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A new cave-dwelling hadzioid amphipod (Senticaudata, Hadzioidea, Melitidae) from sulfidic groundwaters in Iran

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Abstract

Cave systems fed with hydrogen sulfide-rich groundwater are unique chemoautotrophy-dependent ecosystems. Although globally widespread and known to harbor unique subterranean metazoan communities, they have mostly been studied in Europe and North America, less so in Asia. Here, we report on a discovery of a new species of amphipod crustacean from sulfidic waters of Tashan-Chah Kabootari aquifer from Zagros Mountains in Iran. The new species corresponds morphologically to the melitid genus *Tegano* Barnard & Karaman, 1982, and *Tegano tashanensis* sp. nov. is the first amphipod from the superfamily Hadzioidea found exclusively in sulfidic water. Phylogenies derived from the mitochondrial COI and the nuclear 28S rRNA sequences recover *T. tashanensis* sp. nov. in a clade that contains representatives of the currently para- or polyphyletic genera *Barnardomelita*, *Brachina*, *Josephosella*, and *Tegano* indicating the need for a revised melitid genus level systematics. This clade comprises marine, freshwater and subterranean species globally distributed in regions that were connected by the ancient Tethys Sea. As such, these taxa may be suitable biogeographic models for studying past dispersal, vicariance and multiple colonization of inland groundwaters.

Keywords

Amphipoda – groundwater – Iran – Melitidae – phylogeny

Introduction

Groundwater represents the largest freshwater body in the world (Gibert & Deharveng, 2002). Yet, due to being remote and hard to access (Ficetola et al., 2019), groundwater remains one of the most unexplored habitats in the world (Larned, 2012). Groundwater is not a homogenous habitat but rather a palette of diverse habitats (Culver & Pipan, 2019). One of the most interesting groundwater habitats are waterbodies rich in hydrogen sulfide. While the sulfide is toxic to most metazoans, it is also a source of energy to chemolithoautotrophic bacteria, resulting in primary production in the absence of light (Sarbu et al., 1996). On some occasions, chemolithoautotrophic primary production is sufficient to establish and maintain isolated, local ecosystems (Sarbu, 2000). These caves are not as oligotrophic as freshwater caves; despite sulfide toxicity these caves often harbor species-rich

communities (Sarbu, 2000; Hutchins et al., 2016). Apparently, while H₂S may be toxic for most animals, other organisms cope well with it and even thrive in these habitats. To date, more than 19 caves with sulfidic hypogean waters have been reported worldwide (Culver & Pipan, 2019). Metazoan faunas of sulfidic caves have been studied mostly in Europe and North America, including Movile Cave in Romania (Sarbu, 2000; Brad et al., 2021), Frasassi Cave System in Italy (Sarbu et al., 2000), Melissotrypa Cave in Greece (Vaxevanopoulos, 2006; Borko et al., 2019; Popa et al., 2019) and Cueva del Azufre in Mexico (Tobler et al., 2008, 2011; Riesch et al., 2015). In Asia, to our knowledge, metazoan fauna from sulfidic groundwater has only been studied from Ayyalon Cave in Israel (Por et al., 2013) and Tashan Cave in Iran (Fatemi et al., 2019).

The exploration of Tashan Cave in Iran has begun relatively recently. So far, three species

have been described from the cave, including a cave fish *Garra tashanensis* Mousavi-Sabet, Vatandoust, Fatemi & Eagderi, 2016, an isopod *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini & Kuntner, 2018, and a gastropod *Trogloiranica tashanica* Fatemi, Malek-Hosseini, Falniowski, Hofman, Kuntner & Grego 2019 (Mousavi-Sabet et al., 2016; Khalaji-Pirbalouty et al., 2018; Fatemi et al., 2019). Presence of the Tashan Cave fish in outflows of a seasonal karstic spring, five km to the east of its type locality, in syntopy with the stygobiotic form of *Garra rufa* (Heckel, 1843), shows the presence of a unique and big aquifer in that area (Malek-Hosseini et al., 2023). The Tashan-Chah Kabootari aquifer comprises also the Chah-Kabootari Cave. It is in this cave that eyeless and depigmented amphipod crustaceans were found, which we describe in this article.

Crustaceans often dominate metazoan subterranean aquatic communities (Sket, 1999). Amphipods represent a substantial fraction of subterranean crustacean diversity (Väinölä et al., 2008; Zgmajster et al., 2014; Eme et al., 2017). In the Western Palearctic, most subterranean amphipod species belong to the genus *Niphargus* (Schiødt, 1847). *Niphargus*, known from all European sulfidic caves (Flot et al., 2010, 2014; Brad et al., 2015; Fišer et al., 2015; Borko et al., 2019), is represented in Iran with 23 species (Esmaeili-Rineh et al., 2015, 2017; Zamanpoore et al., 2019; Bargrizaneh et al., 2021). The shape of gnathopods, uropods and telson of the newly collected specimens from Chah Kabootari Cave suggests that this species does not belong to *Niphargus*, but rather to an unrelated superfamily Hadzioidea Bousfield, 1973 (Lowry & Myers, 2013; Copilaş-Ciocianu et al., 2020). Amphipods of the superfamily Hadzioidea live in marine and freshwater habitats, including groundwater. Subterranean hadzioid amphipods presumably colonized the subterranean realm from marine ancestors

(Stock, 1980; Holsinger, 2000). Their global distribution in West Indies, Mediterranean, and the Pacific region implies that they may date back to the Tethys (Hou & Li, 2018; Zhao et al., 2021). The Chah-Kabootari Cave, situated proximally to the Persian Gulf, roughly falls within the Tethyan region, and is thus in geographic agreement with previous records of subterranean Hadzioids and our preliminary morphological identification.

The taxonomic position of the herein described species is unclear. The superfamily Hadzioidea comprises eight families of controversial taxonomic status, including families Hadziidae and Melitidae (Lowry & Myers, 2013). Specimens from the Chah-Kabootari Cave could be considered either as a member of the family Hadziidae or, more likely, can be assigned to the melitid genus *Tegano* whose original diagnosis was proposed on a basis of series of reductions, including a minute accessory flagellum, absent inner lobes of the labium, absent mandibular palp, the loss of sexual dimorphism in gnathopods, and the loss of spine at the base of uropod I (Lowry & Myers, 2013); all these character states match the specimens from the Chah-Kabootari Cave. To clarify the taxonomic status of this unexpected finding, we studied the species morphologically and phylogenetically. We show that the new species falls into a clade that contains several paraphyletic melitid “genera”. In the absence of a thorough systematics revision of these genera, and to avoid creating a new monotypic genus that may subsequently need to be synonymized, we here describe the new species as *Tegano tashanensis* sp. nov.

Materials and methods

The studied cave

Chah Kabootari Cave or Chal Kabootari in local language (Persian) means “nest of pigeons”.

A part of the Tashan-Chah Kabootari cave system located close to Sarjowshar village, Tashan district, Behbahan county, Khuzestan Province, Zagros Mountains, southwestern Iran ($30^{\circ}52'10''\text{N}$, $50^{\circ}10'01''\text{E}$, altitude 539 m above sea level) (figs. 1 and 2), it is a natural limestone cave containing sulfidic water. Several neighboring wells and springs are fed with sulfidic water from this vast underlying aquifer. At the time of sampling, the water in Chah Kabootari Cave had 25.6°C and pH 7.1, while the air temperature was 25.3°C , the relative humidity was 99.8 %, and the air contained 768 ppm CO_2 (0.08 %).

We visited Tashan and Chah Kabootari caves during several expeditions, between 2016–2023. The new amphipod species was collected from pools and streams of Chah Kabootari Cave (fig. 2) using a small hand net and a pipette. Specimens were also found in between the white biofilm floating on the sulfidic water in this cave. We preserved two samples in 96% and the others in 75% ethanol.

Morphological analysis

Specimens were immersed in glycerol and the dissected body appendages were mounted on slides using Kaiser's glycerol gelatin (Merck



FIGURE 1 Distribution map of the family Melitidae in Iran, (1) (green circle): Chah-Kabootari Cave, habitat of *Tegano tashanensis* sp. nov.; (2) (red circle): Qeshm Island, type locality of *Melita persia* Momtazi, Sari & Maghsoudlou, 2014; (3) (purple circle) Anzali Port, type locality of *M. mirzajani* Krapp-Schickel & Sket, 2015. Distribution of the genus *Melita* in Iran derived from Momtazi et al. (2014) and Krapp-Schickel and Sket (2015).



FIGURE 2 Chah-Kabootari Cave, part of Tashan-Chah Kabootari aquifer, habitat of *Tegano tashanensis* sp. nov. (A) and (B): ponds at the first part and end of the cave respectively, where the specimens were collected.

KGaA, Germany). The slides were examined under an Olympus SZX16 stereomicroscope equipped with an Olympus SC180 camera, and under an Olympus BX51 microscope with an Olympus SC50 camera. The drawings were performed upon printed photographs by manual inking and continuous inspection of the slides under the microscope. We followed the standard amphipod anatomic terminology (Fišer et al., 2009). The holotype and paratypes are deposited in the collection of Emil Racoviță Institute of Speleology, Cluj, Romania.

Molecular procedures

Genomic DNA was extracted robotically from the pereopod muscle of three specimens

using Mag MAX™ Express magnetic particle processor Type 700 with DNA Multisample kit (Thermo Fisher Scientific kit) following modified protocols (Vidergar et al., 2014). We employed PCR to amplify fragments of two genes, the mitochondrial cytochrome c oxidase subunit 1 gene (CO1) and the nuclear 28S rRNA gene (28S) following established protocols (Malek-Hosseini et al., 2021); for primer pairs, see supplementary table S1 and for PCR amplification protocols, see supplementary table S2. Each fragment was sequenced (Macrogen Europe, Amsterdam) in both directions using PCR amplification primers.

To examine the phylogenetic position of the newly described species, we checked

GenBank for both fragments of all melitid genera from previous publications available as of November 2022 (Tandberg et al., 2012; Chung et al., 2014; Park et al., 2020; King et al., 2022; Tomikawa et al., 2022). We obtained COI fragments for 11 melitid genera, totaling 31 species, and 28S rRNA fragments for six genera and 18 species including our original data (supplementary table S3). As outgroups, we selected two unrelated, freshwater amphipods, *Synurella ambulans* (F. Müller, 1846) (Crangonyctidae) and *Carinurella Paradoxa* (Sket, 1964) (Niphargidae), for which sequences were available (see also Copilas Ciocianu et al., 2020).

To assemble sequences, we used ChromasPro 2.1.3 (Technelysium, Tewantin, Australia). Alignment, the best substitution model and the optimal partitioning scheme for each marker were made using MEGA (Kumar et al., 2018). MUSCLE algorithm was used for alignments. The final length of aligned fragments was 657 bp for COI and 1052 bp for 28S rRNA. Fragments were concatenated in Mesquite version 3.70 (Maddison & Maddison, 2021). Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). The optimal settings for our data involved two partitions, each using an independent nucleotide substitution model (COI: GTR+G+I; 28S rRNA: GTR+I) (supplementary alignments S1–S3). We calculated the ML tree using IQ-Tree v2.1.3 (Minh et al., 2020), and node support was assessed using 1000 ultrafast bootstrap replicates. BI tree and Bayesian posterior probabilities (PP) were estimated using MrBayes v3.2.7a (Ronquist et al., 2012). We ran two independent Bayesian runs, each with four MCMC chains, for 15 million generations with a sampling frequency of 3000. A relative burn-in was set to 25% and checked for the MCMC chain convergence in Tracer 1.7.1 (Rambaut et al., 2018). These analyses were performed for each gene fragment

separately, as well as for a concatenated matrix.

Results

Phylogenetic analyses

Both, ML and BI yielded similar topologies. Detailed comparisons between gene trees are not possible due to different number of species used in different analyses. However, gene trees showed generally congruent topologies (supplementary figs. S1–S5) and thus justify concatenated analysis. The concatenated analysis yielded an overall well supported phylogeny. Melitidae comprises three main clades, but our phylogeny indicates the paraphyly of the genera *Melita*, *Josephosella*, *Megamoera* and *Quasimelita*.

Systematics

The new species from Chah Kabootari Cave is nested in the well-supported clade comprising genera *Josephosella*, *Barnardomelita*, *Tegano* and *Brachina* (fig. 3 and supplementary figs. S1–S5). The Iranian species represents its own, well supported lineage, that might justify the erection of a new genus. However, considering that the overall genus level systematics of the family needs to be revised within a much wider taxon sampling, we feel that an introduction of a new genus would further complicate, rather than resolve, its current taxonomic structure. For this reason, we tentatively place this new species in *Tegano*, although we freely admit that this placement is based on morphological similarities or even absences rather than presumed synapomorphies: the absence of mandibular palp, the shape of gnathopods, the absence of spine at the base of uropod I and of baso-facial spine at uropod I. For the above reason, we refrain from providing a taxonomic diagnosis of the genus *Tegano*.

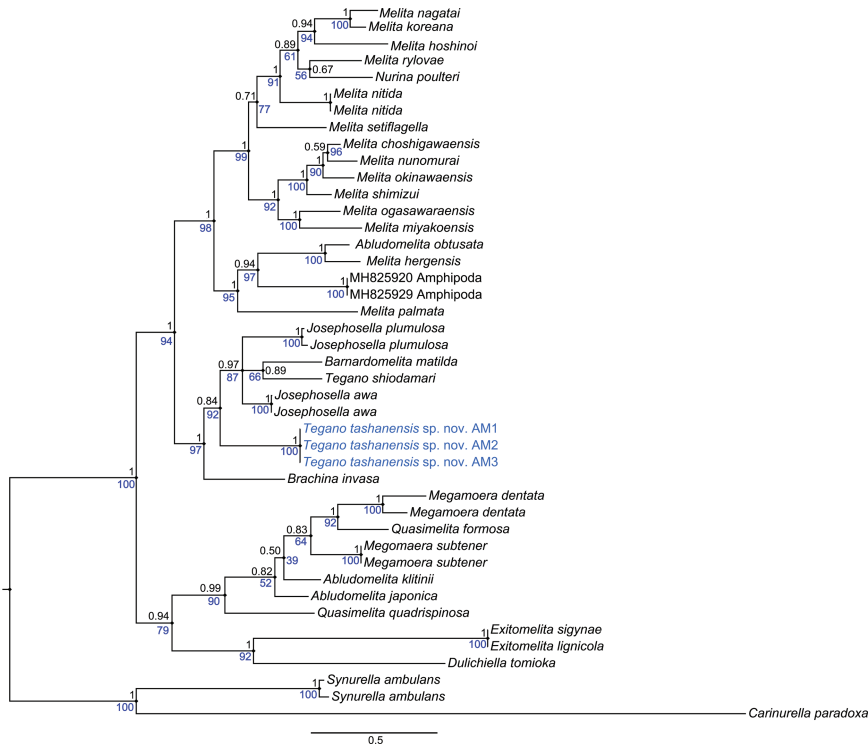


FIGURE 3 Phylogeny of the melitid amphipods including the new species of *Tegano tashanensis* sp. nov. from Chah Kabootari Cave. The topology derives from the Bayesian inference analysis on the concatenated matrix. Node support presents posterior probabilities obtained by Bayesian analysis in MrBayes (in black), and bootstrap support values (in blue) obtained by ML analysis in IQ-Tree.

Taxonomy

Order Amphipoda Latreille, 1816

Family Melitidae Bousfield, 1973

Genus *Tegano* Barnard & Karaman, 1982

Tegano tashanensis sp. nov.

<https://zoobank.org/NomenclaturalActs/614E536F-598E-4B12-8818-DEAF2F6026F7>

Figs. 4–10

Diagnosis. Small-sized stygobiontic amphipod, with robust appearance and clear troglomorphic features. Eyeless, unpigmented, least setose, long antennae and legs relative to body length, labium without inner lobes, mandibles without mandibular palps, exopodite

of uropod III unsegmented. Pleonites 2 and 3 with 2 small setae on the postero-dorsal sides. One seta on each postero-dorsal side of the first two urosomites. Antenna I two thirds of the total body length. Outer lobe of maxilla I with 8 comb-like spines. Inner lobe of maxilliped with 3 stout, 3 serrated and 3 regular setae located apically and 3 serrated setae subapically. Gnathopods scarcely setose, highly dissimilar, propodite of gnathopod I less than half length of propodite of gnathopod II. Uropods I and II with slightly shorter exopodites than corresponding endopodites. Pereopods VI and VII of equal size, more than half of body length. Telson as long as wide, with 4 short apical setae on each lobe.

Material examined. Holotype: ♂ (fig. 4A), Chah Kabootari Cave, Sarjowshar village, Tashan district, Behbahan county, Khuzestan Province, Zagros Mountains, southwestern Iran (30°52'10"N, 50°10'01"E, altitude 539 m a.s.l.), collected on 17.03.2018.

Paratypes: Locality as same as holotype, one dissected ♀ (fig. 4B), 1 ♂ and 2 ♀ undissected; collected on 08.12.2018.

Remark. The morphological description was performed on the body appendages belonging to the dissected holotype male. Sexual dimorphism was described by inspection of one paratype female.

Description (male): The total body length of the male is 4.7 mm (fig. 5A). A chart containing detailed measurements on all diagnosis-relevant body appendages is presented in supplementary table S4.

Head. Head (fig. 5A) with no rostrum; 10 % of the total body length (supplementary table S4).

Antennae. Antenna I (fig. 6A) about two thirds of the total body length, with main flagellum formed of 20 articles. Antenna I articles with short aesthetascs, one third of the respective article. Antenna I peduncle 41 % of the total length of antenna I. Accessory flagellum formed of 1 article and does not exceed the length of the first article of the main flagellum (fig. 6C). Accessory flagellum with 6 apical setae and 1 serrate seta located subapically. A more frequent shape of the accessory flagellum is that present in the paratype male and females (fig. 6D). Here, accessory flagellum is longer, and it is formed of 2 articles. The distal article represents only 5 % of the total length of the accessory flagellum and it bears 2 long setae.

Antenna II (fig. 6A) with the flagellum formed of 10 articles; less than half of the total length of antenna I. The peduncle 60 % of the total length of antenna II.

Mouthparts. Labrum (fig. 6G) trapezoidal, with the width twice as much as its depth. Labium (fig. 6H) without inner lobes. The outer lobes each with one row of fine setae on the outer side. Maxilla I (fig. 6E) with 6 apical and 2 subapical setae on the distal article of the palp. The outer lobe with 8 comb-like spines apically. Inner lobe with 6 apical serrate setae and a row of lateral fine setae. Maxilla II (fig. 6F) with the outer lobe $\frac{3}{4}$ of the inner lobe length. Both lobes with a row of apical setae of various lengths. Outer lobe with a row of fine setae on the outer margin. The outer margin of inner lobe with one row of fine setae and one row of plumose setae. Mandibles lack palps. Left mandible (figs. 7I–7J) with the incisor process formed of two lamellas with 3 and 4 teeth. Lacinia mobilis with 4 teeth; 4 strong serrated setae between lacinia mobilis and molar process. Right mandible (fig. 6K) with 6 teeth on incisor process. Spiniform lacinia mobilis with 2 teeth. 4 larger serrated setae and a row of fine setae between lacinia mobilis and molar process. The molar process present 12 tooth-like structures and an adjacent serrated seta. Maxilliped (fig. 7H) with the palp formed of 4 articles little setose. Article 1 with 1 seta on the inner margin. Article 2 with 13 setae (of which one serrated) aligned along the inner margin over the whole length of the article, and one seta located subapically on the outer margin. Article 3 with one group of 4 apical setae and two groups of 5 setae each on both inner and outer margins. Article 4 with a group of 4 fine setae on the ventral margin and 1 seta on the dorsal margin. The outer lobe of maxilliped (fig. 7I) with 4 serrated setae and 3 flattened spines located apically, a row of 7 setae and 7 denticles aligned over the inner margin. The inner lobe is provided apically with 4 serrated setae, 3 flattened spines and 3 more regular setae. 2 serrated setae located subapically on the inner margin of inner lobe.

Gnathopods. Gnathopod I (fig. 7A) with the coxal plate in the shape of a rectangular trapezoid. The depth of coxal plate I larger than its width (ratio depth:width 1.0:0.5). Basipodite length:width ratio 1.0:0.3. Ischiopodite with one posteroventral group of 4 setae. Basipodite length:carpopodite length ratio 1.0:0.6. Meropodite with a row of fine setae along the whole ventral margin and 4 longer apical setae. Carpopodite with a row of 8 setae located posteroventral, a group of 4 setae located apically and 1 anterodorsal seta. Propodite (fig. 7C) with the length: width ratio 1.0:0.6. Propodite least setose, 2 setae on the ventral margin, a group of 4 setae on the dorsal margin and one group of 6 setae located antero-apically. 2 setae on the lateral surface and 2 small setae in the vicinity of the palmar corner. Palmar spines absent. Dactylopodite with the nail one quarter of the total dactylopodite length. 1 seta on the outer margin of dactylopodite.

Gnathopod II (fig. 7B) highly different compared to gnathopod I. Ovoid coxal plate with the depth: width ratio 1.0:0.6. Basipodite length:width ratio 1.0:0.35. Ischiopodite with on posteroventral group of 3 small setae. Basipodite: carpopodite length ratio 1.0:0.55. Carpopodite with 5 groups of 2–4 setae along its ventral margin, 1 and 2 setae located apically on each side, 1 small seta on the dorsal margin. Long propodite (fig. 7D), length:width ratio 1.0:0.7. Propodite gnathopod II:propodite gnathopod I length ratio 1.0:0.25. Propodite with eight groups of 1–2 setae in its ventral margin, four groups of 2–3 setae on the dorsal margin and one group of 4 setae located antero-apical. On its surface, the propodite has two median groups of 2 setae, and one group of 2 setae close to the palmar corner. Palmar spines missing. Short dactylopodite nail, representing 15% of the total dactylopodite length. One seta on the outer margin of dactylopodite.

Pereopods. Pereopod III (fig. 7E) with a relative rectangular coxal plate, ratio depth:width 1.0:0.55. Dactylopodite with the nail measuring one third of the total length of the dactylopodite, one dorsal seta with plumose tip and one fine seta at the nail insertion. Propodite: dactylopodite length ratio 1.0:0.35. Pereopod III nearly equal in length to pereopod IV (ratio 1.0:0.97).

Pereopod IV (fig. 7F). Coxal plate with depth:width ratio 1.0:0.87. Gill ovoid, almost as large as the coxal plate. Dactylopodite (fig. 7G) with nail measuring one third of the total dactylopodite length, with one dorsal seta with plumose tip and one seta at nail insertion. Propodite:dactylopodite length ratio 1.0:0.35.

Pereopod V (fig. 8A). Coxal plate bilobed. Basipodite ovoid, length:width ratio 1.0:0.83. Short and thick setae present along the anterior and posterior margins of basipodite. Dactylopodite (fig. 8B) with one seta with plumose end on the outer margin and one seta at the insertion of the nail, which represents one quarter of the total dactylopodite length. Propodite:dactylopodite length ratio 1.0:0.38.

Pereopod VI (fig. 8C). Coxal plate of a relatively triangular shape, with a deep concavity in its dorsal margin. Basipodite (length:width ratio 1.0:0.82) with an ovoid shape, 7 groups of 2 setae on the anterior margin and 17 setae on the posterior margin. Dactylopodite (fig. 8D) with 1 plumose seta on the outer margin. The nail length represents one quarter of the total dactylopodite length. Ratio propodite:dactylopodite length 1.0:0.35.

Pereopod VII (fig. 8E). The coxal plate is trapezoidal. Basipodite with an ovoid to trapezoidal shape, ratio length:width 1.0:0.75. Basipodite with nine groups of 2 setae each on the anterior margin, and 21 small setae over the entire length of posterior margin. Dactylopodite (fig. 8F) with one plumose seta

on outer margin. Nail one fifth of the total dactylopodite length. Ratio propodite:dactylopodite length 1.0:0.37. Pereopods V:VI:VII length ratio as 1.0:1.2:1.2

Pereopods VI and VII of equal size, the longest legs of *Tegano tashanensis* sp. nov., measuring 2.5 mm.

Pleopods. Pleopods I, II and III highly similar (fig. 8G), with unequal rami and 2 retinacula (fig. 8H).

Uropods. Urosomites I and II with 1 dorso-lateral seta on each side; dorsal margin of urosomites III with no seta. One spiniform seta on the posteroventral angle of the first urosomite near the insertion of uropod I. Uropod I (fig. 9A) little setose. Endopodite slightly longer than the exopodite; ratio endopodite:exopodite 1.0:0.87. Uropod II (fig. 9B) little setose, endopodite longer than the exopodite; ratio endopodite:exopodite 1.0:0.81. Uropod III (fig. 9C) short, approximately 20% of the total body length. Basipodite with 2 apical stout setae on posterior margin and 2 smaller setae on anterior margin. Short endopodite, half the length of basipodite, with 1 short apical seta. Single-segmented exopodite, with 3 groups of 3 setae on the posterior margin, 5 groups of 2–3 setae on the anterior margin, six robust apical setae (fig. 9E).

Epimeral plates. Epimeral plates (fig. 5B) of different shape and size. Depth ratio of epimeral plates I, II and III is 1.0:1.3:1.2. Epimeral plates with right postero-ventral angle and convex ventral margin. Epimeral plates II and III with 4 setae on the posterior margin. Pleonites II and III with 1 dorsolateral seta on each side.

Telson (fig. 9F) as long as wide. 4 short apical spines on each lobe. The longest spine $\frac{1}{4}$ of total telson length. One mesial spine on each lobe, 1–2 lateral spines on each lobe.

Sexual dimorphism

Sexual dimorphism in *Tegano tashanensis* sp. nov. is negligible. While the male seems

stouter and with slightly longer antennae and pereopods, the diagnostic characters are invariable in the inspected male and female. To test for differences in quantitative traits, like body length (female of 3.86 mm vs. male of 4.77 mm), we would need more individuals.

Etymology

The species is named after the name of Tashan-Chah Kabootari aquifer which Tashan and Chah Kabootari caves are parts of this unique ecosystem. The species epithet is an adjective meaning “of the Tashan cave”.

Taxonomic remarks

The genus *Tegano* was erected by Barnard and Karaman (1982), on the basis of a single species, *Melita seticornis*, primarily due to the reduced mandibular palp. Nowadays, the genus *Tegano* comprises 12 described species distributed from Australia, Sri Lanka (Indian Ocean), and Palau, the Philippine Islands, and the Solomon Islands (all in the tropical western Pacific Ocean), Norfolk Coast of United Kingdom and Curaçao Island (both Atlantic Ocean). *Tegano* includes some obligate cave-dwelling representatives (Sawicki et al., 2005).

The newly described species belongs to a group of *Tegano* species with completely reduced mandibular palps. There are three species in this group, *Tegano vagabundus* from Sri Lanka, where mandibular palp is replaced by a long seta (Karaman, 1984), and *Tegano tashanensis* sp. nov. in Chah Kabootari Cave (Iran) and *Tegano panglaoensis* from Philippines, where the mandibular palp is completely absent. The latter species differs from herein described *T. tashanensis* sp. nov. by presence of eyes and labial lobes, narrow bases of pereopods V–VII (much broader in *T. tashanensis* sp. nov.), single basiofacial spine on uropod I (two in *T. tashanensis* sp. nov.), longer distal article on exopodite of uropod III (short in *T. tashanensis* sp. nov.), and

more pointed telson lobes (subrounded in *T. tashanensis* sp. nov.) (Sawicki et al., 2005).

There is an unclear relationship to hadzioid genus *Fiha*, which we could not analyze phylogenetically, with reduced mandibular palp and inner labial lobes. However, *Fiha* differs from *Tegano tashanensis* sp. nov. in slenderer body (more stout in *T. tashanensis* sp. nov.), accessory flagellum of antenna I (3–4 articles in *Fiha*, 1–2 in *T. tashanensis* sp. nov.), longer propodus in gnathopod I (relatively shorter in *T. tashanensis* sp. nov.), coxal plate IV shallow (deeply incised in *T. tashanensis* sp. nov.), and shallower and more open telson (longer lobes close together in *T. tashanensis* sp. nov.) (Stock, 1988).

Discussion

Higher level classification implications

Ideally, higher level classifications should seek consensus among authors but base the

decisions on numerous factors, such as monophyly, diagnosability, information content, utility and use, and perhaps comparative clade ages (Kuntner et al., 2019, 2023). Melitid genus level systematics is still far from this ideal. The phylogeny (fig. 3) implies the paraphyly or polyphyly of several Melitidae genera underlining the need for a thorough systematics revision as already stressed by other authors (Sawicki et al., 2005; Labay, 2016; Tomikawa et al., 2022). Such a systematics revision is clearly outside of our scope here, and therefore we faced the choice of either creating a new genus for the species we discovered, or placing it in an existing genus. We chose the latter for the above stated reasons. No doubt, future systematics will need to critically evaluate the validity of all melitid genera, *Tegano* included. Rather than on phylogenetic structure, the current taxonomy of melitid genera is based on morphological features that are variable and prone to convergences and parallelisms. Subterranean species are particularly

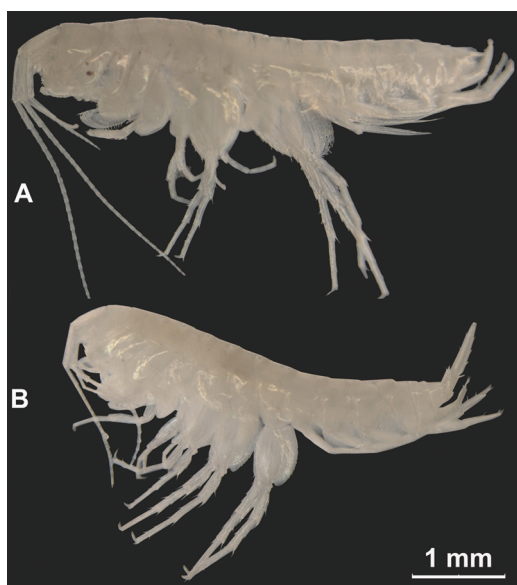


FIGURE 4 Photographs of *Tegano tashanensis* sp. nov.: male (A) and female (B) living in Chah Kabootari Cave, Iran.

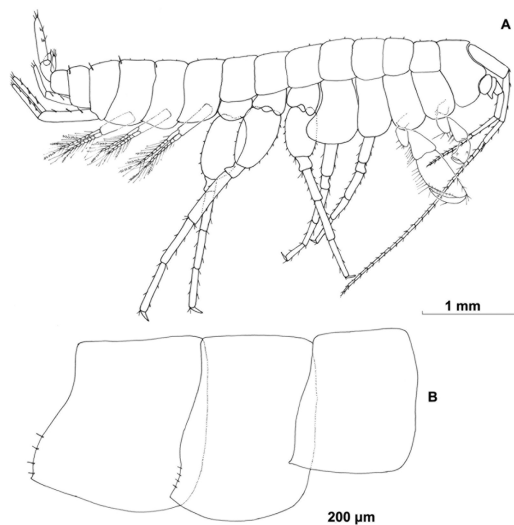


FIGURE 5 *Tegano tashanensis* sp. nov.: (A) holotype, male (4.7 mm), habitus, lateral view; (B) epimeral plates I–III.

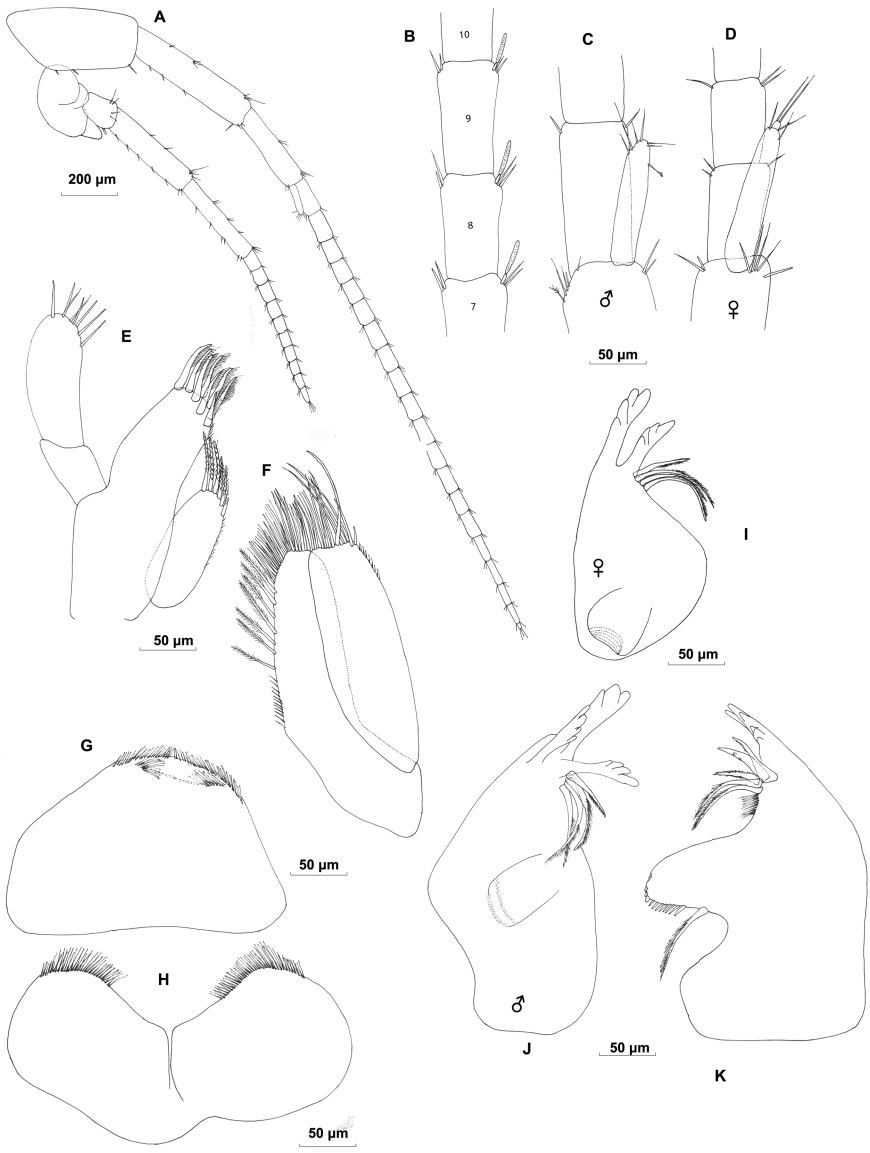


FIGURE 6 *Tegano tashanensis* sp. nov. male: (A) Antennae I and II, (B) aesthetascs on antenna I articles, (C) accessory flagellum of holotype male antenna I, (D) other image of accessory flagellum of female and paratype male antenna I, (E) maxilla I, (F) maxilla II, (G) labrum, (H) labium (note absence of the inner lobes), (I) female left mandible, (J) male left mandible, (K) male right mandible. Note the absolute absence of the palp in both mandibles.

challenging, as many diagnostic characters refer to reduced or absent structures, such as eyes, mandibular palps, baso-facial spines and loss of sexual dimorphism. Trait reduction and loss of sexual dimorphism is an evolutionary

phenomenon accompanying the evolution of subterranean species (Christiansen, 2005; Fišer, 2019). While reductions may in some cases represent synapomorphies (Grandcolas et al., 2001), most of them reflect independent

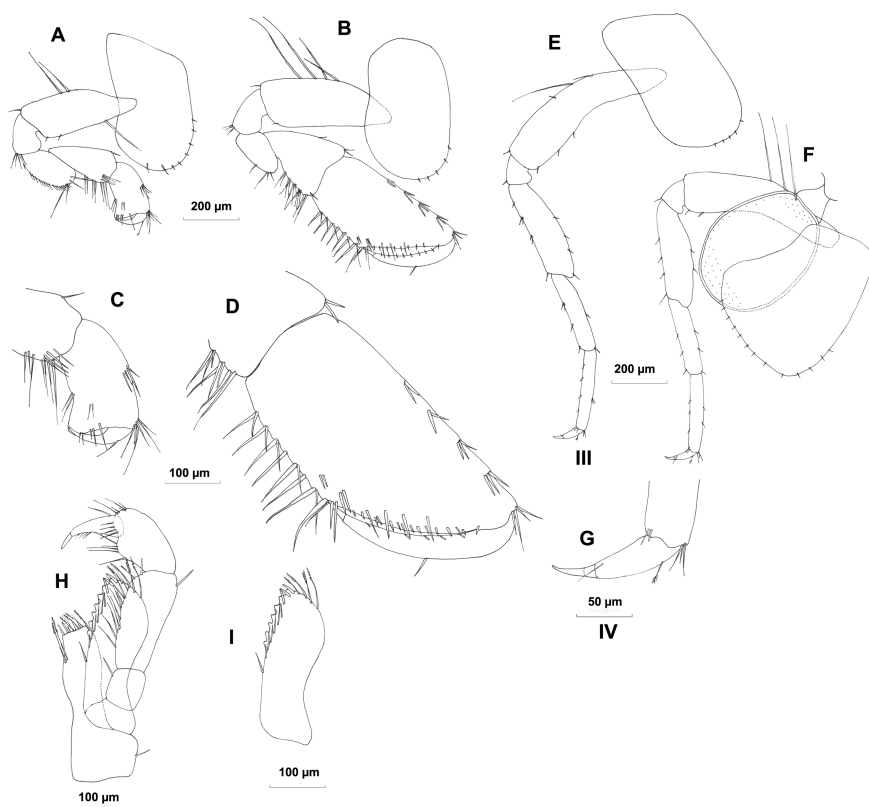


FIGURE 7 *Tegano tashanensis* sp. nov., male: (A) gnathopod I, (B) gnathopod II, (C) propodite of gnathopod I, (D) propodite of gnathopod II, (E) pereopod III, (F) pereopod IV, (G) dactylopodite of gnathopod IV, (H) maxilliped, (I) maxilliped outer lobe.

loss (Recknagel et al., 2023) that can arise via different mechanisms (Culver et al., 2023).

Biogeographic implications

Subterranean melitid genera are widespread around the globe and *Tegano* is no exception (Hamond, 1965; Barnard & Barnard, 1983; Vonk, 1989; Sawicki et al., 2005). A parsimonious explanation of this pattern invokes a common ancestor widespread along the Tethyan seaway, that, according to the stranding hypothesis, may have colonized groundwaters several times independently, analogous to the known cases of continental colonization of caves (Carlini et al., 2009) or colonization

on volcanic islands (Villacorta et al., 2008). If ancestral lineages were not available for sampling or if they have gone extinct, subterranean descendants can falsely be recovered as monophyletic (Cooper et al., 2023) and putative synapomorphies are in fact parallelisms or convergences. We project that the genera *Josephosella*, *Barnardomelita*, *Tegano* and *Brachina* (together monophyletic in our phylogeny) will eventually need to be lumped in a single genus that shows historic radiations in the sea, freshwater, and groundwater. However, as we caution throughout this paper, a thorough systematics revision is needed to solve melitid genus level taxonomy.

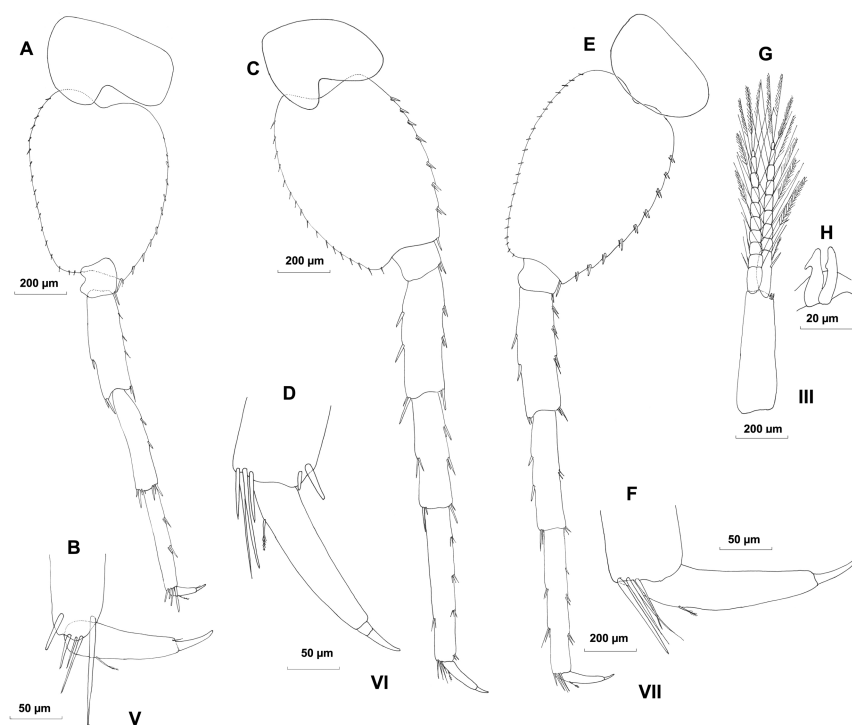


FIGURE 8 *Tegano tashanensis* sp. nov., male: (A) pereopod V, (B) dactylopodite of pereopod V, (C) pereopod VI, (D) dactylopodite of pereopod VI, (E) pereopod VII, (F) dactylopodite of pereopod VII, (G) pleopod III, (H) retinacles on pleopod III.

The significance of the finding

Our description of the new species is important for three reasons. *Tegano tashanensis* sp. nov. is a new subterranean species from Iran. Systematic research of Iranian subterranean fauna has begun a decade ago. Until now, 49 subterranean species have been described, mostly from the Zagros Mountains (Malek-Hosseini & Zamani, 2017; Fatemi et al., 2019; Bargrizaneh et al., 2021; Malek-Hosseini et al., 2021, 2022). Zagros Mountains are an interesting region, a karstic massif in mid-latitudes. Previous studies have suggested that karstic areas in mid-latitudes could present a latitudinal belt of high subterranean species richness (Culver et al., 2006; Zagamajster et al., 2014; Eme et al., 2017), emerging due to habitat availability, high production on surface and reduced impact from Pleistocene glaciations

(Eme et al., 2015). If so, we should expect high species diversity in Zagros Mountains as well. Any progress in understanding Iranian subterranean fauna is a step towards understanding macroecological patterns.

Secondly, finding a hadziid close to the Persian Gulf provides further credibility for the hypothesis of a Tethyan seaway, i.e. that the ancestral sea acted as a dispersal route (Hou & Li, 2018; Zhao et al., 2021), along which many species independently colonized subterranean environments (Holsinger, 2000). Species distributions may be suggestive of biogeographic patterns, but these require phylogenetic testing. Although this question falls beyond the scope of this paper, we showed that herein described *Tegano* comprises its own clade with species distributed in different biogeographic regions and often

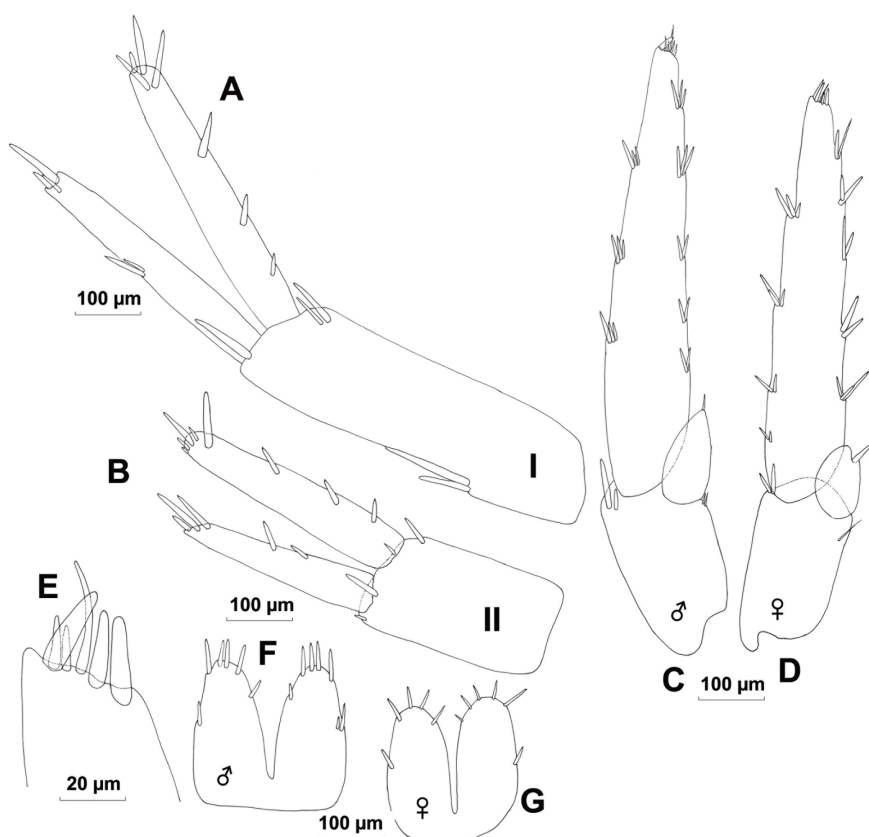


FIGURE 9 *Tegano tashanensis* sp. nov., male: (A) Uropod I, (B) Uropod II, (C) Uropod III male, (D) Uropod III female, (E) Uropod III detail on exopodite tip, (F) Telson (male), (G) Telson (female).

comprise subterranean species. Moreover, recently described *Melita* species from the Persian Gulf (Momtazi et al., 2014), which we could not sequence, is another species from the region that could importantly contribute to biogeographic reconstructions. These data suggest that hadzioids are an interesting model system for testing the hypothesis of Tethyan seaway. *Tegano tashanensis* sp. nov. is the third melitid species from Iran and the first freshwater one. The other two species are from the genus *Melita* including *M. persia* from the Persian Gulf in southern Iran and *M. mirzajani* from the Caspian Sea in northern Iran (Momtazi et al., 2014; Krapp-Schickel & Sket, 2015). Molecular studies on these two

Melita species will give us wider insights into the taxonomic positions and phylogenetic relationships of this group especially Iranian melitids. Synonymies or merging in other genera could be hypothesized for Iranian species.

Finally, water rich in sulfide is an intriguing habitat, and it is not clear how species deal with sulfidic toxicity. Continuous discovery of new, unrelated species from such habitats will facilitate new research directions, for example how these organisms adapt to this extreme environment, and whether the observed adaptations are convergent (Tobler et al., 2008; Riesch et al., 2010; Bauermeister et al., 2013; Fišer et al., 2015).

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Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.24382546>

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