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Are laboratory studies on behavior of troglobitic species always trustful? A case study with an isopod from Brazil

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ABSTRACT

There is a huge lack of information regarding the natural history of subterranean species, particularly focusing on aspects of the behavioral ecology of Brazilian cave fauna. In the present work, we aimed to describe and evaluate the behavioral repertoire of *Xangoniscus itacarambiensis* (Isopoda, Styloniscidae) through observations in the field and laboratory and also by means of complementary experiments. Overall, we recorded 25 spontaneous behaviors. Besides describing the physical habitat, we recorded some intraspecific interactions, agonistic and territorial behaviors, as well as the amphibian habit. There was a direct relationship between the size of travertine dams where they live (measurements of length and width) and the mean number of individuals, although there was no significant correlation with the pool depth. Behaviors observed in the laboratory differed qualitatively and quantitatively from those observed in the field, with individuals more active in the latter. This scenario alerts about the significant behavioral alteration of such isopods when removed from their natural habitat, what must be considered in future behavioral studies including troglobites given their natural sensitivity to environmental changes.

1. Introduction

The typical conditions of subterranean environments such as stable temperature and high moisture, as well as darkness and oligotrophy lead to the establishment of a peculiar fauna, which is specialized and many times restricted to the underground habitats (Culver 1982; Culver and Pipan 2009). The higher environmental stability of subterranean systems when compared to surface ones may influence both ecologically and/or evolutionarily the behavior of organisms that inhabit them. For instance, Hoenen and Gnaspini (1999) observed that epigean species of harvestmen carried food away to feed, while cave species feed where they found the food, which behavior may be attributed to the food scarcity typical of subterranean environments.

The great majority of works regarding behavioral aspects of cave species are conducted in laboratories instead of natural conditions, both due to the risks inherent of the continuous permanence in caves and also due to logistic difficulties for long-term observations in caves. Some subterranean crustaceans like remipedes (Carpenter 1999, Koenemann et al. 2007), crayfishes (Li and Cooper 2002), amphipods (Borowsky 2011; Fišer et al. 2016) and isopods (Harris et al., 2011; Harris et al. 2013) have been studied in laboratories in order to elucidate their behavior mainly regarding feeding, escape and phototatic responses.

Hervant and Renault (2002) studied the responses of hypogean and epigean isopods to food stress and renutrition and proposed an adaptive strategy adopted by these subterranean organisms. Besides physiological traits that provided a longer resistance to fasting for hypogean species, they presented better abilities during refeeding by means of an optimum utilization of the available food and rapid restoration of their body reserves.

In Brazilian caves, most of studies on the behavior of subterranean species were conducted with fishes (Trajano and Bockmann, 2000; Trajano and Menna-Barreto 2000; Bichuette and Trajano 2005; Secutti and Trajano 2009; Rantin and Bichuette 2015) and harvestmen (Machado 2002; Willemart and Gnaspini 2004; Chelini et al., 2012), although there are some studies on other taxa like Schizomida (Oliveira and Ferreira 2014) and Palpigradi (Ferreira and Souza 2012). For Brazilian cave crustaceans, the only work known was conducted by Souza et al. (2015), who recorded for the first time the construction of mud shelters by an Oniscidea, the troglobitic isopod *Iuiuniscus iuiuensis* Souza et al., 2015 (Styloniscidae).

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Among the several invertebrates that occur in Brazilian subterranean systems, isopods are frequently observed in this environment, which currently have 39 recorded species from 10 families (Armadillidae, Armadillidiidae, Dubioniscidae, Philosciidae, Platyarthridae, Porcellionidae, Scleropactidae, Styloniscidae, Trichoniscidae, Pudeoniscidae). Thirteen of them are considered troglobitic, from which six belong to the family Styloniscidae (*Spelunconiscus castroi, Iuiuniscus iuiuensis, Xangoniscus aganju, X. odara, X. itacarambiensis* and *Cylindroniscus flaviae*) (Campos-Filho et al. 2014; Souza et al. 2015; Campos-Filho et al. 2016, Bastos-Pereira et al. 2017, Campos-Filho et al. 2017a, 2017b, Cardoso et al., 2018).

Xangoniscus itacarambiensis Bastos-Pereira et al., 2017, the target of the present study, represents a recently described troglobitic species (Bastos-Pereira et al. 2017), for which there is only taxonomic data available in the literature. We aimed to provide preliminary information on the behavioral ecology of this species that inhabit exclusively the Olhos D'Água cave. We described the habitat and distribution of the population along the cave and quantified spontaneous behaviors both in the field and in the laboratory. Our hypothesis is that behavioral differences are observed between these two environments given the sensitivity of the studied species, which is inherent of troglobites.

2. Methods

2.1. Study area

We conducted the study at Olhos D'Água cave, which is located in the National Park Cavernas do Peruaçu, state of Minas Gerais, Southeastern Brazil. The park is inserted in a transitional region between the biomes Cerrado (Brazilian Savannah) and Caatinga, a semi-arid biome (Ab'Saber, 1977). The climate is hot tropical with mean annual temperature of 24 °C and mean annual rainfall of 876.7 mm. The climate is marked by two well-defined seasons in which the rainy period lasts from November to April (corresponding to the hottest months), while the dry season (which is considerably severe) lasts from May to October (IBAMA 2004).

The Olhos D'Água cave (15º6.813′ S 44°10.176′ W) has about 9 K m of galleries. The main conduct, where the Olhos D'Água stream runs, presents about 5 K m length. The main cave entrance corresponds to the spring. Travertine dams are found in different points throughout the cave. The studied organisms were found in two sets of travertine dams present in the first 300 m of the cave: the upstream travertine (compounded by only one pool) and the downstream travertine set (formed by eight pools) (Fig. 1).

There are six described troglobitic species for Olhos D'Água cave: one fish (*Trichomycterus itacarambiensis* Trajano & de Pinna, 1996), one Amblypygi (*Charinus eleonorae* Baptista & Giupponi, 2003), two Opiliones (*Iandumoema uai* Pinto-da-Rocha, 1996 and *Relictopiolus galadriel* Pérez-González, Monte and Bichuette, 2017) and one cricket (*Endecous peruassuaensis* Bolfarini and Bichuette, 2015) and the currently studied *X. itacarambiensis*. Furthermore, there are at least three species of Colembola, Polydesmida and Isopoda (Styloniscidae) to be described.

2.2. Field observations

We conducted four visits to the Olhos D'Água cave on December 2006. The travertine dams were characterized by measuring the temperature (ºC), maximum depth (cm), maximum width (m) and maximum length (m) and also counting the individuals of *X. itacarambiensis* and other species eventually present in the pools (Fig. 2).

It was not possible to record data blind because our study involved focal animals in the field. The focal-animal method was used to describe the isopods behavior, such as interactions, agonistic relationships and territorial behavior. In this method, all the specific behaviors of one individual are recorded during each sampling period and records are made during the time in which the sampled organism remains in such behavior. We used *ad libitum* observation (Altmann, 1984) as complementary method for the analysis of the isopods general behavior, which corresponds to a method without restrictions in relation to what has been recorded and how long it has been observed, thus allowing opportunistic observations (Martin and Bateson 1986).

Firstly, we qualified all the spontaneous behaviors and interactions based on 12 individuals observed during 30 min each one. The qualitative behaviors were then categorized as "continuous" or "punctual" based on these previous observations. Posteriorly, we chose ten other individuals for which we quantified the behaviors during 30 min of observation. The time of each specific behavior was counted with the aid of a chronometer. Three researchers were present during the observations: one observed and described the behaviors, the second recorded the time spent on each behavior and the third was responsible by writing all the records.

As continuous behavior one can understand those conducts that lasted a minimum period that could be measured, while punctual behaviors were those rapidly performed acts. The punctual behaviors were firstly classified because of their impracticable measurement and also because they eventually corresponded to activities concomitant to

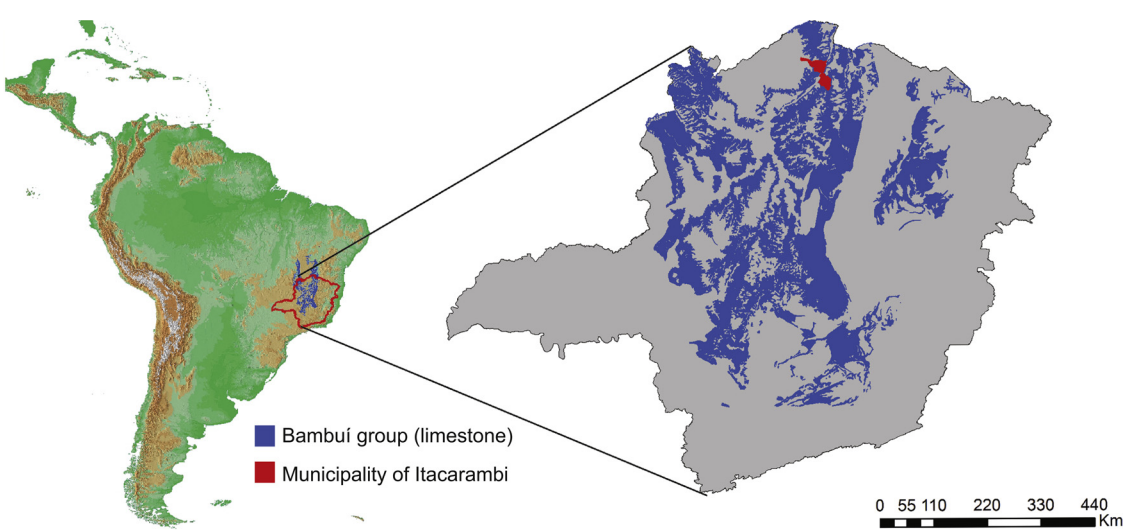

Fig. 1. South America, with the Brazilian state of Minas Gerais highlighted in red. At right the Bambuí limestone group in the state of Minas Gerais is represented in blue. The red region in the Northern Minas Gerais indicates the municipality of Itacarambi, where the National Park Cavernas do Peruaçu is located (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).