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The first record of *Neogammarus* (syn. *Rhipidogammarus) karamani* (Stock, 1971) (Crustacea: Amphipoda) in a marine subterranean cave of Malta

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The crustaceans, with their incredible variety of body plans, are a key group for studying how much anatomical specializations have diversified, revealing the taxonomic and habitat diversity. Remarkably, the amphipod crustaceans are interesting with their diverse morphologies, not been deeply investigated yet, being a model taxon to detect threats to biodiversity as they are often peculiar to specific habitats (Lo Brutto et al. 2021; Lo Brutto et al. 2022).

The Mediterranean Sea includes many habitats still unexplored (Lo Brutto et al. 2024a), rare species (Lo Brutto & Iaciofano 2020) and groups which need to be revised for different taxonomic issues to assess better their distribution and ecology (Lo Brutto et al. 2024b).

A case of a recent synonymy regards *Neogammarus* Karaman, 1969 and *Rhipidogammarus* Stock, 1971. The last became the synonym since the type species – designating *Gammarus rhipidiophorus* Catta, 1878 – which was fixed by Karaman (1969) for *Neogammarus* was successively used by Stock (1971) when erected the genus *Rhipidogammarus* Stock, 1971. The designation of the same type of species created a problematic situation. Thus, as *Neogammarus* Karaman, 1969 had nomenclatural precedence over *Rhipidogammarus* Stock, 1971, all the species included within *Rhipidogammarus* Stock, 1971 have been transferred to *Neogammarus* Karaman, 1969 (Rosas-Ramos et al. 2023).

The *Neogammarus* genus encloses nine species including the no-more-valid genus *Rhipidogammarus* (Özbek & Sket 2020). They are Atlanto-Mediterranean species, distributed mainly in the western part of the Mediterranean basin up to the Canary Islands coast (Stock 1988a; Stock & Sánchez 1990). The names are herein listed in the new genus, plus the synonym as much literature still reports the old name: *Neogammarus adriaticus* Karaman, 1973; *Neogammarus* (*Rhipidogammarus) gomeranus* (Beyer & Stock 1994); *Neogammarus* (*Rhipidogammarus) karamani* (Stock, 1971); *Neogammarus* (*Rhipidogammarus) nivariae* (Stock 1988)b; *Neogammarus* (*Rhipidogammarus)*

rheophilus (Stock & Sànchez, 1990); *Neogammarus* (*Rhipidogammarus) rhipidiophorus* (Catta, 1878); *Neogammarus* (*Rhipidogammarus) triumvir* (Notenboom 1985); *Neogammarus* (*Rhipidogammarus) variicauda* (Stock, 1978).

Recently, a new species *Neogammarus* (*Rhipidogammarus) gordankaramani* (Özbek & Sket 2020) was identified from Turkey, and reported as the first representative of the genus both for Turkey and for the Eastern Mediterranean area.

The species belonging to this genus inhabit freshwater, subterranean, and mixohaline waters.

In 2017, a sampling survey was carried out within the surface waters of the Ħarq Ħammiem cave, which is the Malta's only known fully-submerged terrestrial cave, located along the north-eastern Maltese coast, at the following coordinates: 35°55'37.64"N, 14°29'13.10"E. Sampling was conducted by lowering a bucket within the cave's water column, down to a maximum depth of 2.5m.

The entrance to the cave is at the base of an escarpment, located on the northern flank of the Ħarq Ħammiem valley. The cave constitutes a unique natural feature in the Maltese Islands. It consists of two chambers on different levels, with an interconnecting narrow corridor leading from the upper chamber to a fully submerged lower chamber (Figure 1). The water inside the cave approximates freshwater at the surface, turning brackish with depth and having seawater characteristics at deeper levels (below the floor of the upper chamber).

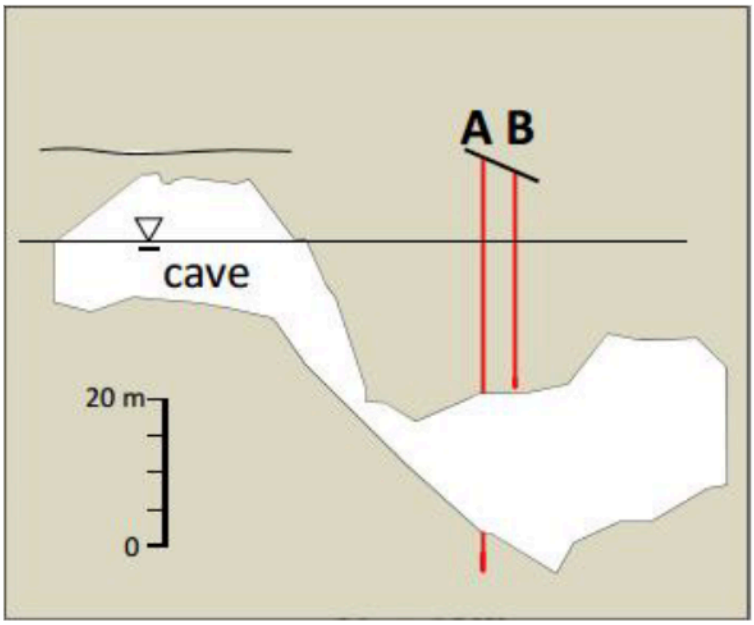

Figure 1. Sampling site. Morphology of Ħarq Ħammiem cave on the north side of St. George's Bay, St. Julians, Malta. Source: Randolph Camilleri Surveys Ltd, 2001.

The cave was formed by a complex mechanism involving intense karstic activity and tectonics. There is ample evidence of both phenomena: (i) dissolution and concretional growth on the walls providing evidence of the karstic activity; and (ii) faults providing evidence of tectonism. An example of the latter is the southern side of the upper chamber which constitutes a sheer vertical wall corresponding to a fault plane aligned along the longitudinal axis of the cave.

Amphipod samples were collected and preserved in EtOH 70%.

The amphipods were identified to the species level as *Neogammarus karamani* (Stock, 1971). Specimens were examined under a stereo microscope and dissected. Body length, from the tip of the rostrum to the apex of the telson, was measured using ImageJ software after placement on graph paper and photographed (FINEPIX S1800, FUJIFILM). The taxonomic identification was performed following Karaman (1991; 1993).

Totally, 10 specimens of *Neogammarus karamani* were collected. The samples were composed of one male (body length 16.46 mm) (Fig. 2A), 7 females (Fig. 2B) (body length 12.16-13.84 mm), and 2 broken specimens.

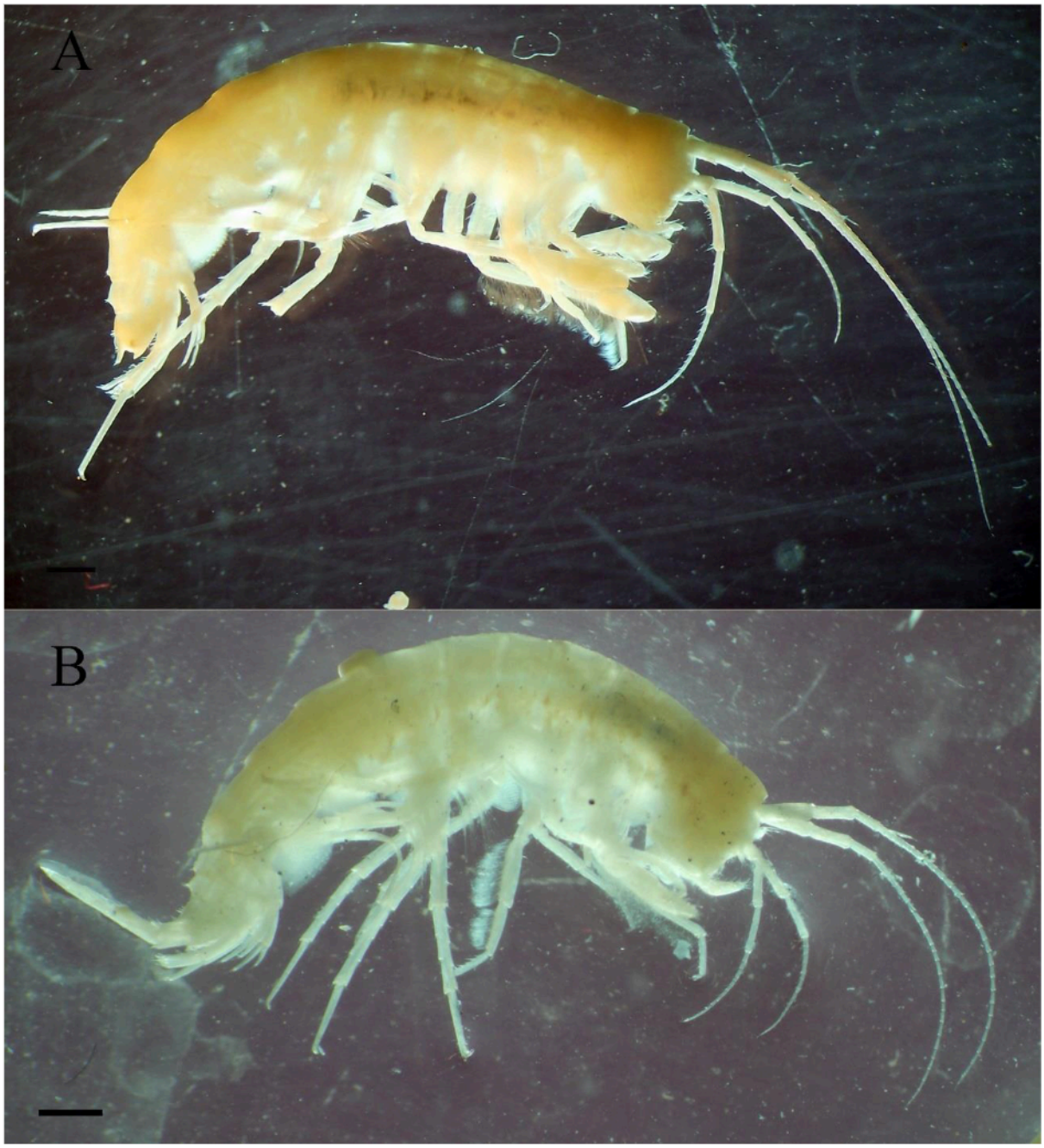

Figure 2. *Neogammarus karamani* (Stock, 1971). A: male; B: female; scale bar 1 mm.

An observation of morphological features was conducted. Lobes of maxilla 2 subequal, bearing only normal distal setae; Mandible with palp. Accessory flagellum developed. Palms of gnathopods 1-2 in males without median spine. Pereopod 3 with a fan of many plumose setae along the posterior margin, at least in males. Coxae 4-5 as long as, or shorter than coxae 1-3. Urosome spiniferous. Rami of uropod 1 partially reduced, without lateral spines, each with only one robust distal spine; Uropod 3 with inner ramus shorter than 1/3 of outer ramus (Figure 3). Outer ramus of uropod 3 2-segmented; both margins of outer ramus of uropod 3 with spines mixed with short setae. According to Stock (1971), the uropod 3 shows the medial margin of the first exopod segment (outer ramus) provided with spines of reduced length. The length of the spines was expressed by Stock (1971) as a function of the exopodal diameter. The ratio exopodal diameter/spine length should be 3.2 - 3.4. The character was herein confirmed, showing to be robust.

Figure 3. *Neogammarus karamani* (Stock, 1971), female, U3.

The absence of eyes was a characteristic feature of the collected specimens. The members of the genus are adapted to inhabit hypogean waters; all of them are known to have more or less developed eyes (Stock, 1988a) and most of them are usually characterized as 'semi-subterranean' animals (Karaman, 2012).

Literature reports *N. karamani* as an endemic species for the central Mediterranean Sea. It is distributed from the Tyrrhenian Sea to the Adriatic Sea and the western coast of Greece (Karaman 1993); a wider area than the one described by Özbek & Sket (2024) which limited its distribution to the Adriatic Sea. All the previous records of the species were listed in Table 1. The record herein reported is the first for the Maltese area and confirms the southernmost range area, together with an old (before 1982) record from Lampedusa Island. It contributes to extending the geographical range of the species useful to implement the World Amphipoda Database (WAD; Horton et al. 2023). In this regard, such a record is consistent with the priorities of the WAD.

Table 1. Records of *Neogammarus karamani* from literature.

Site	Year	Specimens collected	References	Further information
Zenta, Split, Croatia	1946	10	Kaman S., 1950; G. Karaman & Ruffo, 1977	
Pirgy, Corfu, Greece	1957	6	Ruffo, 1960; Stock, 1971	In débris of Posidonia, in a marine erosion cave
Musone River, Macerata, Italy	1964	4	G. Karaman & Ruffo, 1977	
Boka Kotorska Bay, Kotor, Montenegro	1965	2	Karaman, 1969; G. Karaman & Ruffo, 1977	
Skurda River, Kotor, Montenegro	1965	2	Karaman, 1969; G. Karaman & Ruffo, 1977	
Glavati, Montenegro	1965	20	Karaman, 1969; G. Karaman & Ruffo, 1977	
Njivice near Hercegnovi, Montenegro	1966	29	Karaman, 1969; G. Karaman & Ruffo, 1977	
Verige, Montenegro	1966	6	Karaman, 1969; G. Karaman & Ruffo, 1977	
Ljuta near Orahovac village, Montenegro	1966	4	Karaman, 1969; G. Karaman & Ruffo, 1977	

continued on the next page..

Table 1

Donji Morinj, Montenegro	1967	6	Karaman, 1969; G. Karaman & Ruffo, 1977	
Nameless stream discharging on the beach near Miomo, Cap Corse, Corsica, France - *Locus typicus*	1970	85	Stock, 1971	Chlorinity 53 mg/l; salinity 95.695 ‰; on gravel
Nameless stream discharging on the beach near Lavasina, Cap Corse, Corsica, France	December 1970	5	Stock, 1971	Chlorinity 28 mg/l; salinity 50.57 ‰; 9.6°C; pH 4.5; coarse gravel; high water flow
Porto Badisco, Puglia, Italy	1971	3	G. Karaman & Ruffo, 1977	
Nameless stream discharging on the beach near Grigione, Corsica, France	November 1971		Stock, 1972	Chlorinity 27 mg/l; 10-40 m depth; 15.1°C; pH 6; on gravel and sand; very low water flow; site slightly polluted
Lampedusa, Italy	Before 1982		Ruffo, 1982	
Rijeka, Lovran, Croatia			Sket, 1969	
Cavtat, Croatia			Sket, 1969	
Ulcinj, Montenegro			Sket, 1969	

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