

***Stygichthys typhlops* Brittan & Böhlke, 1965 (Teleostei: Characiformes), a phreatobitic fish from eastern Brazil: Comments on Sampaio et al. (2012)**

**Eleonora Trajano<sup>1</sup> & Cristiano Rangel Moreira<sup>2</sup>**

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<sup>1</sup>Departamento de Zoologia, Instituto de Biologia da Universidade de São Paulo, São Paulo, Brasil. [etrajano@usp.br](mailto:etrajano@usp.br) (corresponding author)

<sup>2</sup>Departamento de Ciências Biológicas, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo - Diadema. [moreira.c.r@gmail.com](mailto:moreira.c.r@gmail.com)

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*Stygichthys typhlops* Brittan & Böhlke, 1965 is the only known troglobiotic characiform fish in South America, now only accessible through artificial wells and 'cacimbas' in the Jaíba karst area, State of Minas Gerais, eastern Brazil. The species was described based on a single specimen collected during a well drilling operation (Brittan and Böhlke 1965). Additional specimens were not collected again until 2004, almost 40 years after its description (Moreira et al. 2010). *Stygichthys typhlops* faces a high risk of extinction due to the lowering of the water table caused by groundwater withdrawal for extensive irrigation in the region. This threat promoted the species to be included in the Brazilian Red List of Threatened Species (Ministério do Meio Ambiente 2008). After its rediscovery, information on several aspects of the biology of *S. typhlops* has been published, such as physiology of pigmentation (Felice et al. 2008), chronobiology (Trajano et al. 2011), behavior (Parzefall and Trajano 2010), conservation (Bichuette and Trajano 2010), habitat and distribution (Trajano and Bichuette 2010).

Recently, Sampaio et al. (2012) published a short paper on the diet of this species, and hypothesized that “*S. typhlops* might have retained the ability to consume plant matter from its now extinct surface ancestor to exploit a novel food source in an environment usually poor in such resources.” The authors reached this conclusion based on three observations: (1) the presence of a green mass in the abdominal cavity, seen through transparent abdominal wall in live specimens; (2) the consumption of vegetable matter in captivity; and (3) tooth morphology. We dispute this conclusion due to what we believe were equivocal assumptions on these three aspects. In the absence of supporting data from reliable methods, such as detailed behavioral studies in the laboratory and analyses of stomach contents and morphological studies of the digestive system (see Simões et al. 2013), we consider their hypothesis as unsupported. Moreover, in a recent paper, Simões et al. (2013) presented compelling evidence for a

carnivorous diet, based on the analyses of stomach contents and anatomy of the digestive tract. Each of the three lines of evidence presented by Sampaio et al. (2012) is discussed and refuted below.

### **Feeding in the natural habitat and presence of green masses in the abdominal cavity**

The currently accessible locality where *S. typhlops* have been collected, clearly represents a peripheral habitat that is used by a low percentage of the total population, in a fluctuating way, as indicated by variation in the number and size of individuals observed on different occasions: April 2004, several observed, 21 collected, standard length (SL) from 22.8 to 45.9 mm (Moreira et al. 2010); September 2004, nine collected (Moreira et al. 2010); September 2008, eight collected (Trajano et al. 2011); July 2010, no cavefish observed (M.E. Bichuette, personal communication); and November 2010, several observed, 7 captured, SL from 20.9 to 41.9 mm (Simões et al. 2013). The quick re-colonization of the artificial well is evidence that the small group of *S. typhlops* fish observed in this habitat is a sink population (see Trajano 2012 for the concept of source *versus* sink population integrated to the definition of troglolobiotic species).

Therefore, it is highly unlikely that the particular selective regime in such a spatially restricted, partially illuminated site, over a sink population that is very small and fluctuating, has any relevant effect on the evolution of this species. Furthermore, the high degree of troglomorphy, including the total depigmentation (the tested fish were DOPA(+); Felice et al. 2008), and the high degree of regression of internal time control mechanisms in comparison with other troglolobiotic fishes (Trajano et al. 2011), indicate that the selective regime shaping this species is typically subterranean. The high degree of troglomorphy indicates a long time of genetic isolation and differentiation under special conditions (the regressions mentioned are typically associated with evolution in aphotic environments), but the manmade well is a very recent event in an evolutionary timescale, thus unlikely to have any relevance for the evolution of this species.

Despite Sampaio et al. (2012) reporting of the presence of a high density of macrophytes in a well, the same locality was visited by a colleague (M.E. Bichuette) and us on five other occasions, and in none was this condition observed. This indicates that the presence of macrophytes is a rather rare phenomenon. Moreover, on these five occasions the well was covered and poorly illuminated, which makes it unlikely to sustain a constant population of plants.

The presence of plants in the well, with the observation of a green mass in the abdominal cavity of a few specimens, led Sampaio et al. (2012) to conclude that they were feeding on macrophytes. Despite this bold suggestion, since it is rather unusual for a phreatic fish to feed on plants, the authors made no attempt to dissect the specimens in order to check if the green mass was indeed plant matter in the “stomach and intestines” as suggested. There is, indeed, a green mass in the abdominal cavity shown

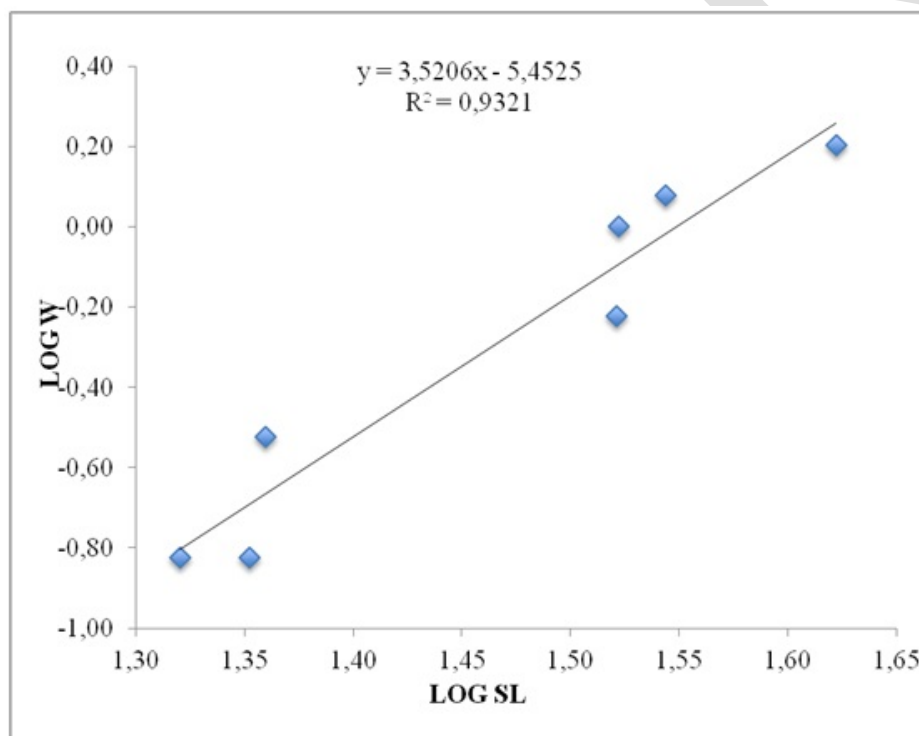
in some photographs presented by Sampaio et al. (2012; see there Figure 1); however, the position and shape of this mass is approximately the same among the specimens illustrated and does not correspond to the position of the stomach. If the green mass was in the intestine, it is expected that its position would be different among the specimens, since it is an elongate organ, looping between the sides of the body (E. Trajano, personal observation). Moreover, it is possible to verify that the green mass region is not included in the food after following the passage of food along the digestive tract at regular intervals (B. Rantin and P. Rizzato, personal communication).

Another inconsistency is the bright green color; it is expected that there should be a color change after the plant material moves from the stomach to the intestine, because chlorophyll would have been mostly transformed into dark green-brownish pheophytins (Lanfer-Marquez 2003). In order to verify our assumptions, we dissected three preserved specimens collected by us (MZUSP 87678) and one specimen collected by Sampaio et al. (2012) (MZUSP 108165). The position of the green masses in Sampaio et al.'s photographs seems to better correspond to the position of the spleen. The presence of an oval, compact organ in the position of the Sampaio et al.'s green mass in a dissected specimen of *S. typhlops* was also observed by C. Oliveira (personal communication). One additional piece of evidence is presented by Simões et al. (2013), based on the analysis of stomach contents and the relative length of the intestine (IRL), a good indicator of diet for many taxa, who demonstrated *S. typhlops* is consistent with a typical generalist carnivore.

Feeding behaviors must be interpreted in terms of their biological meaning. Characids in general are opportunistic pickers (*sensu* Sazima 1986), best defined as omnivores. Sediment and other nonorganic matter are often found in the digestive contents of pickers, such as in the Brazilian blind catfish, *Pimelodella kronei* (Miranda-Ribeiro, 1997) (Trajano 1989), but are obviously not considered food items. Another interesting example refers to *Rhamdiopsis krugi* Bockmann & Castro, 2010, a blind catfish from Chapada Diamantina, northeastern Brazil. This species was initially considered a guanophagous fish due to the presence of a dark-colored mass observed in the stomach through the body wall, but later analysis of stomach contents revealed that it is a generalist carnivore (Mendes 1995). These fish accidentally ingest guano while feeding on invertebrates that concentrate on bat guano. Such behavior also explains the frequent presence of sand and other inorganic matter in the stomach contents of many epigeal and subterranean fishes. *Rhamdiopsis krugi* may receive some nutritional benefits from ingested guano, but this does not make ingestion of guano a feeding adaptation. This might also apply to plant fragments occasionally ingested by accident by *S. typhlops* when foraging for prey on green substrates.

A subterranean carnivorous diet is consistent with fat storage observed in cavefishes (fat content in most plants is too low), such as in the heptapterids *Rhamdia* spp. (Weber 1996), and *Pimelodella kronei* (E. Trajano, personal observation), typically associated with energy-limited ecosystems with a fluctuating availability of nutrients. A progressive

accumulation of fat with growth in *S. typhlops* is shown in **Figure 1**, with pronounced allometric growth partly due to a disproportional accumulation of fat with size increment. Fat cells distributed in the head and body wall are often visible in *S. typhlops* (**Figure 2**). The value of  $b$  (3.5) used to calculate the condition factor in *S. typhlops* is the highest among those recorded for Brazilian cavefishes, including *Pimelodella kronei* (2.7; Trajano et al. 2004), *P. spelaea* (2.3; Trajano et al. 2004), *Ituglanis passensis* (2.9; Bichuette 2003), *I. bambui* (2.1; Bichuette 2003) and *Ancistrus cryptophthalmus* (2.7–3.1 depending on year and population; Trajano and Bichuette 2007). This evidence, in addition to evidence indicating that *S. typhlops* found in the well form a sink population, corroborates the hypothesis that the particularities of the habitat studied by Sampaio et al. (2012), an artificial well with distinct photoperiods, primary productivity, and food represented by small invertebrates, has little importance as a selective regime for this species. Cannibalism observed in laboratory (Moreira et al. 2010), likely a density-dependent mechanism of population regulation (Poulson 1969), is more evidence of a typical subterranean selective regime shaping species evolution.



**Figure 1.** Condition factor for seven specimens of *Stygichthys typhlops* recently collected in the wild, showing the rapid increase in mass with increased length (Simões, Rantin and Bichuette, unpublished data).



**Figure 2.** Detail of a *Stygichthys typhlops* specimen showing fat cells in the head and body wall. Photograph by Dante B. Fenolio.

### **Plant consumption in the laboratory: an abnormal behavior?**

It is worth noting that all 10 specimens collected by Sampaio et al. (2012) died within five months (the cause of death is not specified). Coincidentally, based on our own experience, this is also the average survival time for starved fish. We argue that the experimental conditions (as described by those authors) were less than adequate for this species. Available information in the literature for the long-term maintenance of *S. typhlops* indicates that water temperature was 5–6 °C below that observed in their natural habitat, and that the number of fish maintained in a single aquarium was too high, as indicated by cannibalism observed in the laboratory at different occasions Moreira et al. (2010). Sampaio et al. (2012) do not mention if the aquarium was kept in an isolated, quiet room (as it should be because these fish are highly sensitive to noise and other disturbances). It is widely known that under stress, abnormal behaviors may develop; therefore, any conclusion drawn from these data require validation from other data sources. Poor health conditions of the fish may account for unusual colors of internal organs.

It is widely known that fishes may accept food items not included in their natural diet when in captivity. Opportunistic feeders, such as most subterranean fishes, will consume a large variety of artificial food, including cheese, canned fish and dog food, as has been observed for the blind catfish, *Pimelodella kronei* (E. Trajano, personal observation). The consumption of a particular food item in captivity per se has no biological meaning.

## Tooth morphology

Sampaio et al. (2012) described the dentition of *Stygichthys typhlops* based on scanning electron microscopy of the jaws of a single specimen, which is within the variation already described by Moreira et al. (2010). Sampaio et al. stated that multicuspid teeth “are associated with leaf cutting and herbivory or omnivory in general,” which would explain their observations on the plant matter consumption of *Stygichthys* in captivity. While there is an association with tooth morphology and feeding preference, there is substantial variation in what are considered “multicuspid teeth.” Winemiller (1992), cited by the authors, is very vague in associating multicuspid teeth with feeding habits. He indicates that a multicuspid tooth morphology may be associated with omnivory, insectivory or granivory, three very different feeding habits. Characids with higher consumption of plant matter, especially filamentous algae, tend to have a higher number of cusps with approximately the same size (e.g., Cheirodontinae and Iguanodectinae) than omnivorous characid species. For example, the teeth of *Coptobrycon bilineatus* (Ellis, 1911), a species that is the sister lineage to *Stygichthys* (Oliveira et al. 2011) and that consumes large quantities of plant matter (Langeani and Serra 2010), has up to 10 cusps on its dentary teeth, with cusps approximately of the same size. This is not the case for *Stygichthys*, where no more than five cusps may be present on a single tooth (Moreira et al. 2010), with the central cusp much larger than lateral cusps (Sampaio et al. 2012).

## Final Comments

It should be mentioned that omnivory is not the sum of feeding specializations (which would be contradictory per se), but the lack of such specializations. Specializations for herbivory and iliophagy in fishes are relatively few and usually are associated with increased numbers of teeth (as in armored catfishes, which are periphyton grazers), teeth with greater numbers of cusps or sharp teeth with clear cutting edges (as in many coral reef fishes). In addition, herbivory and iliophagy are typically associated with longer intestines. None of these traits are observed in *S. typhlops*. Omnivorous fishes, and also generalist carnivores, lack such specializations. It is not surprising that subterranean fishes, including both stygobionts and stygophiles, usually belong to taxa characterized by opportunistic feeding, traditionally recognized as a significant preadaptation (or exaptation) for subterranean life (Vandel 1964; Ginet and Decu 1977).

In conclusion, we feel that the available evidence contradicts the conclusions inferred by Sampaio et al. (2012) that *S. typhlops* is herbivorous, possibly as a trait retained from a now extinct surface ancestor evolved to exploit a novel food source. The bold assertion that a phreatic fish species “might have retained the ability to consume plant matter from its now extinct surface ancestor to exploit a novel food source in an environment usually poor in such resources” is not backed by evidence. The presence of green masses in the abdominal cavity observed by the authors is better explained as the spleen, instead of the consumption of macrophytes that were present in the well at

the time of collection. The presence of these macrophytes seems to be a rather rare event. The consumption of plant matter in captivity is not strong evidence since in captivity the ingestion of different food items is more common than in the field. Lastly, tooth morphology in *S. typhlops* is not indicative of a herbivorous species, rather of an omnivorous to invertivorous species. The absence of a proper methodology (e.g., dissection of specimens preserved in the field or isotopic analysis) precludes the authors from being able to properly propose that this species feeds on vegetable matter in its natural habitat. Even if this species was shown to consume plant matter, their evolutionary assertion is a rather weak conjecture, lacking proper data to support it.

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