

Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation

Eleonora Trajano¹, Marcelo R. de Carvalho²

1 *Laboratório de Estudos Subterrâneos, Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, Rodovia Washington Luis, Km 235, 13565-905, São Carlos, Brasil* **2** *Departamento de Zoologia, Instituto de Biociências da Universidade de São Paulo, Rua do Matão, Trav. 14, no. 101, CEP 05508-090, São Paulo, Brasil*

Corresponding author: Eleonora Trajano (etrajano@usp.br)

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Abstract

Subterranean organisms always attracted the attention of humans using caves with various purposes, due to the strange appearance of several among them and life in an environment considered extreme. According to a classification based on the evolutionary and ecological relationships of these organisms with subterranean habitats, first proposed by Schiner in 1854 and emended by Racovitza in 1907, three categories have been recognized: troglobites, troglophles and troglonexes. The Schiner-Racovitza system has been discussed, criticized, emended, the categories have been redefined, subdivided, original meanings have changed, but it is used until now. Herein we analyze in a conceptual framework the main ecological classifications of subterranean organisms, from Schiner to Trajano, in 2012, so far the last author to introduce a relevant conceptual change on the categories definitions, incorporating the source-sink population model. Conceptual inconsistencies are pointed, especially with regards to the generally ill-defined troglonex category, and the correspondence between categories according to the original sense and in alternative classifications is discussed. Practical criteria for distinction between these categories and difficulties for their application are presented. The importance of rightly classifying subterranean populations according to the Schiner-Racovitza system for conservation of these fragile and mostly threatened habitats is discussed.

Keywords

Subterranean biology, troglobites, troglaphiles, troglloxenes, cave conservation

Introduction

The realization that the subterranean realm contains living fauna is probably as ancient as the beginning of the regular use of caves by humans for ritual activities (ceremonies, burials etc.) during prehistoric times (Tolan-Smith 2004). The detailed representation of a raphidophorid cricket carved in a bison bone found in Ariège, French Pyrenees (Richards 1961), is evidence of the good observational abilities of Neolithic humans. Unfortunately, in the historical era, at least in western cultures, the association of caves with the “World of the Dead” and its negative connotations disrupted an engrossed relationship between people and caves that had allowed for their deeper exploration and close observation. Consequently, caves remained an unknown subject for investigation until quite recently.

The presence of animals with very distinctive features, unfamiliar to the general public and conferring an appearance that is usually described as peculiar, bizarre and even fearsome, sometimes depicted as mixing real animals with mythic creatures such as dragons, is the most striking characteristic of the subterranean biota. And more, these creatures coexist in caves with “normal” animals, like those found on surface habitats. So it is not surprising that the first attempts to classify cave animals were based on their differences to surface inhabitants. A traditional classification, still used, is that by Schiner, published in 1854 and emended by Racovitza in 1907 (Racovitza 1907) that encompassed three categories, troglobites for those distinctive, peculiar cave animals, and troglaphiles and troglloxenes for animals also found on the surface, but with different relationships with caves.

The classification of organisms living in subterranean habitats according to their ecological and evolutionary relationships is a central issue in subterranean biology because it provides the starting point for many other questions. However, underlying concepts are not well understood and definitions of these categories have been changing through time, such that the same term is used for different situations and *vice-versa*. Because authors very rarely make reference to the system they used, or the practical criteria for its implementation, the general application of a classification to cave animals is frequently unreliable.

Here we present a review of the most used ecological/evolutionary classification of subterranean organisms, the Schiner-Racovitza classification, analyzing it from historical and conceptual points of view, and detail a recent proposal incorporating the source-sink population model. We also discuss practical criteria for its application and its importance for conservation of the fragile subterranean ecosystems.

The Schiner-Racovitza classification: a critical review

According to Racovitza's classic publication, several attempts had been made to establish divisions of the cave fauna based on diverse criteria, such as type of preferred habitat of cavernicoles. The latter criterion was used in 1854 by Schiner to classify these organisms into: 1) *hôtes occasionels* (occasional visitors): "*animaux qu'on rencontre dans les grottes mais aussi à la surface, partout où sich die ihrer Lebensart entsprechenden Beggungen vorfinden*"; 2) *troglophiles* (troglophiles): "*animaux habitant les régions où la lumière du jour pénètre encore, qu'on peut, exceptionnellement, rencontrer à la surface ou qui ont seulement des formes représentatives lucicoles*"; 3) *troglobies* (troglobites): "*animaux exclusivement cavernicoles, qu'on ne rencontre jamais dans les régions épiées, sauf dans le cas d'événements exceptionnels comme les crues*" (Racovitza 1907) (translated in Table 1).

According to Racovitza (1907), Schiner distributed his examples in these three categories in a rather arbitrary way, but he recognized that this would be the *moins mauvaise* ('least bad') among the available classifications. Therefore, Racovitza adopted Schiner's categories but slightly modified the definitions, especially the first two, aiming for a classification reflecting the degree of adaptation to subterranean life as shown by taxonomic and anatomical characters of cave organisms, as well as their relationship with the habitat: 1) *trogloxènes* (trogloxenes, a new term, created by Racovitza 1907, p. 437): "*ce sont des égarés ou des hôtes occasionels, ces derniers attirent soit par l'humidité, soit par la nourriture, mais n'y habitent pas constamment et n'y reproduisant pas*"; 2) *troglophiles* (troglophiles): "*habitent constamment le domaine souterrain, mais de préférence dans ces régions superficielles; ils s'y reproduisent souvent, mais ils peuvent être aussi rencontrés à l'extérieur*"; 3) *troglobies* (troglobites): "*ont pour habitat exclusif le domaine souterrain et se tiennent de préférence dans ces parties les plus profondes*" (Table 1).

The latter category, the troglobites, is basically that of Schiner and has remained mostly unaltered to the present. On the other hand, and assuming that Racovitza (1907) accurately translated Schiner's classification, the definition of troglophiles was significantly changed. Schiner's troglophiles apparently encompassed two different, incongruent groups: animals restricted to the entrance zone, exceptionally found outside caves, and animals belonging to photophilous taxa. In contrast, Racovitza's troglophiles are typically photophobic ("*Ce sont des Lucifuges très caractérisés, ayant subi souvent des réductions de l'appareil optique, et d'autres adaptations à la vie obscuricole*"; Racovitza 1907, p.437). Therefore, completely different animals would fulfill the criteria for troglophily according to these two classifications. Racovitza's definition is the one currently employed.

In addition to creating a new term, Racovitza (1907) redefined the first category, using objective ecological characteristics. As a matter of fact, Schiner's definition of *hôtes occasionels* is so vague that it would also apply to the troglophiles in the current sense.

Since the beginning there has been a consensus about the definition of troglobites as animals confined to subterranean habitats. However, many authors, including Racovitza, mistakenly made a necessary linkage with the presence of morphological

Table 1. A comparison of definitions of the Schiner-Racovitza categories (except for the troglobites, var. troglobionts) by different authors.

	Schiner 1854	Racovitza 1907	Thinès and Terafs 1972	Holsinger and Culver 1988 (also Barr 1967, 1968)	Sket 2008 (based on Ruffo 1957)	Trajano 2012
Trogloxenes (Schiner's <i>hôtes occasionels</i> = occasional visitors)	Animals found in caves, but also at the surface, everywhere 'when one finds those constraints typical of their life style	Animals lost or occasional visitors of caves attracted by humidity or food, but that do not live continuously or reproduce in caves	Organisms that live in the surface but, due to very precise reasons, they colonize temporarily the subterranean environment ("hôtes temporaires")	Species habitually found in caves or similar cool, dark habitats outside caves, but they must return periodically to the surface or at least to the entrance zone of a cave for food.	Subtroglophiles Need to utilize the surface environment for at least one vital function (e.g., reproduction or feeding)	Source populations in epigean habitats, with individuals using subterranean resources
Troglophiles	Animals inhabiting regions where day-light still penetrates, which can exceptionally be found at the surface or that only have photophilous forms	Permanently inhabiting the subterranean domain, but preferably in superficial regions; they frequently reproduce there, but may also be found outside	Organisms that live in the subterranean environment as well as in the surface ("hôtes élecltifs")	Species able to complete their life cycles within a cave but may also occur in ecologically suitable habitats outside caves	Eutroglophiles Essentially epigean species able to establish more or less permanent subterranean populations	Source populations both in hypogean and epigean habitats, with individuals regularly commuting between these habitats, promoting the introgression of genes selected under epigean regimes into subterranean populations (and <i>vice-versa</i>)
Accidentals				Species that wander, fall or are washed into caves and generally exist there temporarily.	Trogloxenes	Organisms introduced into caves by mishap or entering in search of a mild climate; may survive temporarily, but the inability to orient themselves and to find food leads to their eventual demise. Not evolutionary units responding to subterranean selective regimens.

cave-related traits, termed *trogglomorphisms* by Christiansen (1962, 2012). Christiansen emphasized the lack of eyes and dark pigmentation but, presently, the term has been expanded to include any autapomorphy of exclusively subterranean species that may be directly related to the subterranean selective regime (Bichuette et al. 2015). Although troglomorphisms are frequently present in exclusively subterranean species, these two phenomena (troglomorphisms and being a troglobite) may be the result of independent biological phenomena (troglomorphisms may result from modifications *within* a lineage, i.e. autapomorphies, whereas troglobites may be the result of modifications *leading to* separate lineages, i.e. synapomorphies). Therefore, they cannot be consistently equaled in any definition. Logically, one may restrict the other; for instance, one may consider as troglobites only the exclusively subterranean populations that present troglomorphisms, but they are not the same.

The absence of organisms in epigean habitats is a definition by itself, independent of the cause of the absence. At least theoretically, because there are very few experimental studies on the subject, troglomorphisms would hinder epigean life, but it is not the only possible cause for it. The maintenance of ecological, hydrological and/or geological barriers may also account for the troglobitic status (i.e. restriction to caves) without the onset of troglomorphisms (i.e. of cave-related autapomorphies).

Racovitza's imprecise definition of troglaphiles persisted in Europe until the 1960's – Vandel (1964) used it in his classic book *Biospéologie. La biologie des animaux cavernicoles*, including, among other examples, raphidophorid orthopterans, which are mostly troglloxenes (in the modern sense, see below) in Europe and North America. In fact, the categories referred to as "troglloxenes", with subdivisions in some classifications (detailed below) have always been ill defined, including animals with different kinds of ecological relationships with caves, or even none at all.

Modern, biologically meaningful definitions taking into account the Schiner-Racovitza categories were published in the late 1960's and early 1970's. The most important advancement was the troglloxene concept, which excluded accidentals, i.e. animals without an ecological relationship with caves (Table 1). In Barr's definition, troglloxenes frequent caves for shelter and a favorable microclimate, but must return periodically to the surface for food (Barr 1968). According to Thinès and Tercafs (1972, p. 53), "*ces organismes vivent dans le milieu extérieur mais pour diverses raisons très précises colonisent temporairement le milieu souterrain*" (see Table 1). However, according to these authors, their activity in caves is generally very reduced or even absent, and they rarely reproduce there; their presence in caves being mostly due to hibernation and aestivation. In fact, caves may be used by quite active troglloxenes as reproduction and feeding sites (e.g. raphidophorid orthopterans), routes for predator escape, etc., at different times of the year.

Thinès and Tercafs (1972, p.53) definition for troglaphiles (*ces organismes vivent également dans le milieu extérieur... Ils choisissent ce milieu.... certaines de leur potentialités... les prédisposent à vivre dans le milieu souterrain.... Ils se reproduisent dans les cavernes et y ont une activité permanente. Ce sont des hôtes électives...*) also corresponds to its current sense: facultative species which commonly inhabit caves and complete their life

cycle there, but also occur in sheltered, cool, moist, epigeal microenvironments (Barr 1968) (Table 1). The role of preadaptations (or exaptations *sensu* Arnold 1994) for the successful colonization of subterranean habitats is evidenced in Thinès and Tercafs's mention to potentialities (physiological and ethological) that would predispose these animals to live in these habitats. It is noteworthy that Sket (2008) states that “the definition of this group [troglophiles] has never been very clear”. This is wrong. Since the early 1960's, with Poulson (1963), Barr (1967, 1968) and others, the concept has stabilized.

The Shiner-Racovitza classification, understandably in view of its importance as a central theme in subterranean biology, has been subject to much debate and criticism in the last century. Several proposals have been elaborated, either as modified versions of the original classification, or more detailed versions with subdivisions, redefinitions, or with alternative meanings, and with new categories based on distinct criteria (Camacho 1992). We will not discuss each and every classification, only those that received more attention and had some impact on speleobiology.

Christiansen (1962, p.77) proposed four categories, troglloxenes being the only one retaining the original term and sense: “*touts les animaux trouvés soit accidentellement dans les grottes soit passant régulièrement une partie seulement de leur existence dans les grottes*” (all animals found by accident in caves or regularly spending there a part of their life). For typical epigeal animals that live and reproduce in caves, without showing morphological modifications for subterranean life, he created the term *epigeomorphs*, which would be equivalent to troglloxenes in the modern sense (see below). Finally, trogllobites in Schiner's sense, which is strictly distributional (restriction to the subterranean habitat), were subdivided into *ambimorphs*, for those with some modifications but maintaining most features of epigeal forms, and *troglo-morphs* for animals clearly modified for cave life, totally different from their surface-dwelling counterparts. This is a very unpractical classification because differentiation is a continuous process and, as discussed, trogllobitic status and troglomorphism are conceptually distinct.

Another good example of unnecessary complication leading to classifications devoid of biological sense is the essentially theoretical system proposed by the Italian speleobiologist M. Pavan in the late 1940's, a hierarchical dichotomous system based on the ability to live and reproduce in the subterranean environment (Vandel 1964; Thinès and Tercafs 1972). It resulted in seven categories, the first three (eutroglloxenes, subtroglloxenes, and aphyletic troglloxenes) corresponding to accidentals in the modern sense. Two terms – subtroglloxenes and eutroglloxenes – have been used by modern authors, however in different senses. In Pavan's sense, both subtroglloxenes and eutroglloxenes choose to live in subterranean habitats but are facultative there (in opposition to trogllobites that are obligatory subterranean); however, the former do not reproduce in these habitats whereas the latter do. Therefore, eutroglloxenes would correspond to the troglloxenes in the modern sense, and subtroglloxenes and possibly phyletic troglloxenes (animals that enter caves by accident but live there without difficulty and may reproduce) would correspond to troglloxenes.

Contrary to assertions by Sket (2008), and endorsed by Culver and Pipan (2009), Pavan's subtroglaphiles do not correspond to Racovitza's troglaphiles (which, in turn, do not correspond to Schiner's, as already discussed), because, by definition, the latter *s'y reproduisent souvent* [frequently reproduce in caves], whereas the former *ne se reproduit pas* (they do not reproduce) (Vandel 1964, p.25). Therefore, these are completely different instances of animals inhabiting and utilizing caves. In fact, the definition of eutroglaphiles is so vague that it encompasses both troglaxenes and troglaphiles in the modern sense. Pavan's classification is unclear and biologically meaningless because it contains both artificial and superfluous categories.

The subdivision of troglaphiles into eutroglaphiles and subtroglaphiles, as recently defended by Sket (2008) (Table 1), and the use of the latter instead of the term troglaxenes *sensu* Barr (1968), Thinès and Tercafs (1972, and Trajano (2012), among others, is unnecessary and confusing. The same is true for the use of the term troglaxenes ("animals with no special inclination to occupy/inhabit hypogean habitats") as a synonym of accidentals in Barr's sense. As discussed by Trajano (2012), accidentals (*égarés* – troglaxenes in part, according to Racovitza 1907) cannot be considered subterranean organisms, thus should not be included in the Schiner-Racovitza ecological classification (see below). Therefore, Barr's and Thinès and Tercafs's definitions of troglaxenes are consistent with that of Racovitza, and the term has an historical precedence over others.

It is noteworthy that the Schiner-Racovitza system applies to organisms living in the subterranean environment in general, i.e. in networks of heterogeneous inter-communicating spaces of the subsoil, characterized by permanent absence of light, moderate annual amplitude of temperature and, for the terrestrial component, relative humidity close to 100% (Juberthie 1983), which result in the many singularities of subterranean ecosystems and their component fauna. The subterranean environment encompasses a variety of subsurface habitats, such as the MSS (Mesovoid Shallow Substratum, *sensu* Juberthie 2000) that may form in talus slopes; the epikarst (network of small cavities in the uppermost part of karstified rock); the hyporheic zone (interstitial spaces in sediments of the stream bed, constituting a transition zone between surface and groundwater – Gibert et al. 1994); seepage springs draining hypotelminorheic habitats (e.g., Culver and Pipan 2008), etc. According to the classic, operational definition, spaces large enough to admit a human being are called caves. For the sake of simplicity, and considering that the great majority of data on subterranean ecosystems were obtained in caves, from now on we will refer to subterranean habitats in general as caves.

A new approach to the Schiner-Racovitza classification of subterranean organisms

A first conceptual problem with these definitions refers to the organizational level of the categories. In many definitions the reference used is "animals" or "cavernicoles". These terms are too vague, and may refer to individuals, populations or species. In others, the reference is the species, at least for troglobites and troglaphiles, as in Barr

(1968). However, each condition operates at a different biological level: the troglobitic condition applies to species, the troglophilic condition refers to populations and the troglaxene condition encompasses populations or individuals (each individual must leave the cave at some point).

Another apparent inconsistency comes from the occasional observation of troglobites in surface habitats. Schiner had acknowledged this possibility as an exceptional event, exemplified by the presence of European blind salamanders *Proteus anguinus* outside caves as a consequence of flash floods (Racovitza 1907; see above). However, there are rare instances of troglobites that leave caves periodically under special circumstances, as is the case of the blind catfish, *Pimelodella kronei*, which feeds at night in the Bombas Resurgence, Southeast Brazil (Pavan 1945). If the definition of troglobite were taken at the individual level, then these examples would challenge it.

Another issue concerns groups of individuals in habitats where they would not form self-sustained populations, i.e., in habitats where reproduction would not be sufficient to balance local mortality (sink habitats). Such populations might only persist if maintained by immigration from more-productive sources, i.e., from populations with excess production that would continue to grow if isolated (source population) (Fong 2004).

Hence, Trajano (2012, p.277) redefined the Schiner-Racovitza categories, adapting them to the source-sink model: “1. Troglobites (var. troglobionts) correspond to exclusively subterranean source populations; sink populations may be found in surface habitats; 2. troglophiles include source populations both in hypogean and epigean habitats, with individuals regularly commuting between these habitats, promoting the introgression of genes selected under epigean regimes into subterranean populations (and *vice-versa*); 3. troglaxenes are instances of source populations in epigean habitats, but using subterranean resources (the so-called obligatory troglaxenes, all individuals are dependent on both surface and subterranean resources)” (Table 1). Troglaxenes would function as sink-populations of epigean source populations. Some authors use the terms stygobites (var. stygobionts), stygophiles and stygoxenes (Gibert et al. 1994: Fig. 1) for aquatic subterranean organisms. These terms have been initially coined for groundwater fauna in non-cave areas, such as spaces in hyporheic habitats (see, for instance, Gibert, Danielopol and Stanford 1994).

These categories apply to subterranean organisms (cavernicoles *sensu lato*) defined as evolutionary units responding to subterranean selective regimens. Subterranean habitats would provide resources, e.g. food, shelter, substrate, climate, which affect survival/reproductive rates. Such units have an historical connectivity, therefore may be classified as systematically meaningful biological systems. Therefore, “accidentals”, i.e., organisms introduced into caves by mishap (by being washed into caves or falling through upper openings, for instance) or when entering in search of a mild climate are excluded; although such organisms can survive temporarily, their inability to properly orient themselves and to find food leads to their eventual demise. From an ecological point of view, accidentals are potential resources for subterranean organisms (food, substrate, etc.). Resources *per se* have no historical connectivity, and when an organism is just a resource, it makes no sense to classify it into a taxonomic system, based on



Figure 1. *Trichomycterus itacarambiensis* (Siluriformes: Trichomycteridae), troglolitic catfish from eastern Brazil, showing intrapopulation variation in pigmentation and eye development (Photos: Dante Feno-lío). **A** pigmented individual, with reduced eyes and pigmentation **B** albino (DOPA (-) individual, with very reduced eyes, not visible externally).

phylogeny. Moreover, accidentals are grouped by a negative trait (i.e., they are not subterranean organisms, as herein defined). In conclusion, it is clear that the “accidental” concept has a different nature, and therefore should not be included in the Schiner-Racovitza system (Trajano 2012).

It is noteworthy that troglobites, troglaphiles and troglloxenes are all subterranean, i.e., they are all adapted to subterranean life, each in their own way. It is a common mistake to refer to troglobites in the speleological literature as **the** cave-adapted organisms, as a distinction from other subterranean animals, i.e., from troglaphiles and troglloxenes (e.g. Ginet and Decu 1977: “non-pigmentation... est l’indice d’une réelle adaptation pour une vie... monde souterrain.” [lack of pigmentation indicates a real adaptation for life in the subterranean world]; for Culver and Pipan 2009, cave adaptation starts after isolation in this habitat). As a consequence, many authors treat cave animals/species and troglobites as the same entity (e.g. Jeffery 2001, p. 2 – “Cave animals are sometimes dismissed as entirely degenerate and unable to provide”; Juan et al. 2010: 3865 – “Cave animals...attracted the attention...because of their bizarre ‘regressive’ characters...”), and studies reportedly on subterranean biodiversity are commonly restricted to troglobites (e.g. Schneider and Culver 2004). Hence, the existence of troglaphiles as cave animals, acknowledged during the last 150 years (Racovitza 1907, Vandel 1964, Thinès and Tercafs 1972, Holsinger and Culver 1988, Trajano 2012), is disregarded. And, as shown by Trajano (2001b), regions in which troglobites are diverse do not necessarily coincide with those of total subterranean diversity (troglobites + troglaphiles + troglloxenes), because the latter would be mainly related to present-day ecological factors, whereas richness in troglobites is better explained by historical factors.

The origin of such errors is probably the equivocated notion that the presence of autapomorphies (such as troglomorphisms, in the case of troglobites) is a necessary condition for adaptation to certain ways of life. However, by definition, troglaphiles are self-sustained (source) subterranean populations, recognizable as such and distinguishable from troglobites exactly by the lack of autapomorphies due to the introgression of genes maintained by stabilizing selection in epigean populations. In fact, vicariance models, which would explain the origin of troglobites in most cases (Barr 1968, Trajano 1995, Gibert and Deharveng 2002, among others), are based on isolation of well-established troglaphilic populations that are able to survive frequently under conditions harsher than during the colonization phase. According to the paleoclimatic model, vicariance would be due to the arrival of environmental conditions so severe that they restrict survival in epigean habitats, followed by differentiation allowed by the interruption of genetic flow from the outside. It is highly unlikely that populations without a “real” adaptation to subterranean life could survive long enough to accumulate all the autapomorphies generally required to be recognized as troglobites (but see Trajano and Bichuette 2010, and other publications by these authors for a different approach, as discussed below).

Moreover, according to the neutral hypothesis for character regression, most troglomorphisms are not adaptive but neutral; the modern alternative hypothesis, that of pleiotropic effects due to selection of some beneficial traits, proposed for eye and pigmentation regression in Mexican cavefish, genus *Astyanax*, lacks validation from genetic studies (Wilkens 2010, 2011) and is not corroborated for other species (Secutti and Trajano 2009, unpubl. data).

The paleoclimatic model implies isolation of troglophilic populations in subterranean habitats due to exclusion of the epigeal population living in the area, as a consequence of climatic changes that render surface habitats unsuitable for a species (Barr 1968, among others). In times of cyclic, alternating contrasting climates, the original environmental conditions may be restored in the next favorable climatic phase. If the epigeal species survived somewhere else, there could be the re-establishment of surface populations in the area, with a new colonization event followed by the formation of troglophilic populations, and so on. In some cases, such populations coexist with congeneric troglobites originated in earlier vicariant events (e.g., the Brazilian blind catfish, *Pimelodella kronei* and its putative sister-species, *P. transitoria*, that forms, by secondary dispersion, a troglophilic population syntopic with the former in Areias cave; Trajano 1991).

On the other hand, depending on the degree of differentiation achieved, the troglobitic species might or might not be able to return to the surface when environmental conditions that were previously favorable to the ancestral populations are reinstated. Therefore, conceptually there are two modalities of troglobitic status: 1) troglobites that are unable to survive in any superficial habitat, and 2) troglobites that are not found in the epigeal area connected with their subterranean habitat, because the environment is unfavorable, but which could re-colonize the surface if the original conditions were to be restored. It is reasonable to hypothesize that the most specialized, highly troglomorphic troglobites are included in the first case. On the other hand, although no such case has been demonstrated so far, it is not unreasonable to suppose that among troglobites showing individual variability of troglomorphisms (Fig. 1), which in the most derived condition would impair epigeal life (e.g., regressed eyes and pigmentation, reduced phobic reactions and cryptobiotic habits), some could re-colonize the surface.

Since, especially for troglobites with intrapopulational variability, it is not possible to anticipate the level of differentiation achieved, we propose an amendment to the definition of troglobites: *troglobites correspond to exclusively subterranean source populations; sink populations may be found in surface habitats, but they are unable to turn into source populations under present-day conditions.*

The application of the Schiner-Racovitza classification modified by Trajano (2012): difficulties and pitfalls

Criticisms on the Schiner-Racovitza classification, resulting in proposals to modify or abandon it, are generally based on difficulties for its application. Most frequently, such difficulties are due to: 1) poor understanding of the conceptual framework, 2) use of inadequate methods, especially insufficient sampling effort, and/or 3) missing data on the distribution and biology of taxa of interest. By definition, troglobites are distinguished from troglophiles and troglloxenes by their geographic isolation. Hence, the primary criterion for separation of troglobites from other subterranean organisms

is habitat restriction. However, it is not possible to prove an absence, only to raise its probability by repeatedly searching for the absent item, until such absence may be statistically accepted or dismissed. In our case, acceptance of a statistically significant probability of absence in epigean habitats contiguous to the subterranean one inhabited by the putative troglobite depends on extensive surveying of the surface until sampling sufficiency is demonstrated. Except for large animals, such as fishes and large arachnids, this condition is rarely achieved.

Therefore, in practice, troglobite status is recognized after morphological differentiation has occurred. It is expected that relatively small populations, isolated in environments that highly contrast with the ancestral one, present high rates of divergence (e.g. Trajano 2007). So, isolation is probably closely followed by morphological differentiation, resulting in autapomorphies including, but not restricted to, troglomorphisms. According to the model of allopatric speciation, which is credited as explaining diversification in most animal taxa (Coyne and Orr 2004, Futuyma 2005), the presence of autapomorphies indicate geographic isolation (i.e. *becoming* a troglobite), followed by or concomitant with genetic differentiation that is expressed in the phenotype as morphological, physiological, biological and/or behavioral derived character states (and therefore recognized as a troglobite). Character polarization usually depends on out-group comparisons, thus the comparative method must be used to infer troglotic status (Trajano 1993) (Fig. 2).

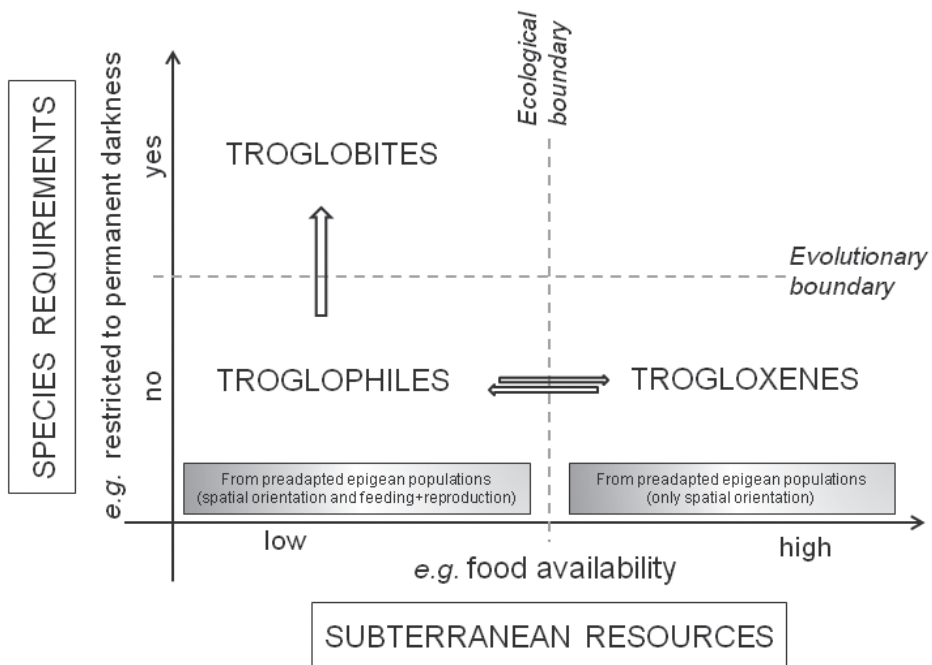


Figure 2. Interrelationships between evolutionary (historical) and ecological (present-day) factors, defining the conditions of troglloxenes *versus* trogllophiles *versus* trogllobites for subterranean organisms.

Assigning a subterranean organism to any of these categories, a procedure that has not only scientific but also conservational consequences, is not a trivial matter. With few exceptions, it is not possible to do so with an acceptable degree of confidence after a single or a few instances of field observation, and especially without a thorough taxonomic study. Ideally, the inclusion of a troglomorphic species in the most robust phylogenetic proposal available, allowing for a more complete understanding of character evolution, would be sufficient for a well-based hypothesis of troglobitic status. One may think that if there were no other troglomorphic representative in the genus or higher-level taxa then the question would be solved. However, due to the dynamic nature of systematics, where the finding of new taxa or more detailed analyses may change ideas about phylogenetic relationships, and therefore classification, the question is never closed and troglobitic status must remain a hypothesis.

Many authors, partially following Racovitza's (1907) definitions ("*Troglobies... sont très modifiés et ils offrent les adaptations les plus profondes à la vie obscuricole*"; "*Troglophiles... ayant subi souvent des reductions de l'appareil optique,... et d'autres adaptations à la vie obscuricole*") [Troglobites... are very modified, presenting the deepest adaptations to life in darkness; Troglophiles ... frequently show reduction of the visual apparatus... and other adaptations to life in darkness]), distinguish troglobites from troglophiles by "degree of adaptation". This notion is equivocated for several reasons. First, as aforementioned, according to the neutral hypothesis for character regression, most troglomorphisms that are regressive characters are not adaptive. Most importantly, continuous characters such as "degrees" are not very useful for distinguishing lineages (taxa). In practice, species or OTUs (Operational Taxonomic Units) are distinguished by differences recognized by specialists in that particular taxonomic group. These differences are the result of fixation, throughout the population, of derived character states or of unique combinations of these that result from isolation at some point of its evolutionary history. The first and necessary step to apply the Schiner-Racovitza classification is to identify species (or OTUs), otherwise the habitat concept (presence *versus* absence in epigean habitats) cannot be used. Troglobites are, then, identified as species by differences in relation to their closest epigean relatives, usually including those commonest and most conspicuous troglomorphisms, which are reduction of visual structures and dark or lack of pigmentation. Differences are differences, and any reduction of eyes and/or pigmentation consistently observed throughout the population and that allow for the recognition of its individuals distinguishing them from those of other populations, is enough for species recognition no matter the degree. Any recognizable troglomorphism means time in isolation and genetic and morphological response to the subterranean niche.

The correlation between permanent absence of light and regression of visual structures and melanic pigmentation has been established several decades ago, indicating the same evolutionary mechanisms (e.g. Thinès 1969, Thinès and Proudlove 1986), based on the observation of blind and depigmented animals belonging to unrelated taxa living in diverse aphotic habitats, such as caves and other subterranean habitats (e.g., MSS - Mesovoid Shallow Substratum, hyporheic zone), soil, deep sea and bot-

tom of large rivers, and even inside other organisms, as is the case of internal parasites. Soil animals pose special problems for classification into the Schiner-Racovitza system for two reasons: first, because several non-subterranean species are troglomorphic, and also because soil may be a dispersal route between subterranean habitats.

The small Brazilian prodidomid spider, *Brasilomma enigmatica* (Brescovit et al. 2012), provides an illustrative example of such difficulties. This eyeless species, characterized by elongation of legs, was recorded in an iron, a quartzite and a limestone cave, separated by more than 180 km of distance and different types of rock formations, rendering subterranean connections extremely unlikely. Therefore, the most parsimonious explanation is dispersal through soil and, as a logical consequence, *B. enigmatica* is not a troglobite according to the Schiner-Racovitza definition. On the other hand, a plausible explanation for eye regression and elongation of legs is differentiation in some subterranean locality (probably a limestone or quartzite system) in isolation during a past dry phases (paleoclimatic model), when the epigeal soil would be incompatible with the spider way of life; under the present-day wetter conditions, a moister, enriched soil, representing a dark habitat required by the cave species, could have provided conditions for dispersal of the otherwise exclusively subterranean species. Under this hypothesis, the species was a troglobite, and may still be in part of its distribution, where soil did not provide epigeal connections.

In conclusion, the morphological approach alone, not associated with extensive epigeal surveys, is particularly inadequate when the objective is a conclusive classification of typical soil organisms into the Schiner-Racovitza system.

It is also noteworthy that finding troglomorphic specimens considered in epigeal habits is not enough to dismiss the troglotic status at once. As epigeal individuals may be stranded in caves, and thus becoming ecological accidentals, the opposite is also true. So, this may be a case of sink population, expected according to Trajano's (2012) concept of troglobites. A population-level genetic study is required, with additional sampling, to test if those individuals are part of a sink or of a source population.

Troglophiles versus troglloxenes

The separation between the troglphilic and troglloxene status is ecological, not evolutionary, since it may depend on food availability (Fig. 2). Indeed, there are instances of species with troglloxenic individuals in most caves, but which may give rise to troglphilic populations in particularly food-rich caves (Holsinger and Culver 1988, Trajano and Moreira 1991). The difference lies in the fact that troglphilic animals *may* leave caves, and troglloxenes *must* leave them, therefore individual records of specimens leaving or entering caves are not, *per se*, evidence for any of these conditions. Thus, in order to ascertain whether individuals are troglphilic or troglloxene, it is necessary to perform long-term studies using chronobiological methods, allowing for the detection of possible cyclic patterns of movements between epigeal and subterranean habitats that, if present, indicate troglloxene status (Menna-Barreto and Trajano 2015). Brazil-

ian examples include obligatory troglloxenes, such as the goniosomatine harvestmen *Acutisoma spelaeum*, which presents well-defined patterns of leaving/returning to the home caves as part of their foraging cycles (Santos 1998), in opposition to the phalangopid cricket *Strinatia brevipennis*, for which no circadian rhythms of movements to and from the entrance of the studied cave were detected (Hoenen and Marques 1998) as expected for a troglophile.

It is noteworthy that troglophiles are not less modified cavernicoles in a continuum of cave adaptation towards troglobites. In fact, the mosaic distribution of troglomorphic character states in several subterranean taxa demonstrates that such a *continuum* does not exist. Among fishes, this is well illustrated for the North-American amblyopids (Poulson 1963, 1985; although the author did not use this concept, his data clearly show a mosaic distribution of troglomorphisms among species), Thai nemacheilids and Brazilian siluriforms (Trajano and Bockmann 1999, Parzefall and Trajano 2010, Trajano and Bichuette 2010). Troglophiles are populations of epigean species, so considered because they cannot be taxonomically distinguished from the latter (i.e. there is no recognizable evidence of isolation in their subterranean environment).

Once a subterranean specimen is assigned to a known species or OTU, the following step is to find evidence that it belongs to a source population. In the case of non-troglomorphic subterranean animals that cannot be identified, either due to a lack of taxonomic expertise or because it is a new species (very common in tropical countries), it is especially difficult without an extensive surface survey and comparative taxonomic study to distinguish between the status of troglophile and troglobite without troglomorphisms. Because few cases of non-troglomorphic troglobites have been reported for areas where epigean habitats are relatively well known, in a first moment such animals should be considered troglophiles without further consideration, except when epigean habitats are clearly unsuitable for their survival.

Evidence of self-sustained, source populations in subterranean habitats include the presence of all age/size classes throughout the cave, throughout the year. Troglloxenes, on the contrary, are usually found not far from contacts with the surface, at distances compatible with their locomotor capacity allowing for regular commuting between epigean and hypogean habitats without losing much energy (the trade-off between the advantages of using subterranean resources, mainly for shelter, and the energy spent for movements). Moreover, several troglloxenes use caves seasonally, being absent during part of the year. Therefore, a definitive distinction between troglophiles and troglloxenes depends on populational studies conducted on an annual basis.

Among troglloxenes, recognition of obligatory troglloxenes depends on good data on biology, population ecology and distribution of the species, indicating that the epigean distribution is always correlated to the presence of rocky shelters in the area. *Hadenoeus* camel crickets (Rhaphidophoridae) from karst areas in Kentucky, USA, have long been recognized as obligatory troglloxenes, based on visual censuses from several seasons revealing the existence of circadian rhythms (with activity in the night phase) and analyses of food items showing that most have epigean procedence. These crickets may be found deep in caves, but usually during the reproductive phase. Also,

two species of *Euhadenoecus* from the Appalachians are obligatory troglloxenes that must reproduce in caves but spend time in the forest, always being found in karst areas; camel crickets of two other species are forest-dwellers that may frequent cave entrances (Hubbell and Norton 1978).

In Brazil, the only obligatory troglloxene so far recognized with basis on scientific data is the harvestman *Acutisoma spelaeus* (Fig. 3), distributed in the Alto Ribeira karst area, south São Paulo State. Population studies based on mark and recapture have shown that, like *Hadenoecus* crickets, these harvestmen always reproduce in caves or rocky crevices, and all individuals forage by night in the forest, leaving the cave according to well-defined cycles whose period decreases with age (Gnaspini 1996, Santos 1998, referring to “strict troglloxenes”). Individuals of other species in this subtropical genus may shelter during the day in cave entrances, when available, otherwise they hide in the vegetation. Briefly, confirmation of the status of obligatory troglloxene depends on confirmation that every individual of the species must not only leave caves regularly, but also return periodically to these habitats in order to complete their life cycle.

Among bats, species from temperate regions, such as *Myotis sodalis* and *M. lucifugus*, which have a relatively wide distribution in North America, are dependent on a small number of caves for hibernation (Menzel et al. 2001, Kunz and Reichard 2010, among others), and therefore are obligatory troglloxenes.



Figure 3. *Acutisoma spelaeum* (Arachnida: Opiliones), an obligatory troglloxene from caves in southeastern Brazil: female taking care of eggs (Photo: Renata Nunes).

It is important to emphasize that observation of individuals entering or leaving caves is not, *per se*, evidence of troglone status, because, as mentioned, both troglones and trogloniles move across contacts between epigean and subterranean habitats, the former because they have to and the latter because they may do so. Also, isolated individuals are frequently classified as troglones due to the erroneous notion that they are always rare in caves whereas trogloniles are common. Population densities are not criteria for distinction between Schiner-Racovitza classes, because this parameter is dependent on current ecological factors and presents the same range of variation for populations within each of these classes (variations in population sizes and densities are even observed within the same species, as in the troglobitic armored catfish, *Ancistrus cryptophthalmus*; Trajano 2001a, E Trajano pers. obs. in the 2000's)

Another pitfall in the application of Schiner-Racovitza classification is borne from the fact that, in many cases, population densities of trogloniles are considerably higher than those of conspecific epigean populations. On the surface, where other species with similar ecological requirements are present, intraspecific competition would maintain low population densities. In caves, the absence of competitors and predators allows for greater population densities. As a consequence of low densities, and also the usually higher structural complexity in epigean habitats, sampling surface populations would demand higher collecting efforts than in caves. When epigean surveying is insufficient, trogloniles may be mistaken for non-troglomorphic troglobites or something else. For instance, not having collected epigean specimens, Gnaspini and Hoenen (1999) coined the term "strict troglonile" for the cricket *Strinatia brevipennis*; this term would apply to populations found only in caves and presenting a disjunct distribution, so that some individuals should be in the epigean environment at least for enough time to reach other caves. However, the advocated absence in surface habitats was actually due to insufficient collecting efforts by these authors (epigean specimens had been collected by other researchers; F. Pellegatti Franco pers. comm. 2004). Likewise, wandering spiders, *Ctenus fuscatus* (Figure 4) are common in caves of the Alto Ribeira karst area, southeastern Brazil, but rarely observed on the surface. The species was described in 1943 based on a single specimen from Iporanga Co. (possibly from a cave), but additional specimens, all from caves, were obtained only in the early 1970s by speleologists (epigean individuals were found much later as a result of collecting efforts targeting Ctenidae spiders; F Pellegatti-Franco pers. obs. 2004). In conclusion, the criterion of habitat occupation may only be applied when knowledge of epigean habitats is sufficient.

Two biological elements of the subterranean environment that are considered quite spectacular call the attention of the general public: the bizarre looks of the most specialized troglobites and the presence of relicts, still called "living fossils". Relicts are generally defined as troglobites without known close living relatives in the regional epigean area, either because these relatives became extinct (phylogenetic relicts) or because they were excluded from that area for some reason (for instance, due to climate change) but survived somewhere else (distributional or geographic relicts) (e.g. Holsinger 1988). This is, to say the least, a vague definition. The notion of closeness



Figure 4. *Ctenus fasciatus* (Arachnida: Araneae), a common troglophile in caves from southeastern Brazil (Photo: Renata Nunes).

is dependent on state-of-the art systematics. Hypotheses of phylogenetic relationships among taxa at all taxonomic levels change with inclusion of new taxa (the main factor of change) and/or new characters in the analysis, application of different techniques and theoretical approaches in the study, etc. Moreover, the concept of relict is based on absence, which, as discussed above, cannot be proved, only dismissed. For instance, extant peracarida crustaceans of the order Calabozoa have so far been found exclusively in subterranean waters, thus being considered phylogenetic relicts. Different species were recorded in Venezuela and in Brazil; in the latter, they inhabit limestone caves located in far apart sedimentary basins, which have never been connected by aquifers. Hence, dispersion through epigean waters is likely to have occurred, possibly leaving extant descendents that have yet to be found due to the paucity of studies directed to minute crustaceans. The same is true for spelaeogriphaceans, currently restricted to subterranean waters in Gondwanan regions (Brazil, South Africa and Australia). Finding epigean relatives is proof that the status of phylogenetic relict is false, possibly leaving that of distributional relict.

Another example of the volatility of the concept of relict is the highly troglomorphic heptapterid catfish from Toca do Gonçalves, Campo Formoso karst area, Bahia State, northeastern Brazil (Figure 5). Fifteen years ago, the most recent taxonomy of the Heptapteridae led to its assignment in the genus *Taunayia*. Because the only other species of this genus was restricted to epigean streams in southeastern Brazil, the Toca



Figure 5. Highly troglomorphic catfish, genus *Rhamdiopsis* (Siluriformes: Heptapteridae), a relict from Campo Formoso karst area, northeastern Brazil (Photo: Dante Fenolio).

do Gonçalves catfish was treated as a distributional relict (as in Trajano and Bockmann 2000). Recently, with the revision of the genus *Rhamdiopsis* (F. Bockmann in progress), it was clear that the troglobitic catfish would be better allocated in *Rhamdiopsis* (see, for instance, Mattox et al. 2008). This progress in systematics completely changes the evolutionary model proposed for the species. In conclusion, the status of relict must always be considered a hypothesis and treated accordingly.

Implications for conservation of subterranean ecosystems

Even the greatest optimist knows that it is impossible to save all and each ecosystem and that many natural habitats will be lost for the sake of human interests. The goal of conservation is to minimize such losses by setting priorities based on scientific criteria that take into account the relative importance of areas in terms of biodiversity representativeness, not only in terms of diversity (taxonomic, phylogenetic, ecological) but also in relation to the processes that produce it. Therefore, one of the main focuses of conservation is singularity, i.e., sets of exclusive characteristics accountable for biodiversity loss if the ecosystem is irreversibly impacted.

Due to their many particularities, and although normally presenting taxonomic diversities considerably lower than that observed on the surface, subterranean ecosystems are generally characterized by high phylogenetic, morphological and functional

diversities (Trajano et al. 2016). Likewise, as a consequence of their high frequency of genetic divergence, expressed as morphological, physiological, and/or behavioral apomorphic characters (mostly related to subterranean life, i.e. troglomorphisms), troglobites contribute significantly to global diversity. Troglaphiles and troglloxenes are also singular in extreme ecological plasticity, with modifications to two very contrasting environments. Models of evolution in caves assume that troglbolic species originate from isolated (at least genetically) troglphilic populations, justifying the protection of the latter as potential ancestors of troglobites. Moreover, the high population densities achieved by many troglphilic that are rare on the surface opens the possibility of cave populations as sources of colonizers for epigean habitats, especially after long periods of adverse climate. “Classic” troglloxenes, such as bats, provide important and essential ecological functions (e.g. Cleveland et al. 2006, Kunz et al. 2011), but these animals must be protected in their totality, and not only the troglloxenes.

There are enough reasons justifying the protection of all subterranean organisms, but troglobites and obligatory troglloxenes are matters of greater concern, not only because of their higher degree of singularity (especially the former), but also in view of their much higher vulnerability to environmental disturbances due to their dependence on the integrity of a fragmented, frequently spatially restricted and intrinsically fragile environment (e.g. Tercafs 1992, Proudlove 2001, Trajano 2000; among others). Furthermore, obligatory troglloxenes are also highly vulnerable to anthropic interference in epigean habitats.

The main challenges facing conservation of subterranean populations are: 1) to distinguish accidentals, which have no importance for conservation at all, from subterranean organisms with low population densities that require large areas for maintenance of minimum viable effective populations; it is noteworthy that sparse populations and/or small ranges, a frequent trait of troglobites, are conditions in two out of three ecological axes (habitat requirements, local abundance and geographic range) which, combined, result in the seven Rabinowitz’s forms of rarity conferring priority for conservation (Espeland and Eman 2011, among others); 2) to separate troglbolic from troglphilic populations belonging to epigean troglomorphic taxa; 3) to recognize the troglloxene condition, identifying obligatory troglloxenes, also a priority for conservation.

Classifying subterranean organisms according to a biologically meaningful, unambiguous, consistent Schiner-Racovitza system is highly relevant for the preservation of fragile subterranean ecosystems because it will direct conservation policies. Such policies are based on speleobiological studies which, to be reliable for this purpose, should incorporate methods allowing for a more clear distinction between the Schiner-Racovitza classes.

For many subterranean populations, caves are only part of their natural habitat. These animals may migrate between large caves and the network of small spaces around them on seasonal and/or non-seasonal bases (Giachino and Vailati 2010). Infra-annual variations, i.e. fluctuations with a period longer than an annual cycle, have also been reported (Trajano 2013). Therefore, to classify subterranean organisms according to the Schiner-Racovitza system in a study, its experimental design should: 1) sample dur-

ing three or more years to account, at least, for seasonal variations in the community composition (in order to uncover any cyclical pattern, the length of a study must be at least three times the period of the cycle; 2) include collections in epigean areas; 3) test for sampling sufficiency. When employing classifications of subterranean organisms, especially for conservation purposes, these conditions should be checked for reliability of the status attributed to them. Misplacing these organisms within the Schiner-Racovitza categories impairs the efficiency of such policies.

Summary

Since its first proposition, in the mid 1850's, the Schiner-Racovitza system of classification of subterranean organisms, primarily with three categories based on their ecological-evolutionary relationships with the hypogean environment, has been subject to much debate, criticism and redefinitions. Therefore, it is always necessary to make reference to the system followed.

Aiming at a biologically meaningful classification, which would account for the apparent observed inconsistencies, Trajano (2012) incorporated the source-sink population model into the Schiner-Racovitza system, redefining the three original categories.

Troglophiles are not less modified cavernicoles in a continuum of cave adaptation, with troglobites at the extreme end; troglophiles and troglobites are equally adapted to the subterranean life.

Troglobites and obligatory troglloxenes are especially fragile because they depend on the integrity of the subterranean habitat for their survival. Therefore, determination of their status is relevant for conservation purposes.

Major difficulties and pitfalls in the application of the Schiner-Racovitza classification are: separation of subterranean organisms (defined as evolutionary units responding to subterranean selective regimens) from accidentals; use of troglomorphisms to infer the troglobitic status; distinction between troglophiles and troglloxenes; detection of obligatory troglloxenes. In order to overcome such difficulties and avoid the pitfalls, one should take into consideration the following points:

- A regular use of subsurface habitats is the first criterion to distinguish subterranean organisms from accidentals, thus isolated observations are insufficient. Repeated observations, supported by data on distribution, ecology and biology of the taxa of interest, are needed for a conclusive classification into the Schiner-Racovitza system.
- The use of troglomorphisms, such as the reduction of visual organs and dark pigmentation, to infer the troglobitic condition requires the comparative method in order to confirm their autapomorphic state.
- Distinction between troglophiles and troglloxenes is not trivial because in both cases individuals move between the subterranean environment and the surface. Evidence of subterranean source populations characterizing the first ones

includes the presence of all age/size classes throughout the cave, throughout the annual cycle.

- Except for mammals and birds, for which the high energetic demands of endothermy naturally implies the troglloxenic status, to establish this condition is usually difficult because it requires demonstrating that each individual leaves the subterranean habitat in a cyclical way. Thus, for a conclusive classification, long term ecological studies using a chronobiological approach are necessary.
- Recognition of obligatory troglloxenes depends on good data on biology, population ecology and distribution of the species indicating that the epigeal distribution is always correlated to the presence of rocky shelters in the area.
- The condition of relict (taxon without living epigeal relatives) may be an artifact of the state-of-art of the group systematics and biogeography, hence it must be treated with caution.
- The dynamics of troglphilic populations may be different from that of epigeal populations, with higher densities observed in caves. Collecting efforts in epigeal habitats even higher than in the subterranean ones may be required to distinguish between non troglomorphic troglobites and troglophiles with very low population densities in the surface.

A robust, consistent conceptual framework is very important for a proper application of the Schiner-Racovitza ecological classification of subterranean organisms. Misplacing these organisms within these categories impairs the efficiency of conservation policies aiming for protection of the fragile subterranean ecosystems.

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Typhlocirolana longimera sp. n. (Crustacea, Isopoda, Cirolanidae) from north-western Algerian ground waters with notes on Algerian *Typhlocirolana*

Abdelhakim Mahi¹, Amina Taleb¹, Nouria Belaidi¹, Giuseppe Messina²

1 Ecology and Environment Dept, University of Tlemcen, B.P. 119, DZA-13000 Tlemcen, Algeria **2** Istituto per lo Studio degli Ecosistemi (CNR – ISE), Via Madonna del Piano, 10, 50133, Sesto F.no, Florence. Italy

Corresponding author: Abdelhakim Mahi (eco_hakim@yahoo.fr)

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Abstract

A new species of hypogean cirolanid isopod, *Typhlocirolana longimera* sp. n. is reported and described from a region located in north-western Algeria. *Typhlocirolana longimera* sp. n. can be distinguished from all other species of the genus especially by the peculiar shape of the merus of pereopod I longer than in any other *Typhlocirolana* species, and for the presence of 6 molariform robust conical robust setae, the bottle shape of uropods and the aesthetasc formula of flagellum in antennulae. The presence in the same region of the two already known species *T. fontis* and *T. gurneyi* is also discussed.

Keywords

Ground waters, Crustacea, Cirolanidae, *Typhlocirolana*, Algeria

Introduction

The Cirolanidae is one of the most speciose isopod families, with more than 497 species belonging to 61 genera. Approximately 89 species in 26 genera are inhabiting subterranean waters (Botosaneanu et al. 1986, Botosaneanu and Vilorio 1993, Coineau et al. 1994, Holsinger et al. 1994, Botosaneanu 2001, Coineau and Boutin 2015).

One of the most interesting taxa of cirolanid isopods is the western Mediterranean stygobitic genus *Typhlocirolana* Racovitza, 1905. Widely spread in the area, it has colonized the continental groundwater of Sicily, Iberian Peninsula, Balearic Islands, Tunisia, Algeria and Morocco with several species (Racovitza 1912, Monod 1934, Boutin et al. 2002, Baratti et al. 2004). The genus occurs in the western Mediterranean with ten described species and several as yet undescribed species, whose phylogenetic relationships have been investigated (Baratti et al. 2004) and still need deeper investigation, especially after the recent transfer of the species *T. leptura* Botosaneanu et al. 1985 to a new genus *Botolana* Coineau and Boutin, 2015 (Coineau and Boutin 2015).

During a survey of the subterranean waters of north-western Algeria, several specimens of the *Typhlocirolana* were collected from several wells of the region. Most of the specimens collected in one of the wells were attributed to the already described species *T. fontis* (Gurney 1902) and *T. gurneyi* Racovitza, 1912.

The aim of this paper is to describe a new species of the North African *Typhlocirolana* and comment on the presence of other Algerian species of the genus.

Methods

The specimens were collected (Fig. 1) using Cvetkov's net (Cvetkov 1968) and baited traps. Dissected specimens were pencil drawn and the figures composed using the GIMP 2.8.14 program (Montesanto 2015).

Results

Suborder Cymothoida Wägele, 1989

Family Cirolanidae Harger, 1880

Genus *Typhlocirolana* Racovitza, 1905

Typhlocirolana Racovitza 1905: 74–76; Racovitza 1912: 226–249; Monod 1930: 134, 139–141, 145–153

Typhlocirolana longimera Mahi & Messana, sp. n.

<http://zoobank.org/0344B98E-B980-4A7E-B20C-EFF0AE285256>

Figs 2–5

Material examined. Holotype: 1♂, 9.8 mm wells in Ghazaouet, north-western Algeria, 35°04'34.53"N, 001°50'11.64"W; April 2011, A. Mahi legit, MZUF Coll. Crust. 4750. **Paratypes:** 3 ♂♂ and 4 ♀♀ (dissected and mounted on 40 slides), MZUF

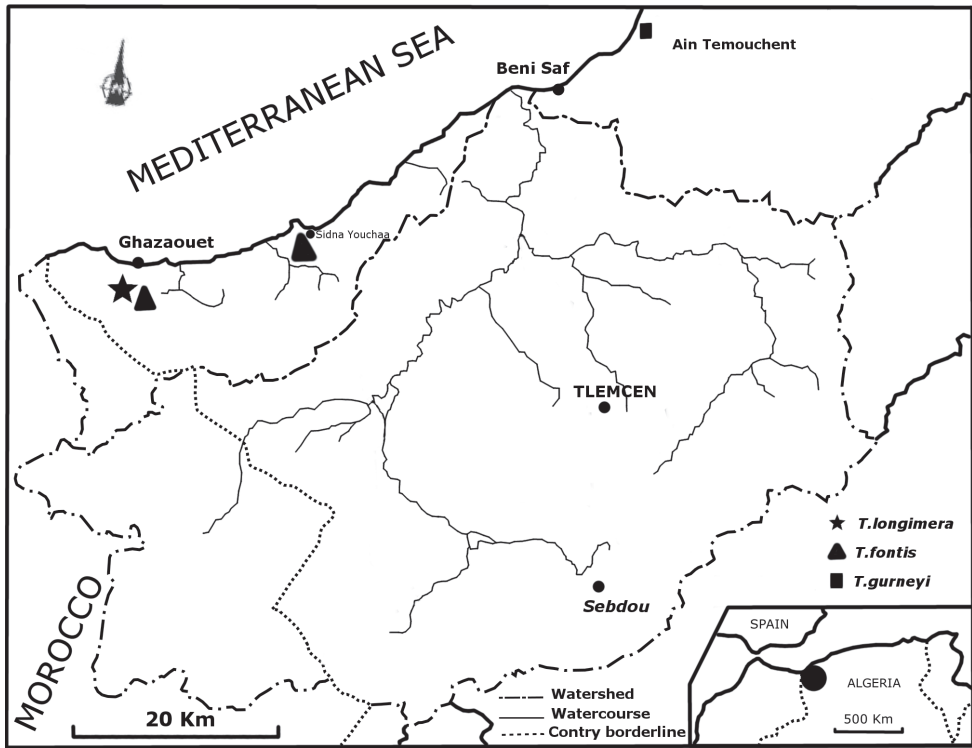


Figure 1. Map showing collection localities of the genus *Typhlocirolana* from the north-western Algeria.

Coll. Crust. 4751; 38 ♂♂ and 129 ♀♀, MZUF Coll. Crust. 4752; 55 ♂♂ and 2 ♀♀, same locality and collector, coll. Mahi.

Male dimensions. length between 9.8 mm and 10.9 mm.

Female dimensions. length between 10.1 mm and 12.2 mm.

Etymology. the specific name refers to the distinctive shape of the merus of pereopod 1. with its inner margin extending to half of propodite, and thus being longer than in any other species of *Typhlocirolana*.

Description. A small-medium *Typhlocirolana*, epimera II–VII carinate, merus of I pereopod long, surpassing carpus and reaching 2/3 the length of propus, Penial processes about (1/4) length of pereonite 7. Pleotelson triangular, bearing 8–13 short simple setae on distal margin and 5–10 short simple sub-marginal setae (Fig. 2).

Lamina frontalis (Fig. 3g): lanceolate-clavate and strongly tridimensional, laterally flattened, tip rounded. Clypeus: flatly triangular with lateral margins rounded, labrum subrectangular, rounded margins.

Antennula (Fig. 2e): Antennula short, reaching mid-length of pereonite 1. Flagellum shorter than peduncle with 6 articles, with few simple setae, aesthetascs present from second to fourth segment. Aesthetascs formula is 022210 in male and 012220 or 012210 or 011110 in female.

Antenna (Fig. 2d): Antenna reaching the distal margin of pereonite 5, flagellum extending to posterior part of pereonite 4. Flagellum nearly 2 times longer than peduncle, with about 32 segments in male and 35 in female. Segment length regularly decreasing from the base to the apex; all segments with 5 to 9 setae, mainly long and simple setae. 1–3 long plumose setae in each of segment number 4 and 5 of peduncle, and one tufted setae in segment number 4 of peduncle.

Mandibles (Fig. 3a): incisor with three strong teeth in right mandible (Fig. 3a) and 4 in left (Fig. 3b). Lacinia mobilis bearing 13–16 toothed robust setae. Left pars molaris provided with 24 (male) to 33 (female) strong short and regular robust setae.

Palp article 1 with 1 distal simple setae; article 2 with 16–20 setae (2–3 distal long simple setae, 2–4 basal simple setae, 11–13 medial barbed setae); article 3 with 9–11 barbed setae (the 3 last one are longest); article 3 shortest.

Maxillules (Fig. 3d): internal lobe bearing 3 strong and plumose setae and 2 small simple setae. Lateral lobe with 10–11 strong and conical teeth (3 of which toothed) and 2 barbed (on one side) setae.

Maxillae (Fig. 3c): Outer lobe with 3–4 barbed setae on one side in the distal part. Inner lobe with 3–4 similar setae. Basal endite with 9 setae of different lengths, delicately plumose on the two sides and at the tip. Propod with a short simple setae in the middle of distal margin.

Maxilliped (Fig. 3e,f): Palp with 5 articles provided with setae and a well-developed endite with only one coupling hook. Article 1 with one inner simple setae at apex; article 2 with 2 on outer corner margin and 6 on inner margin; article 3 with 4 setae on outer margin and 14–18 on inner margin; article 4 with 2 setae on outer margin and 12 on inner margin (2 of which are plumose in one side); article 5 with 13–17 distal setae, all setae are simple excepted 4–5 which are plumose in one side. Endite with single hook and 4 plumose setae.

Pereiopod 1 (Fig. 4a) Basis with one tufted setae and one simple setulae on outer margin. On inner margin, 2 medial small setae and 2 small setae on distal angle. Ischium 5 setulae, 3 of which on inner margin and 2 distal near outer corner and one spine on inner distal corner. Merus with inner margin elongate, surpassing carpus and reaching the $\frac{2}{3}$ length of propodus, with 3 setulae on distal outer angle. Inner margin with 6 molariform robust conical robust setae and 2 short, robust setae with additional setule and 1 long simple setae. This armature is constant and similar in male and female. Two to three simple small setae are close to the three proximal molariform robust setae. Carpus with 2 simple setae and 1 single spine with additional setula on distal inner corner. Propodus with inner margin proximally crenulate, with 2 distal spines with additional setula (one which is stronger) and a bunch of 5 apical setae (one of which is barbed on one side). Two simple setae at distal corner on outer margin. One setula on outer side. Dactylus with 5 short setae and 1 spine with additional setula on distal inner corner, 4 subdistal setae on lateral surface.

Pereiopods 2–7 (Fig. 4b–g) similar to each other and progressively growing in length from 2 to 7. Pereopods 2 and 3 exhibit the propodial organ in both males and females. These pereiopods differ by their chaetotaxy, bearing 2 to 5 tufted setae

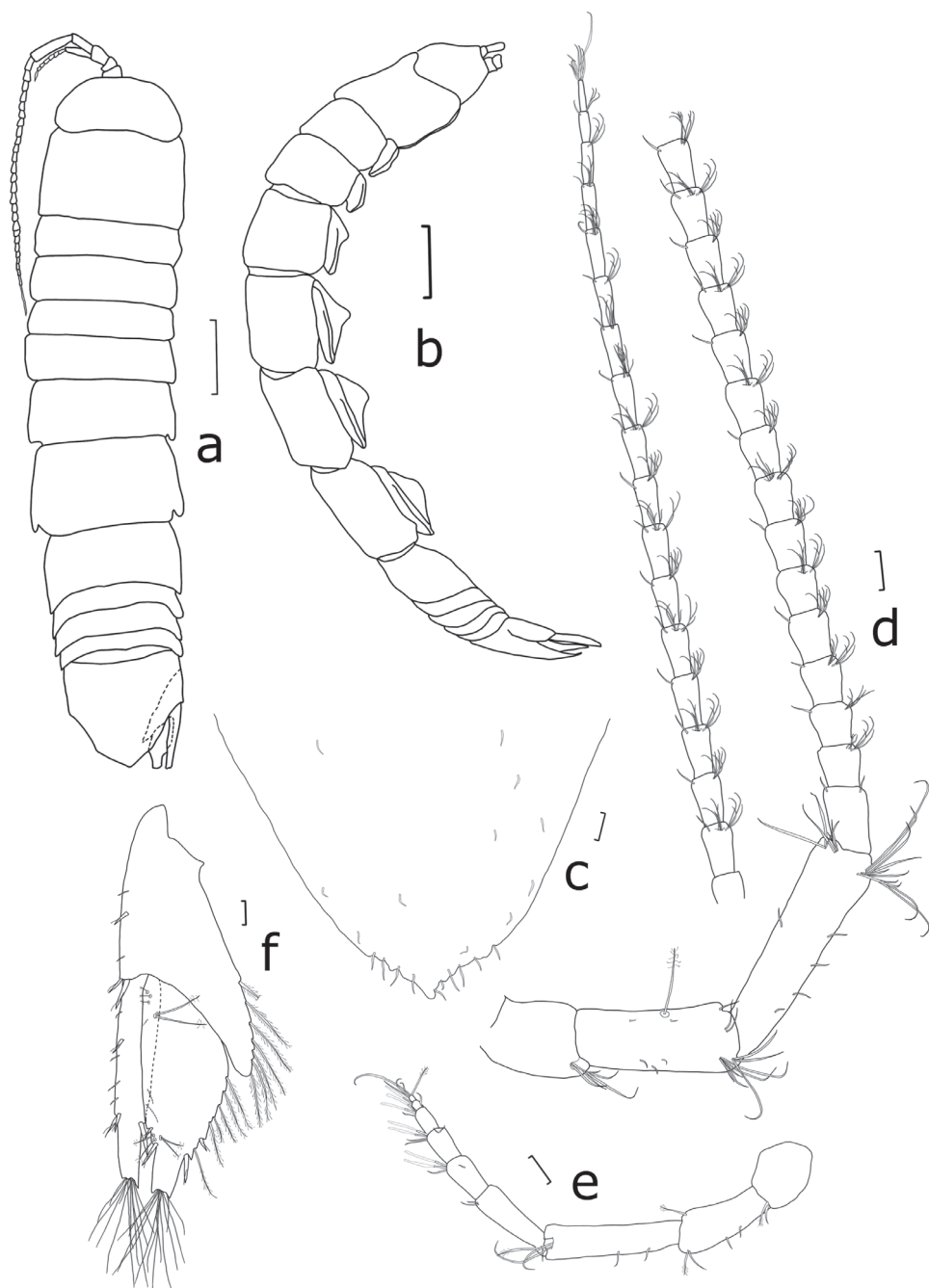


Figure 2. *Typhlocirolana longimera* sp. n. Male. 10.9 mm. **a** habitus **b** lateral view **c** posterior margin of pleotelson **d** antenna **e** antennula **f** uropod. Scale: **a**, **b** = 1 mm; **c**-**f** = 0.1 mm.

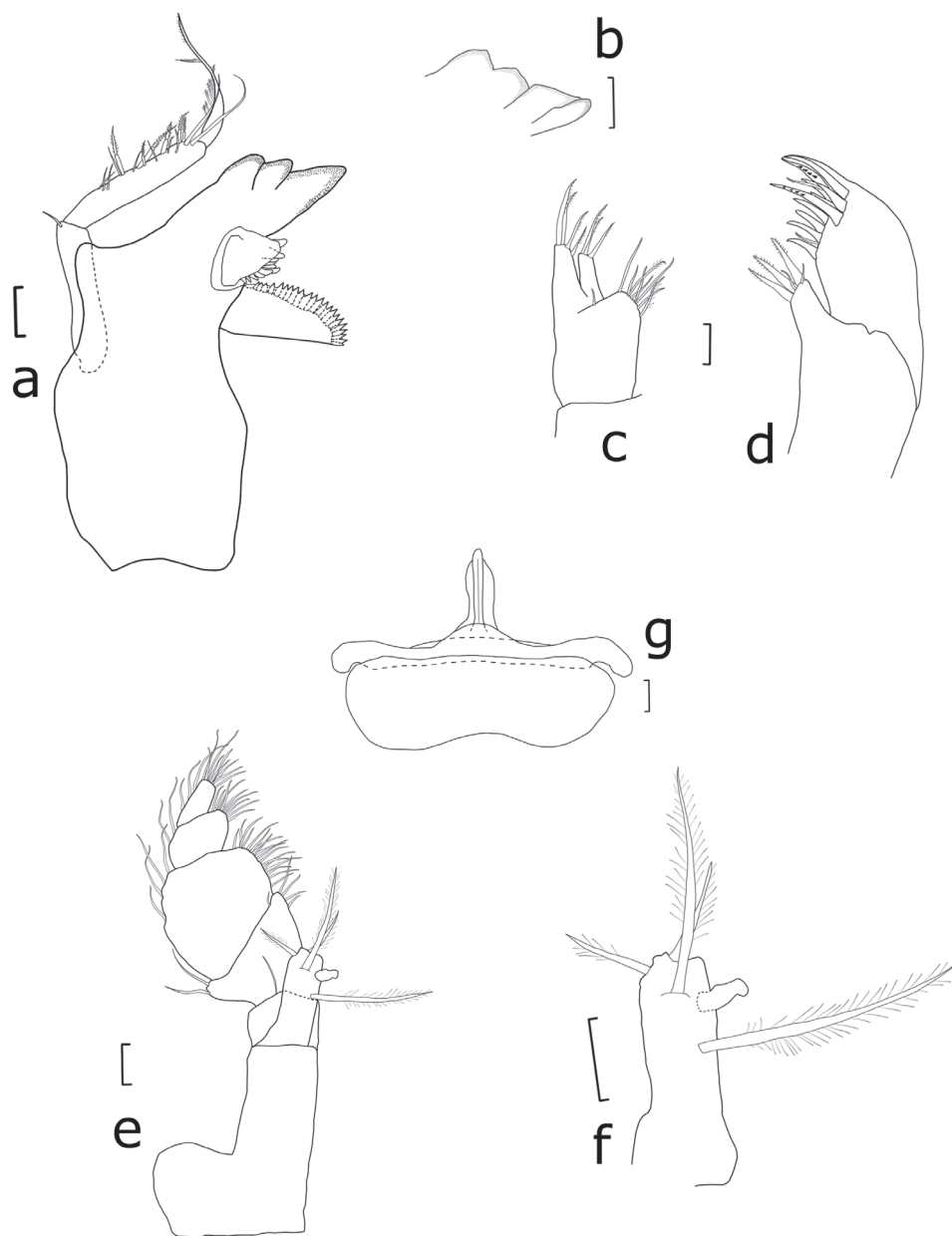


Figure 3. *Typhlocirolana longimera* sp. n. **a** right mandible **b** incisor, left mandible **c** maxilla **d** maxillule **e** maxilliped **f** endite of maxilliped **g** frontal lamina. Scale: **a–g** = 0.1 mm.

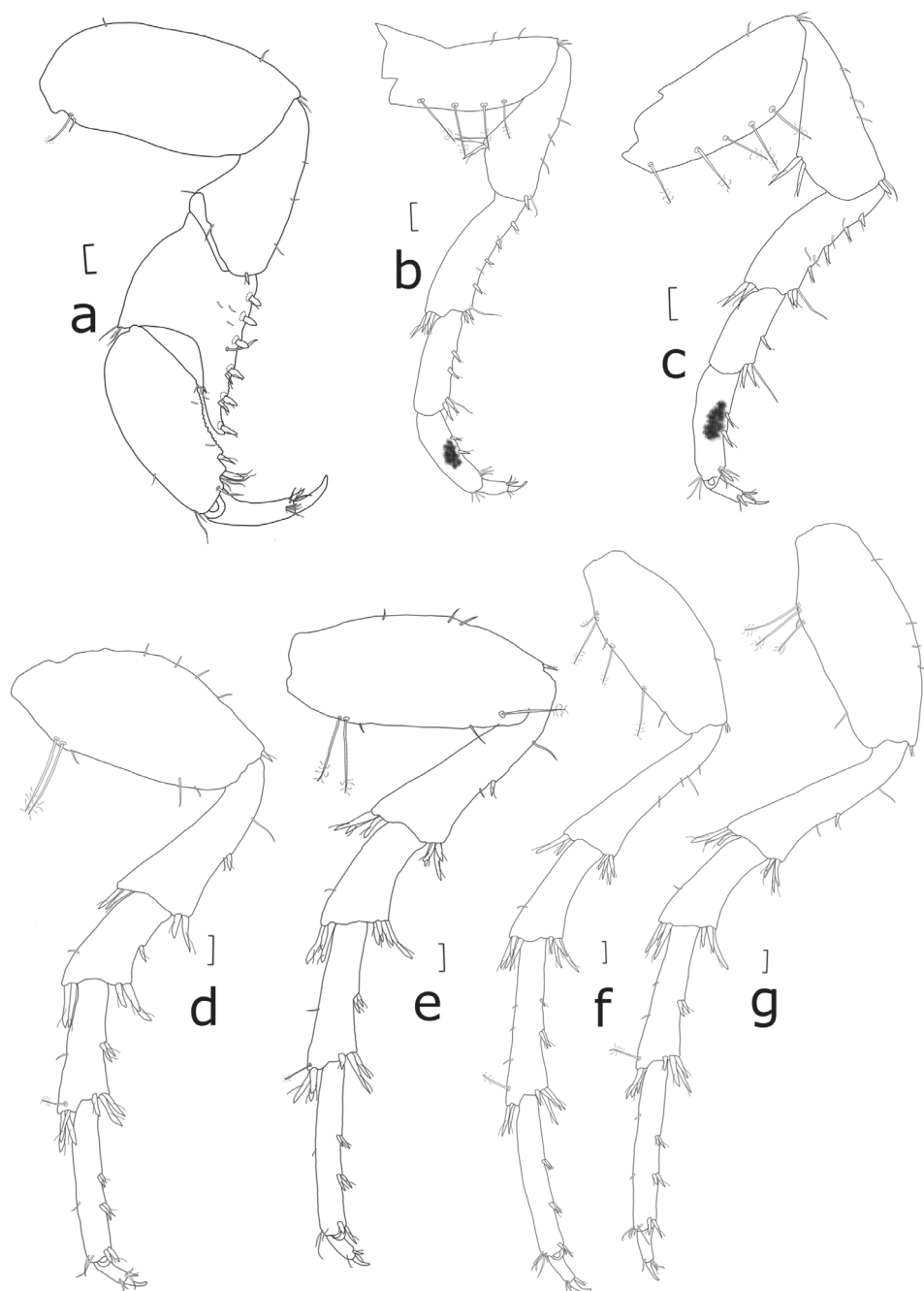


Figure 4. *Typhlocirolana longimera* sp. n. **a–g** pereopods 1–7. Scale: **a–g** = 0.1 mm.

on outer margin of basis; 4–5 along the margin of P2–P3, 2 proximal setae on P4, 2 proximal with 1 distal setae on P5, 4 setae along margin of P6 and 3 proximal setae with penial processes on P7 and about $\frac{1}{4}$ the length of the same.

Pleopod 1 (Fig. 5a) with 5–8 coupling hooks on subdistal inner margin of sympod, single short and simple seta on outer distal angle; endopod narrower about half than exopod. Endopod with 14–16 plumose setae in male (16–20 in female), exopod with 28–29 plumose marginal setae in male (28–36 in female).

Pleopod 2 (Fig. 5b,c) sympod with 4–6 coupling hooks, and 0–2 plumose setae on lateral subdistal angle, single short and simple seta on distal external angle; exopod oval, with 31–34 plumose setae on distal margin in male and (32–39 in female). Endopod with 9–14 plumose setae. Appendix masculina scimitar shaped externally directed, exceeding exopod by $\frac{2}{3}$ of length.

Pleopod 3 (Fig. 5d) sympod with 3–5 coupling hooks and 0–4 plumose seta on inner subdistal margin, single short and simple seta on distal outer angle. Exopod oval, with 24–31 plumose setae on distal margin in male (29–37 in female), a few scales and 1–3 short simple setae on inner lateral margin. Transversal suture incomplete.

Pleopod 4 (Fig. 5e) sympod with 2–3 coupling hooks and 1–3 plumose setae on inner subdistal margin, and one simple seta on distal outer angle. Exopod with 8–9 distal inner plumose setae in male (10–11 in female), a few scales and 1–2 short simple setae on internal lateral margin, and 0–2 plumose setae on external lateral margin. Transversal suture complete.

Pleopod 5 (Fig. 5f) sympod has one simple setae on distal outer angle; exopod with 5–7 distal inner plumose setae. A few scale-spines and 2–3 short simple setae on inner lateral margin. Transversal suture complete.

Uropods (Fig. 2f) Sympod subtriangular. Lateral margin with 3 spines with additional setula, 1 medial and 2 on distal corner. Two small and simple setae on the outer margin. Seven to eight plumose setae distal on mesial margin in male and 8–12 in female. Exopod styliform, shorter than endopod, regularly slender from base to apex, with 2 strong robust setae with additional setula on lateral margin and 1–2 on mesial margin accompanied by 1 to 2 long simple setae and 1–2 small simple setae; outer margin with 1 small proximal spine with additional setula and one line of 3–4 small simple setae. Apex with about 13 long simple setae of different length. Endopod bottle shaped, clearly wider than exopod. Slightly longer than exopod, with 6 plumose setae on internal margin in male and 5–8 in female and 2–3 strong robust setae with additional setula. Nine tufted setae on external margin grouped by 2 or 3 proximally and distally. Apex bearing about 12 simple distal setae of different lengths.

Remarks. *Typhlocirolana longimera* is different from all other *Typhlocirolana* species described by the combination of the following characters:

- The peculiar shape of pereopod 1 merus, which is longer than in any other species, surpassing carpus and reaching the $\frac{2}{3}$ length of propus.
- The presence of 6 molariform conical robust setae and 2 short strong robust setae usually 4 molariform robust setae;

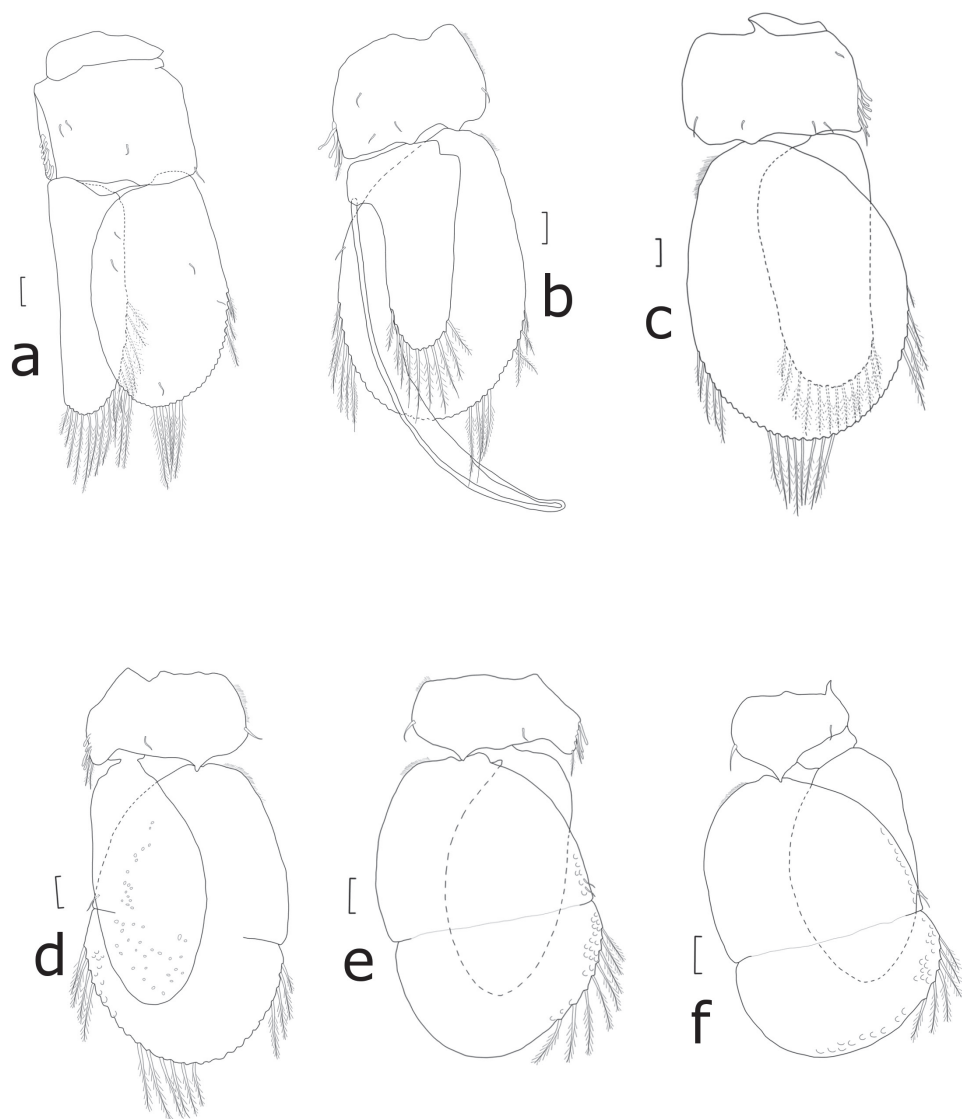


Figure 5. *Typhlocirolana longimera* sp. n. **a** pleopod 1 **b** pleopod 2 **c** female pleopod 2 **d-f** pleopods 3–5. Scale: **a-f** = 0.1 mm.

- Propodus of pereopod I with only 2 distal robust setae with accessory seta on internal margin, which is proximally crenulated and devoid of setae.
- The bottle shape of uropods;
- The chaetotaxy of pleopods 1–5 more abundant.

Differential diagnosis with other material examined. Based on the general morphological aspect, as well as some characters, *Typhlocirolana longimera* sp. n. exhibits

the greatest affinities with *T. fontis* and *T. haouzensis* Boutin, Boulanouar, Coineau & Messouli, 2002. However there is a mix of characters approaching *T. longimera* to several other *Typhlocirolana* species.

In addition to the main characters that make the difference with the other species of the genus *Typhlocirolana*, as cited above, *T. longimera* differs greatly from the others according to the aesthetasc formula of flagellum in antennulae: 12222222212 in *T. buxtoni* Racovitza, 1912, 12222212 in *T. fontis*, 12222212 in *T. gurneyi*, 121221 in *T. ichkeuli* Ghlala, Della Valle & Messana, 2009, 22210 in *T. rifana* Margalef, 1953.

Dentition and number of robust setae in propodus of pereopod I: in *T. gurneyi* not toothed with 2 or 3 short strong robust setae, in *T. buxtoni* not toothed with 3 strong robust setae, in *T. fontis* we don't have information, in *T. haouzensis* not toothed with 3 medio distal robust setae, in *T. ichkeuli* not toothed with 3 robust setae.

Propodial organ is present in both male and female in *T. longimera* as well as *T. buxtoni*, but it is present only in the male in *T. fontis*, *T. gurneyi*, *T. haouzensis* and *T. tiznitensis* Boulal, Boulanouar & Boutin, 2009, while, it is absent in *T. ichkeuli*.

The basal palp article of the mandible in Algerian species (*T. buxtoni*, *T. fontis* and *T. gurneyi*) including *T. longimera*, exhibits a strong plumose seta. On the contrary this article is bare in Moroccan (*T. haouzensis* and *T. tiznitensis*) and in the Tunisian species *T. ichkeuli*.

Article III of the mandibular palp has 30 plumose setae in the Algerian species and only 13–16 in the Moroccan one. Whereas, *T. longimera* has an intermediate position with 9–11.

Pleopod 1 exopod of *T. longimera* with 28 or 29 distal setae in male and 28 to 36 in female is different to *T. buxtoni* (40), *T. fontis* (24 in male and 20 in female), *T. gurneyi* (27), *T. haouzensis* (23–26 in male and 27 in female), *T. tiznitensis* (26–30) and *T. ichkeuli* (24–26).

A complete transversal suture is present in pleopod 3, 4 and 5 in Moroccan (*T. haouzensis* and *T. tiznitensis*) and in the Tunisian species (*T. ichkeuli*), while it is present only in pleopod 4 and 5 in Algerian species (*T. buxtoni*, *T. fontis* and *T. gurneyi*) including *T. longimera*.

Uropod shape of *T. longimera* is similar to *T. buxtoni*, *T. fontis*, *T. haouzensis* and *T. tiznitensis*.

Distal margin of the pleotelson with plumose setae in Algerian species (*T. buxtoni*, *T. fontis* and *T. gurneyi*), versus simple setae in *T. longimera* such as Moroccan (*T. haouzensis* and *T. tiznitensis*) and Tunisian species (*T. ichkeuli*).

During several surveys in the years 2010–2013 many specimens of the taxon *Typhlocirolana* where collected in the wells of Ain Temouchent, SidiYouchaa and Ghaz-aouet, which do not belong to the new described species. In fact the examination of several specimens led us to consider that they are related to the two species described by Racovitza (1912): *T. fontis* and *T. gurneyi*. The examination of these specimens gave the following results:

***Typhlocirolana* cf. *gurneyi* Racovitza, 1912**

Typhlocirolana gurneyi, Racovitza 1912: 261–266, figs 54–63; Monod 1930: 148, 152–155; Nourisson 1956:103, 110–113, 121.

Material examined. Ain Temouchent 8 ♂♂, 4 ♀♀, July 2012, A. Mahi legit, MZUF Coll. Crust. 4753.

Remarks. The specimens of the Ain Temouchent region are most similar to *T. gurneyi* by the shape of uropod that is shallower, the pleotelson bearing 12 long plumose setae on the distal margin in male, 3 strong setae with additional setula on propodus of pereopod 1 and the endite of maxilliped with 1–2 hooks and 3–4 plumose setae (Fig. 6).

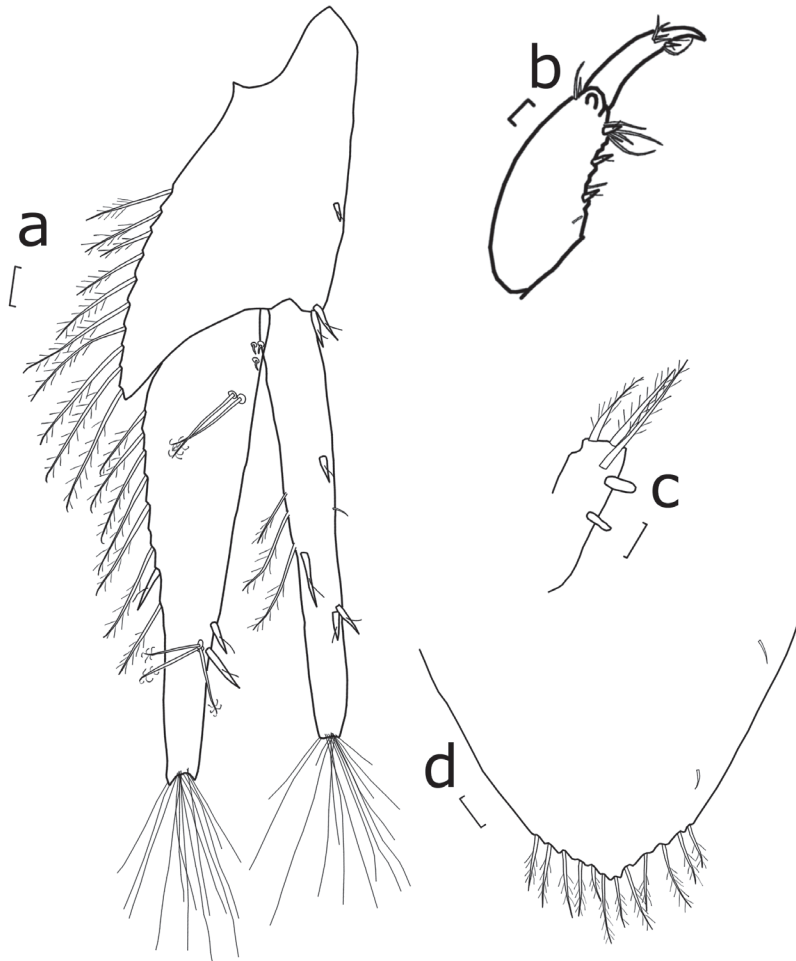


Figure 6. *Typhlocirolana* cf. *gurneyi*. **a** uropod **b** propodus and dactylus of pereopod I **c** endite of maxilliped **d** apex of pleotelson. scale: **a–d** = 0.1 mm.

***Typhlocirolana cf. fontis* (Gurney, 1908)**

Cirolana fontis, Gurney 1908: 682–685

Typhlocirolana fontis, Racovitza 1912: 254–261, figs 49–53 ; Monod 1930: 139, 143, 144, 146–150, 152, 153, 155; Nourisson 1956: 103, 113–116, 121.

Material examined. Sidna Youchaa 181 ♂♂, 138 ♀♀, Octobre 2010, A. Mahi legit; Ghazaouet, 32 ♂♂, 42 ♀♀, date, A. Mahi legit, MZUF Coll. Crust. 4754.

Remarks. These specimens are most similar to *T. fontis* by the presence of propodial organ in male, endite of maxilliped with 1–2 hooks, chaetotaxy of pleopods 1 (19 plumose setae on exopod and 11 plumose setae on endopod of Ghazaouet collection; and 23 plumose setae on exopod and 14 plumose setae on endopod of Sidna Youcha) and pleopod 2 (21 plumose setae on exopod and 7 plumose setae on endopod of Ghazaouet collection; and 26 plumose setae on exopod and 6 plumose setae on endopod of Sidna Youcha) (Fig. 7).

Discussion

The first surveys of the underground aquatic fauna in Algeria run by Gurney (1908) and Racovitza (1912), lead to the discovery of three species of *Typhlocirolana*: *T. fontis*, *T. gurneyi* and *T. buxtoni*. However, Monod (1930, 1934) and Nourisson (1956), argued the status of the two species, *T. fontis* and *T. gurneyi*, suggesting they might be a single species. These authors underline the great variability of the characters used by Racovitza (1912). *Typhlocirolana buxtoni* has been maintained as an independent species probably by the presence of propodial organ in the two sexes (Por 1962). Later, other authors suggest to maintain the separation between the three Algerian species (Botosaneanu et al. 1985, Boutin et al. 2002). We do agree about this point, because we have not enough arguments to separate the three species. On the other hand, we need other new observations for an exhaustive comparison.

The history of *Typhlocirolana* evolution and colonization of subterranean waters, such as that of several other stygobitic crustaceans (Baratti et al. 2010), is the result of multiple vicariance events, which happened in the Mediterranean basin in the last 90–15 MYA. In particular the western Maghreb region has experienced extensive marine incursions in different periods that allowed a connection between the Tethyan basin and the Atlantic Ocean. The articulated paleogeographic history of the region resulted in a complex of species strictly related morphologically and genetically whose position is not easy to elucidate (Boutin et al. 2002, Baratti et al. 2004, 2010, Ait Boughrouss et al. 2007, Boulal et al. 2009).

The Algerian situation is rather complicated and will need an accurate revision of the taxa both morphological and molecular. As has been pointed out by other authors (Nourisson 1956), many of the characteristics examined do not correspond to the descriptions that have been given or are common to several species.

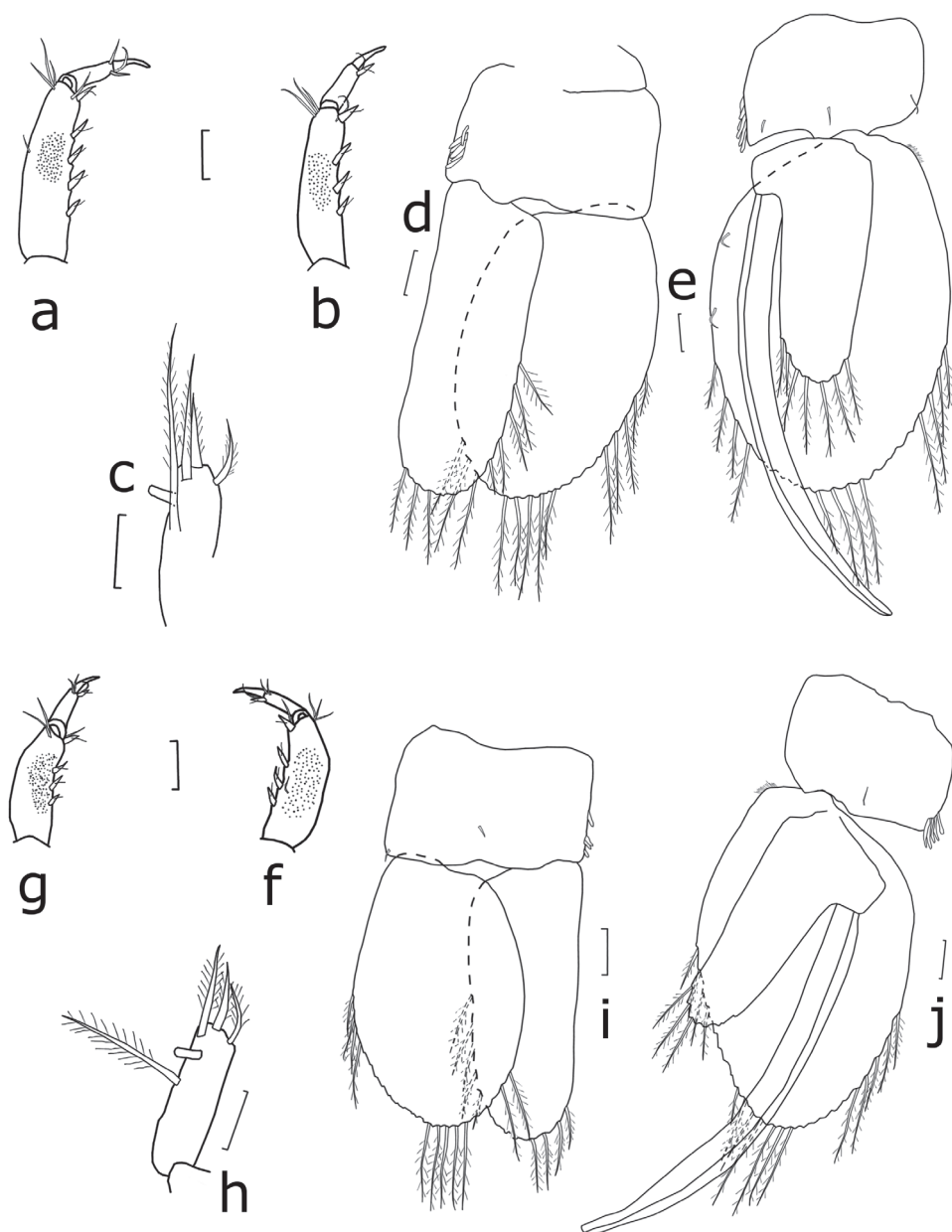


Figure 7. *Typhlocirolana cf. fontis*, Ghazaouet (a–e): a pereopod 1 b pereopod 2 c endite of maxilliped d pleopod 1 e pleopod 2 Sidna Youcha (f–j): f pereopod 1 g pereopod 2 h endite of maxilliped i pleopod 1 j pleopod 2. Scale: a–j = 0.1 mm. (The armature of pleopods has been partially omitted)

Acknowledgements

This research and paper was supported by the PNE program of the Ministry of Higher Education and Scientific Research of Algeria. We are especially grateful to M. Boulanouar who provided precious help giving his advice on the study of other specimens of *Typhlocirolana* and to Dr. Nicole Coineau who critically revised the manuscript. G. Montesanto is gratefully acknowledged for his critical effort to teaching us the GIMP software for drawing.

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The description of a new species of *Niphargus* from Iran based on morphological and molecular data

Somayeh Esmacili-Rineh¹, Seyyed Ahmad Mirghaffari¹, Mozafar Sharifi¹

¹ Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

Corresponding author: Somayeh Esmacili-Rineh (sesmaeili@razi.ac.ir)

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Abstract

The freshwater amphipods of the genus *Niphargus* Schiödte, 1849 are widespread in subterranean waters of the western Palearctic. The eastern half of the genus range has been incompletely studied despite the presence of karstic areas and large aquifers. In this paper, we describe a new species from Hamedan Province in Iran and name it as *N. hakani* **sp. n.** This species hypothesis is based on the analysis of morphological characters and 28S ribosomal DNA sequences. The taxonomic status of the new species within the genus is discussed in comparison to the known Iranian species. Results revealed that this species is phylogenetically close to *N. khwarizmi* and is morphologically similar to *N. borisi*.

Keywords

Niphargus, Hamedan Province, Taxonomy, 28SrDNA, Iran

Introduction

The members of genus *Niphargus* Schiödte, 1849 are widespread in subterranean freshwaters of West Palearctic (Meleg et al. 2013). Most of the species of this genus occur in subterranean waters and constitute an important part of biodiversity in this environment (Fišer 2012). Few studies of this genus in the Middle East indicate that about 24 species live in this area, representing only a small fraction of over 350 known species.

Iran presents the eastern-most boundary of the *Niphargus* range. The first species is record of *N. valachicus* Dobreanu & Manolache, 1933 in Iran, a widely distributed species (Karaman 1998). According to previous studies, 10 species have been found in the northern and western parts of Iran. Species inventory includes *N. valachicus*, *N. khayyami* Hekmatara et al., 2013, *N. khwarizmi* Hekmatara et al., 2013, *N. alisadri* Esmaili-Rineh & Sari, 2013, *N. daniali* Esmaili-Rineh & Sari, 2013, *N. bisitunicus* Esmaili-Rineh et al., 2015, *N. borisi* Esmaili-Rineh et al., 2015, *N. darvishi* Esmaili-Rineh et al., 2015, *N. sharifi* Esmaili-Rineh et al., 2015, and *N. kermanshahi* Esmaili-Rineh et al., 2016. In this paper, we describe a new species from karstic spring in north of Hamedan Province.

Materials and methods

Morphologic and morphometric studies

The specimens were collected using a small hand net in Kheder-Goli Spring in Hakan Village close to Razan City (see Figure 1). Details of individual landmarks were measured according to Fišer et al. (2009) and then mounted on slides in a Euparal medium. Digital photos were taken with an Olympus LABOMED iVu 7000 camera fitted on an LABOMED Lx500 stereomicroscope. Measurements and counts were made using the computer program ProgRes CapturePro 2.7. The specimens used for the present study are deposited in the Zoological Collection of Razi University (ZCRU).

Molecular and phylogenetic analyses

Genomic DNA was isolated from a part of animal using the Tissue Kits (GenNet-Bio™), according to the manufacturer's instructions (Seoul, South Korea). Amplification and sequencing of the 28S ribosomal DNA (rDNA) fragment were performed using the forward primer from Verovnik et al. (2005) and the reverse primer from Zakšek et al. (2007).

PCR amplifications were done in 25 µl volumes, containing, 2.5 µl of 10× PCR buffer (100 mM Tris-HCl (pH 8.8), (500 mM KCl), 0.2 µl of each primer (10 µM), 0.5 µl of dNTP (10 mM), 0.75 µl MgCl₂ (50 mM), 50–100 ng of genomic DNA template, and 1 unit of Taq DNA polymerase. Cycling parameters were as follows: initial denaturation of 94°C for 7 min, 35 subsequent cycles of 94°C for 45 s, 55°C for 30 s and 72°C for 1 min, and a final extension of 72°C for 7 min. Sequencing reactions were done in MacroGen Korea Laboratories.

In order to figure out the phylogenetic position of the new discovered species, we analyzed the acquired sequences (GenBank accession numbers are KY629001 and KY629002) within the data set of Esmaili-Rineh et al. (2015a) and Esmaili-Rineh et al. (2016). Three species including *Synurella ambulans* (F. Müller, 1846), *Obesogam-*

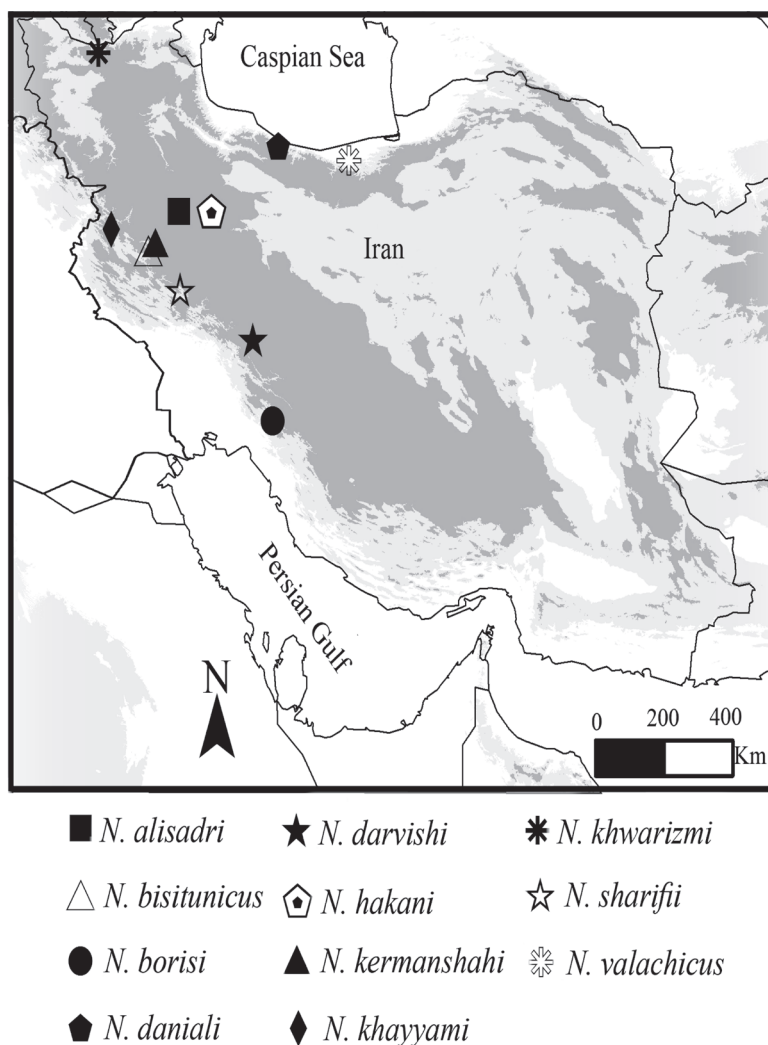


Figure 1. Distribution map of the members of genus *Niphargus* in Iran.

marus crassus (Sars G.O., 1894), and *Gammarus fossarum* Koch, 1836, were used as outgroup (KF719240, KF719242 and KF719244). All the sequences were edited and aligned using ClustalW (Thompson et al. 1994), as implemented in the Bioedit program sequence alignment editor (Hall 1999) using the default settings.

Phylogenetic reconstruction was performed using the Bayesian inferences in MR-BAYES, version 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). The Bayesian inferences analysis used the TVM+G model of nucleotide substitution. Estimates for the model parameters included estimated base frequencies A = 0.1949; C = 0.2733; G = 0.2807; T = 0.2511 and gamma distribution shape parameter ($\alpha = 0.3650$) that was selected as the most appropriate substitution model using

the Akaike information criteria ($-\ln L = 8493.8093$; $AIC = 17215.6186$) implemented in jModelTest, version 0.1.1 (Posada 2008).

To assess interspecific divergence between the Iranian species of *Niphargus*, we calculated the genetic distances corrected with Kimura two-parameter (K2P) model (Kimura 1980) as implemented in MEGA ver. 5 (Tamura et al. 2011).

Bayesian Inference was performed with two simultaneous runs and four search chains within each run (three heated chains and one cold chain) for 10,000,000 generations, sampling trees every 1000 generations. The first 2500 sampled trees were discarded as burn-in, and subsequent tree likelihoods were checked for convergence in Tracer 1.5.0 (Rambaut and Drummond 2009). A consensus tree with posterior probabilities was generated and visualized using the FigTree v1.4.0 software. Data on analyzed species are available in the Electronic Supplement of Esmaeili-Rineh et al. (2015a).

Results

Systematics

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry & Myers, 2013

Family Niphargidae Bousfield, 1977

Genus *Niphargus* Schiödte, 1849

***Niphargus hakani* sp. n.**

<http://zoobank.org/13DEBDBC-0EF8-44C3-AC3E-A5F2ADDE6F17>

Material examined and type locality. Holotype, male specimen (9 mm) from Kheder-Goli spring, Razan city, Hamedan Province, Iran; coordinates 35°27'N, 49°07'E. Specimens were collected by S. A. Mirghaffari in September 2014. Holotype with two paratypes is stored under catalogue number ZCRU Amph.1010 in the Zoological Collection of Razi University, Iran (ZCRU).

Diagnosis. At the base of uropod I observed only one robust seta. The palpus of maxilla I is slightly longer than outer lobe. Urosomites I–III bear two, five and two robust setae on dorso-lateral margin. Epimeral plates distinctly pointed and have three and four robust setae in ventral margin of plates II–III. The propodi of gnathopods I–II are trapezoid. Dactylus of gnathopod I does not reach to posterior margin of propodus. Third article of mandibular palp bears a single group of two A-setae.

Description of holotype. Total length of specimen 9 mm. Body strong and stout. Head length 11% of body length (Figure 2). Antennae I (Figure 2A) 0.6 of body length. Peduncular articles 1–3 progressively shorter; peduncular articles 2: 3 (ratio 1 : 0.75); main flagellum with 19 articles (most of which with short setae); accessory flagellum biarticulated and reaching 1/3 of article 4 of main flagellum, both articles with two simple setae, respectively (Figure 2A). Length ratio antenna I : II as 1 : 0.46.

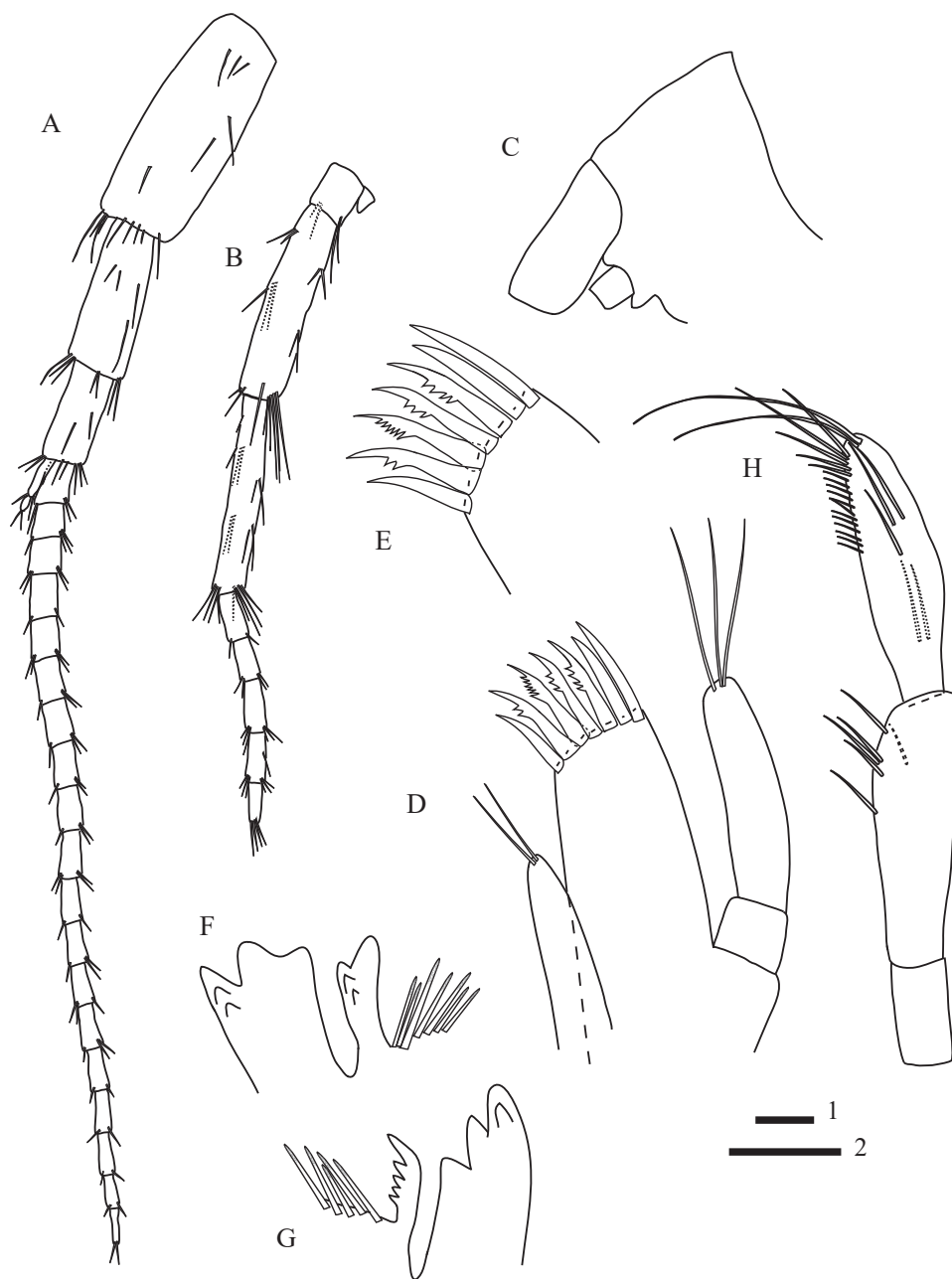


Figure 2. *Niphargus hakani* sp. n., male 9 mm (holotype, ZCRU Amph.1010). **A** Antenna I **B** Antenna II **C** Head **D-E** Maxilla I **F** Left mandible. **G** Right mandible. **H** Mandibular palp. Scale bars: 1=0.25 mm (**F-G**). 2=0.5 mm (**C-E, H**). 3=1 mm (**A-B**).

Peduncular article 4 slightly as long as article 5, each with seven groups of simple setae; flagellum with five articles. Length of flagellum: length of peduncle article 4 + 5 as 0.57 : 1 (Figure 2B).

Labium (Figure 3D) with inner lobes and setae on the tip of lobes. Inner plate of maxilla I with two long simple setae; outer plate with seven bi-, pluri or without lateral projections; palp biarticulated, slightly longer than outer lobe, with three long distal simple setae (Figure 2D–E). Both plates of maxilla II with numerous distal simple setae (Figure 3E).

Incisor in left mandible with five teeth, lacinia mobilis with four teeth; seven setae with lateral projections between lacinia and triturative molar (Figure 2F). Incisor in right mandible with four teeth, lacinia mobilis pluritooth; five setae with lateral projections between lacinia and triturative molar (Figure 2G). Mandibular palp articles in ratio 1 : 2 : 3 as 1 : 2.46 : 2.76. The proximal article has no setae, the second article with six setae along inner margin and the third article with one group of two A-setae, two groups of B-setae, no C-setae, 15 D-setae and four E-setae (Figure 2H).

Maxilliped with short inner plate on which are four distal robust setae intermixed with five distal simple setae; outer plate exceeding half of the posterior margin of palp article 2, with 11 robust setae along inner margin and three simple setae distally. Palp article 3 of maxilliped with one proximal, inner and outer group of long simple setae at outer margin; terminal article of palp with one simple seta at outer margin, nail shorter than pedestal (Figure 3C).

Coxa of gnathopod I shorter than gnathopod II. Coxa I rectangular, longer than broad, ventral to anterior margin with four and three simple setae, respectively. Basis with setae on anterior and posterior margins; ischium and merus with posterior group of setae. Carpus with one group of three setae anterodistally, a bulge with long simple setae; carpus 0.42 of basis length and 0.79 of propodus length. Propodus slightly longer than broad; anterior margin with seven setae in two groups in addition to anterodistal group of six simple setae. Palm slightly convex, with one strong long palmar robust seta, one short supporting robust seta on inner surface and two robust setae with lateral projections on outer surface; two simple setae under supporting robust seta in palmar corner. Dactylus not reaching posterior margin of propodus, outer and inner margins with a row of three and five simple setae, respectively; nail short, 0.23 of total dactylus length (Figure 3A).

Coxa of gnathopod II slightly rounded, with six setae along antero-ventro-posterior margins. Basis with setae in groups and single setae along anterior and posterior margins; posterior margins of ischium and merus with one posterior group of setae each. Carpus 0.43 of basis length and 0.67 propodus length. Carpus with one group of four setae anterodistally. Propodus in gnathopod II larger than gnathopod I, trapezoid shape and broader than long; anterior margin with seven setae in one group in addition to anterodistal group of six simple setae. Palm nearly convex, with one strong palmar robust seta, one supporting robust seta without lateral projections on inner surface, and one robust seta with lateral projections on outer surface; two setae under supporting robust setae in palmar corner. Dactylus reaching posterior margin of propodus,

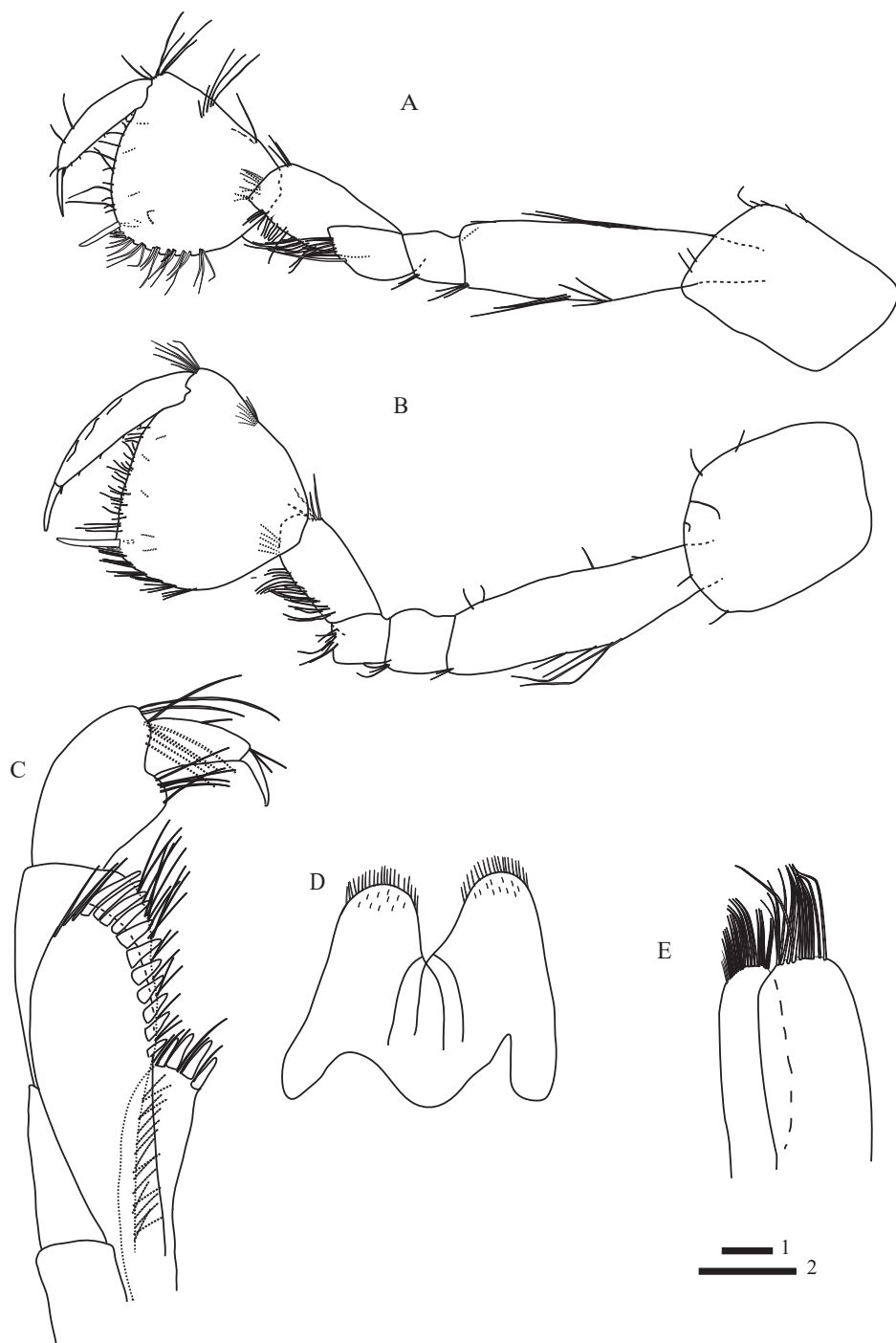


Figure 3. *Niphargus bakani* sp. n., male 9 mm (holotype, ZCRU Amph.1010). **A** Gnathopod I **B** Gnathopod II **C** Maxilliped **D** Labium **E** Maxilla II. Scale bars: 1=0.5 mm (**C-E**). 2=1 mm (**A-B**).

outer and inner margins of dactylus with three and four simple setae, respectively. Nail length 0.25 of total dactylus length (Figure 3B).

Coxa III rectangular, length to width ratio as 1.29 : 1; antero-ventral margin with five simple setae. Coxa IV rectangular, length to width ratio as 1 : 1.04, antero-ventral margin with seven simple setae, posterior concavity shallow and approximately 0.1 of coxa width (Figure 4A–B). Coxa V with anterior lobe, with five and three simple setae on anterior and posterior lobe, respectively. Coxa VI with anterior lobe, with one simple seta on posterior lobe. Coxa VII with one simple seta (Figure 4C–E).

Pereopod III : IV lengths ratio as 1 : 1.35 (Figure 4A–B). Dactylus IV short, length of dactylus 0.40 of propodus, nail shorter than pedestal (Figure 4B). Pereopods V: VI: VII length ratios as 1 : 1.35 : 1.30, respectively. Pereopod VII 0.63 of body length. Pereopod bases V–VI each with seven simple setae along posterior margin and six and seven groups of robust setae along anterior margins, respectively. Pereopod basis VII with 10 simple setae and six groups of robust setae along posterior and anterior margins, respectively (Figure 4C–E). Postero-ventral lobe of ischium in pereopods V–VII developed. Ischium, merus and carpus in pereopods V–VII with several groups of robust and simple setae along anterior and posterior margins; propodus of pereopod VII longer than these in V–VI, dactyli of pereopods V–VII with one robust and one short simple seta at the base of nail on inner margin, nail length of pereopod VII 0.29 of total dactylus length (Figure 4C–E).

Epimeral plates I–III (Figure 5G) with angular postero-ventral corner, anterior and ventral margins convex; postero-ventral corners of plates I–III with one robust seta each, and with two, three and two simple setae posteriorly, respectively. Epimeral plates II–III with three and four robust setae along of ventral margins, respectively. Peduncle of pleopod I with one simple seta and two-hooked retinacles at distal part of inner margin (Figure 5A); peduncle of pleopods II–III with two-hooked retinacles at distal part of inner margin; rami of pleopods I–III each with seven to 10 articles (Figure 5A–C).

Pereonites I–VI without setae. Pereonite VII with two simple setae. Pleonites I–III each with one long simple seta along dorsal surface. Urosomites I–III with two, five and two robust setae laterally, respectively.

Peduncle of uropod I with six and three large robust setae along dorsolateral and dorsomedial margins, respectively. Rami of uropod I with equal length (ratio 1 : 1); inner ramus with three groups of robust setae laterally and five robust setae distally; outer ramus with three groups of six robust setae laterally and five robust setae distally (Figure 5D). Outer ramus in uropod II longer than inner, both rami with lateral and distal long robust setae (Figure 5E). Uropod III long, almost 0.45 of body length. Peduncle of uropod III with five robust setae, Outer ramus biarticulated, distal : proximal article as 1 : 1.92. The proximal article of outer ramus bearing five groups of robust setae along inner and outer margins (Figure 5F); distal article with simple setae laterally and seven simple setae distally. Inner ramus short, with one robust and one simple distal seta. Telson two times as long as broad, lobes slightly narrowing; each lobe with three robust setae distally, with one long robust and one plumose seta marginally (Figure 5H).

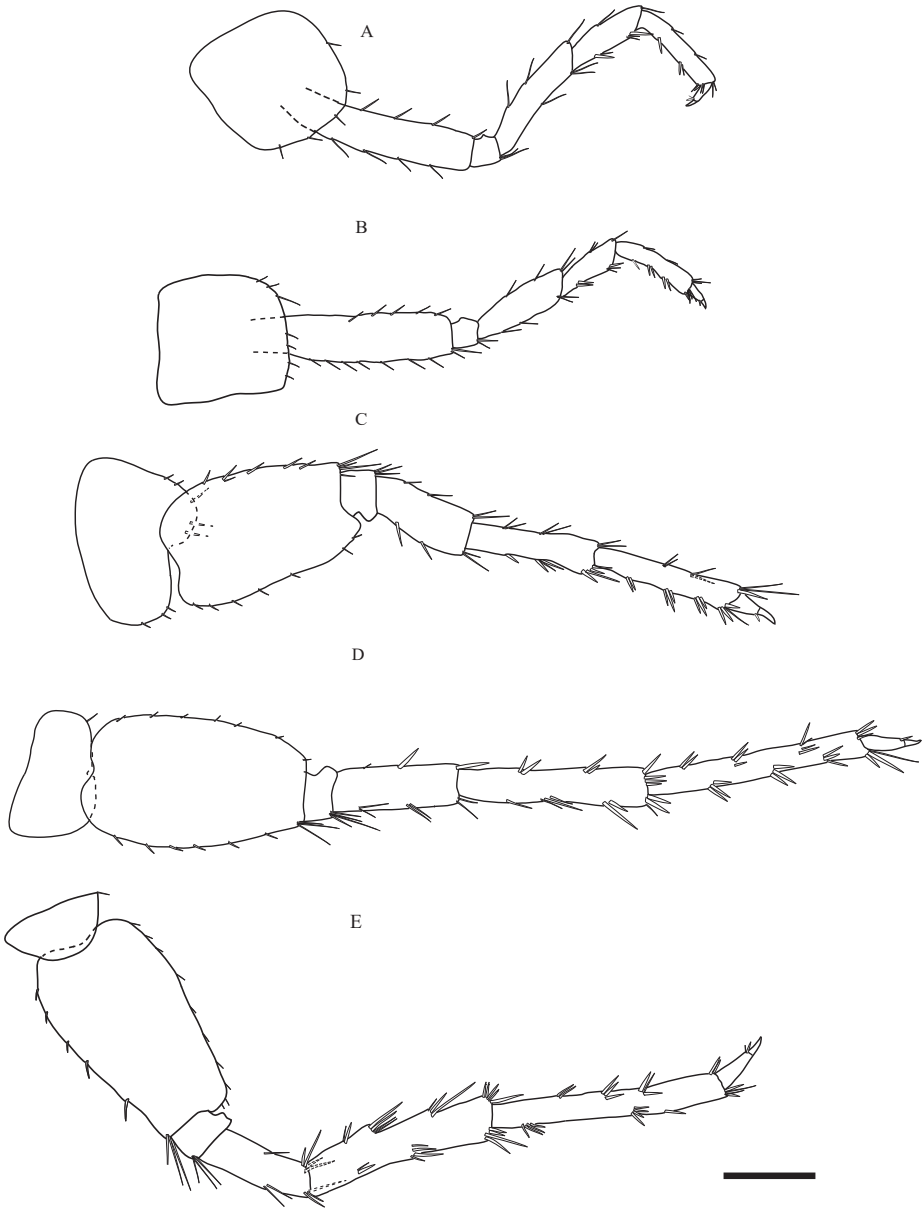


Figure 4. *Niphargus hakani* sp. n., male 9 mm (holotype, ZCRU Amph.1010). **A** Pereopod III **B** Pereopod IV **C** Pereopod V **D** Pereopod VI **E** Pereopod VII. Scale bars: 1mm (**A–E**).

Etymology. The name “*hakani*” refers to Hakan village where specimens were found.

Phylogenetic position of *N. hakani* sp. n. species. This species is nested within the main Iranian clade and apparently shares the nearest common ancestor with

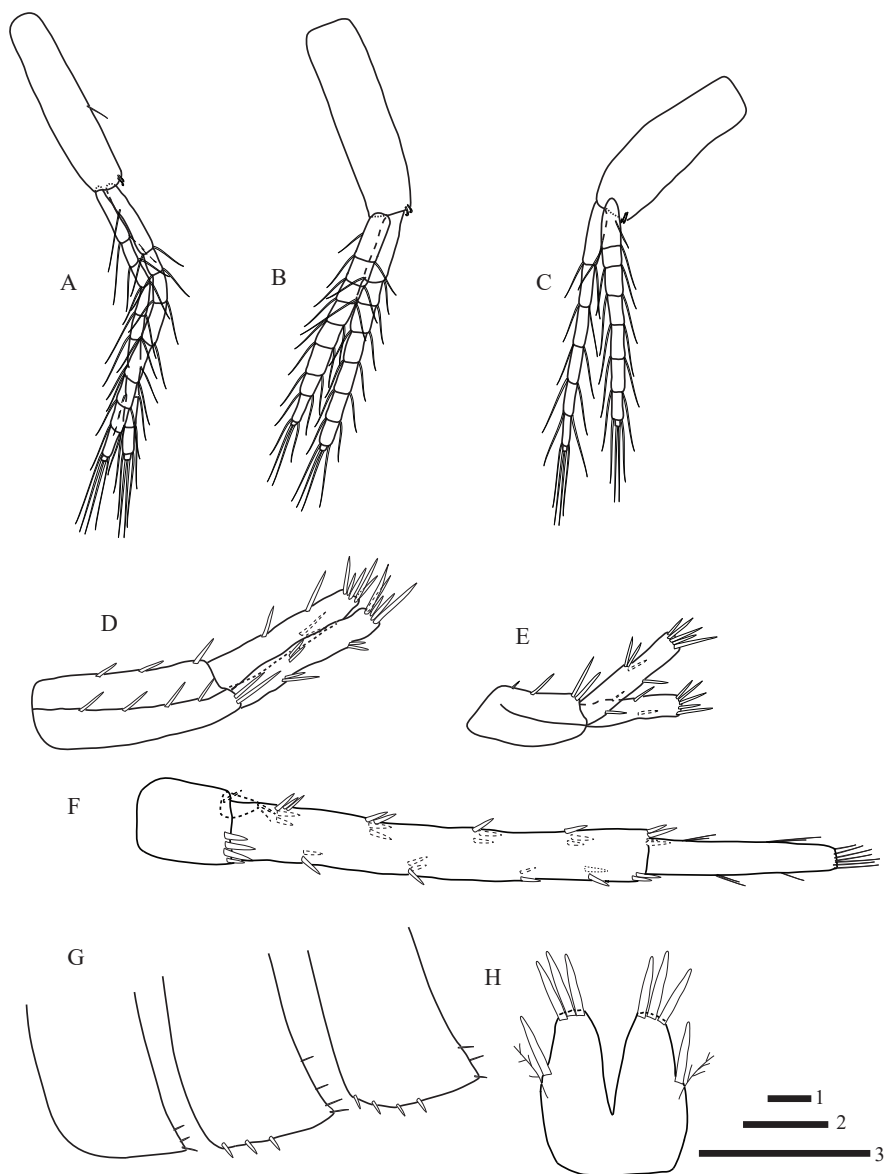


Figure 5. *Niphargus bakani* sp. n., male 9 mm (holotype, ZCRU Amph.1010). **A** Pleopod I **B** Pleopod II **C** Pleopod III **D** Uropod I **E** Uropod II **F** Uropod III. **G** Epimeral plates **H** Telson. Scale bars: 1=0.5 mm (**G-H**). 2=1 mm (**A-E**). 3=2mm **F**.

N. khwarizmi, *N. khayyami*, *N. kermanshahi* and *N. bisitunicus* (Figure 6, please note weak node support). Among these, this species is genetically the most similar to *N. khwarizmi* (1.93% K2P divergence in the studied 28 rDNA gene fragment) and the most divergent species from *N. bisitunicus*, (2.20% K2P divergence).

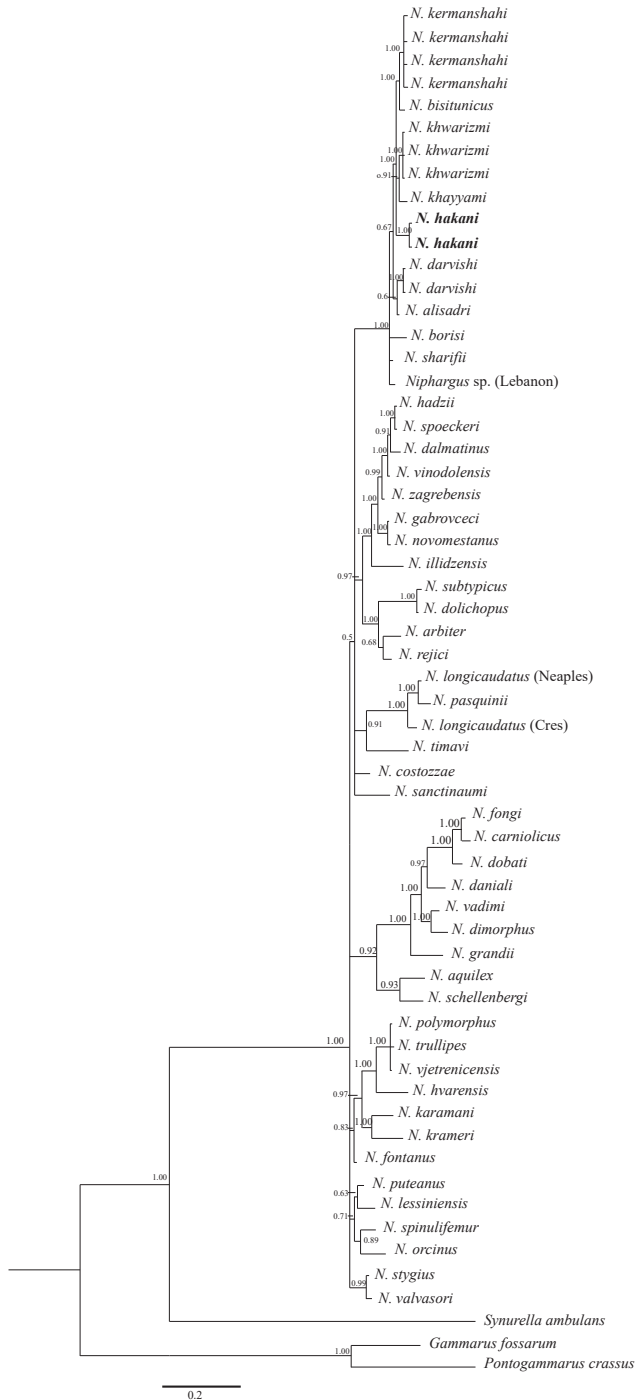


Figure 6. Bayesian consensus tree of 49 *Niphargus* species (48 taxa from Esmaili-Rineh et al. 2015a, 2016), based on the 28S ribosomal DNA sequences. Species are identified and named according to the valid taxonomic description. Posterior probabilities are indicated on main branches.

Table 1. Diagnostic characters in identification of Iranian species of the genus *Niphargus*.

Characters	Species	Urosomites I-III (number of dorso-postero- lateral setae)	Telson setal pattern (A – apical robust setae, L – lateral robust setae)	Size of coxa of GNI to GNII	Maxilla I -number of denticles on R. S of outer plate (from outer towards inner)	Uropod I (ratio of uropod rami)
<i>N. hukani</i> sp. n.		I: 2 R.S II: 5 R.S III: 2 R.S	3 A, 1 L	smaller	0,0,0,2,3,4,more than 5	Inner>outer
<i>N. kermanshahi</i> Esmaili-Rineh et al. 2016		I: 3 S.S II: 3 S.S, 2R.S III: No setae	3 A, 1 L	of similar size	0,1,1,1,2,2,5	Inner<outer
<i>N. alisadri</i> Esmaili-Rineh & Sari, 2013		I-III: 6 R. S	3 A, 2 L	smaller	0,1,1,1,2,2,5	Inner>outer
<i>N. daniali</i> Esmaili-Rineh & Sari, 2013		I-II: 1 S.S III: No setae	4 A, 2 L	smaller	0,0,0,1,1,1,3	Inner>outer
<i>N. valachicus</i> Dobreanu and Manolache, 1933		I-II: 1 R.S III: No setae	3 A, 1 L	smaller	1,1,1,1,1,1,1	Inner<outer
<i>N. khayami</i> Hekmatara et al. 2013		I: 1- 2 S.S II: 2- 3 S.S	3 A	smaller	1, others more than 5	Inner>outer
<i>N. khwarizmi</i> Hekmatara et al. 2013		I: 1 S.S II: 2 R.S III: No setae	3 A, 2 L	smaller	0,1,1,1,1,1,1	Inner<outer
<i>N. sharifii</i> Esmaili-Rineh et al. 2015		I: 1 S.S II: 3 R.S III: No seta	3 A	smaller	5,5,5, others more than 5	Inner>outer
<i>N. bistunicus</i> Esmaili-Rineh et al. 2015		I: 1R.S,2S.S II: 3R.S,2 S.S III: No setae	3 A, 1 L	smaller	0,0,1,1,1,1,2	Inner<outer
<i>N. borisi</i> Esmaili-Rineh et al. 2015		I-II: 4 R.S III: 2 R.S	3 A, 1 L	smaller	0,0,0,1,1,2,2	Inner>outer
<i>N. darvishi</i> Esmaili-Rineh et al. 2015		I-II: 2R.S, 2S.S	3 A	smaller	0,2,2,3,3,4,5	Inner>outer

Table 1. Continued.

Characters					
Species	The number of supporting robust setae in palmar corner of GNII	Length of maxilla I palpus	Shape of propods of GNII	Shape of propods of GNII	Uropod III (length of distal article of outer ramus.)
<i>N. hubani</i> sp. n.	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	approximately 0.52 of the first article
<i>N. kermanshahi</i> Esmaili-Rineh et al. 2016	2	shorter than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	approximately 0.5 of the first article
<i>N. alisadri</i> Esmaili-Rineh & Sari, 2013	3	as long as outer lobe of maxilla I (without R. S)	rectangular	triangular	as long as the first article.
<i>N. daniadi</i> Esmaili-Rineh & Sari, 2013	1	longer than outer lobe of maxilla I (without R. S)	rectangular	sub quadrangular	approximately 0.33 of the first article
<i>N. vulachicus</i> Dobreanu and Manolache, 1933	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	approximately 0.33 of the first article
<i>N. khayyami</i> Hekmatara et al. 2013	1	as long as outer lobe of maxilla I (without R. S)	trapezoid	rectangular	0.1 of the first article
<i>N. khvarizmi</i> Hekmatara et al. 2013	1	longer than outer lobe	trapezoid	trapezoid	0.65 of the first article
<i>N. sharifi</i> Esmaili-Rineh et al. 2015	2	as long as outer lobe	rectangular	rectangular	0.16 of the first article
<i>N. bistunicus</i> Esmaili-Rineh et al. 2015	2	longer than outer lobe	rectangular	rectangular	approximately 0.33 of the first article
<i>N. borisi</i> Esmaili-Rineh et al. 2015	3	shorter than outer lobe of maxilla I (without R. S)	rectangular	rectangular	0.14 of the first article
<i>N. darvishi</i> Esmaili-Rineh et al. 2015	1	longer than outer lobe of maxilla I (without R. S)	trapezoid	trapezoid	0.08 of the first article

A., apical; GNII-II., gnathopods I-II; L., lateral; R. S., robust seta; S. S., simple seta.

Discussion

Niphargus hakani sp. n. is a member of the main Iranian clade (Esmaeili-Rineh et al. 2015a) and shares common ancestry with *N. khwarizmi*, *N. khayyami*, *N. kerman-shahi* and *N. bisitunicus*. The closest relative seems to be *N. khwarizmi*. Although *N. hakani* sp. n. shares relative length of palpus of maxilla I, shape of propodus of gnathopods and ratio of second to first article of outer ramus of uropod III. However, this species differs from *N. khwarizmi* in the fewer lateral robust setae in telson, ratio of inner to outer ramus of uropod I and ornamentation of lateral projection of robust setae in outer plate of maxilla I (Hekmatara et al. 2013). A shortened list of diagnostic traits for the Iranian species is presented in Table 1. *Niphargus khayyami* differs from *N. hakani* by ratio of distal to proximal article of outer ramus of uropod III, number of apical robust setae on telson and multidenticulated robust setae on the outer plate of maxilla I.

An important diagnostic traits of *N. hakani* sp. n. is the presence of robust setae on urosomite III. This character was observed in *N. borisi* and *N. alisadri* from Iran and some species from Europe including *N. croaticus* Sket, 1958, *N. trullipes* (Jurinac, 1887), and *N. hercegovinensis* S. Karaman, 1950 (Karaman 1984). *Niphargus borisi* resembles *N. hakani* sp. n. also in shape of postero-ventral angle of epimeral plates; but differs from herein described species in higher number of robust setae at the base of uropod I, the shape of propodus of gnathopods and in the elevated number of supporting robust setae in palmar corner of gnathopod I (Esmaeili-Rineh et al. 2015b). *Niphargus alisadri*, the second species sharing spiny urosomite III with *N. hakani* sp. n. differs from this species in ratio of distal to proximal article of outer ramus in uropod III, the increased number of lateral robust setae in telson and the ratio of palpus to outer plate length in maxilla I (Esmaeili-Rineh and Sari 2013).

Conclusion

We described new taxon of Iranian *Niphargus*. Iranian niphargid fauna now counts 11 species, all but *N. valachicus* being endemic to Iran. We expect that further explorations will unveil additional new species from rich biodiversity in this area.

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Biospeleological activities in Central Europe – a status report

Dieter Weber^{1,2}

1 *Evolutionary Biology & Ecology, CP 160/12, Université libre de Bruxelles, Avenue F.D. Roosevelt 50, B-1050 Brussels, Belgium* **2** *Kirchgasse 124, D-67454 Hassloch, Germany*

Corresponding author: Dieter Weber (dieter.weber124@gmx.de)

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Abstract

Catalogues of cave fauna from Belgium, Switzerland, Austria, Germany (Swabian Alb, Franconian Alb, Westfalia, Hesse, Harz, Rhenish Palatinate and Saarland), and Luxembourg are available. Several activities deal with public relations, education, and training: the cave animal of the year, a camp for young cavers, the day nature, and the biospeleological workgroup. The German Barcoding of Life is a project which aims to obtain CO1 barcodes from every species in Germany with a sub-project on cave fauna. Special projects deal with *Bythiospeum*, niphargids, diplurans, sphaerocerids, and the biodiversity and ecology of cave invertebrates in the Central European Uplands.

Zusammenfassung

Es gibt Höhlenfaunenkataloge von Belgien, der Schweiz, Österreich, Deutschland (Schwäbische Alb, Fränkische Alb, Westfalen, Hessen, Harz und Rheinland-Pfalz/Saarland) und Luxemburg. Verschiedene Aktivitäten befassen sich mit Öffentlichkeitsarbeit und Schulungen; Das Höhlentier des Jahres, ein Trainingslager für junge Höhlenforscher, der Tag der Natur und eine biospeläologische Arbeitsgruppe. Das Projekt „German Barcoding of Life“ versucht CO1-Barcodes aller deutschen Arten zu erstellen. Es hat ein Unterprojekt zur Höhlenfauna. Tiergruppenspezifische Projekte behandeln *Bythiospeum*, Niphargen, Dipluren, Sphaeroceriden und Biodiversität und Ökologie von Höhlenevertebraten der zentraleuropäischen Mittelgebirge.

Keywords

Biodiversity assessment reports, public relations, education, *Bythiospeum*, Niphargidae, Diplura, Sphaeroceridae

Introduction

This text gives an overview of important past and recent biospeleological activities in “Central Europe”. The Netherlands, Belgium, Luxembourg, Germany, Switzerland and Austria belong to “Central Europe” in this context. The area is therefore not identical to the geographic definition of Central Europe. Activities on bats are not included in this paper.

Biodiversity assessment of cave fauna

In many karstic and non karstic areas in Central Europe summarizing biodiversity assessment reports have been published: Belgium (Leruth 1939 with 600 species), Switzerland (Strinati 1965 with 513 species), Austria (Strouhal and Vornatscher 1975), Swabian Alb (Dobat 1975 with 289 species), Franconian Alb (Dobat 1978 with 491 species), Westfalia (Weber 1991 with 1084 species), Hesse (Zaenker 2001 with 3259 species, ongoing), Harz (Hartmann 2004 with 224 species), Luxembourg (Weber 2013 with 390 species online available [<https://www.mnhn.lu/science/2013/03/15/ferrantia-69/>], ongoing), Rhenish Palatinate and Saarland (Weber 1988, 1989, 1995, 2001, 2012 with 2600 species, ongoing).

In addition, many smaller publications on the cave fauna of other areas, dealing mostly with one specific animal group, are available and contain additional information.

The assessment of the cave fauna and its documentation in Central Europe is therefore comprehensive, although in some areas it is unfortunately not up to date.

Public relations, education, and trainings

Cave animal of the year

The idea of a cave animal of the year arose during the yearly conference of the Society of German Cave and Karst explorers in 2008. It has the following aims: inform the public that caves are sensitive and fragile biotopes, raise the importance of caves to authorities and NGOs, cave fauna and their protection, motivate cavers working on biospeleology, and protection of subterranean ecosystems.

Since then, one species has been selected every year as “Cave Animal of the Year,” to indicate the importance of caves not only to their permanent inhabitants but also to hibernating species. Eutroglobiontic, eutroglophilie and subtroglophile species have alternated.

Every year, posters and flyers are printed. A presentation on the cave animal of the year and an internet homepage (<http://www.hoehlentier.de/>) are available. The homepage contains information on the species, photos and a press release.



Figure 1. Homepage of the cave animal of the year.

Table 1. Cave animals of the year from 2009 until 2017.

2009	<i>Niphargus</i> sp.
2010	<i>Scoliopteryx libatrix</i>
2011	<i>Myotis myotis</i>
2012	<i>Meta menardi</i>
2013	<i>Speolepta leptogaster</i>
2014	<i>Proasellus cavaticus</i>
2015	<i>Oxychilus cellarius</i>
2016	<i>Amilenus aurantiacus</i>
2017	<i>Diphyus quadripunctorius</i>

JuHöFoLa – Camp for young cavers

The “JuHöFoLa” (<http://www.juhoefola.de/>) is a training camp for young cavers with participants from all over Europe. It is held in Germany and is conducted in English. It consists of two weeks training with three days on biospeleology. The biospeleological part consists of short collecting trips to caves and springs in the morning, sorting/determination of the collected specimens and a theoretical session in the afternoon.

The next JuHöFoLa is planned for summer 2018.



Figure 2. Determination of cave animals in the “lab” during the JuHöFoLa (Photo: Otto Schwabe).

Day of nature

The day of nature (previously: day of biodiversity; <http://www.geo.de/natur/tag-der-artenvielfalt/9274-rtkl-das-projekt-geo-tag-der-artenvielfalt-2016>) is sponsored by the journal GEO and the KfW foundation. It aims to identify as many species as possible in one day and is held once a year in alternating regions.

For the last 5 years, biospeleologists have been offering collecting trips to caves, mines or springs and have published the results (Blick et al. 2014; Fritze et al. 2014).

Biospeleological workgroup

The biospeleological workgroup, created in 2016 at Eurospeleo in the Yorkshire Dales, is an e-mail information exchange system for all biospeleologists. As of the end of 2016, it had 36 participants. E-mails can be sent by every participant on all biospeleological topics anytime.

All biospeleologists are invited to join (hannes@bigwalls.de)!

DNA barcoding

“The GBOL = German Barcoding of Life” (<https://www.bolgermany.de/>) is a project in cooperation with several German museums and institutes, with the target to obtain

CO1 barcodes from 10 specimens of every species that has been found in Germany (the barcodes need not be from specimens collected in Germany).

A special sub-project under the head of Alexander Weigand, University of Duisburg-Essen (WeigandA@gmx.net) deals with cave fauna. As of December 2016, 381 cavernicolous species and several thousand specimens have been barcoded.

Topics on special animal groups

Bythiospeum

A project at the Staatliches Museum für Naturkunde Stuttgart deals with the cavernicolous snail genus *Bythiospeum*, with the aim to learn about the phylogenetics, biogeography and diversity of this genus in Europe. First results have been published (Richling et al. 2016). Ira Richling is in charge (ira.richling@smns-bw.de).

Niphargids

A project at the Université libre de Bruxelles, under the head of Jean-François Flot, to resolve various questions on the cavernicolous shrimp family Niphargidae started in 2016. It aims to compare the phylogeny and taxonomy of the niphargids, estimate species richness, find cryptic species, identify distributional patterns delineation and to analyze the effects of the last Quaternary glaciation on both species richness and distribution. Central Europe, where specimens are still needed from the constituent countries is managed by Dieter Weber (dieter.weber124@gmx.de).

Diplura

The target of the Diplura project, a cooperation of several universities and museums, is to compile a catalogue of all cave diplurans in Central Europe, including their phylogenetic description. Alberto Sendra (Alberto.Sendra@uv.es) is in charge.

Sphaeroceridae

After knowledge was gained of the cave dwelling fly family Sphaeroceridae in certain regions (Rhenish Palatinate and Saarland, Bährmann and Weber, 2008; Luxembourg, Bährmann and Weber 2013), the intention of this project is to improve the knowledge of sphaerocerids in caves within the missing regions. Point of contact is Dieter Weber (dieter.weber124@gmx.de).

Biodiversity and ecology of cave invertebrates in the Central European Uplands

A comprehensive project in cooperation with the University of Duisburg-Essen and the National Museum of Natural History Luxembourg deals with the biodiversity and ecology of selected species of cave invertebrates in the Central European Uplands. One target is to compare subtroglophile species (*Limonia nubeculosa*, *Scoliopteryx libatrix*, *Triphosa dubitata*) with eutroglophile species (*Meta menardi*, *Metellina merianae*, *Gammarus pulex*, *Discus rotundatus*, *Oxychilus draparnaudi*, *Speolepta leptogaster*), and eutroglabiontic species (*Niphargus schellenbergi*, *Porrhomma convexum*, *Trichoniscoides helveticus*). Alexander Weigand (WeigandA@gmx.net) is in charge of this project.

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Notes on predator-prey relationships among Tanypodinae larvae (Diptera, Chironomidae) and mites (Acariformes) in Brazilian subterranean aquatic environments

Bárbara Goulart Costa¹, Thais Giovannini Pellegrini^{1,2},
Leopoldo Ferreira de Oliveira Bernardi^{2,3}, Rodrigo Lopes Ferreira¹

1 Centro de Estudos em Biologia Subterrânea, Setor de Zoologia Geral, Departamento de Biologia (DBI), Universidade Federal de Lavras (UFLA), Minas Gerais, CEP: 37200-000, Brazil **2** Programa de Pós-Graduação em Ecologia Aplicada, Setor de Ecologia, Universidade Federal de Lavras (UFLA), Minas Gerais, CEP: 37200-000, Brazil **3** Bolsista do Programa Nacional de Pós-Doutorado (PNPD/CAPES)

Corresponding author: Thais Giovannini Pellegrini (thais.g.pellegrini@gmail.com),
Leopoldo Ferreira de Oliveira Bernardi (leopoldobernardi@gmail.com)

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Abstract

Chironomidae larvae and mites are abundant and diversified groups that coexist in several environments. However, little importance has been attributed to their ecological relationships (predator–prey, parasitism, etc.). Therefore, the present study aimed to report the predation of mites by Tanypodinae larvae in Neotropical quartzite caves.

Keywords

Gut content, cave; interaction, predation

Chironomidae represents an abundant and diverse family of the order Diptera. Its larval forms are one of the most important groups of aquatic insects, representing an abundant part of the fauna present in lake and river biotopes (Trivinho-Strixino 2014). Also, many species are commonly found in habitats with environmental conditions

that are unfavorable for other organisms (e.g., polluted or with low oxygen content) (Silva et al. 2008). Hence, chironomids are considered as good indicators of water quality (Saether 1979, Quinlan and Smol 2001). They have also been found in terrestrial habitats and caves, where conditions are distinct from those in external habitats, particularly with respect to permanent absence of light. Darkness imposes a strong selective pressure on the organisms that inhabit caves, and it is impossible for photosynthetic organisms to survive in these habitats, indirectly indicating the oligotrophic conditions of these environments (Simon et al. 2007).

Independent of their habitat, chironomid larvae play an important role in the trophic chains of aquatic communities (Schmid-Araya and Schmid 2000). These organisms ingest various food items, such as algae and detritus and their associated microorganisms, besides some invertebrates (Silva et al. 2008). They also serve as food for many other aquatic invertebrates (Schmid-Araya and Schmid 2000). Among the several chironomid subfamilies, the Tanypodinae larvae comprise the main predators in this family (Merritt and Cummins 1984).

Studies have highlighted that Tanypodinae larvae show a well-defined food preference, mainly consuming larval forms of other Chironomidae species (Baker and McLachlan 1979). However, in unfavorable foraging conditions, they may adopt generalized and opportunistic strategies, using a variety of foods that are available in the environment (Silva et al. 2008). Mites are among the food items that may be consumed by Tanypodinae (Blakely et al. 2010).

Although some studies have reported this trophic interaction between mites and Tanypodinae (e.g., Smith and Oliver 1986, Proctor and Pritchard 1989), they have only identified mite as the group present or absent in the gut of Chironomidae larvae, without any accurate identification of the prey (Blakely et al. 2010). Furthermore, these reports have only focused on the epigeal environment and not on subterranean habitats. Therefore, given the lack of information regarding the predator–prey relationship among Tanypodinae larvae and mites in caves, the present work aimed to report and illustrate this interaction, providing more detailed data on the identification of involved specimens and the frequency of its occurrence.

For this purpose, the specimens were collected across eight sampling events (from June 2013 to January 2014). The study area comprised three subterranean allogenic streams that run through the following quartzite caves: Mandembe cave (21°32'38.1"S, 44°47'57.3"W), Serra Grande cave (21°33'33.5"S, 44°49'10.7"W), and Toca cave (21°28'24"S, 44°40'02"W), all of which are located in southern Minas Gerais, Brazil. Specimens were collected along a 100-m stretch of each stream divided into 11 transects, with three replicates per transect, using a Surber net of 400 cm². Tanypodinae larvae were separated from other organisms and mounted on slides using Hoyer medium. The predator and prey specimens were identified using an optical microscope ZEISS Primo Star, with identification keys presented by Walter et al. (2009), Smith et al. (2009), Cook (1988), and Trivinho-Strixino (2014).

The gut content of 287 Tanypodinae specimens collected across all sampling events were analyzed; only seven of them had preyed on mites (Figures 1–3) (Pellegrini 2016).

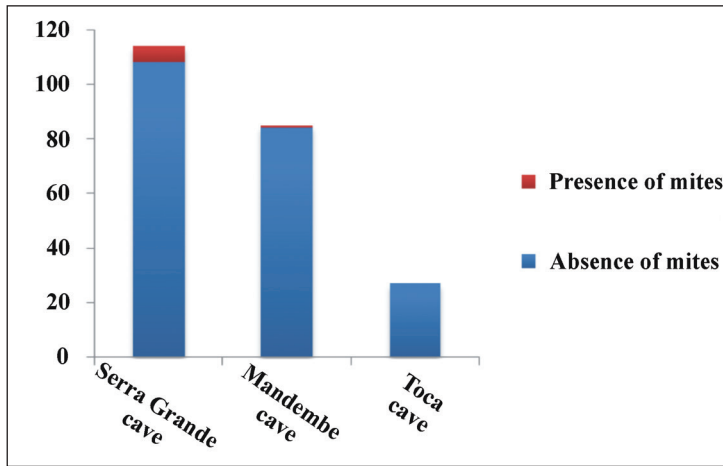


Figure 1. Tanypodinae preying on mites at each studied cave.

Among all analyzed specimens, 114 were found from Mandembe cave, 85 from Serra Grande cave, and 27 from Toca cave. The gut contents of these specimens showed the presence of mainly other chironomid larval and some copepod species. Among the specimens that preyed on mites, 85% (6 individuals) were found from Mandembe cave, 15% (1 individual) from Serra Grande cave, and none from Toca cave (Figure 1). These specimens belonged to the following Tanypodinae species: four to *Pentaneura* spp., two to *Ablabesmyia* (Karelia) spp., and one to *Parapentaneura* spp., all belonging to the Pentaneurini tribe. The following prey taxa were identified in the gut content: one of the Limnesiidae family (Acariformes: Trombidiformes), probably of the *Limnesia* genus (Fig. 2A), one of the Teratopiidae family (Acariformes: Sarcoptiformes) (Fig. 3), one of the *Tyrophagus* sp. (Acariformes: Sarcoptiformes: Acaridae) (Fig. 4A and 4B), and one possibly of the Frontipodopsidae family (Acariformes: Trombidiformes) (Fig. 4C) as well as unidentified parts of Hydrachnidia specimens (Trombidiformes: Parasitengona) (Fig. 2B–D).

These results suggested that Tanypodinae larvae had no preferences for mite species. Because these chironomids are opportunistic organisms (Silva et al. 2008), they can consume a variety of food items in oligotrophic environments, thus consuming mites at random.

The greatest number of individuals preying on mites were observed at the Mandembe cave, which may be due to greater abundance of Tanypodinae in this cave. The greater abundance of such organisms in a single cave compared with other caves may be related to the small size of the Mandembe cave. The short allogenic stream stretch running through this cave allows higher connectivity with the epigeal environment (Miller 1996), thus favoring greater abundance and colonization by a large number of surface species (Watson 2010).

Although a less intense prey–predator relationship was observed between Tanypodinae and mites, such relationship has been reported for the first time in a hypogean

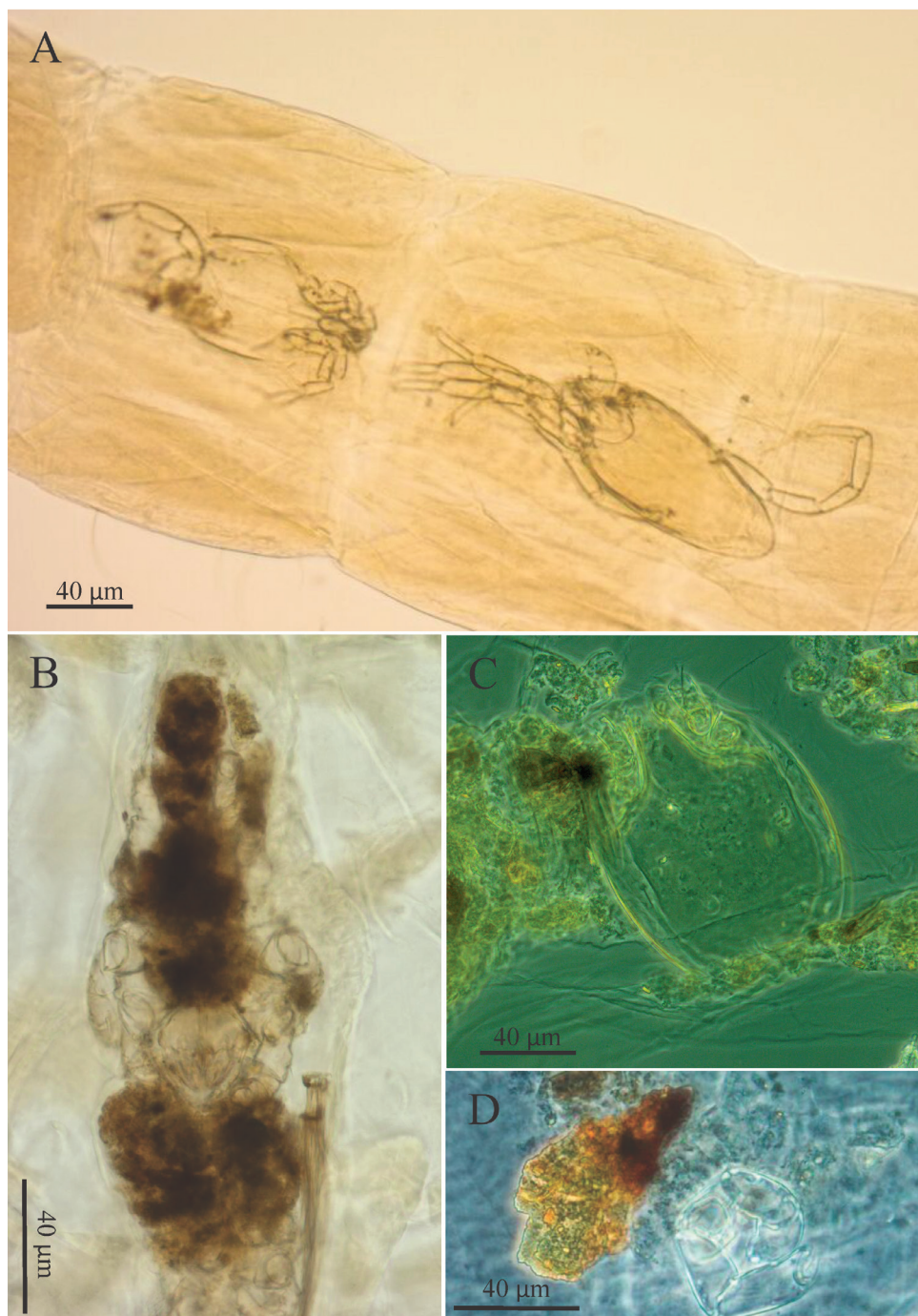


Figure 2. Mites found in gut contents of four specimens of Tanypodinae (Chironomidae). **A** Two examples of Limnesiidae larvae (Acariformes: Trombidiformes), probably belonging to the genus *Limnesia* **B–D** partially digested mites, probably Hydrachnidia.

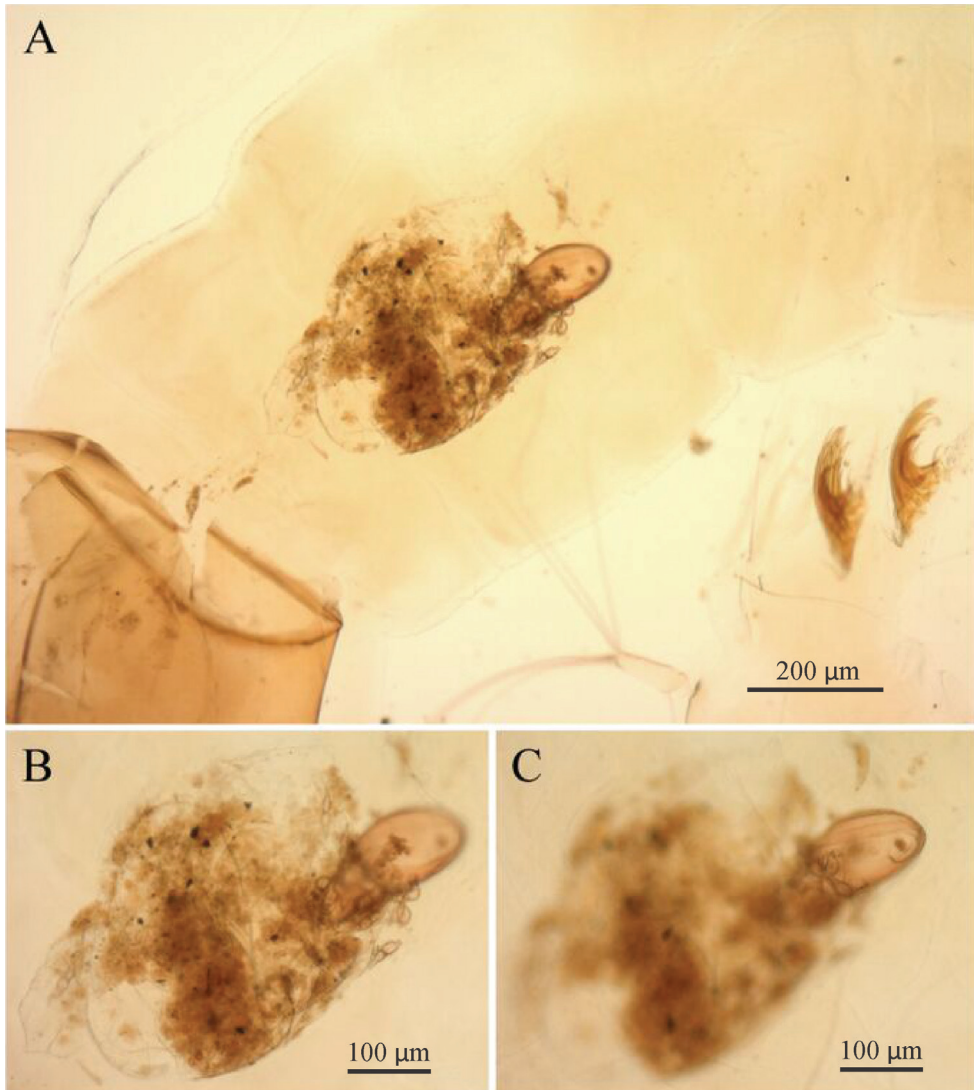


Figure 3. A–C Teratopiidae (Acariformes: Sarcoptiformes) found in gut contents of four specimens of Tanypodinae (Chironomidae).

environment in this study. Theoretical ecologists assume the importance of such weak interactions (with few occurrences in a community) due to their stabilizing effect on the trophic dynamics of systems (e.g., Pimm 1984, Paine 1992, Gorman and Emerson 2009). Empirical studies have confirmed that such interactions allow food-web flexibility, thus preventing destabilization of all the trophic dynamics during disturbances (e.g., McCann et al. 2005, Navarrete and Berlow 2006, Eveleigh et al. 2007, McCann and Rooney 2009), including in benthic communities (Chase 2003).

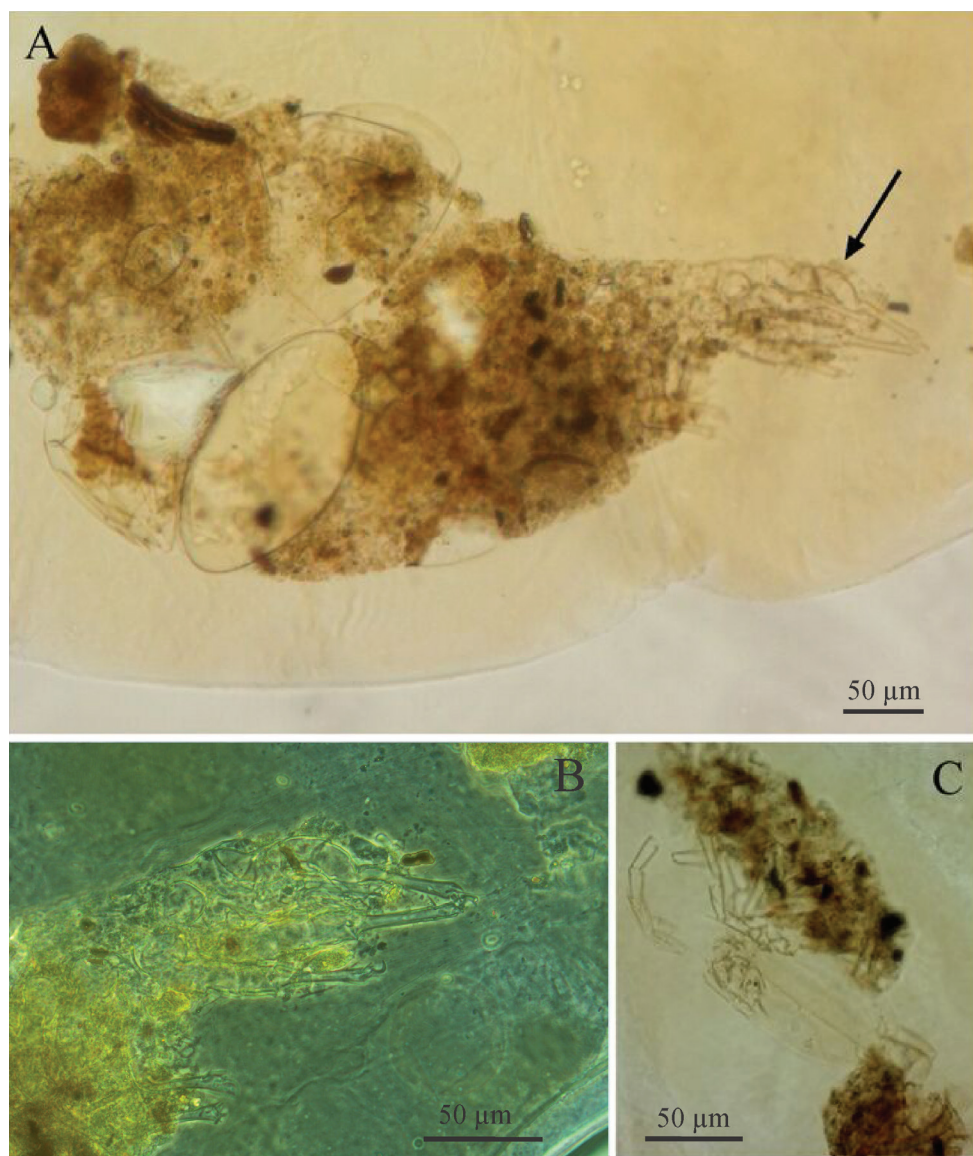


Figure 4. **A** General view of gut contents from a specimen of Tanypodinae (Chironomidae), where the arrow shows an specimen of *Tyrophagus* sp. (Acariformes: Sarcoptiformes: Acaridae) among alimentary items **B** Detail view of the partially digested *Tyrophagus* sp. **C** Detail of specimen already partially digested which possibly belongs to the family Frontipodopsidae (Acariformes: Trombidiformes).

Therefore, the comprehension of possible food-web routes, including systems with omnivorous organisms, has important implications for the management and conservation of natural communities (Paine 1992), mainly when considering poorly understood communities, such as those associated with subterranean environments.

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