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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

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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 trogloxenes. 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 trogloxene 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, troglophiles, trogloxenes, 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 rhaphidophorid 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 troglophiles and trogloxenes 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 'wo sich die ihrer Lebensart entsprechenden Bengungen vorfinden*'; 2) *troglophiles* (troglophiles): "*animaux habitant les régions où la lumière du jour pénètre encore, qu´on peut, excepcionallement, rencontrer à la surface ou qui ont seulement des formes répresentatives lucicoles*"; 3) *troglobies* (troglobites): "*animaux exclusivement cavernicoles, qu´on ne rencontre jamais dans les regions épigées, sauf dans le cas d´événements excepcionnels 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 attires soit par l´humidité, soit par la nourriture, mais n´y habitant pas constamment et n´y reproduisant pas*"; 2) *troglophiles* (troglophiles): "*habitent constamment le domain souterrain, mais de preference dans ces regions 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 preference 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 subis souvent des reductions de l´appareil optique, ... et d´autres adaptations a 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 ocasionnes* 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 Tercafs 1972**	**Holsinger and Culver 1988 (also Barr 1967, 1968)**	**Sket 2008 (based on Ruffo1957)**	**Trajano 2012**
Trogloxenes (Schiner´s *hôtes occasionels* = 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 suface 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 fuction (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* électifs”)	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 *vice-versa*)
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 *troglomorphisms* 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 troglophiles 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, rhaphidophorid orthopterans, which are mostly trogloxenes (in the modern sense, see below) in Europe and North America. In fact, the categories referred to as “trogloxenes”, 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 trogloxene concept, which excluded accidentals, i.e. animals without an ecological relationship with caves (Table 1). In Barr´s definition, trogloxenes 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 organisms vivent dans le milieu extérieur mais pour diverses raisons très precises colonisent temporairement le milieu souterraine*” (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 trogloxenes as reproduction and feeding sites (e.g. rhaphidophorid orthopterans), routes for predator escape, etc., at different times of the year.

Thinès and Tercafs (1972, p.53) definition for troglophiles (*ces organismes vivent également dans le milieu extérieur…Ils choisissent ce milieu…. certaines de leur potentialities… les prédisposent à vivre dans le milieu souterrain…. Ils se reproduisent dans les cavernes et y ont une activité permanente. Ce sont des hôtes electives…*) 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, epigean microenvironments (Barr 1968) (Table 1). The role of preadaptions (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, trogloxenes 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 epigean animals that live and reproduce in caves, without showing morphological modifications for subterranean life, he created the term *epigeomorphs*, which would be equivalent to troglophiles in the modern sense (see below). Finally, troglobites 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 epigean forms, and *troglomorphs* 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, troglobitic 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 (eutrogloxenes, subtrogloxenes, and aphyletic trogloxenes) corresponding to accidentals in the modern sense. Two terms – subtroglophiles and eutroglophiles – have been used by modern authors, however in different senses. In Pavan´s sense, both subtroglophiles and eutroglophiles choose to live in subterranean habitats but are facultative there (in opposition to troglobites that are obligatory subterranean); however, the former do not reproduce in these habitats whereas the latter do. Therefore, eutroglophiles would correspond to the troglophiles in the modern sense, and subtroglophiles and possibly phyletic trogloxenes (animals that enter caves by accident but live there without difficulty and may reproduce) would correspond to trogloxenes.

Contrary to assertions by Sket (2008), and endorsed by Culver and Pipan (2009), Pavan´s subtroglophiles do not correspond to Racovitza´s troglophiles (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 eutroglophiles is so vague that it encompasses both trogloxenes and troglophiles in the modern sense. Pavan´s classification is unclear and biologically meaningless because it contains both artificial and superfluous categories.

The subdivision of troglophiles into eutroglophiles and subtroglophiles, as recently defended by Sket (2008) (Table 1), and the use of the latter instead of the term trogloxenes *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 trogloxenes ("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* – trogloxenes 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 trogloxenes 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% (Juberthie1983), 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 troglophiles, 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 trogloxene 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. trogloxenes are instances of source populations in epigean habitats, but using subterranean resources (the so-called obligatory trogloxenes, all individuals are dependent on both surface and subterranean resources)" (Table 1). Trogloxenes 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), troglobitic catfish from eastern Brazil, showing intrapopulation variation in pigmentation and eye development (Photos: Dante Fenolio). **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, troglophiles and trogloxenes 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 troglophiles and trogloxenes (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 troglophiles 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 + troglophiles + trogloxenes), 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, troglophiles 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 troglophilic 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 epigean 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 epigean 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 epigean 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 epigean life (e.g., regressed eyes and pigmentation, reduced phobic reactions and cryptobiotic habits), some could recolonize 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 trogloxenes 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 troglobitic status (Trajano 1993) (Fig. 2).

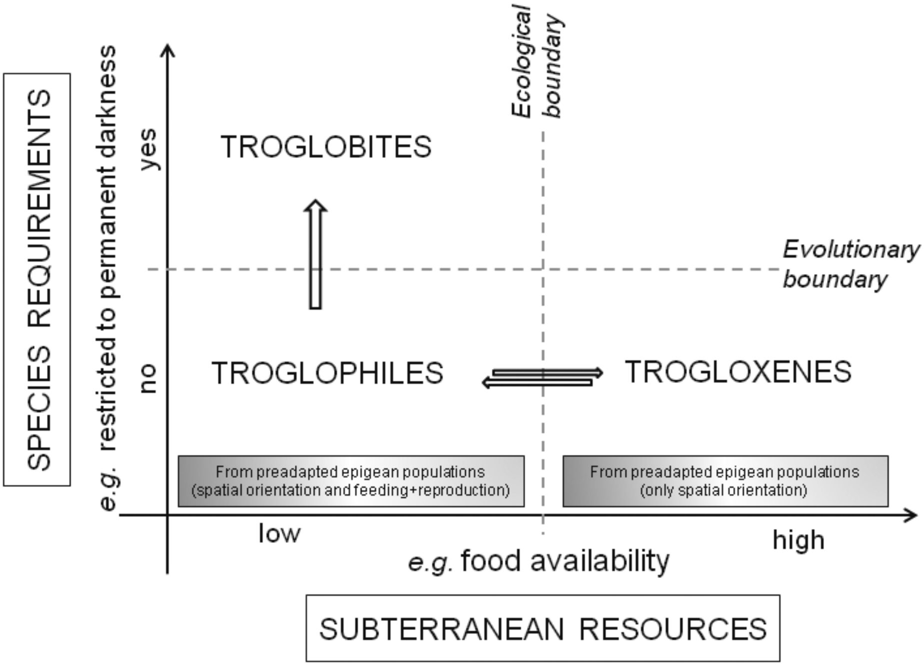

Figure 2. Interrelationships between evolutionary (historical) and ecological (present-day) factors, defining the conditions of trogloxenes *versus* troglophiles *versus* troglobites for subterranean organisms.

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 bottom 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 quartize system) in isolation during a past dry phases (paleoclimatic model), when the epigean 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 epigean connections.

In conclusion, the morphological approach alone, not associated with extensive epigean 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 epigean habits is not enough to dismiss the troglobitic status at once. As epigean 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 trogloxenes

The separation between the troglophilic and trogloxene status is ecological, not evolutionary, since it may depend on food availability (Fig. 2). Indeed, there are instances of species with trogloxenic individuals in most caves, but which may give rise to troglophilic populations in particularly food-rich caves (Holsinger and Culver 1988, Trajano and Moreira 1991). The difference lies in the fact that troglophilic animals *may* leave caves, and trogloxenes *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 troglophilic or trogloxene, it is necessary to perform long-term studies using chronobiological methods, allowing for the detection of possible cyclic patterns of movements between epigean and subterranean habitats that, if present, indicate trogloxene status (Menna-Barreto and Trajano 2015). Brazilian examples include obligatory trogloxenes, 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 phalangopsid 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 amblyopsids (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. Trogloxenes, 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 trogloxenes use caves seasonally, being absent during part of the year. Therefore, a definitive distinction between troglophiles and trogloxenes depends on populational studies conducted on an annual basis.

Among trogloxenes, recognition of obligatory trogloxenes 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. *Hadenoecus* camel crickets (Rhaphidophoridae) from karst areas in Kentucky, USA, have long been recognized as obligatory trogloxenes, 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 trogloxenes 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 trogloxene 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 trogloxenes"). 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 trogloxene 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 trogloxenes.

Figure 3. *Acutisoma spelaeum* (Arachnida: Opilioes), an obligatory trogloxene 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 trogloxene status, because, as mentioned, both trogloxenes and troglophiles 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 trogloxenes due to the erroneous notion that they are always rare in caves whereas troglophiles 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 troglophiles 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, troglophiles 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 troglophile” 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 fasciatus* (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 Gonçalo, 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 Gonçalo 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. Troglophiles and trogloxenes are also singular in extreme ecological plasticity, with modifications to two very contrasting environments. Models of evolution in caves assume that troglobitic species originate from isolated (at least genetically) troglophilic populations, justifying the protection of the latter as potential ancestors of troglobites. Moreover, the high population densities achieved by many troglophiles 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" trogloxenes, 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 trogloxenes.

There are enough reasons justifying the protection of all subterranean organisms, but troglobites and obligatory trogloxenes 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 trogloxenes 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 troglobitic from troglophilic populations belonging to epigean troglomorphic taxa; 3) to recognize the trogloxene condition, identifying obligatory trogloxenes, 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 during 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 trogloxenes 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 trogloxenes; detection of obligatory trogloxenes. 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 trogloxenes 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 trogloxenic 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 trogloxenes 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.
- The condition of relict (taxon without living epigean 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 troglophilic populations may be different from that of epigean populations, with higher densities observed in caves. Collecting efforts in epigean 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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