## **RESEARCH ARTICLE**

ARAŞTIRMA MAKALESİ

# A Second New Amphipod species from the Peynirlikönü Cave (EGMA Cave): *Gammarus egmao* sp. nov. (Crustacea: Amphipoda)

Peynirlikönü Mağarası (EGMA Mağarası)'ndan bir diğer yeni amphipod Türü: *Gammarus egmao* sp. nov. (Crustacea: Amphipoda)

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**Abstract:** Another new amphipod species, *Gammarus egmao* sp. nov., from Peynirlikönü Cave [Evren Günay Mehmet Ali Özel (EGMA) Cave] was identified. The specimens of the new species were sampled from 350 m inside of the cave entrance. This is the second species identified from the cave in addition to *Gammarus ustaoglui* Özbek and Güloğlu, 2005. The newly identified species has the following characteristic features: a) small eyes, b) 2- segmented accessory flagellum of antenna II, c) aesthetascs on the flagellar segments of antenna I, d) reduced armaments in distal part of palp of maxilla I e) short inner ramus of uropod III and reduced setation on margins of rami of uropod III. A detailed description of holotype female and illustrations of the appendages were presented. Additionally, differences from the related species were discussed.

Keywords: Amphipoda, new species, EGMA Cave, troglobitic amphipod, Turkey

Öz: Peynirlikönü Mağarası'ndan [Evren Günay Mehmet Ali Özel (EGMA) Düdeni] bir diğer yeni amphipod türü, *Gammarus egmao* sp. nov., tanımlanmıştır. Yeni türe ait bireyler mağaranın girişinden 350 m içeriden örneklenmiştir. Bu, *Gammarus ustaoglui* Özbek ve Güloğlu, 2005 türüne ilave olarak, mağaradan tanımlanan ikinci yeni türdür. Yeni tanımlanan tür şu karakteristik özelliklere sahiptir; a) küçük gözler, b) ikinci antende iki segmentli yardımcı flagella, c) I. antenin flagellasında astetasklar (çubuk şeklinde yapılar), d) I. maksil palpinin uç kısmında azalmış diken sayısı, e) çok kısalmış III. üropodun iç lobu ve III. üropodun her iki lobunun iç ve dış kenarlarında azalmış tüylenme. Holotip dişinin detaylı tanımlanması ve ekstremitelerin çizimleri çalışmada sunulmuştur. İlave olarak, benzeri türlerle olan farklılıklar tartışılmıştır.

Anahtar Kelimeler: Amphipoda, yeni tür, EGMA Mağarası, troglobitic amfipod, Türkiye

### INTRODUCTION

Caves and sinkholes are very special habitats (e.g. permanent darkness, constant temperature, resource scarcity, etc), especially for aquatic invertebrates. *Niphargus* species are the most common amphipod inhabitants of these habitats in the western Palearctic region. In some cases, there can be more than one water layer (as epikarsts, cave streams, and cave lakes) present in a single cave and they can be special habitats for different species (Trontelj et al., 2012). *Gammarus* species are also distributed in this kind of special environments also preferring springs and other

similar freshwater biotopes (Sidorov et al., 2018), but the number of reported species is much smaller than that of *Niphargus*.

Turkey is rich in terms of karstic habitats. About one-third of its geographic area consists of carbonate rocks. The Taurus Mountain Range is the biggest and most extensively karstified area in Turkey (Baba and Tayfur, 2011). There are several studies conducted on the taxonomy of freshwater amphipod species of Turkish caves (Andreev and Kenderov, 2012; Fišer, 2009; Karaman, 1973, 2012, Özbek, 2005, 2010, 2013). Peynirlikönü Cave (EGMA Cave) is a vertical cave and has a special importance in terms of amphipod distribution: there are several water layers and two of them have their own species. One of them is located around 350 m inside of the entrance while the other one is located in deeper part, 650 m, of the cave. *G. ustaoglui* Özbek & Güloğlu 2005, which is a blind species, was identified from the pool placed 650 m inside of the entrance. The present study is carried out on the other amphipod samples collected from 350 m inside the cave.

#### **MATERIAL AND METHODS**

Discovered in 1993, Peynirlikönü (or EGMA) Cave is the deepest cave in Turkey and one of the deepest caves in the world (1,429 m depth and 3,118 m length).

EGMA Cave is located on the Çukurpınar Upland of the Taşeli Plateau of the Anamur District of Mersin (Figure 1). The entrance is 1900 m above sea level. Small pools of water can be found at different elevations throughout the cave.

The present specimens (2 adult females) were sampled at a pond 350 m inside the entrance by the second author and fixed in 4% formalin solution. In order to measure the body length of the specimens, they were kept straight and the distance between the rostrum and telson were measured under an Olympus SZ61 model stereo microscope. The photos of the holotype female were taken with a digital camera attached to the stereomicroscope. Then the holotype female was dissected and permanent slides were prepared using CMCP-10 high-viscosity mountant under the stereomicroscope. Each of the appendages was photographed under a binocular microscope (Olympus CKX41) and the taken photos were transferred into a PC in order to make detailed drawings. The drawings were made with a Wacom PTH-451 model digitizer board which has a USB connection with PC. During the drawings, pertinent papers of O. Coleman were followed (Coleman, 2003, 2006, 2009). The sampled materials were stored in the Museum of the Faculty of Fisheries of Ege University, İzmir, Turkey (ESFM).

#### RESULTS

#### Gammarus egmao sp. nov. (Figures 2-6)

Holotype: Female, 16.0 mm (ESFM-MALI/07-20), Anamur District, Mersin Province, Turkey (36°18'54"N 32°46'44"E), 01.vii.2007; collected by M. O. Güloğlu.

*Paratype:* 1 female, 13.0 mm (ESFM-MALI/07-21), same data as holotype.

Diagnosis: Gammarus egmao sp. nov. has the following characteristic features: rounded minute eyes,

2-segmented accessory flagellum, rod-like structures on the flagellar segments of antenna I, reduced armaments in the distal part of maxilla I, slightly elongated extremities, short inner ramus of uropod III (extremely less than half of inner ramus) and reduced setation on margins of rami of uropod III.

Description of holotype female: Antennal sinus deep (Fig. 6H). Eyes reduced, ovoid; much shorter than the diameter of first peduncular segment of antenna I (Figure 2).

Antenna I (Figure 4A) is shorter than half of the body length with weak setation both on peduncle and flagellar segments. The length ratio of peduncular segments is 1:0.6:0.4. Main flagellum with 17 articles, accessory flagellum with 2 articles, bearing small short simple setae in distal parts of each article. Aesthetascs present through 3<sup>rd</sup> to 16<sup>th</sup> flagellar segments of antenna I.

Antenna II (Figure 4B) shorter than antenna I. Gland cone straight and short, hardly reaches to the distal end of the third peduncular segment. Antenna I to antenna II length ratio is about 1:0.6. Peduncular segments have a few groups of simple setae both on dorsal and ventral margins. Setae on ventral side slightly longer than those on the dorsal side. Length ratio of the peduncular segments is 1:2.0:1.7 respectively. Flagellum with 8 segments; not swollen and have more (and longer) setae than those of Antenna I. Rod-like structures and calceoli absent in the flagellar segments of antenna II.

Left mandible (Figure 3D) with 5 toothed incisor, lacinia mobilis with 4 dentitions, molar triturative. The first article of palp without setae, the second one with 4 short and 4 longer setae. The third segment has 15 D-setae, 5 E-setae, one group of A- and one group of B-setae. C-setae absent.

Right mandible (Figure 3E) has 3 teethed incisor and bifurcate lacinia mobilis.

Right maxilla I (Figure 3B, C) has 11 plumose setae along the inner margin of the inner lobe in addition to some tiny setules. Outer lobe bears 11 distal stout serrate spines and some tiny setules on the inner margin. Palp of the outer lobe with no seta in the first segment and three stout spines and a simple seta on the distal part of the second segment. The spine on the outer corner is bigger and stronger. The outer margin has no seta. The second article of left palp bears 4 spines and two simple setae on its distal part and no seta along the outer margin.

Lower lip (Figure 3A) without inner lobe and bears numerous small simple setae along the distal margins of both lobes.

Upper lip (Figure 3G) with numerous minute setules in distal part.



**Figure 1.** The entrance (1) and inner parts (2, 3) of EGMA Cave and the type locality (4) of *Gammarus egmao* sp. nov. (Photos: M. O. Güloğlu)

Maxilla II (Figure 3H) has 10–12 simple setae in the distal part of the outer lobe and a few tiny hairs along the outer margin. Inner lobe also has 8–10 simple setae in distal part in addition to 10 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.

Inner plate of maxilliped has 3 tooth-like spines and a spine in the distal part and in the distal corner, respectively. Additionally, there are 7 plumose setae along the inner margin of the lobe. Outer plate armed with 3–4 serrate stout setae in the distal part and 8–10 spines along its inner margin.

Coxal plate I (Figure 4E) rectangular and bears 2 setae and 1 seta on anterodistal and posterodistal

corners, respectively. Coxal plate II (Figure 4F) narrowed in distal part; bears a seta on both distal corners. Coxal plate III (Figure 4C) rectangular and armed with 2 setae on its distal corners. Coxal plate IV (Figure 4D) has a slightly convex distal margin and bears 2 and 3 setae on the anterior and posterior corners, respectively. Coxal plates V and VI (Figure 5A, B) bilobate. Coxal plate V bears 1 seta on the anterior lobe and 4 setae along the posterior margin of posterior lobe. Coxal plate VI with 2 setae on the posterior lobe and the anterior lobe with no seta. Coxal plate VII (Figure 4D) bears 3 setae along the posterior margin.

Gnathopod I (Figure 4E, E') has 5-6 very long simple setae along both margins of the basal segment; the length of the setae can be up to twice of the diameter of the basis. In the distal corners, there are a few short simple setae. Ischium and merus have a group of simple setae on their posterior corners. Carpus triangular; anterior margin bears three groups of longer simple setae which are shorter than the diameter of the segment; posterior margin with numerous simple



**Figure 2.** Habitus and head of the holotype female of *Gammarus egmao* sp. nov. (16.0 mm)



**Figure 3.** Mouth parts of *Gammarus egmao* sp. nov. (holotype female). (A: lower lip; B: right maxilla I; C: palp of left maxilla I; D: left mandible; E: right mandible; F: maxilliped; G: upper lip; H: maxilla II).

setae. Propodus pyriform, anterior margin with two groups of simple setae; mid-distal spine is absent; posterodistal corner armed with a strong spine in addition to some small spines; inner surface with three groups of short simple setae. Dactylus reaches to the posterodistal corner and bears a simple seta along the outer margin in addition to a small setule around the distal part of the inner margin.

Gnathopod II (Figure 4 F, F') bears 4 or 5 long setae along the anterior margin of basis in addition to some shorter simple setae; the length of the setae can be up to twice length of the diameter of the segment. Ischium and merus bear a group of simple setae along their posterior margins. Carpus triangular with two groups of setae along the anterior margin in addition to many groups of setae along the posterior margin. Propodus is in a sub-rectangular shape and bears three groups of simple setae along the anterior margin; inner surface with 4 groups of short simple setae; three spines occur on the disto-posterior corner; posterior margin with numerous setae. Dactylus bears a simple seta along the outer margin and a small setule near the distal end of inner margin.

Basal segment of pereopod III (Figure 4C) bears 12 and 6 long simple setae along the anterior and posterior margins respectively. The length of the setae can be



**Figure 4.** Extremities of *Gammarus egmao* sp. nov. (holotype female). (A: antenna I; B: antenna II; C: pereopod III; D: pereopod IV; E: gnathopod I; F: gnathopod II; E': inner view of gnathopod I propodus; F': inner view of gnathopod II propodus).

2.5 times of the diameter of the segment. Posterior margins of merus and carpus bear long setae (not curled) in addition to a few spines. Propodus with three groups of spines accompanied by a few short setae along the posterior margin. Dactylus not elongated, a minute plumose seta occurs on outer margin; inner margin with two small setules.

Pereopod IV (Figure 4D) bears several long setae on both margins of the basal segment; they can be 4.5 times longer than the diameter of the segment. The armament of both pereopod III and IV are similar to each other but the setae along the posterior margins of merus and carpus are shorter in pereopod IV. Pereopods V to VII (Figure 5A-C) slightly prolonged and without long setae along their anterior margins. Dactylus bears a minute plumose seta on outer margin and 2 minute setae in distal part.

Pereopod V (Figure 5B) has a rectangular basis (max. length/width ratio 1.4) with 7 setules and 3 spines on posterior and anterior margins, respectively.

Basal segment of pereopod VI (Figure 5A) is in an elongated rectangular shape (max. length/width ratio 1.8) and armed with 11 setules and 4 spines on posterior and anterior margins, respectively.

Pereopod VII (Figure 5C) has a semi-triangular basal segment (max. length/width ratio 1.7) and there are 12



**Figure 5.** Extremities of *Gammarus egmao* sp. nov. (Holotype female). (A: pereopod VI; B: pereopod V; C: pereopod VII; D: telson; E: uropod III).

setules along the posterior margin and 3 spines on its anterior margin.

Epimeral plates (Figure 6D-F) not pointed. Epimeral plate I bears 7 long setae on the anterior margin and 2 setules along the posterior margin. Both epimeral plates II and III armed with 3 spines on their ventral parts and bears 3-4 setules along their posterior margins.

Peduncle segments of pleopods (Figure 6A) bear several simple setae on both sides in addition to 2 retinacula and 2 accompanied setae. Rami with 10-14 segments and bear numerous plumose setae.

Urosomites 1-3 (Figure 6G) with no elevation, flat. Armament weak. Uropod 1 (Figure 6C) has longer peduncle than rami. Peduncle with a spine in proximal part in addition to 6 spines along the inner margin and 3 spines in distal part. Outer ramus slightly shorter than the inner one (0.9:1 respectively). Inner ramus bears a spine along the inner margin, outer margin bare. Outer ramus bears two groups of spines (2x2) along the inner margin and no spine on the outer side. Both rami bear 4-5 spines in their distal tips.

Uropod 2 (Figure 6B) much smaller than the first one (0.6:1 respectively). Peduncle longer than the rami and bears 2 spines along the inner margin, outer margin bare. The length and armaments of both rami



Figure 6. Extremities of Gammarus egmao sp. nov. (Holotype female). (A: pleopod II; B: uropod II; C: uropod I; D: epimeral plate I; E: epimeral plate II; F: epimeral plate III; G: urosomites; H: head).

look very similar to each other, they bear one spine along their inner and outer margins in addition to 4-5 longer spines on their distal tips.

Uropod 3 (Figure 5E) has one of the characteristic features of the present species which is the shape and setation of the rami. Peduncle bears 4 spines in the distal part. Inner ramus much shorter than the outer one (0.35:1 respectively). It has 1 spine and 2 plumose setae along the outer margin and 2 distal spines and accompanied 2 simple setae. Outer ramus has 3 groups of spines (1-2-1) and a short simple seta accompanied to each group along the outer margin. Inner margin with 5 plumose setae and a spine. The distal part with

4-5 spines and accompanied 4-5 simple setae. Article 2 of the outer ramus shorter than the surrounding spines and has a few simple setae on distal tip.

Telson lobes (Figure 5D) cleft, each lobe bears 1-2 spines and 2 simple setae in the distal part. The setae are shorter than the spines. The length/width ratio of the lobes is about 1:0.5 respectively.

*Etymology:* Evren Günay and Mehmet Ali Özel were the cave researchers and passed away during the exploration of Peynirlikönü Cave. The epithet "*egmao*" is the capital letters of their names and the newly identified species is devoted in their memory.



Figure 7. Comparison of the females of *G. ustaoglui* Özbek & Güloğlu, 2005 (left) and *G. egmao* sp. nov. (right). A, A': maxilla 1; B, B': uropod 3; C, C': pereopod 3 and pereopod 4.

Habitat: The specimens were sampled from a pool (Cadikazani) in the dark zone of the cave. The pool is mainly fed by drip water and located at 350 m depth below the entrance (see Figure 1). Seasonal flooding brings organic materials via cave channels, no other animals were observed during the cave research. Water temperature was around 6 °C when the specimens sampled.

#### DISCUSSION

*Gammarus egmao* sp. nov. has reduced eyes and slightly elongated extremities, which are usually observed in several troglophile/troglobite amphipod species, in addition to aesthetascs on the flagellar segments of antenna 1. These characters can be a result of an adaptation mechanism from the surface to groundwater habitats.

The number of the armaments present on distal parts of the left and right palps of maxilla 1 reduced (Figure 3B, C). There is only one tooth-like robust spine on the palp of right maxilla 1; the other two spines are slender and not tooth-like. Similarly, armament on the palp of left maxilla 1 is also reduced. This character is not frequently seen in the genus *Gammarus*. In the other species identified from the locality, *G. ustaoglui* Özbek & Güloğlu 2005, the number of armaments on the distal parts of both palps in maxilla 1 is higher than the present species. Additionally, the shape and length of antenna 1 and the endopod/exopod ratio of uropod 3 are different. A comparison between the new species and *G. ustaoglui* Özbek & Güloğlu 2005 is presented in Figure 7.

*Gammarus egmao* sp. nov seems like a morphospecies of *Gammarus balcanicus*-group (see Karaman and Pinkster, 1987) because of shape and setation of uropod 3 but the presence of long setae along the posterior margins of pereopod 3 and 4 are unusual for the group. However, for several species in the *Gammarus balcanicus*-group, the females have more setose pereopod III and IV, (e.g. *G. hamaticornis* (Copilaş-Ciocianu et al., 2018).

To date, six blind *Gammarus* species have been reported from Europe and Turkey (Özbek, 2010), and there are only a few in the genus that have minute or reduced eyes. *Gammarus microps* Pinkster & Goedmakers, 1975 is one of them which has minute eyes but it belongs to the *pulex*-group. Additionally, it has more elongated extremities and setose antenna II and uropod III. The number of flagellar segments of antenna 1 is quite different in *G. microps* (up to 46 segments) and the present species (17 segments). Additionally, the present species has less setose uropod 3 (in both rami) and peduncle segments of antenna 2, and shorter flagellum of antenna 2 (7 flagellar segments) than that of *G. microps* (16 flagellar segments). The endopod/exopod ratio of uropod is more than 0.5 in *G. microps* while much lower (about 0.35) in the present species.

Gammarus abscisus G. Karaman, 1973 also has rounded small eyes and was identified from Kırşehir, Turkey but the species differs from *G. egmao* sp. nov. by the presence of numerous short setae on the surface of metasome segments in addition to higher endopod/ exopod ratio of uropod 3.

*G. accolae* G. Karaman, 1973 is another amphipod species having small and round eyes and was identified from Kırkgöz Springs near Antalya province, Turkey. *Gammarus accolae*, which belongs to the *balcanicus*-group, differs from the present species by having crenulated and slightly bulging metasome segments, prolonged antenna 1 (up to 44 flagellar segments), sharply pointed epimeral plates and setose anterior margins of pereopods 5 to 7. Additionally, there are spines on the posterior margins of the mentioned segments. The metasome segments of *Gammarus egmao* sp. nov. are not crenulated and their posterior margins bear only a few short setae.

*Gammarus dulensis* S. Karaman, 1929 (belongs to *G. balcanicus*-group) has small eyes and identified from Dulo Spring, Montenegro. The species has several setae on the inner surfaces of basal segments of both pereopod 6 and 7. *Gammarus egmao* sp. nov. has no setae on the inner surfaces of basal segments of pereopod 5 to 7 (in females) and lower endopod/ exopod ratio of uropod 3, which is about 0.5 in *G. dulensis* while 0.35 in the present species. Additionally, the shape and the armaments of epimeral plates differ from *Gammarus dulensis*.

Gammarus pljakici G. Karaman, 1964 is the other species having small eyes in the *balcanicus*-group. It differs from *G. egmao* sp. nov. by having long setae on the ventral margins of peduncular segments of antenna 2, by having less setose posterior margins of pereopods 3 and 4 and by having shorter and sparsely armed uropod 3. Additionally, the endopod/exopod ratio of uropod 3 (about 0.5) is higher than the present species.

*Gammarus halilicae* G. Karaman, 1969 was identified from the Republic of Macedonia and has small eyes. It has elongated pereopods and weak setation in all of the extremities which are some of the different features than the present species.

Gammarus longipedis Karaman & Pinkster, 1987 was identified from Su Çıktığı Cave, Hadim, Turkey. The type locality of the species is close (~110 km air distance) to the newly identified species because both of them were placed in southern Anatolia. Gammarus longipedis has elongated pereopods with weak setation in addition to higher endopod/exopod ratio of uropod 3. The setation of pereropod 3 and 4 is also weak in the females of *G. longipedis* which is distinctly from the newly identified species.

Gammarus hamaticornis Copilaș-Ciocianu et al., 2018 was identified from SE Europe. It differs from the present species by having well developed eyes, by having latero-distally bent antennal gland cone and

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by presence of setae on the inner surface of basis of pereopod VII.

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