below the diagonal) and 28S dataset (above the diagonal)

Authors: Murat Özbek, Hazel Baytaşoğlu, İsmail Aksu
Data type: xls

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