Spontaneous behavior of basal Copionodontinae cave catfishes from Brazil (Teleostei, Siluriformes, Trichomycteridae)

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Abstract
Cave animals are particularly interesting due to their behavioral specializations, resultant from evolution in isolation. We present data from a spontaneous behavior study (spatial distribution and preference for microhabitats) of two troglobitic catfish from Brazil: Glaphyropoma spinosum and a new species of Copionodon. We compared the data with those obtained of a sympatric epigean species, Copionodon pecten. These Trichomycteridae species belong to a basal and apparently monophyletic subfamily – Copionodontinae, endemic to Chapada Diamantina, central Bahia state, eastern Brazil. We observed the fishes in natural and laboratory conditions through ad libitum and focal animal methods. Each spatial behavioral category (hidden, bottom, midwater, surface and wall swimming and stationary in the bottom) was timed individually, with a sample of 12 specimens per species. Unlike most troglobitic fishes, cave copionodontines tested herein did not extend exploratory behavior to midwater, with benthonic and thigmotactic-related exploratory behavior. This behavior is possibly related to its feeding behavior specializations, strong territorialism and photophobic behavior. The epigean Copionodon species is also benthonic. The spatial behavior of the cave Copionodontinae could be interpreted as a retained and plesiomorphic character-state in relation to other trichomycterid catfishes.

Keywords
Spontaneous behavior, subterranean environment, Neotropical region, Glaphyropoma, Copionodon
Introduction

Stygobites are organisms restricted to the subterranean aquatic environment, which had evolved isolated in a peculiar selective regime, distinct from that of their ancestors: total absence of light, a tendency to environmental stability, lack of primary production and low energy intake (Culver 1982, Poulson and Lavoie 2000, Culver and Pipan 2009). In order to survive and effectively colonize the hypogean realm, subterranean organisms must reproduce, defend their territories and find food and mates in this environment, regardless of vision (e.g. Trajano 1989, Wilkens 1992, Moore and Sullivan 1997, Soares and Niemiller 2013). Several specializations related to life in the subterranean environment have been reported in literature - autapomorphies, called troglomorphisms (Arnold 1994).

These cave specializations include morphological troglomorphisms (for instance, reduction of eyes and melanic pigmentation and elongation of body and appendices) and behavioral ones (such as increase of midwater activity and reduction of photophobia, cryptobiotic behavior and circadian rhythms) (Trajano 2003). Among behavioral specializations, the spontaneous behavior is well studied for Brazilian troglobite fish (e.g. Trajano 1989; Trajano and Bockmann 1999; Bichuette and Trajano 2005; Bichuette 2003). Spontaneous behavior encompasses use of space, i.e., preference for microhabitats, spatial distribution (bottom, midwater or surface), burrowing, hiding and other behavior expressed in the absence of stimuli.

Considering cave fish with putative bottom-dwelling ancestors, living in an environment characterized by food scarcity, such as caves, it is beneficial to extend the spatial distribution, since it means increasing the probability of finding food in conditions of low prey densities, especially if associated with low predation rates and low competition (Trajano 1989). Thus, increasing midwater activity, as observed in catfishes and balitorids (Parzefall and Trajano 2010), could be considered adaptive (Trajano 2003).

In turn, the reduction of cryptobiotic behavior, namely, decreasing the hiding behavior, may be explained by the absence of visually guided predation in the hypogeal realm: the pressure for maintaining the hiding behavior would be relaxed, and the character would regress (Wilkens 1992). In this case, reduction of behavior traits could be analogous to regression of morphologic ones, such as eyes and pigmentation, and probably correspond to characters that became neutral in subterranean habitat (Wilkens 1992; Culver and Wilkens 2000; Trajano 2003).

Most troglobitic fishes belong to epigean taxa which show cryptobiotic behavior, and there is a general tendency among such troglobitic species towards reduction of cryptobiotic behavior along with an increase of midwater activity (Parzefall and Trajano 2010). Among Brazilian troglobitic fishes we observed this tendency for Heptapteridae catfishes Pimelodella kronei Miranda Ribeiro 1907 (Trajano 1989), Rhamdiopsis sp. from Toca do Gonçalo, Campo Formoso (= Tauxnayia sp.) and Rhamdiopsis krugi Bockmann and Castro 2010 (formerly cited as Rhamdiopsis sp. from Chapada Diamantina) (Parzefall and Trajano 2010).
Brazil has 25 species of troglobitic fishes, most of them belonging to the siluriforme families – Callichthyidae, Heptapteridae, Loricariidae and Trichomycteridae (Proudlove 2010, Trajano and Bichuette 2010). Among Trichomycteridae, there are two troglobitic representatives of the Copionodontinae subfamily, both in caves from Bahia State – *Glaphyropoma spinosum* Bichuette, Pinna and Trajano 2008 and a new species undescribed of *Copionodon*. Copionodontinae is a basal taxon within the Trichomycteridae, lacking many synapomorphies (that is, characters shared by two or more taxonomic groups and derived from a common ancestral species) previously used to diagnose the family (Pinna 1992). It is probably a monophyletic group, diagnosed by several unambiguous synapomorphies, including a unique dentition composed of spatulate teeth not seen anywhere else in Trichomycteridae (Bichuette et al. 2008). They are represented by five species in two genera, *Glaphyropoma* and *Copionodon* (Pinna 1992, Bichuette et al. 2008).

*Glaphyropoma spinosum* and *Copionodon* new species are syntopic and coexist in sandstone/quartzitic caves. Cases of cave fish coexistence are rare in global terms and present an excellent opportunity to compare behavioral studies, since intraspecific competition for space, feeding and/or mating territory may occur (Trajano et al. 2002). These two species were studied with focus on feeding and agonistic behavior and reaction to mechanical stimuli, comparing to a sympatric epigean species, *Copionodon pecten*. A previous study of phototactic behavior showed that *Glaphyropoma spinosum* is highly photophobic, even at low intensities (ranging from 40 up to 1,700 lux), while *Copionodon* sp. n. is photophobic under low light intensities (40 and 170 lux) and indifferent to high intensity (1,700 lux). The epigean related species, *Copionodon pecten*, showed indifference to light, even at high ones (Rantin and Bichuette 2013).

The comparison of the spontaneous behavior of Copionodontinae catfishes occurring in distinct habitats (subterranean and epigean typical headwaters streams) will bring more evidence to understand the possible modifications related to the adoption of a subterranean way of life.

In order to understand whether the observed spontaneous behavior consist of specialization traits for Copionodontinae, we considered its basal phylogenetic position within Trichomycteridae clade. Therefore, we established the following question: Do the cave copionodontine *Glaphyropoma spinosum* and *Copionodon* sp. n. show reduction of cryptobiotic behavior and/or extended spatial exploratory behavior?

**Materials and methods**

**Study area**

Copionodontine catfishes are endemic to Paraguaçu river basin, in Chapada Diamantina, situated in central Bahia, eastern Brazil (Fig. 1). The climate is tropical semi-arid, with distinct wet/dry periods, Aw category (tropical wet and dry) according to Köp-
pen’s (1948) classification. Rainy season is from November to March/April and the dry season between May and September (Brasil 1981).

The caves where subterranean copionodontines coexist are formed mainly by sandstone and quartzite rocks and are situated in the surroundings of Igatu village, within the limits of Chapada Diamantina National Park (CDNP) and are, therefore, protected by law. Specimens occupy waters with slow current, in pools and/or runs of subterranean streams (Fig. 2a, b). Apparently, the subterranean streams from Igatu do not have any connection with epigean rivers.

The epigean copionodontine, *C. pecten*, inhabits streams with interconnected pools of dark waters, with a high amount of organic matter, with sand, boulders and bedrock in the bottom (Fig. 2c).

**Studied species**

*Glaphyropoma spinosum* catfish show little intraspecific morphological variation: its cutaneous melanic pigmentation is homogeneous, light yellow to pale pinkish; the eyes are reduced and, in some individuals, covered by skin and not externally visible (Fig. 3).

*Copionodon* sp. n., on the contrary, presents a high degree of intraspecific variability in morphological traits: the degree of melanic pigmentation varies from pale gray to dark gray, sometimes with lateral and dorsal stripes. Eyes may vary from reduced and covered by skin to developed eyes as observed for epigean copionodontines. Occasionally, the left or the right eye is larger than the other, showing an individual asymmetry in this character-state (Fig. 4).

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**Figure 1.** Copionodontinae occurrence area. Map of Brazil, indicating the Chapada Diamantina region, endemic area of Copionodontinae catfishes. In green, the Chapada Diamantina National Park (CDNP), eastern Brazil. Author: Diego Monteiro Von Schimonsky.
Figure 2. Spatial behavior and natural habitats of copionodontine catfishes from Chapada Diamantina. a Glaphyropoma spinosum (black arrow) swimming close to the bottom in a pool (Photography: Maria Elina Bichuette) b small stream in the cave environment, place where Copionodon sp. n. occurs (Photography: Jonas Eduardo Gallão) c natural pool where Copionodon pecten forages under the day-light (Photography: Bianca Rantin).
The epigean species, *Copionodon pecten*, is clearly homogeneous in morphological traits (for morphological details see Pinna 1992): body is grayish in color, with iridescent spots and conspicuous lateral stripes; eyes well developed (Fig. 5).
Specimens collecting and laboratory maintenance

Copionodontinae catfish were hand-netted and transported in 3-liter containers, filled with water from the place of collection. The containers were conditioned in thermal boxes, with constant aeration, and low temperature was achieved with addition of recycled hypergel.

Intraspecific groups up to 10 individuals were maintained in 50-liter aquaria with continuous filtration, aeration and available shelters, made with sandstone rocks and big enough to fit the whole body. Aquaria were installed at the Laboratório de Estudos Subterrâneos (LES) at the Departamento de Ecologia e Biologia Evolutiva of Universidade Federal de São Carlos (UFSCar). Room conditions were manipulated to simulate aspects of cave natural environment, in order to maximize welfare and survival: it remained in permanent darkness (except during maintenance activities), water temperature was stabilized at 27 °C with air conditioning and pH was maintained near to natural habitat using rocks brought from the collection site. Epigean Copionodontinae were also maintained in the LES, but in a separate compartment, covered with a black shield, where a lamp attached to a timer simulated the daily light cycle (12:12 light/dark hours). The catfish were fed once a week with commercial food for carnivorous fishes (dry or frozen *Artemia salina*) at no fixed days and times.

Figure 5. *Copionodon pecten* – fixed specimen, left lateral view and dorsal view (Photography: Pedro Pereira Rizzato). Standard length: 43.2 mm.
Animal rights

There are no specifications regarding behavior research in Brazilian Animal Ethics Law (11.794/2008). However, our financial support (Fundação de Amparo à Pesquisa no Estado de São Paulo (FAPESP - a public sponsoring agency) approved and supported our research (process numbers 2008/08910-8 and 2008/05678-7). We also have a collection and transportation permission (license number 20165-1), provided by the Brazilian environment governmental agency ICMBIO (Instituto Chico Mendes de Biodiversidade). Furthermore, experiments were delineated prior to collection, in order to maximize the scientific value of individuals captured: reaction to mechanical stimuli, feeding, spontaneous and phototactic behavior (Rantin and Bichuette 2013) and were also assessed.

Field observations

The study of behavior in natural environment was performed by direct observations in four field trips: May 2009, August 2009, May/June 2010 and August 2010 (Fig. 2c), with a total of 40 hours. Behavioral categories exhibited in natural habitat were posteriorly compared with laboratory observations.

In the field, the focal-animal-method was used when individuals were found isolated in pools. Whenever in groups, fish were observed by *ad libitum* mode (Altmann 1974). Their behavior was classified into categories, in order to glimpse the species general pattern behavior in nature. These behavioral categories were based in protocols proposed in other studies (e.g., Buck 2000): A. hidden (hiding behavior on under blocks and striped pebbles), B, bottom swim (swimming with ventral region touching substrate); C, midwater swim; D, surface swim and E, stationary (at the bottom). Additionally, we observed gregarious (group formation) and solitary behavior.

Environmental physical characteristics were also recorded: fast or slow water current; bottom formed by silt, sand, gravel, pebbles, rock boulders and/or blocks. Studied individuals were observed/captured on/from the following localities: Parede Vermelha cave, Rio dos Pombos cave, Torras cave, Morro de Alvo cave, Coisa Boa river and Xavier river.

Spontaneous behavior analysis

Spontaneous behavior tests were performed a month after the fish collections. Twelve adult specimens from each studied species were tested individually: the troglobites – *Glaphyropoma spinosum* and *Copionodon* sp. n.; the epigean – *Copionodon pecten*.

Each specimen was placed in a 50 l test-aquarium, with a single den, with no filtering or aeration systems. After one hour of acclimatization, we started the tests,
always between 8:00 am and 6:00 pm. The observations were conducted under the same luminosity, for one hour. Considering that the epigean species is indifferent to light, and that both cave species react the same towards low light intensities (both are photophobic), a 140 lux light intensity was used (enough for visualization of behavioral units) for the tests.

The behavioral categories were divided as follows: A. hidden (at least the anterior part of the body covert, inside den, with no attempts to dig the substrate), B. bottom swim, C. midwater swim (swimming in water column), D. wall swim (ventral region touching walls), E. surface swim and F. stationary (in the bottom, outside of the den). Swimming in water column was separated from swimming in wall due to field observations: these animals are used to swim close a reference (bedrock in the bottom or walls), but rarely in water column. The time spent by each fish in each behavioral category was summed.

**Statistical analysis**

The time of each behavioral category were organized in tables and individual estimated frequencies graphs. To verify variability in categorical data we made a box-plot showing medians and standard deviations of frequencies. In order to analyze the similarity between behavioral patterns, that is, whether the proportions (time spent in each behavioral category) are the same in different species, a one-way ANOVA was performed, followed by post-hoc Tukey’s pairwise comparisons. We used frequency means for calculations and reliability index of 0.05 (Zar 1996). Statistical analysis was performed using PAST software (Hammer et al. 2001) and box-plot graph with Statistica software (version 12.0).

**Results**

**Field observations**

Table 1 presents the compared environmental and behavioral characteristics showed by copionodontine species.

The habitats of the troglobitic species are similar considering the physical characteristics. Water was generally dark/reddish, due to high levels of organic matter (Fig. 2a, b). Both solitary individuals and groups of fish, even in distinct caves, presented the same behavioral pattern: calm and exploratory swimming at the bottom (Fig. 2a), sometimes outside of the dens (exposed), or hidden between pebbles.

Regarding the epigean species, *Copionodon pecten*, we also observed similar physical characteristics in Coisa Boa and Xavier rivers. Both solitary and gregarious fish exhibited basically the same behavioral pattern: calm and exploratory swimming, mainly at the bottom, exposed or under a shelter formed by bedrock (see the habitat at Fig. 2c).
Table 1. Description of habitat and space use by Copionodontinae. General description of habitat and space use of Copionodontinae catfishes from Chapada Diamantina, eastern Brazil. O.M., organic matter.

<table>
<thead>
<tr>
<th>Troglobites (Glaphyropoma spinosum and Copionodon sp. n.)</th>
<th>Epigean (Copionodon pecten)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Habitat</strong></td>
<td></td>
</tr>
<tr>
<td>Pools with 0.2 to 0.5 m of depth. Water current from weak to moderate. Fig. 2a, b.</td>
<td>Pools or river stretch with 0.2 to 0.7 m of depth. Water current from weak to moderate. Fig. 2c.</td>
</tr>
<tr>
<td><strong>Bottom river</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Activity</strong></td>
<td></td>
</tr>
<tr>
<td>Stationary or exploratory swimming, sometimes counter-current.</td>
<td>Preference for exploratory swimming, sometimes counter-current or stationary.</td>
</tr>
<tr>
<td><strong>Spatial distribution</strong></td>
<td></td>
</tr>
<tr>
<td>Exposed in the bottom or hidden under pebbles, seldom in midwater, preference for shallow waters.</td>
<td>Exposed in the bottom or hidden under the shelf formed by bedrock, seldom in midwater, preference for shallow waters.</td>
</tr>
<tr>
<td><strong>Gregarious/solitary habits</strong></td>
<td></td>
</tr>
<tr>
<td>Solitary or in small groups.</td>
<td>Groups from three to 70 individuals, seldom solitary.</td>
</tr>
</tbody>
</table>

Spontaneous behavior

Statistical analysis shows that the space use behavior between copionodontine species is significantly different (F=16.78, p<0.0005), result corroborated by post hoc tests Tukey test (Table 2).

Time frequencies for each behavioral category, resultant from the spontaneous behavior, were organized in graphs of frequencies (Fig. 6). We produced a box-plot graph to explore the intraspecific/individual variation in these time frequencies (Fig. 7). The troglobitic Glaphyropoma spinosum exhibited the lowest variability (Fig. 6a, 7) in almost all categories, with exception to the hidden category, which was extremely wide and variable (Fig. 7). Copionodon sp. n. and the epigean Copionodon pecten, however, had higher intraspecific/individual variation (Fig. 6b, c) and wider data distribution (Fig. 7), with few outliers. All individual timings were summed up and are shown in Table 2.

Glaphyropoma spinosum is the most cryptobiotic species, having an evident preference for the hidden behavior category (76.9%, Table 2 and Fig. 6a). Only a single specimen did not show such behavior, remaining stationary almost all the time (specimen 11 - 98.5%). This species did not display midwater swimming.

In a general way, as observed for Glaphyropoma spinosum, the new Copionodon species also showed a hiding tendency (Table 2 and Fig. 6b – 44.4%). Nevertheless, it also remained for a great part of the time swimming in contact with the substrate (bottom + wall = 49.6%). If calculated together, bottom and wall swimming exceeds the time spent hiding, indicating a preference for swimming with the ventral region touching the substrate, a typical benthonic behavior.

The epigean Copionodon pecten also spent much time swimming in contact with the substrate (bottom + wall = 39.4% – Table 2) and hidden (32.6%). However, just like Copionodon sp. n., it shows a slightly preference for swimming with the ventral region touching the substrate. This species did not display midwater swimming.
Figure 6. Individual frequencies of the behavioral categories recorded for copionodontine catfishes from Chapada Diamantina. Dark blue, hidden; light blue, stationary; dark green, bottom swim; light green, wall swim; dark yellow, midwater swim; light yellow, surface swim a Glaphyropoma spinosum b Copionodon sp. n. c Copionodon pecten.
Table 2. Frequencies of behavioral categories. Frequencies (%) of behavioral categories for Copionodontinae catfishes from Chapada Diamantina, eastern Brazil.

<table>
<thead>
<tr>
<th>Species</th>
<th>Hidden</th>
<th>Bottom swim</th>
<th>Wall swim</th>
<th>Midwater swim</th>
<th>Surface swim</th>
<th>Stationary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glaphyropoma spinosum</td>
<td>76.9</td>
<td>5.3</td>
<td>3.4</td>
<td>0</td>
<td>0</td>
<td>14.4</td>
</tr>
<tr>
<td>Copionodon sp. n.</td>
<td>44.4</td>
<td>20.1</td>
<td>29.5</td>
<td>3.5</td>
<td>0.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Copionodon pecten</td>
<td>32.4</td>
<td>24.7</td>
<td>14.7</td>
<td>0</td>
<td>1</td>
<td>27.2</td>
</tr>
</tbody>
</table>

Discussion

Most studied troglobitic fish with bottom-dwelling ancestors, show a trend in increasing midwater exploratory behavior in parallel with decreasing cryptobiotic behavior, that are considered behavioral specializations to cave life (Trajano 1993, Parzefall and Trajano 2010). However, both cave copionodontines herein studied presented cryptobiotic and benthonic behavior. The same pattern (strong cryptobiotic and benthonic) was described for other heptapterid cave catfishes: *Pimelodella spelaea* Trajano, Reis and Bichuette 2004 and a species of *Rhamdia* from northeastern Brazil (Bichuette and Trajano 2005). Considering the cryptobiotic behavior, three populations of the
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...armored cave catfish *Ancistrus cryptophthalmus* show burrowing behavior (Bessa and Trajano 2001), resembling that observed for subterranean species of Copionodontinae studied herein.

The strong cryptobiosis presented by the subterranean species *Glaphyropoma spinosum* is corroborated by field and laboratory observations, where only rarely the specimens were observed exposed, stationary on the aquarium bottom, and seldom exploring walls and water column. Despite the fact that hiding is a well-known strategy against visually oriented predators, it is not an effective tactic in environments with absence of light, such as caves. Moreover, in a high intraspecific competition situation hiding behavior may be disadvantageous, since it increases the time of search of food (Trajano 1989). However, *G. spinosum* cryptobiotic behavior may be related to the strong territorialism and photophobia. In fact, dens are apparently highly valuable resources, since it consists of one of the determinant factors for establishing dominancy in *G. spinosum*, maybe allied to spawning periods (hypothesis to be tested), which always displays patrolling behavior as observed in laboratory.

For *Copionodon* sp. n., despite the great intraspecific variation, some behavioral patterns were detected, also exhibiting cryptobiotic and benthonic behavior. The same was described for troglobitic trichomicterid *Ituglanis passensis* Fernández and Bichuette 2002, *I. bambui* Bichuette and Trajano 2004 and *I. ramiroi* Bichuette and Trajano 2004 (Bichuette 2003). Despite the weak surface or midwater exploratory behavior, *Copionodon* sp. n. displays an extended space use when compared to *G. spinosum*, since it expands its exploratory behavior to the aquaria walls. Phototactic and agonistic tests corroborate this trend: *Copionodon* sp. n. is less photophobic than *G. spinosum*, and less territorial as well.

With respect to the variability in the spontaneous behavior, it is clear that *Glaphyropoma spinosum* shows less intraspecific variation than *Copionodon* sp. n., also observed in their morphology: *G. spinosum* is more homogenous regarding melanic pigmentation and eyes size than *Copionodon* sp. n. The same trend was observed in phototactic behavior tests, in which *G. spinosum* exhibited very small individual differences (Rantin and Bichuette 2013).

The strong benthonic behavior displayed by the epigean species, *Copionodon pecten* could be related to its foraging behavior and diet. Zanata and Primitivo (2013) observed a shift in *Copionodon pecten* diet and feeding behavior according to its ontogenetic stage: juveniles swim more on water column, feeding on drifting algae and other items; adults, on the other hand, are mainly benthonic, scratching algae and other items from substrate. Our laboratory observations were made only with adult specimens and we observed the same pattern. However, unlike proposed by Zanata and Primitivo (2013) we also observed many juveniles of *C. pecten* foraging in the substrate in field, with strong benthonic behavior.

Moreover, in a general way, it seems that the walls/substrate play an important role on the copionodontines’ exploratory behavior: they probably use positive thigmotaxis, that is, the tendency to remain close to surfaces, to better choose where/how to explore the environment. Whenever exploring away from the den, all species preferred
to swim on aquarium walls, instead of extending it to midwater (“open field”), that is, they probably use thigmotaxis to choose the most secure areas of the test aquarium to explore. The highly photophobic spring cave fish *Chologaster papilliferus* Forbes, 1882 also uses thigmotactic responses to find secure dens, and usually seeks several levels of depth in order to carry out the touch responses (Weise 1957).

Therefore, *G. spinosum* and *Copionodon* sp. n. are cryptobiotic and benthonic, unlike most cave fish. Whether this pattern was retained from ancestors or consist of new acquisitions, such behaviors are probably important to help these species to cope with cave pressures and to successfully colonize the subterranean environment: both species form great groupings, with extremely high population densities (Bichuette 2012).

It is noteworthy that, unlike the great majority of troglobitic fish, the subterranean copionodontines, *Glaphyropoma spinosum* and *Copionodon* sp. n., alternate solitary/gregarious habits. The sympatric epigean *Copionodon pecten* is rarely observed alone, usually only when trapped in isolated pools (Zanata and Primitivo 2013). The spring cave fish *Chologaster papilliferus* display gregarious behavior, explained by a thigmotactic response: the specimens use this positive sthigmotactic behavior in order to recognize other individuals (sexual dimorphism in head and pelvis), and apparently, this is dependent on their sensory papillae distributed on body and mainly on head (Weise 1957). However, so far, most Brazilian cave catfishes are believed to be solitary, a plesiomorphic condition to some species (such as *Rhamdiopsis krugi* – Trajano and Bockmann 1999) and an autapomorphy for others, like *P. kronei* (Trajano 1989). Our results show that the comparisons must be more specific always considering the phylogenetic context.

Considering a more inclusive comparative context (Copionodontinae clade), cave copionodontine spontaneous behavior cannot be interpreted as specialization to isolation in the cave environment, since they do not present extended spatial exploratory behavior allied to reduction of cryptobiotic behavior, as observed for many cave fishes (Parzefall and Trajano 2010). Moreover, it is possible that after isolation in the subterranean environment, *Glaphyropoma spinosum* and *Copionodon* sp. n. retained the benthonic behavior and their spontaneous behavior can be interpreted as a plesiomorphic character-state in relation to Trichomycteridae clade, in according to the observed for the morphological traits, observed by Pinna (1998).

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