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Biology and behavior of *Eigenmannia vicentespelaea*, a troglobitic electric fish from Brazil (Teleostei: Gymnotiformes: Sternopygidae): a comparison to the epigean species, *E. trilineata*, and the consequences of cave life

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We compared the behavior, including spatial distribution, reaction to stimuli, activity phases, and agonistic interactions, as well as diet and reproduction, of the troglobitic Eigenmannia vicentespelaea and that of its epigean relative, E. trilineata, both from São Domingos karst area, central Brazil. We utilized ad libitum underwater observations, complemented by physicochemical water variables, habitat descriptions, and collections of specimens. Differences in behavioral aspects include the absence of cryptobiotic habits and an extended spatial and temporal activity in E. vicentespelaea that were not present in *E. trilineata*, and the foraging angle, which was approximately 30° in E. vicentespelaea and 90° in E. trilineata. The agonistic behaviors recorded for the troglobitic E. vicentespelaea could be related to the preservation of a character state that is present in its epigean relatives. Eigenmannia vicentespelaea and E. trilineata might be considered benthophagous invertivores, similar to other Eigenmannia electric fishes, with no evidence of seasonality in the volume and diversity of prey items in their stomachs, suggesting that there is a food spectrum common to the two species. Both epigean and troglobitic *Eigenmannia* fish from Goiás reproduce during the dry season, with no indication of reproductive peaks during this period.

Keywords: cave fish; feeding; reproduction; spatial distribution; threatened species

Introduction

Neotropical electric fishes, Order Gymnotiformes, are an important component of the night-active ichthyofauna from South and Central America. Because of their ability to utilize electrolocation for topographic orientation, feeding, and social communication, these fishes are able to colonize permanently darkened habitats, such as highly turbid waters and the bottom of large Amazonian rivers (Albert and Campos-da-Paz 1998; Albert 2001), as well as the subterranean (hypogean) realm.

Gymnotiforms have been recorded in several Brazilian caves (Bichuette and Trajano 2003; Mattox et al. 2008). The majority are most likely troglophiles, i.e. species with source populations in both hypogean and epigean (surface) habitats, with individuals regularly moving between them, promoting the introgression of genes selected under epigean regimes into subterranean populations (Trajano 2012). Up to now, the only electric fish reported as being a troglobite (species with exclusively subterranean source populations; Trajano 2012) is the sternopygid *Eigenmannia vicentespelaea* Triques, 1996, from the São

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Domingos karst area, in central Brazil. This species has an absence of eyes and noticeably reduced melanic cutaneous pigmentation (Bichuette and Trajano 2006), with these character states being classical troglomorphisms observed in many restricted subterranean organisms (*sensu* Christiansen 2012).

Few data on the basic aspects of gymnotiform natural history have been reported till date, with the majority considering aspects of reproduction biology and feeding regimes (Lissmann 1961; Steinbach 1970; Hagedorn 1988; Westby 1988; Alves-Gomes 1997; Crampton 1998; Giora et al. 2005; Giora and Fialho 2009; Schaan et al. 2009; Giora et al. 2011). Recently, Bichuette and Trajano (2015) published a study discussing the population aspects of the troglobitic *E. vicentespelaea*, which is considered the first study to focus on an electric fish population living in a cave environment.

Among the approximately 170 troglobitic fishes recorded in the world (Proudlove 2010; G. Proudlove, pers. comm.) and approximately 30 in Brazil (M. E. Bichuette, pers. obs.), only a few have been studied in detail with focus on behavior, feeding, and reproductive traits (Trajano et al. 2010; Rantin and Bichuette 2013; Simões et al. 2013; Rantin and Bichuette 2015). However, such knowledge is not scientifically relevant for the establishment of conservation policies.

Considering the behavioral aspects, the majority of Brazilian subterranean fishes have been investigated in the laboratory. These studies have focused on spontaneous feeding and reproductive behavior, reaction to light, chemical communication, social interactions and agonistic behavior, and biological rhythms, which have allowed for detection of caverelated patterns. The majority of species that have been investigated include Heptapteridae, Trichomycteridae, and Loricariidae catfishes (Siluriformes), and the only South American troglobitic characiform, *Stygichthys typhlops* Brittan & Böhlke, 1965 (Trajano and Bockmann 1999; Parzefall and Trajano 2010; Rantin and Bichuette 2013; Trajano et al. 2009, 2012). However, data from direct observations in the species' natural habitats are still scarce, and few studies have included comparisons with closely related epigean species. This imposes limitations to evolutionary interpretations, which has been seen in Rantin and Bichuette (2015) study on Copionodontinae catfishes (Trichomycteridae). The present study is, to our knowledge, the first to focus on behavior in the natural habitat and biological aspects of the troglobitic cave electric fish *E. vicentespelaea*.

Most cave life specializations involve character regression, frequently assigned to the loss of function or to the relaxing of stabilizing selective pressure (Wilkens 1992). Besides the classic troglomorphisms (i.e. changes in morphological characters), including regressions in the eyes and melanic pigmentation until total loss of these characters occurs, there are also regressions of several behavioral patterns (Parzefall and Trajano 2010). Therefore, subterranean organisms are excellent models for studies of ecology/behavior and evolution (Trajano and Bockmann 1999).

The São Domingos karst area, Upper Tocantins River basin, central Brazil, is identified as a location having high diversity of troglobitic fishes, with seven troglobitic species recognized so far. Biological and behavioral aspects have been studied for five of them: *Ancistrus cryptophthalmus* Reis, 1987 (Trajano and Souza 1994; Bessa and Trajano 2002), *Ituglanis passensis* Fernández & Bichuette, 2002, *I. bambui* Bichuette & Trajano, 2004, *I. ramiroi* Bichuette & Trajano, 2004 (Bichuette 2003), and *E. vicentespelaea* (Bichuette and Trajano 2015). *Eigenmannia vicentespelaea*, which is the focus of this present study, is under threat and considered vulnerable (category VU D2 on the Brazilian Red List) (Bichuette 2008; Gallão and Bichuette 2012). This fish occurs in a single cave system in the São Domingos karst area and shows low population densities (Bichuette and Trajano 2015).

(b)

Figure 1. *Eigenmannia vicentespelaea* in the natural habitat (a) river gallery showing the sinkhole of the São Vicente Cave system (b) São Domingos karst area, Goiás state, central Brazil. Source: (a) M.E. Bichuette; (b) A. Gambarini.

Here, we present data on behavior observed in the natural cave habitat (spatial distribution, feeding tactics, reaction to light and mechanical stimuli, and activity phases, and agonistic interactions) and on biological aspects studied in the laboratory (diet spectra and reproduction) of *E. vicentespelaea*, compared to the epigean species, *E. trilineata* López & Castello, 1966, which occurs in the same region. The following questions have been proposed: 1. Is there a spatial extension of the habitat in the troglobitic fish compared to that of its epigean relative? 2. Do *E. vicentespelaea* fish show reduced cryptobiotic habits and photonegative reactions as observed in other troglobitic fishes? 3. Do *E. vicentespelaea* fish show reduced mechanical stimuli reactions? 4. Do *E. vicentespelaea* fish show differences in behavior, specifically agonistic interactions, compared to *E. trilineata*? 5. Are the diet spectra of the two species similar? 6. Are the reproduction aspects of *Eigenmannia* species seasonal?

Materials and methods

Study site

The study area is located in the São Domingos karst area, within the limits of the Terra Ronca State Park (46°10′–46°30′ S, 13°30′–13°50′ W), in the Upper Tocantins river basin, state of Goiás. The vegetation is the Cerrado phytophysiognomy (savannah-like vegetation) and the climate is tropical semi-humid, with 5–6 dry months (Nimer 1989).

Eigenmannia vicentespelaea (Figure 1(a)) has been recorded in two caves from a single integrated cave system: São Vicente I cave, upstream, and São Vicente II cave, downstream, separated by a 500 m long epigean stream reach. We studied the accessible population of *E. vicentespelaea* from the São Vicente II cave $(13^{\circ}58'37'' \text{ S}, 46^{\circ}40'04'' \text{ W})$ at the cave entrance, which is the stream sinkhole. For the majority of its extent, this is a fast-flowing stream with strong currents and has water spilling over rocky blocks, gravel, and sandy patches (Figure 1(b)). The water is limpid and transparent, which is favorable for underwater observations.

Eigenmannia trilineata electric fish are found in the epigean reach of the Rio da Lapa (13° 38'44" S; 46° 38' 08" W), upstream of the sinkhole in the Terra Ronca cave, and in

(a)



Figure 2. Local observation of *Eigenmannia trilineata* at Rio da Lapa, São Domingos karst area, Goiás state, central Brazil. Source: M.E. Bichuette.

several subterranean stream reaches of the São Mateus III and the Angélica caves (Bichuette and Trajano 2003, 2015), which belong to different microbasins in the São Domingos karst area. Near the sinkhole, moderate water currents characterize the Rio da Lapa and the bottom substrate is formed from sand, boulders, and rocky blocks, along with logs and branches, and large quantities of vegetal debris at the margins.

Behavioral observations

Underwater observations of *E. vicentespelaea* and *E. trilineata* were carried out from July 1999 to August 2001 by the first author, using snorkel, mask, and diving spotlights with light intensity of approximately 100 lux (halogen light) at the water surface. A 12 m transect, with 1 m average depth and an area of 18 m² was established in the São Vicente II cave stream for *E. vicentespelaea*. This transect was located 600 m downstream from the cave entrance, in the aphotic zone, with vegetal debris on the silty bottom (Figure 1(a)).

The sampled transect area for *E. trilineata* in Rio da Lapa was 40 m long, with an average width of 10 m and depth varying from 0.8 to 1.5 m, and was located 100 m upstream from the large entrance of the Terra Ronca cave (the stream sinkhole). The bottom substrate was composed of silt, sand, gravel, and branches, with logs carried by floods and rocky blocks at the margins (Figure 2). A rope was anchored just upstream of the sampled area to keep the observer in position.

Eigenmannia vicentespelaea was observed randomly (five hours of free-running observations, day and twilight phase), and *E. trilineata* was observed during twilight (crepuscule) and the first hours of the nocturnal period of the daily cycle (from 5:20 to 11:50 pm). The animal-focal method was used (Altmann 1974), with variable sampling times, owing to difficulties in keeping account of some of the individuals.

The following behavioral categories were recorded for both species, based on Trajano (1989, 1991a) and Rantin and Bichuette (2015): cryptobiotic habits (hidden under trunks, rocks, or neither); spatial distribution (bottom, midwater, or surface); reaction to light and mechanical stimuli; foraging behavior (body angle in relation to the bottom and feeding

tactics); agonistic interactions (submissive and aggressive components), and gregarious habits (groups of fishes swimming close and interacting or not interacting). Physical characteristics of the habitat were also recorded: water current (slow, moderate, or fast) and river bottom complexity (silt, sand, gravel, boulders, rock blocks, and presence of vegetal debris).

To quantify potential variations in the activity of *E. trilineata* within the nighttime period, on two occasions (April and August 2001) a transect 20 m long and 5 m wide was established in the epigean stream reach of Rio da Lapa, where the number of active specimens (swimming or foraging on the bottom) was counted every ten minutes between 5:20 and 5:40 pm (just before sunset) and again at 11:50 pm. Physicochemical variables–pH, conductivity, dissolved oxygen, temperature, and salinity–were recorded hourly using an Horiba apparatus (model U50) for Rio da Lapa. Activity data are presented in bar graphs.

Feeding habits and reproduction

Owing to their high degree of endemism and low population densities, which are associated with a decreased capacity for recovery from population losses (Bichuette and Trajano 2010), many troglobites are highly vulnerable to disturbance. This is the case for *E. vicentespelaea*, which has an estimated abundance of only 270 ± 89.1 individuals in the entire São Vicente II cave area (Bichuette and Trajano 2015).

Therefore, to obtain information on diet and reproduction aspects, we sampled 14 specimens of *E. vicentespelaea* from the São Vicente II cave (collected from May 1999 to August 2001), and 11 specimens of *E. trilineata* from Rio da Lapa (from July 1999 to May 2001, excluding May 2000). This number was sufficient to determine their food spectrum and to verify differences in the gonadal conditions within the dry seasons; in view of the low variation of food items among individuals, these numbers were considered adequate. Fish were euthanized via a benzocaine overdose, fixed in 10% formalin for a minimum of three days, preserved in 70% ethanol, and dissected to determine macroscopically the stage of gonadal development and to analyze the stomach contents.

Prey items in the stomach were identified to the lowest taxonomic level possible with the aid of specialized literature: Pennak (1978), and Borror et al. (1989). Stomach contents were analyzed via frequency of occurrence, percentage composition (Hynes 1950), and volumetric frequency (measured in mm³ and transformed to mL unit) methods (Luiz et al. 1998). The feeding index, which is a compound method expressed as percentages proposed by Natarajan and Jhingran (1961) and modified by Kawakami and Vazzoler (1980) and Hahn et al. (1997) was then calculated.

Sex and maturation stage were recorded by direct observation of the gonadal morphology: immature (IM), gonads occupying less than a third of the abdominal cavity; undergoing maturation (InM), gonads occupying between one third and two thirds of the abdominal cavity; and mature (MA), gonads occupying more than two thirds of the abdominal cavity and with visible ovocytes in the females (*sensu* Vazzoler 1996).

Results

Table 1 shows behavioral aspects and habitat preferences of the two species studied. Both species form small groups with polarized swimming, showing a gregarious habit. Remarkable reactions to flashes from the diving spotlights were recorded, with the cave fish always exhibiting an escape reaction, moving backward very quickly (10–12 s after the flashes), then turning their body 180° and swimming away from the light, while the epigean fish

	Eigenmannia vicentespelaea	Eigenmannia trilineata	
Habitat	Slow to moderate water current	Slow to moderate water current	
Substrate	Clay/sand/vegetal debris	Clay/sand/rocky blocks/vege- tal debris	
Activity phase (observed)	11:00 am to 4:00 pm	Bimodal: 6:00 to 7:00 pm and 10:00 to 00:00 pm	
Spatial distribution	Bottom/midwater (deep areas)	Mainly in the bottom, rarely at midwater	
Cryptobiotic habits	No	Yes	
Foraging angle in relation to substrate	Predominantly 30°	Predominantly 90°	
Reaction to light stimuli	Fast escape (about 10–12s after stimuli)/slow backward swimming	Fast and sudden escape at twi- light (approximately 3 s) and indifferent to light at night/fast side and backward swimming	
Social interactions	Agonistic behavior (two occasions)	No records	
Gregarious habit	Seven individuals/polarized swimming	Five individuals/polarized swimming	
Total time of observations	180 min	210 min	

Table 1. Behavioral components and habitat description of *E. vicentespelaea* and *E. trilineata* from the São Domingos karst area, central Brazil.



Figure 3. Number of active individuals of *Eigenmannia trilineata* from Rio da Lapa, São Domingos karst area, central Brazil. (a) April 2001; (b) August 2001. *, sunset.

swam away immediately after the stimuli at dusk, but did not react during the night phase. In contrast to these observations for light stimuli, after a gentle mechanical stimuli (a touch with a pencil on the water surface), all observed individuals of *E. vicentespelaea* escaped very quickly, also observed for *E. trilineata* (Suplemmentary material: https://figshare. com/s/e7da6c29261eb6f61972). However, a few seconds after the stimuli ceased, they



Figure 4. Frequency of occurrence (FO), volume (V) and feeding index (FI), expressed as a percentage, for *Eigenmannia vicentespelaea* (a) and *E. trilineata* (b) from the São Domingos karst area, central Brazil. n.i., not identified; l., larvae; n., nymph; p., pupae; a., adult.

returned and resumed foraging, and, after 2–3 min, the fish approached and touched the observer with their snout, apparently exploring the new stimuli.

Agonistic interactions were observed in two occasions for *E. vicentespelaea*. In both cases, the pair of individuals had similar body sizes. During the interactions, the resident



Figure 5. Stomach volumes (in mL) for *Eigenmannia vicentespelaea* (a) and *E. trilineata* (b) from the São Domingos karst area, central Brazil. The number of analyzed stomachs per month is in parenthesis.

was chased away by the intruder via pushing of its snout into the side of the resident, after which the intruder started to forage in the area displaced by the resident. No agonistic interactions were observed for the epigean species, *E. trilineata*.

In general, *E. trilineata* started its activity, mainly foraging, at the beginning of dusk. In April 2001, the observations were made during the full moon, and the night was clear; on this occasion, the seven observed individuals started their foraging activity at 5:37–5:45 pm, under a large rock (Figure 3(a)). In August 2001, the observations were carried out on a dark night during the waning moon, when the five observed fish started being active at approximately the same time as during April 2001 (i.e. 5:20–5:45 pm, Figure 3(b)), but were exposed and not hidden under any objects. In April 2001, the first peak of activity was observed around 6:00 pm, with a sudden decrease in the number of active individuals between 8:00 and 10:10 pm. After this, a second peak of activity was recorded at approximately 11:00 pm (Figure 3(a)). However, in August 2001 the first peak of activity occurred later, at approximately 8:00 pm, and a decrease in the number of active individuals was recorded between 9:20 and 10:20 pm, followed by a second peak close to 11:00 pm (Figure 3(b)). On the two occasions (April and August 2001), greater than 10 individuals were observed as being isolated, i.e. retreating to their shelters under rocks and logs after 11:00 pm. During the six hours of observations, the temperature decreased from 25.8 to 23.8 °C in April 2001 and from 24.0 to 22.4 °C in August 2001. Variations in pH (around 7.5) were negligible and null in salinity (always 0%). Conductivity showed a high variation (10–16 µS.cm-1 in April 2001 and 5 µS.cm-1 in August 2001). Dissolved Oxygen showed small variations (around 8.0 mg.l⁻¹) and normoxic conditions.

Feeding habits of E. vicentespelaea and E. trilineata

Both species catch prey items by turning up the substrate (vegetal debris and sand) with their snouts ("hunting speculation" feeding tactic, *sensu* Curio 1976 and "grubbers excavating while moving", *sensu* Sazima 1986). However, *E. vicentespelaea*, the troglobitic species, also explores the midwater and walls of the rocky blocks, touching them with the anterior tip of its snout whereas *E. trilineata* does not.

Species	Date	п	Females	Males	NI
<i>E. vicentespelaea</i> (São Vicente II cave)	May 1999	2		2 MA	
	July 1999	1		1 IM	
	September 1999	2	1 MA	1 IM	
	May 2000	2		2 MA	
	July 2000	2		2 MA	
	September 2000	1	1 MA		
	May 2001	1	1 MA		
	August 2001	3	2 MA	1 MA	
E. trilineata (Rio da Lapa)	July 1999	2	2 MA		
	September 1999	2	1 IM	1 IM	
	August 2000	2	1 MA	1 IM	
	September 2000	2	1 MA	1 MA	
	May 2001	3	2 MA		1

Table 2. Sex ratio and maturation status of *Eigenmannia* spp. from the São Domingos karst area, central Brazil. *n*, number of analyzed gonads per month; MA, mature; InM, undergoing maturation; IM, immature; NI, not identified sex.

In total, the stomach contents of 14 specimens of *E. vicentespelaea* and 11 specimens of *E. trilineata* were analyzed. In general, the diversity of items was almost identical between these species. Their diet consists of insects (Figures 4(a) and (b)), with the majority of these being aquatic (68.8% of items for *E. vicentespelaea* and 75.0% for *E. trilineata*). The most frequently ingested items were fragments of larvae, especially trichopteran larvae, and adult insects.

For *E. vicentespelaea*, 10 out of 14 stomachs analyzed were full or partially full, with 16 items recorded. Larvae of an unidentified trichopteran and insect fragments were the most frequently ingested items according to both the volumetric and feeding index methods (Figure 4(a)). There was a decrease in the total volume of stomach contents per month during the dry seasons in 1999 and 2000 (Figure 5(a)), with a difference observed during the dry season in 2001 (August), when the stomach content volumes were larger than that observed in May 2001.

For *E. trilineata*, 9 out of 11 analyzed stomachs were full or partially full, with 16 items recorded. Larvae of the undetermined trichopteran and insect fragments were the most frequently ingested items according to both the volumetric and feeding index analysis methods (Figure 4(b)), similar to the observations for *E. vicentespelaea*. Although the absence of collections during several months and the small sample size did not allow a more accurate analysis of seasonality, there was no tendency detected for the total volume per dry month to change (Figure 5(b)).

A significant volume of the stomachs of *E. vicentespelaea* was composed of sand, probably ingested during their non-selective foraging, indicating that this species explores more effectively the substrate searching for hidden prey than does *E. trilineata*. *Eigenmannia trilineata* is apparently more selective, since no sand or plant fragments were recorded in their stomachs. In terms of feeding categories, both species are invertivores, with preference for insects, and the benthophagous habit is clearly present, especially in *E. vicentespelaea*.

Reproduction of E. vicentespelaea and E. trilineata

The sex ratio in *E. vicentespelaea* was 9:5 (males:females); a total of 12 out of 14 gonads examined were MA, with no records of females and/or males that were InM during the study period. No seasonal patterns in the maturation condition were detected, because there were records of MA individuals during the entire study period (Table 2). The minimum body size (total length, TL) recorded for the MA individuals were 91.1 mm for females and 105.1 mm for males. Considering the maximum body size recorded for this population was 210.7 mm TL, this means that this fish species reaches maturity at half the maximum size recorded.

For *E. trilineata*, the difference in the sex ratio was smaller (2:5) (males:females). From the eleven gonads examined, seven were MA and, as observed for *E. vicentespelaea*, no females or males InM were recorded (Table 2), and no seasonal pattern was detected. The minimum body size for the MA females was 67.9 mm TL and for the MA males was 85.6 mm TL. The maximum body size recorded for this population was 108.4 mm TL, so these fish would reach the adult phase (maturity) at approximately two thirds of their potential maximum size.

Discussion

The epigean and hypogean *Eigenmannia* species occupy microhabitats with similar physical characteristics. Contrary to results reported for other epigean Eigenmannia species (Alves-Gomes 1997), E. trilineata from Rio da Lapa appears to prefer sites with rocky substrate, such as the entrance to the Terra Ronca cave, even when riparian vegetation, trunks, and logs are available. This particular trait of *E. trilineata* may be a preadaptation (exaptation) for adoption to subterranean life, explaining why it is the only Eigenmannia species that is probably closely related (probably a sister species) to a troglobitic one. The habit of hiding under rocky blocks and among branches and logs during the daytime that was observed in E. trilineata in the present study, and has also been seen in other gymnotiforms, might arise from competition for food, space, and other resources, or might be a response to diurnal predators (Lissmann 1961). However, this is a hypothesis that still needs to be tested. In contrast, E. vicentespelaea did not exhibit cryptobiotic habits on any occasion, suggesting a relaxed visually oriented predation pressure, as in several other troglobitic fishes, such as the Brazilian heptapterids, Pimelodella kronei (Miranda-Ribeiro, 1907) (Trajano 1989), Rhamdiopsis krugi Bockmann & Castro, 2010 (Trajano and Bichuette 2010), and the not described yet Rhamdiopsis sp. (Trajano and Bockmann 2000); the loricariid Ancistrus cryptophthalmus Reis, 1987 (Bessa and Trajano 2002); and Thai balitorids, such as Schistura jaruthanini Kottelat, 1991 (Parzefall and Trajano 2010).

Comparing the spatial distribution, there is an apparent extension in the use of spatial resources in *E. vicentespelaea*, with *E. trilineata* being observed mainly on the bottom substrate while *E. vicentespelaea* also explored the midwater. Exploration of the midwater and surface water in addition to the bottom substrate has been reported for other troglobitic fish species: the Brazilian catfishes *P. kronei*, *R. krugi*, *Rhamdiopsis* sp., *Trichomycterus itacarambiensis* Trajano & de Pinna, 1996, and *Glaphyropoma spinosum* Bichuette, de Pinna & Trajano, 2008 (Trajano 1989; Trajano and Bockmann 2000; Parzefall and Trajano 2010; Rantin and Bichuette 2015), and the Thai balitorids, *S. jaruthanini* and *S. spiesi* Vidthayanon & Kottelat, 2003 (Parzefall and Trajano 2010). This character state may be an adaptive response to a selective regime generally characterized by a scarcity of nutrients and absence of important fish predators, because an extended volume of habitat explored

enhances the chances of finding food without the risk of being preyed upon (Trajano 1993). However, to know whether this character state represents an autapomorphy of *E. vicentespelaea*, it is necessary to obtain similar data for a larger number of *Eigenmannia* species, in combination with a phylogenetic analysis of the group.

The gregarious habit expressed in darkness by the two studied *Eigenmannia* species contrasts with that reported for other gymnotiforms, including other *Eigenmannia* species, which are gregarious only during the daytime, when they are stationary under logs, branches, or roots of the riparian vegetation (Steinbach 1970; Hopkins 1972; Hagedorn 1988; Alves-Gomes 1997). We did not find any explanation for this difference. It is note-worthy that epigean catfish, *Pimelodella* spp. (Heptapteridae), are also gregarious during the nighttime (like the presently studied gymnotiforms) when foraging, but are solitary during the daytime when they are hidden, whereas the troglobitic *P. kronei* is always solitary (Trajano 1989).

For the period of the present study (crepuscule and the first six hours of night for *E. trilineata*, and five free-running hours for *E. vicentespelaea*), the latter species showed a more evenly distributed activity pattern than the former species, which presented two marked activity peaks. Gymnotiformes in general are described as predominantly noc-turnal. For instance, Lissmann (1961), using electrodes for the detection of knife-fishes, observed nocturnal activity in gymnotiforms from Pará state, northern Brazil. Steinbach (1970) used similar methodology studying knife-fishes from the Rio Negro Amazonas basin and did not record electrical signals at the diurnal phase, which means that the fish could be in a stationary phase that produces only weak signals or perhaps the fish were distant from the electrodes. Therefore, although no observations on *E. trilineata* were carried out during the daytime, it is reasonable to assume that this species is nocturnal, but with two or more activity peaks during the nighttime interspersed with short episodes of less activity. A similar pattern was not observed for *E. vicentespelaea* under the natural free-running conditions.

Agonistic interactions were observed for *E. vicentespelaea*, with apparent competition for feeding sites. To our knowledge, only one detailed study focusing on agonistic interactions in *Eigenmannia* fish has previously been published (Hopkins 1974). This author, studying *E. virescens* specimens from Guyana, detected at least six agonistic components, and discussed the function of this behavior to establish hierarchy during the night, when the fish forage, probably for optimized capture of prey. However, the author provided electrical stimuli to potentiate a response. We observed such behavior on only a few occasions for *E. vicentespelaea*, indicating that agonistic interactions are not common in the natural habitat without artificial stimuli. The predominance of females in the studied *E. trilineata* population might explain the absence of agonistic interactions observed for these fish, as males tend to be more territorial than females. The maintenance or enhancement of aggressiveness has also been described for other Brazilian troglobitic fishes, such as *P. kronei*, *A. cryptophthalmus* and *G. spinosum* (Trajano 1991a; Trajano and Souza 1994; B. Rantin, unpubl. data), probably as a consequence of competition for food and potential mates (Parzefall and Trajano 2010).

Differences in the body angle in relation to the bottom substrate between *Eigemannia* species are close to those reported for the Mexican troglobitic characin *Astyanax jordani* (Hubbs & Innes, 1936) (mean = 55.7°) and its epigean congener, *Astyanax mexicanus* (De Filippi, 1853) (mean = 81.8°) (Schemmel 1980) and might be related to the number and distribution of taste buds in the head. In *A. jordani*, taste buds are concentrated in the mouth region, but also extend over the lower jaw and cover ventral areas of the head. In contrast, in *A. mexicanus* the taste buds are restricted to the mouth region (Schemmel

1973), as is observed for the epigean *E. humboldtii* (Steindachner, 1878) and *E. virescens* (Albert 2001). However, the shape of the anterior portion of the body is clearly different between the two species here studied, with *E. vicentespelaea* showing a more pronounced snout than that seen in *E. trilineata* (Bichuette and Trajano 2006), which must influence the distribution and arrangement of other sensorial organs, the tuberous electroreceptors and ampullary electroreceptors (involved in the active and passive electrolocation, respectively). This hypothesis requires further testing with detailed morphological studies.

According to our data, both *Eigenmannia* species studied may be classified as benthophagous invertivores, with similar feeding tactics ("hunting speculation" *sensu* Curio 1976 and "grubbers excavating while moving" *sensu* Sazima 1986), with a preference for insects. Invertivory appears to be common for *Eigenmannia* fishes, as observed by Marlier (1968), Knöpell (1970), Soares (1979), Ferreira (1984), Lowe-McConnell (1987), Marrero (1987), Goulding et al. (1988), Peretti (1997), and Giora et al. (2005).

In addition, the troglobitic species also showed foraging in the rocky walls, parallel to the water column, which could be an extended tactic to locate prey items. The preference for rocky substrates of the *E. trilineata* fish species is related to the generalist invertivore diet typical of the genus and to the nocturnal activities and electric communication characteristics of gymnotiforms in general, and represents character states enabling the epigean *Eigenmannia* population from the São Domingos karst area to colonize the subterranean environment.

The record of potentially reproductive or MA individuals of epigean and troglobitic *Eigenmannia* species in almost all months of the study period suggests a frequent and year-round reproduction cycle. Similar results were recorded for the troglobitic catfish, *P. kronei* (Trajano 1991b). However, the absence of reproductive peaks for *E. trilineata* in the present study disagrees with that observed for other epigean *Eigenmannia* species, which show reproductive peaks during the rainy season or just before the beginning of rain (Hopkins 1972, 1974; Kirschbaum 1975, 1979, 1984; Alves-Gomes 1997), and for *E. trilineata* from other regions. Giora and Fialho (2009) analyzed 428 gonads of *E. trilineata* from south Brazil and recorded a differentiated reproductive period for this species during the rainy, high temperature season.

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