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# Unrevealing Parasitic Trophic Interactions—A Molecular Approach for Fluid-Feeding Fishes

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Fish diets have been traditionally studied through the direct visual identification of food items found in their stomachs. Stomach contents of Vandeliinae and Stegophilinae (family Trichomycteridae) parasite catfishes, however, cannot be identified by usual optical methods due to their mucophagic, lepidophagic, or hematophagic diets, in such a way that the trophic interactions and the dynamics of food webs in aquatic systems involving these catfishes are mostly unknown. The knowledge about trophic interactions, including difficult relation between parasites and hosts, are crucial to understand the whole working of food webs. In this way, molecular markers can be useful to determine the truly hosts of these catfishes, proving a preference in their feeding behavior for specific organisms and not a generalist. Sequences of *cytochrome oxidase subunit 1* (*COI*) were successfully extracted and amplified from mucus or scales found in the stomach contents of two species of stegophilines, *Homodiaetus anisitsi,* and *Pseudostegophilus maculatus*, to identify the host species. The two species were found to be obligatory mucus-feeders and occasionally lepidophagic. Selection of host species is associated to host behavior, being constituted mainly by substrate-sifting benthivores. Characiformes are preferred hosts, but host choice depends on what characiform species are available in their environments, usually corresponding to the most abundant species. This is the first time that host species of parasitic fishes bearing mucophagous habits are identified, and demonstrates the effectiveness of the extraction and amplification of mitochondrial DNA from the ingested mucus in gut contents. The molecular markers effectively allowed determine parasite preferences and helps in better understanding the food web and trophic interaction on which fish species are involved. Despite, the methodology applied here can be used for an infinitive of organisms improving ecological trophic studies.

**Keywords: annealing blocking primer, DNA barcode, food webs, parasite-host interaction, stegophilinae, vandeliinae**

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## INTRODUCTION

The role of parasites in food webs has been largely disregarded (Sukhdeo, 2012). As an example, Winemiller and Polis (1996) was the most important contribution of food web studies but not approached the parasite-host interaction as part of the trophic webs. Since Elton insights (Elton et al., 1931) it is known that parasites are very important links in the food webs and are capable of externing major effects on ecological interactions. Today, there is no longer the need to argue that

parasites must be included in all models of ecosystem function (Sukhdeo, 2012). However, many questions remain as how parasites might fit in food webs, if it should be included or excluded in the food webs, what is the role of parasites in host population regulation, and what are the evolutionary and ecological implications of parasite mediation in trophic interactions (Sukhdeo and Hernandez, 2005). Nevertheless, small alteration in the position of parasites and host can change the food chain length, connectance, and the establishment of food patterns (Huxham et al., 1995; Leaper and Huxham, 2002).

Indeed, only dietary studies allow the comprehension of the trophic interactions and of the dynamics of food webs specific for fishes in aquatic systems (Winemiller and Polis, 1996; Carreon-Martinez and Heath, 2010; Murray et al., 2011). For these purposes, food items are traditionally identified through visual analysis of stomach, gut, or fecal contents under microscope (Hyslop, 1980; Taguchi et al., 2014) and through stable isotope analysis (DeNiro and Epstein, 1978, 1981; Post, 2002; Mercado-Silva et al., 2015). None of these methods, however, allow the identification of items that are easily digested or of specific parasitic-host (or predator-prey) interactions, which may cause a bias in data interpretation (Sheppard and Harwood, 2005; Paquin et al., 2014). This may be especially problematic when dealing with small species (Sheppard and Harwood, 2005; Jo et al., 2014; Paquin et al., 2014).

Since the 1940's many methods of diet analysis have been developed with a recent use of molecular tools (Symondson, 2002; King et al., 2008; Hardy et al., 2010; Pompanon et al., 2012; Taguchi et al., 2014). Molecular techniques are efficient for the identification of small preys or digested items that cannot be identified through traditional methodologies. It is also presumed to allow a higher taxonomic resolution (Carreon-Martinez et al., 2011) through the use of sequences of specific regions of the mitochondrial genome to identify any species (DNA Barcode). In most animal groups, the *cytochrome oxidase subunit 1* (*COI*) is the reference for the DNA barcoding system (Hebert et al., 2003). *COI* sequences libraries are available through on-line systems (such as GenBank, Bold), enabling its use in the identifications of host or prey species (Valentini et al., 2009; Corse et al., 2010; Leray et al., 2013a; Jo et al., 2014). DNA barcode techniques have been used to determine host-parasitoids webs in arthropods (Hrček and Godfray, 2014), as in Lepidoptera (Janzen et al., 2009) and Hemiptera (Gordon and Weirauch, 2015). However, in fish species the establishment of parasitic interaction has been reported as difficult (Sazima, 1983; Lima et al., 2012). According to Paine (1980) "*The central significance of webs is derived from the fact that the links between species are often easily identified and the resultant trophic scaffolding provides a tempting descriptor of community structure*." Thus, the no comprehension of the parasite/host interaction leads to the impossibility of understanding food webs and the trophic ecology at the ecosystems.

The Neotropical catfish family Trichomycteridae is composed of eight subfamilies, two of which consist exclusively of fishes referred to as parasites, the Stegophilinae ,and Vandeliinae (de Pinna, 1998, 2016; Datovo and Bockmann, 2010; Ferrer and Malabarba, 2013). The vandeliines feed exclusively on blood from the gills of other fishes (Kelley and Atz, 1964; Machado and Sazima, 1983) while the stegophilines have been reported as scale and mucus-feeders (Eigenmann and Allen, 1942; Roberts, 1972; Baskin et al., 1980; Machado and Sazima, 1983; Winemiller and Yan, 1989; Neto and de Pinna, 2016). The feeding habits of the representatives of a third subfamily, the Tridentinae, remains uncertain, but there are circumstantial records of semi-parasitic (scale-eating) or predation of small invertebrates among its species. Certainly, their biology is key to understanding the evolution of parasitic feeding behavior of stegophilines and vandeliines (de Pinna, 2016).

The species of the stegophiline genus *Homodiaetus* are small (maximum 42.0 mm of standard length) and translucent when alive, except the head and abdominal region (Koch, 2002). *Homodiaetus anisitsi* Eigenmann and Ward, 1907 (**Figure 1**) is found in lakes and rivers in the lower Paraná-Paraguay System and coastal river drainages of the Rio Grande do Sul State, Brazil (Koch, 2002). The stegophiline *Pseudostegophilus maculatus* (Steindachner, 1879; **Figure 1**) reaches a maximum size of 60.0 mm of standard length and is found in the lower Paraná and Uruguay river basins, South America (de Pinna and Wosiacki, 2003). Specimens of both species inhabit fine grain sandy bottom environments. In aquarium observations, specimens of *H. anisitsi* remain buried in sandy bed except for the eyes. They quickly move out from the substrate and attach to the side of fishes passing by for mucus and occasionally scale ingestion (LRM pers. obs.).

Host identification of mucophagous, lepidophagous, and hematophagous trichomycterids cannot be made by conventional analysis of the stomach contents due to the nature of the ingested items. This is notably exemplified by Winemiller and Yan (1989) that examined 245 specimens of the stegophiline *Ochmacanthus alternus* Myers, 1927. They were able to identify the presence of ingested mucus in 95% of the stomachs, but were not able to identify a single hosts species among the 88 fish species found syntopic with this catfish. Likewise, there is no information available about the host species exploited by *H. anisitsi*, *P. maculatus* or other stegophilines in natural environments. The same is true for most vandeliines, except for

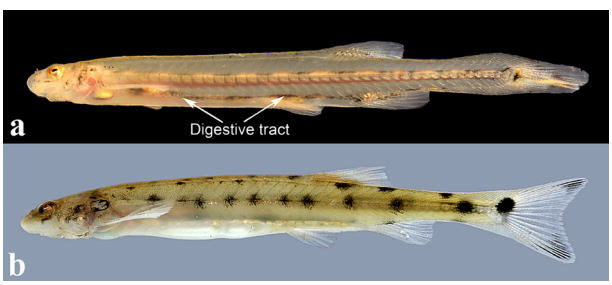


**FIGURE 1 |** Studied species of Stegophilinae. **(a)** *Homodiaetus anisitsi*, 32 mm standard length, specimen from Lagoa dos Quadros, rio Tramandaí basin, Rio Grande do Sul State, Brazil. **(b)** *Pseudostegophilus maculatus*, 38 mm standard length, specimen from rio Ibicuí, rio Uruguay basin, Rio Grande do Sul State, Brazil. Arrows indicate the digestive tract, visible by transparency as a relatively straight tube from the mouth to the anus.

a few visual records of specimens observed attached to gill arches of some large fishes (Zuanon and Sazima, 2005) or experiments in aquaria or observation on large fishes tied to river banks (Machado and Sazima, 1983; Zuanon and Sazima, 2004).

Considering mucophagous or hematophagous catfishes, molecular techniques provide an alternative to determine the origin of the food items ingested by mucus-, scale- and blood-feeders, being considered as a powerful tool for studying feeding ecology (King et al., 2008). However, this tool has not been used yet to identify the species to which belong these items found in the stegophiline stomachs. Therefore, our hypothesis is that molecular markers can be useful to determine the truly hosts of these catfishes, proving a preference in their feeding behavior for specific organisms and not a generalist. Notwithstanding, the aims of this study are: (1) to verify the viability of DNA extraction and amplification of the *cytochrome oxidase subunit 1* gene (*COI*) from the mucus and occasionally scales ingested by *H. anisitsi* and *P. maculatus*; (2) the identification of their hosts, and (3) establishment of specific parasitic-host interactions.

# METHODS AND MATERIALS

## Ethics Statement

This study was approved by the Animal Ethics Committee of the Universidade Federal do Rio Grande do Sul (Permit Number: 24434) and was conducted in accordance with protocols in their ethical and methodological aspects for the use of fish.

## Analysis

Sixty three specimens of *H. anisitsi* and 18 specimens of *P. maculatus* (**Tables 1**, **2** and Supplementary Table 1) were analyzed and vouchers cataloged in the fish collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS; CGEN Process # 02000.002101/2007-25) (Supplementary Table 2).

The digestive tract of *H. anisitsi* and *P. maculatus* is a relatively straight tube from the mouth to the anus (**Figure 1**) with a very thin wall. The entire digestive tract was opened and all the content was examined under a stereomicroscope to identify the food items. Immediately after that, the ingested items and a tissue sample of the dissected specimens were moved to separate and empty sterilized tubes of 0.2 μl to proceed with the DNA extraction. DNA was extracted from all stomach contents using the "Phire Animal Tissue Direct PCR Kit" by Thermo Scientific, following manufacturer instructions. Extracted DNA samples were then submitted to two different protocols for gene amplification.

The PCR reactions were carried out in a reaction volume of 20 μL [12.9 μL of H20, 2 μL of 10 × reaction buffer (Platinum®Taq), 0.6 μL of MgCl2 (50 mM), 2 μL of dNTPs (2 mM), 0.2 μL of each primer (10 μM), 2.0 μL of the blocking primer, 0.1 μL (5 U) of Platinum® Taq (Invitrogen), and 100 ng of template DNA. A versatile PCR primer (mlCOIintF/jgHCO2198 Geller et al., 2013; Leray et al., 2013b) was used to amplify a 313 bp region of the mitochondrial COI region. In *H. anisitsi* samples, this primer was associated to a parasite-specific annealing blocking primer (blocking primer sequence: 5′ CGAARAATCARAAYARRTGTTG3SpC3 3′). Because the predator DNA co-amplification is known to inhibit prey DNA detection in stomach contents (Vestheim and Jarman, 2008), the blocking primer was included at ten times the concentration of versatile primers (Leray et al., 2013b). The PCR cocktail and touchdown temperature profile used in this study follow Leray et al. (2013b).

The quantification of the amplified gene was carried out in agarose gel using a Low Mass Ladder 100 pb by Ludwig Biokee as comparison parameter. The PCR products were purified by enzymatic methods (ExoSap) and sequencing was performed on a sequencing platform of Actgene (Porto Alegre, Brazil) and Laboratory of analytical biology at National Museum of Natural History, Smithsonian Institution (Washington DC). The chromatogram qualities of the generated sequences were checked in MEGA 6.0 (Tamura et al., 2013), and were aligned using Clustal W (Higgins et al., 1994).

The sequences obtained from stomach contents were compared to the GenBank on-line platform; and to local Barcode inventories (sequences deposited in Genbank; access numbers in Supplementary Table 2). The genetic distance between the stomach contents and the local Barcode inventories were estimated using p-distance in MEGA 6.0 (Tamura et al., 2013) software using as molecular evolution model Kimura-2 parameters.

# RESULTS

Stomachs of three specimens of *H. anisitsi* contained sand only, and 15 stomachs of *H. anisitsi* and eight of *P. maculatus* were empty, being not submitted to DNA extraction. The stomachs of 42 specimens of *H. anisitsi* and 10 specimens of *P. maculatus* contained ingested items identified as mucus and occasionally scales, sometimes associated with sand (**Tables 1**, **2** and Supplementary Table 1). DNA was successfully extracted from gut contents of all these specimens, but amplification gave positive results only for 10 specimens of *H. anisitsi* and 7 specimens of *P. maculatus*.

The BLAST search (GenBank) based on the sequences obtained from stomach contents generated identifications with values of identity ranging from as low as 80% and up to 100%. Values of BLAST identity lower than 98% were not considered (Supplementary Table 1), since it generates spurious identifications, including fish species absent in the hydrographic regions sampled (e.g., *Astyanax altiparanae*) or even marine species of Balistidae and Apogonidae. Further comparison to local barcode data allowed a second and independent identification, allowing the confirmation of the identity of the species spoiled by these stegophilines, or identification of species whose sequences are not available in GenBank (**Tables 1**, **2**).

The *COI* sequences amplified from stomach contents resulted always in the identification of a single species per stomach. Six host species were identified in the contents of eight stomachs in *H. anisitsi* (**Table 1**), five freshwater species belonging to

**TABLE 1 |** Results of the identification of the collected samples from stomach contents of *Homodiaetus anisitsi*.

| Voucher UFRGS | SL (mm) | Drainage | Stomach Contents | BLAST identity (>95%) | *p* distance to local barcode |
|---|---|---|---|---|---|
| 13611A | 29.4 | Patos Lagoon | Scales | *Bryconamericus iheringii* (98%) | *Bryconamericus iheringii* ($p = 0.01$) |
| 13611B | 30.9 | Patos Lagoon | Scales | *Bryconamericus iheringii* (98%) | *Bryconamericus iheringii* ($p = 0.01$) |
| 13937 | 34.9 | Patos Lagoon | Mucus | – | *Homodiaetus anisitsi* ($p = 0.01$) |
| 16715 | 28.7 | Tramandaí River | Sand and mucus | *Mugil liza* (100%) | *Mugil liza* ($p = 0$) |
| 19372 | 33.7 | Tramandaí River | Mucus and scales | *Cyphocharax voga* (99%) | *Cyphocharax voga* ($p = 0$) |
| 19373 | 28.4 | Tramandaí River | Mucus | *Mugil liza* (100%) | *Mugil liza* ($p = 0$) |
| 13807 | 36.3 | Uruguay River | Mucus | – | *Astyanax fasciatus* ($p = 0.03$) |
| 14658 | 36.1 | Uruguay River | Mucus | – | *Acestrorhynchus pantaneiro* ($p = 0.12$) |
| 17927A | 34.8 | Uruguay River | Detritus and mucus | *Astyanax altiparanae* (98%) | *Astyanax jacuhiensis* ($p = 0.00$ |
| 17927B | 31.4 | Uruguay River | Mucus and scales | | *Homodiaetus anisitsi* ($p = 000$) |
| 17927C | 42.3 | Uruguay River | Mucus | Failed | Failed |

*Sequences were compared to GenBank (BLAST identity), to local Barcode data (p distance) and to the parasitic catfish COI sequence. UFRGS, catalog number of vouchers in the Fish Collection, Universidade Federal do Rio Grande do Sul; SL, standard length in mm.*

**TABLE 2 |** Results of the identification of the collected samples from stomach contents of *Pseudostegophilus maculatus*.

| Voucher UFRGS | SL (mm) | Stomach contents | BLAST identity | *p* distance to local barcode |
|---|---|---|---|---|
| 20127-5 | 41.8 | Mucus | *Pseudostegophilus maculatus* (98%) | *Pseudostegophilus maculatus* ($p = 0.00$) |
| 20127-6 | 40.4 | Mucus | *Pseudostegophilus maculatus* (100%) | *Pseudostegophilus maculatus* ($p = 0.00$) |
| 20127-8 | 38.3 | Mucus | *Prochilodus lineatus* (100%) | |
| 20127-11 | 35.0 | Mucus | *Pseudostegophilus maculatus* (100%) | *Pseudostegophilus maculatus* ($p = 0.00$) |
| 20127-11 | 35.0 | Scale | *Bryconamericus turiuba* (98%) | *Piabarchus stramineus* ($p= 0.01$) |
| 20127-13 | 33.8 | Mucus | *Prochilodus lineatus* (100%) | |
| 20127-14 | 33.1 | Mucus | *Prochilodus lineatus* (100%) | |

*Sequences were compared to GenBank (BLAST identity), to local Barcode data (p distance) and to the parasitic catfish COI sequence. UFRGS, catalog number of vouchers in the Fish Collection, Universidade Federal do Rio Grande do Sul; SL, standard length in mm.*

the order Characiformes (six stomachs) and one species of the diadromous mugilid *Mugil liza* (two stomachs). Two host species were identified in the contents of eight stomachs in *P. maculatus* (**Table 2**), both belonging to the freshwater order Characiformes (four stomachs).

Both species were mucophagous and lepidophagous, and parasitized organisms that live close to the bottom or that feed on the bottom.

## DISCUSSION

The analysis of guts contents of *H. anisitsi* and *P. maculatus* suggests they are obligatory mucus feeders, as also observed in the stegophiline *O. alternus* by Winemiller and Yan (1989). This diet is possible by the morphological specializations shared by the members of the subfamily Stegophilinae that possess a wide and ventral mouth with numerous teeth in both jaws, arranged

in several rows (Baskin et al., 1980). This allows mucus scraping of their hosts and may function as a sucker (Winemiller and Yan, 1989).

A scale-feeding behavior has been also described for the stegophilines (Eigenmann and Allen, 1942; Roberts, 1972; Baskin et al., 1980; Machado and Sazima, 1983; Winemiller and Yan, 1989), but we found lepidophagy in the two studied species to be non-obligatory and related to the body size and consequently scale size of the host species (Supplementary Table 1). Even though we do not have information on the body size of the hosts whose mucus were found in the stomachs (adult size or juveniles), none of the stomachs containing mucus of the largest host species (*M. liza* and *Prochilodus lineatus*) showed the presence of scales (**Figures 2**, **3**). Instead, scales were found associated with DNA of *Bryconamericus* and *Cyphocharax*, both with smaller body sizes (**Figures 2**, **3**).

It is remarkable the preference for characiforms as hosts in the two stegophiline species. In a recent inventory of the freshwater fish species from the rio Uruguay, rio Tramandaí and laguna dos Patos drainages, that embraces the areas of collection of the samples studied herein, Siluriformes was found to be the dominant group, corresponding to 42% of the species found in these drainages (Bertaco et al., 2016), but none correspond to the stomach contents. Instead, except for two stomachs containing one species of mullets (Mugilidae), all stomachs contained species of Characiformes, that corresponds to nearly 1/4 (28%) of the freshwater species found in these drainages (Bertaco et al., 2016). In addition and further supporting the preference for characiforms, several species of Siluriformes and Cichlidae (Cichliformes) were collected along with the specimens of *H. anisitsi* or *P. maculatus* analyzed here (Supplementary Table 3), but were not found in their stomachs.

Host species of *H. anisitsi* varied according to the locality of collection, and seems to be related to hosts that live close to the bottom or that feed on the bottom, and to the species available. *M. liza* is very abundant in coastal lagoons of southern Brazil (Reis and D'Incao, 2000), including the locality sampled in the rio Tramandaí drainage, being found in two stomachs of *H. anisitsi*. The second species found in stomach contents in this drainage is *Cyphocharax voga*, one of the five most abundant species in these coastal lagoons (Schifino et al., 2004). Both host species are detritivorous and edentulous (Dualiby, 1988; Oliveira and Soares, 1996; Corrêa and Piedras, 2008). In the laguna dos Patos drainage the two identified stomach contents included scales of *Bryconamericus iheringii*, a characid species with inferior mouth that feeds in the substrate (Orciolli and Bennemann, 2006) and is very abundant in creeks of this drainage (Corrêa et al., 2015). In the rio Uruguay drainage, three host species were found (*Astyanax jacuhiensis*, *Astyanax fasciatus,* and *Acestrorhynchus pantaneiro*), but in this case none have feeding habits associated to bottom feeding. Instead, *A. lacustris* and *A. fasciatus* normally inhabit the column of water and surface and can inspect the bottom for feeding (Casatti et al., 2001).

A similar result was obtained from *P. maculatus*, being host species related to host behavior and to the species availability. *P. lineatus*, the most common host found in three stomachs, feeds on algae and detritus grasping the substrate (Fugi et al., 2001). *Piabarchus stramineus* found in one stomach inhabits the water column and can inspect the bottom for feeding (Casatti et al., 2003; Brandão-Gonçalves et al., 2009). Likewise, most hosts of *H. anisitsi*, *P. lineatus* is very abundant in the Rio de la Plata basin,

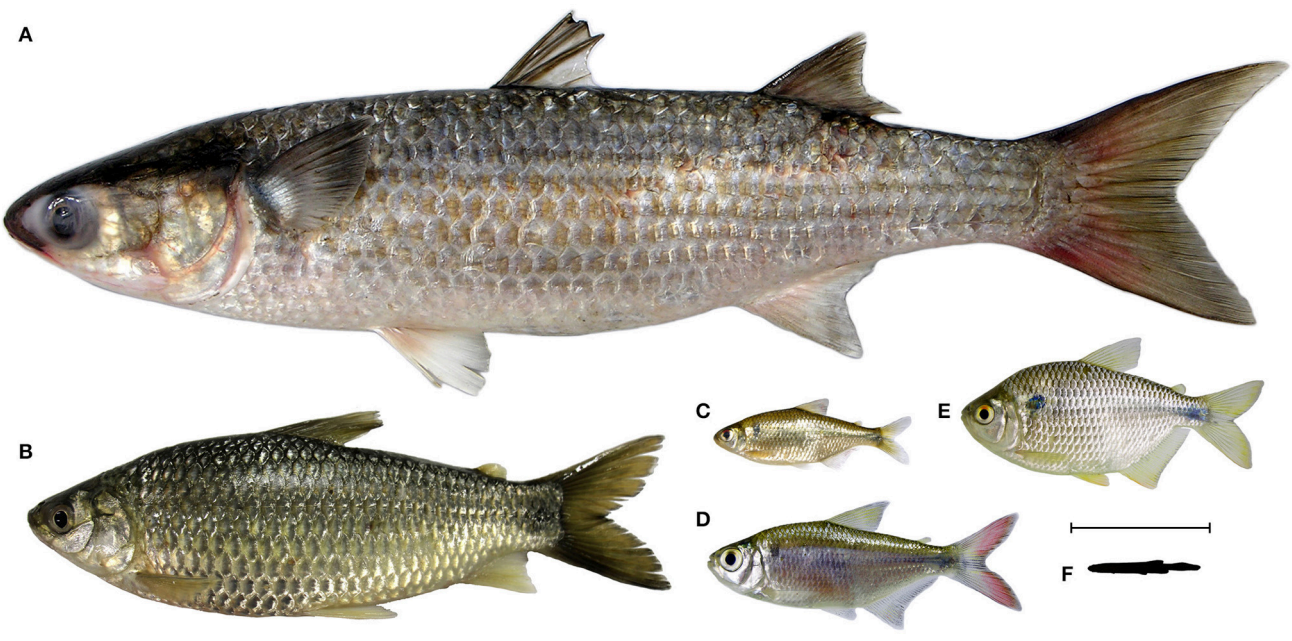


**FIGURE 2 |** Host species of *Homodiaetus anisitsi* represented in scale. **(A)** *Mugil liza*; **(B)** *Cyphocharax voga*; **(C)** *Bryconamericus iheringii*; **(D)** *Astyanax fasciatus*; **(E)** *Astyanax jacuhiensis*; **(F)** *Homodiaetus anisitsi* represented in black. Scale bar 50 mm.


**FIGURE 3 |** Host species of *Pseudostegophilus maculatus* represented in scale. **(A)** *Prochilodus lineatus*; **(B)** *Piabarchus stramineus*; **(C)** *Pseudostegophilus maculatus* represented in black. Scale bar 50 mm.

including the rio Uruguay, and dominates the biomass, being the target of the principal freshwater fishery and is the main prey item for large predatory fish (Speranza et al., 2013).

When studying trophic ecology, one of the most difficult concepts is the classification of organisms as specialists or generalists. Parasites are usually highly host-specific (Sukhdeo and Hernandez, 2005), but the problem is why some species appear to not specialize as consistently on a single host species (Thompson, 1982). In the best revision about this approach Sukhdeo and Hernandez (2005) indicated that phylogenetic history as morphologic characters and feeding behaviors of both players are able to promote parasite-host specificity. Authors argue that if you base your definition of specificity on the number of higher taxa on which a parasite feeds it allows inferences about the extent to which a parasite is tracking it host taxon phylogenetically. Our results suggest that these two species of stegophilines show host-specificity, because they boot feed mainly on species belonging to the same order. The study of the feeding behavior of other stegophilines may test if the evolution of mucous and scale feeding behavior of these catfishes has been associated to a coevolutionary interaction with the order Characiformes.

This is the first time the mucous found in the stomachs of mucophagic species were identified through DNA extraction and sequencing, and this approach may be used to any parasite/host interactions in any group of organisms. It can also test hypotheses based on empiric observations that lack testing support. For example, Sazima (1983) reports the characid fish *Probolodus heterostomus* as lepidophagous and associates the scales found in its stomach with the shoal that this species is mimetic. Since then, no study has been done to determine the origin of scales commonly found in the stomachs of *P. heterostomus*: are them from its shoal or can be originated from other species, preferentially not belonging to the shoal?

In other examples, Lima et al. (2012) reports a behavior of "mutilating predation" for *Odontostilbe pequira* preferentially attacking *Leporinus friderici*, but do not show any support as molecular identification of food items. Based only in field observations of the putative attack, *O. pequira* is currently classified as omnivore. Other problem is related to the determination of species in piscivorous fish diets. In most cases it is possible to determine only the genus or family of prey by bone remains of semi digested preys in the stomachs due the similarity of structures or the stage of fragment digestibility (Hansel et al., 1988). In such cases, the DNA identification of hosts or preys is the best alternative to solve these questions, and can be applied to any parasite/host or predator/prey interaction.

Thus, in our case, regardless the relative small samples from which stomach contents were positively identified through DNA extraction, some patterns are clearly discernible in the two stegophiline species: (1) they are truly parasites once they are obligatory mucophagous and lepidophagous; (2) Characiformes are preferred hosts event thought they are not the most abundant order in their environments; (3) host choice is related to the habits of hosts, choosing those that feed and live in or near the bottom. The unanswered question now is: why the preferential choice for Characiformes?

Finally, the accurate analysis of parasite/host interactions of the stegophilines allowed us to better understand their function on the freshwater ecosystem where these are inserted. From this point, we conclude that molecular helps in reveal any trophic interaction, including the identification of food items not allowed by usual methods, permitting a whole comprehension of the interactions inside of the food webs and any trophic ecology of the system.

## AUTHOR CONTRIBUTIONS

KB, PS, and LM: Conceived and designed the experiments; KB and PS: Performed the experiments; KB, PS, and LM: Analyzed the data; PS and LM: Contributed reagents, materials, analysis tools; PS: Drawn the blocked primer; KB, PS, and LM: Wrote the paper; KB, PS, and LM: Reviewing the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00022/full#supplementary-material

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