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Original Article

# Characterizing functional morphology and trophic niches in a neotropical Characiforms (Actinopterygii: Teleostei) assemblage in middle Munim River basin, Maranhão, Brazil

## Caracterizando a morfologia funcional e os nichos tróficos em uma assembleia de Characiformes (Actinopterygii: Teleostei) neotropicais na bacia do médio Rio Munim, Maranhão, Brasil

E. S. Oliveira[a,b], J. South[c], E. C. Guimarães[d], L. O. Vieira[a,b], D. S. Campos[a,b] and F. P. Ottoni[a,b]*

[a]Universidade Federal do Maranhão – UFMA, Programa de Pós-graduação em Rede de Biodiversidade e Biotecnologia da Amazônia Legal, São Luís, MA, Brasil

[b]Universidade Federal do Maranhão – UFMA, Laboratório de Sistemática e Ecologia de Organismos Aquáticos, Chapadinha, MA, Brasil

[c]University of Leeds, Faculty of Biological Sciences, School of Biology, Leeds, United Kingdom

[d]Universidade Federal do Oeste do Pará – UFOPA, Instituto de Ciências da Educação, Programa de Pós-graduação em Sociedade, Natureza e Desenvolvimento, Santarém, PA, Brasil

**Abstract**

Understanding how functionally similar species segregate resources to minimize competition is vital for predicting evolutionary factors and patterns of coexistence. We conducted a study in Mata de Itamacaoca, in the middle Munim River basin, Maranhão, northeastern Brazil, to characterize the functional morphology and trophic niches of five coexisting Characiform species in this area - including a recently described species, and to investigate whether their functional morphology is a key determinant of their trophic niches. Our analysis of functional morphology and diet, employing linear measurements to predict dietary specializations, showed that these species are predominantly generalist insectivores with a significant morphological overlap. This study underscores the influence of species' natural history on their ecological characteristics, contributing to more effective conservation strategies.

**Keywords:** functional morphology, fish, Munim river, niche overlap, resource partitioning.

**Resumo**

Compreender como espécies funcionalmente semelhantes segregam recursos para minimizar a competição é vital para prever fatores evolutivos e padrões de coexistência. Conduzimos um estudo na Mata de Itamacaoca, na bacia do médio rio Munim, Maranhão, nordeste do Brasil, para caracterizar a morfologia funcional e nichos tróficos de cinco espécies de Characiformes coexistentes nesta área - incluindo uma espécie recentemente descrita, e investigar se a morfologia funcional dessas espécies é determinante para seus nichos tróficos. Nossa análise da morfologia funcional e da dieta, empregando medidas lineares para prever especializações alimentares, mostrou que essas espécies são predominantemente insetívoras generalistas com sobreposição morfológica significativa. Este estudo ressalta a influência da história natural das espécies em suas características ecológicas, contribuindo para estratégias de conservação mais eficazes.

**Palavras-chave:** morfologia funcional, peixe, rio Munim, sobreposição de nicho, partição de recursos.

## 1. Introduction

Freshwater ecosystems play a pivotal role in ecological research by offering a window into the complex dynamics that shape biological communities (Bower and Winemiller, 2019; Melo et al., 2021). Understanding the processes that shape communities is fundamental to ecology. Freshwater fish assemblages provide an excellent model system for addressing fundamental questions, especially in regions with a high diversity of functionally similar species, such as in the Neotropical region (Reis et al., 2016). Speciation in these regions often depends on factors such as niche opportunities, migratory constraints, and intricate species interactions (Garcia et al., 2020; Melo et al., 2021). Niche partitioning, as a consequence of diversification, can alleviate interspecific and intraspecific competition across trophic (Brandão-Gonçalves and Sebastien, 2013; Lubich et al., 2022), spatial or temporal niches (Gomiero et al., 2010), and is often mirrored in morphological characteristics of species (Garcia et al., 2020).

*e-mail: fpottoni@gmail.com
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Morphological traits serve as invaluable tools for understanding how fish species delineate their trophic and habitat niches across various scales, from macrohabitats to microenvironments (Bower and Winemiller, 2019). This approach has played a crucial role in unraveling niche partitioning within assemblages (Sibbing and Nagelkerke, 2000), assessing responses to environmental changes and predation pressure (Santi et al., 2020), comprehending susceptibility to parasitism (Pegg et al., 2015) and evaluating aspects of ontogeny (Villares Junior and Goitein, 2016). However, it is essential to recognize that morphological specialization alone does not rigidly dictate trophic niches, especially in resource-rich systems. In such ecosystems, even specialized species may adopt a degree of trophic generalism, a phenomenon known as "Liem's Paradox" (Robinson and Wilson, 1998). Species often exhibit dietary flexibility based on resource availability or adjust the breadth of their niches in response to competitive pressure (Dominguez-Almela et al., 2021).

Characiform species are present in Afrotropical and Neotropical freshwater systems. This order is highly specious with vast variations in body shape and size (Burns and Sidlauskas, 2019) and trophic guild (Barbosa et al., 2017). The Neotropical region alone boasts over 1,700 described Characiform species (Reis et al., 2016), marking it as a hotspot of diversity and ecological complexity. They have repeatedly transitioned between different trophic ecologies according to the resources available in the environment, such as invertivory, omnivory, herbivory, piscivory, and detritivory (Burns and Sidlauskas, 2019).

Despite their prominence, the fish inhabiting the river basins of the State of Maranhão in northeastern Brazil remain relatively enigmatic, with sparse ecological data (Abreu et al., 2019). The Munim River basin is one of the main hydrological units in the state of Maranhão, Brazil (Koerber et al., 2022; Vieira et al., 2023), has approximately 16,000 km$^2$ and is located in a transitional zone between the Amazonia and Brazilian Cerrado (its lower portion), and in a zone with a phytophysiognomy typical from the Brazilian Cerrado (Upper portion) (UEMA, 2016; Vieira et al., 2023), a biome considered a biodiversity hotspot (Myers et al., 2000). Within this basin, the Mata de Itamacaoca, a protected area amid urbanization, located in the middle Munim River basin according to Vieira et al. (2023), shelters a diverse assemblage of Characiforms, characterized by their small size and functional similarity (Oliveira et al., 2020). The prevalence of functionally similar species suggests a finely balanced ecosystem shaped by ongoing interspecific competition (Burns and Sidlauskas, 2019).

Our research was motivated by the Characiform species inhabiting the Mata de Itamacaoca, where their predominance hints at a central role in shaping aquatic communities, which is consistent with findings from broader Neotropical studies (Burns and Sidlauskas, 2019). Specifically, we hypothesized that the functional morphology of these Characiform species is a key to understanding their trophic niches. Through a comparative analysis of functional morphology and diet, we characterized the functional morphology and trophic niches of the coexisting characiform species in this area. The anticipated outcomes of our study provide crucial insights into the ecological dynamics of these species, ultimately contributing to the formulation of essential conservation measures necessary to address ongoing environmental changes.

## 2. Material and Methods

### *2.1. Study area and field sampling*

This study was conducted in Mata de Itamacaoca, a protected urban area spanning 460ha within the Cerrado biome, situated at an elevation of approximately 90 meters above sea level in the municipality of Chapadinha, State of Maranhão, Brazil (3°44'55.16"S 43°19'57.10"W) (see Figure 1, Table 1). The study area encompasses a diverse array of plant formations, incorporating riparian and gallery forests alongside watercourses, as well as various stream springs, sheltering a diversity of fish communities (Oliveira et al., 2020; Vieira et al., 2023). It also includes closed forest formations, characterized by trees exceeding 10 meters in height (Silva et al., 2008). The establishment of the protected area aimed to sustain the city's water supply, necessitating the preservation of vegetation integrity around springs, water bodies, and reservoirs (Silva et al., 2008). It is pertinent to highlight that this area has been acknowledged as an Area of Relevant Ecological Interest for the conservation of fauna and flora by Municipal Decree No. 05/2018 (Maranhão, 2018). Additionally, Mata de Itamacaoca plays a pivotal role in maintaining environmental equilibrium and contributes to local climate regulation, soil conservation, and water quality improvement (Silva et al., 2008). Fish were collected from August 2014 to February 2020, amounting to a total of 22 sampling events. These collections occurred at five distinct sampling sites (C1-C5), Including an environment altered by a dam (C4), which was described by Oliveira et al. (2020), distributed across the Mata de Itamacaoca, within the middle Munim River basin (Vieira et al., 2023) (see Figure 1, Table 1). Fish were captured using dip nets "*redes de mão*" (80 cm long by 54 cm wide, 2 mm mesh) and trail nets "*redes de arrasto*" (240 cm long x 100 cm high, 2 mm mesh), following the methods outlined by Auricchio and Salomão (2002). The collection procedures adhered to the animal welfare guidelines, established by Underwood and Anthony (2020). Specimens were euthanized in a solution of ethyl-3-amino-benzoate-methanesulfonate (MS-22) at a concentration of 250 mg/L until opercular movements ceased. Following euthanasia, the specimens were preserved in formalin (10%) and transferred to a 70% ethanol solution after 10-15 days. The specimens were deposited in the Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais of Universidade Federal do Maranhão (CICCAA) (see Supplementary Material 1).

### *2.2. Diet characterization*

Gut content was examined in 161 individuals representing five species of Characiforms: *Astyanax* cf. *bimaculatus* (n=23; Characidae); *Hemigrammus* cf. *ocellifer* [*Hemigrammus* sp. 1 *sensu* Oliveira et al. (2020)] (n=40; Characidae); *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni, 2018 (n=39; Characidae); *Characidium* sp. (n=20;

Crenuchidae); and *Nannostomus beckfordi* Günther, 1872 (n= 39; Lebiasinidae). We calculated the occurrence frequency (FO) of each food item, based on the number of times each item appeared in the stomach divided by the number of stomachs analyzed (Hyslop, 1980). The volume (V) of each food item in the stomach was determined by the volumetric method (Hellawell and Abel, 1971; Hyslop, 1980). These values were used to calculate an adapted feeding index (IAi) for each species following the method of Kawakami and Vazzoler (1980).

To assess diet overlap within the Characiform assemblage, we performed an analysis of Non-Metric Multidimensional Scaling (nMDS) using the Bray-Curtis dissimilarity coefficient and Wisconsin Double standardization via vegan::metaMDS (Oksanen et al., 2019). The stress values were <0.2, indicating that the ordination interpretation on the first two axes was appropriate. A PERMANOVA (999 permutations) was conducted to test for differences in the diets of Characiforms following the assessment of homogeneity of variance between groups.

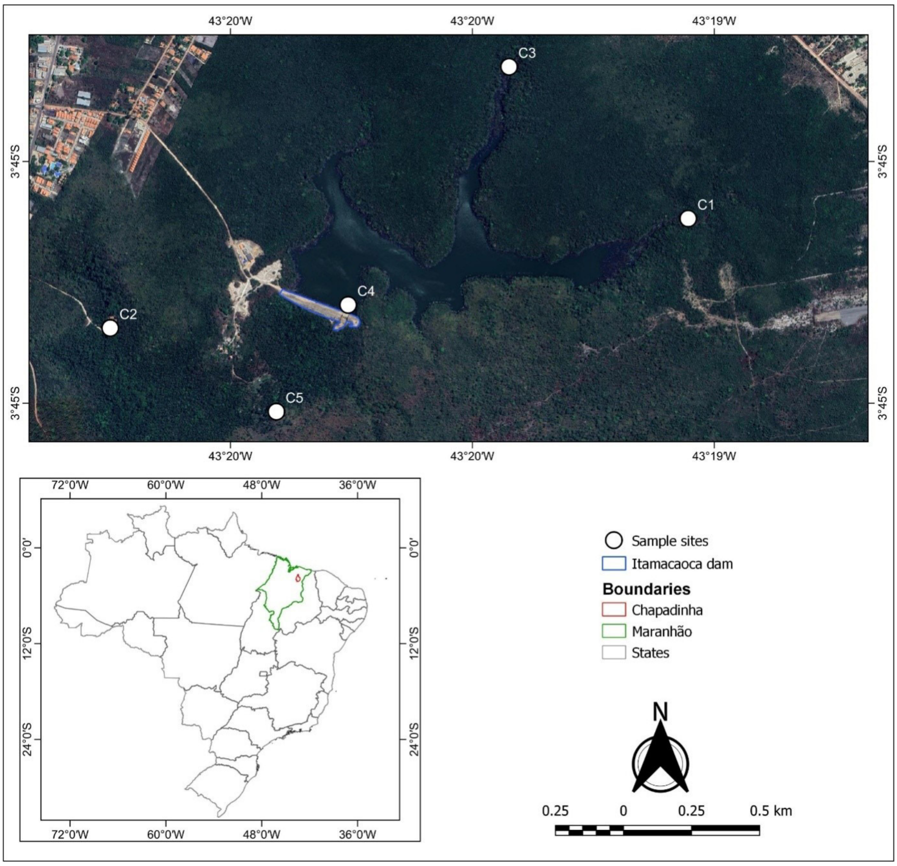


**Figure 1.** Location of the sampling sites (C1-C5) distributed across the Mata de Itamacaoca, within the middle Munim River basin, Brazil.

**Table 1.** Sample sites at the Mata de Itamacaoca, middle Munim River basin, State of Maranhão, Brazil.

| Collecting Site | Coordinates | Remarks |
|---|---|---|
| C1 | 3°44'45.20"S 43°19'15.10"W | Stream near spring, surrounded by a gallery and riparian forest, within Mata de Itamacaoca, situated in the Municipality of Chapadinha, State of Maranhão. Please note that surveys on this location encompassed approximately 200 meters along the watercourse. |
| C2 | 3°44'58.24"S 43°20'23.91"W | Stream situated within the area known as Repouso do Guerreiro, located in Mata de Itamacaoca, part of the Municipality of Chapadinha, in the State of Maranhão. |
| C3 | 3°44'27.1"S 43°19'36.4"W | Stream close to a natural water source, featuring a gallery and riparian forest, located in Mata de Itamacaoca, within the Chapadinha Municipality, in the State of Maranhão. |
| C4 | 3°44'55.16"S 43°19'57.10"W | Itamacaoca dam, situated in the Chapadinha Municipality, within the State of Maranhão. |
| C5 | 3°45'8.20"S 43°20'4.13"W | Stream, after the dam located in Mata de Itamacaoca, within the municipality of Chapadinha, in the State of Maranhão. |

Initially, a full model with interactions was included and then simplified stepwise for the most parsimonious model. ANOSIM was performed to determine whether differences in diet between species exceeded variations within species. We also conducted an indicator species analysis using indicspecies::multipatt (De Cáceres et al., 2010) to identify statistically more abundant resources (alpha = 0.05) in each species diet. For these analyses, the gut contents were categorized into functional groups that reflected the properties of aquatic food types, such as size, shape, and escape characteristics: Insects (Coleoptera, Diptera, Ephemeroptera, Hymenoptera, Hemiptera, Isoptera, Collembola, Trichoptera, Insect remains), Insect larvae (Coleoptera larvae, Diptera larvae, Hemiptera larvae, Odonata larvae, Trichoptera larvae), Plant material (Leaves, Flowers, Filamentous algae, Plant remains), Zooplankton (Cladocera, Hydracarina), Araneae (Spiders), Worms (Nematodes), Crustaceans (Decapoda), Fish (Fish scale).

*2.3. Functional morphology*

We selected 40 adult individuals from each of the five studied species, with similar standard lengths and well-preserved fins. In cases where the total number of individuals for a particular species in the collection was less than 40, we measured a minimum of 20 individuals (Table 2). We adapted here measurements based on the protocol established by Balon et al. (1986), Sibbing and Nagelkerke (2000), and Breda et al. (2005) to obtain the 20 linear morphological measurements on the captured fishes (see Supplementary Material 2). These morphological measurements were selected based on their relevance to feeding capacity, habitat use and locomotion, all of which are key factors influencing the ecological niche of each of the five studied species (see Supplementary Material 2). Accurate measurements of morphological traits were obtained using a digital caliper (two decimals of precision) and observed using microscopic stereoscopy. To correct for the overall size difference, we applied Mosimann corrections to each of the morphological traits as this provides the most accurate detection of shape differences after controlling for size variation (Jungers et al., 1995). This was performed by calculating the geometric mean and dividing the individual trait values by it to produce size-corrected values. The geometric mean (GM) was then included as a conglomerate trait variable to represent the overall body size/shape instead of the standard length (SL) (Luger et al., 2020). First, using all measured specimens, Principal Component Analysis (PCA) was completed on the correlation matrix to determine species overlap in morphospace. All statistical analyses were performed using R software (R Core Team, 2021).

## 3. Results

The consumption of food items by the five characiform species is presented in Tables 3 and 4. A total of 25 distinct food items were recorded in the respective diets (see Table 3). Insects were the most proportionately represented in the diets of all the species diets (see Table 3). We also found a significant number of insect larvae in the diets of *Characidium* sp. and *H. piorskii* (see Table 3, Table 4, Figure 2). *Astyanax* cf. *bimaculatus* was the only species to consume other fish parts (fish scale), crustaceans, and spiders (see Table 3, Table 4, Figure 2). Seeds comprised 21.5% of *A.* cf. *bimaculatus* gut contents. *Hemigrammus* cf. *ocellifer* had the highest proportion of plant material (33.8%) (see Table 3, Table 4, Figure 2). Seeds comprised 11.1% of *N. beckfordi* gut volume. Zooplankton comprised only small amounts in *H. piorskii* and *Characidium* sp. (see Table 3, Table 4, Figure 2). Worms (nematodes) were found in the gut contents of two individuals of *Characidium* sp. (see Table 3, Table 4, Figure 2).

The nMDS showed a diet overlap between the Characiform species (see Figure 3). PERMANOVA indicated a weak but significant difference between species diet compositions, where species identity was responsible for 27% of the variance in the dataset ($R^2 = 0.27$, $F_{4, 82} = 7.42$, $p<0.001$; see Figure 3). Similarly, ANOSIM showed weak but significant differences in diet dissimilarity between the species ($R=0.31$, $p < 0.001$). The consumption of Coleoptera, Coleoptera larvae, Hemiptera, insect remains,

**Table 2.** Trophic guild, Sample size of the five Characiform species employed in the characterization of functional morphology and trophic niche, mean ± standard deviation (SD), median and range of all sampled Characiform standard length (SL).

| Family/Species | Trophic Guild | Sample size | SL Mean ± SD (mm) | SL Median (mm) | SL Range (mm) |
|---|---|---|---|---|---|
| **Characidae** | | | | | |
| *Astyanax* cf. *bimaculatus* | Omnivorous-Generalist | 23 | 56.22 ± 9.8 | 57.22 | 27.50 – 70.25 |
| *Hemigrammus* cf. *ocellifer* | Insectivore-Generalist | 40 | 29.75 ± 2.50 | 29.75 | 24.72 – 35.50 |
| *Hyphessobrycon piorskii* | Insectivore-Generalist | 39 | 24.88 ± 3.19 | 25.15 | 16.80 – 31.50 |
| **Lebiasinidae** | | | | | |
| *Nannostomus beckfordi* | Insectivore-Generalist | 39 | 27.12±1.01 | 27.1 | 25.02 – 30.3 |
| **Crenuchidae** | | | | | |
| *Characidium* sp. | Insectivore-Generalist | 20 | 24.52±1.16 | 24.58 | 21.95 – 26.84 |

**Table 3.** Alimentary importance index (IAi) of food items of each of five Characiform species in Mata de Itamacaoca, middle Munim River basin, Brazil.

| Food items | *Astyanax* cf. *bimaculatus* | *Hemigrammus* cf. *ocellifer* | *Hyphessobrycon piorskii* | *Nannostomus beckfordi* | *Characidium* sp. |
|---|---|---|---|---|---|
| Insects | | | | | |
| Coleoptera | 1.48 | 9.75 | 4.48 | 1.47 | 3.72 |
| Diptera | 3.35 | 3.49 | 2.80 | 1.67 | |
| Ephemeroptera | 4.01 | | | | 3.52 |
| Hymenoptera | 1.56 | 2.50 | | 2.87 | |
| Hemiptera | 4.14 | 9.85 | 9.16 | | |
| Isoptera | 1.01 | 2.55 | | 9.83 | 4.68 |
| Collembola | 5.01 | | 1.19 | | |
| Trichoptera | 3.34 | | | | |
| Insect remains | 1.05 | 5.69 | 3.54 | 6.25 | 9.49 |
| Insect larvae | | | | | |
| Coleoptera larvae | 8.53 | 1.10 | 2.32 | 2.40 | 5.48 |
| Diptera larvae | 4.51 | 2.51 | 6.79 | 2.85 | 2.10 |
| Hemiptera larvae | | | | | |
| Odonata larvae | 1.62 | | | | 2.93 |
| Trichoptera larvae | | | | | 1.01 |
| Plant material | | | | | |
| Leaves | | 4.72 | | | |
| Flowers | | 1.38 | 2.12 | | |
| Filamentous algae | | 3.81 | | 2.82 | |
| Seeds | 6.64 | 1.92 | 7.01 | 1.68 | |
| Plant remains | 3.77 | 1.35 | 1.56 | 1.15 | |
| Zooplankton | | | | | |
| Cladocera | | | 1.46 | 8.88 | 1.98 |
| Hydracarina | | | 1.79 | 2.66 | 3.40 |
| Araneae | | | | | |
| Spiders | 2.18 | | | | |
| Worms | | | | | |
| Nematodes | | | | | 2.91 |
| Crustaceans | | | | | |
| Decapoda | 2.90 | | | | |
| Fish | | | | | |
| Fish scale | 2.68 | | | | |

**Table 4.** All significant indicator resources of feeding items are grouped into categories related to of each the five Characiform species.

| Species | Resource | r.g. | p |
|---|---|---|---|
| *Astyanax* cf. *bimaculatus* | Insects | 0.711 | <0.001 |
| | Fish | 0.613 | <0.001 |
| | Crustaceans | 0.508 | <0.001 |
| | Spiders | 0.418 | <0.01 |
| *Hyphessobrycon piorskii, Characidium* sp. | Insect larvae | 0.406 | <0.01 |
| *Astyanax* cf. *bimaculatus*, *Hemigrammus* cf. *ocellifer*, *Nannostomus beckfordi* | Plant material | 0.494 | <0.001 |

r.g.= the point biserial correlation coefficient where higher values indicate stronger association.

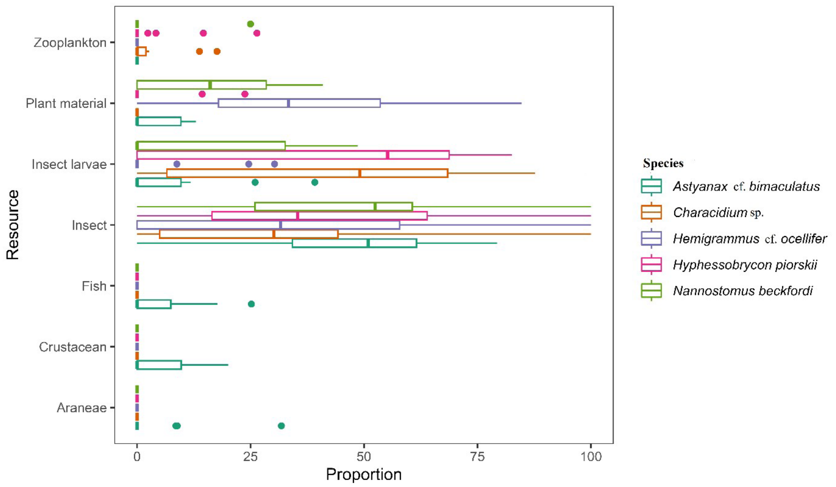


**Figure 2.** Proportional contribution (volume) of feeding items grouped into categories related to the five Characiform species, Mata de Itamacaoca, middle Munim River basin.

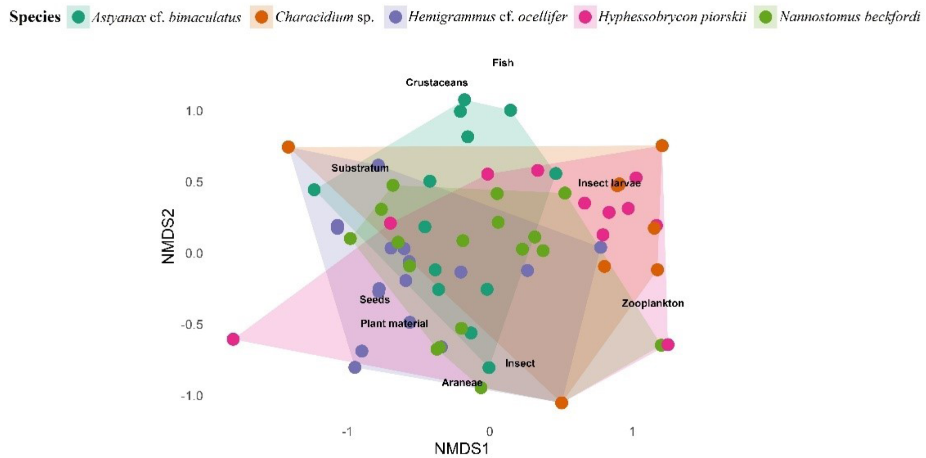


**Figure 3.** Non-metric multidimensional scaling of the resource use of Characiform species of the Mata de Itamacaoca, middle Munim River basin. Shaded areas indicate trophic niche overlap.

filamentous algae, and seeds contributed the most to this dissimilarity (see Table 2, Table 3). Insects, seeds, fish, crustaceans, and spiders were strongly associated with *A.* cf. *bimaculatus* (see Table 4). Insect larvae were moderately associated with *Characidium* sp. and *H. piorskii* (see Table 4). Plant materials were strongly associated with *A.* cf. *bimaculatus*, *N. beckfordi,* and *H.* cf. *ocellifer* (see Table 4).

The first and second axes of PCA captured 40.9% of the variance (see Figure 4A, Figure 4B). There was some separation along PC1 by *A.* cf. *bimaculatus* indicating deeper body depth, body width, head depth, and geometric mean. Whereas *N. beckfordi* and *Characidium* sp. are separated along PC1 from the rest of the assemblage (see Figure 4A, Table 5). They were thus distinguished from the rest of the assemblage by increasing the gill raker distance and caudal peduncle depth on PC1. *H. piorskii* and *H.* cf. *ocellifer* occupy the center of the morphospace showing wider plasticity (see Figure 4A, Table 5).

## 4. Discussion

In this study, we conducted a functional morphological analysis in five Characiform species (*A.* cf. *bimaculatus*, *H.* cf. *ocellifer*, *H. piorskii*, *N. beckfordi*, and *Characidium* sp.), belonging to three families (Characidae, Lebiasinidae and Crenuchidae), which coexist in a protected area in Mata de Itamacaoca, middle Munim River basin, Maranhão, Brazil (see Table 2, Table 3). However, most of these species examined here (three) belong to the family Characidae (*A.* cf. *bimaculatus*, *H.* cf. *ocellifer* and *H. piorskii*), while the remaining two species (*N. beckfordi* and *Characidium* sp.) belong to the families Lebiasinidae and Crenuchidae, respectively (Fricke et al., 2023).

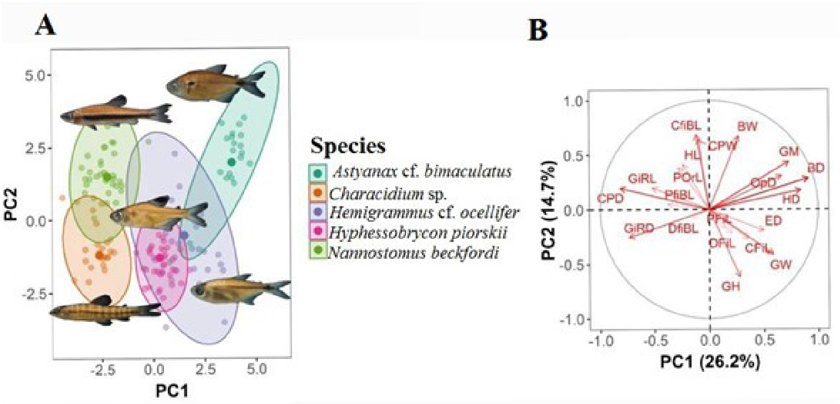


**Figure 4.** Biplot of Principal Component Analysis (PCA) of: (A) morphological trait space between Characiforms species; and (B) variable loadings on the PC axes.

**Table 5.** Abbreviation and Kendall correlation (tau Kendall) values were obtained for each morphological attribute in the two first axes (PC1 and PC2) of the principal component analysis (PCA).

| Morphological attribute | Abbreviation | PC1 | PC2 |
|---|---|---|---|
| Body depth | BD | **0.9088** | 0.2852 |
| Body width | BW | 0.2685 | 0.6705 |
| Head length | HL | -0.2239 | 0.4120 |
| Head depth | HD | **0.8402** | -0.1769 |
| Pectoral fin length | PFiL | 0.1454 | -0.1737 |
| Dorsal fin length | DFiL | 0.2075 | -0.2203 |
| Caudal fin length | CFiL | 0.3565 | -0.2679 |
| Pectoral fin base | PfiBL | -0.3783 | 0.0478 |
| Dorsal fin base | DfiBL | -0.1495 | -0.0829 |
| Caudal fin base | CfiBL | 0.3565 | -0.2679 |
| Caudal peduncle depth | CPD | **-0.8159** | 0.1962 |
| Caudal peduncle width | CPW | -0.0991 | 0.6422 |
| Oral gape width | GW | 0.5862 | -0.4109 |
| Geometric mean | GM | **0.7300** | 0.4376 |
| Oral gape height | GH | 0.2809 | -0.6170 |
| Eye diameter | ED | 0.4992 | -0.1941 |
| Postorbital length | POrL | -0.2837 | 0.3819 |
| Operculum depth | OpD | 0.6626 | 0.3089 |
| Gill raker length | GiRL | -0.5232 | 0.1983 |
| Gill inter-raker distance | GiRD | **-0.7325** | -0.2547 |

Values > 0.7 are in bold because they explain most of the morphological patterns for both species.

Closely related sympatric species may present high competitive potential owing to niche conservatism; therefore, they are expected to exhibit niche differentiation to reduce the effects of interspecific competition (Sampaio et al., 2013). The Characiform assemblage of the Mata de Itamacaoca has considerable trophic niche overlaps and clustered representations in morphospace. Similar results were found by Mise et al. (2013), where three Characiform species had similar trophic and morphological niches, with variation in resource contribution to diet depending on size and trophic position. Therefore, resource partitioning may occur between the habitat preferences of the five studied species.

The characiform assemblage is dominated by generalist species. *Astyanax* cf. *bimaculatus* is the largest and most omnivorous species whereas the other species are insectivorous. Therefore, dietary plasticity and generalist trophic profiles explain the trophic niche overlap in our results. However, *Astyanax* cf. *bimaculatus* separates from the rest of the assemblage in morphology and shows a larger overall size (GM), deeper body, wider body, and deeper head. These traits are all related to the exploitation of larger prey and fish ambush strategies; hence, a more predatory trophic behavior (Nagelkerke et al., 2018; Luger et al., 2020). This is reflected by the proportion of insects, seeds, fish parts (fish scales), crustaceans, and spiders in its diets, which drives the dissimilarity in the trophic niche between the species. Other investigations into *A.* cf. *bimaculatus* trophic niche concluded similar results of a generalist omnivorous diet (Silva-Camacho et al., 2014) and a lack of morphological specialization throughout the genus *Astyanax* Baird and Girard 1854 (Casatti et al., 2001). The intraspecific morphospace occupied by *A.* cf. *bimaculatus* was the broadest, although, it was likely driven by ontogenetic differences in shape.

All species relied heavily on insect prey, this may be due to chitinous bodies taking longer to digest and biasing the results, but regardless shows the importance of the riparian aquatic trophic linkage in the Mata de Itamacaoca. However, some species, such as *Characidium* sp. and *H. piorskii*, have diets that mainly include insect larvae. This may be related to specific morphological adaptations, such as the distance between the gills (GiRD), which may allow efficient capture of insect larvae at the expense of other types of prey. Species such as *H. piorskii* and *Characidium* sp. have reduced amounts of zooplankton in their diets and worms (nematodes) have even been found in some individuals. These feeding habits may be related to their morphological characteristics, which may not be well adapted for the efficient capture of zooplankton compared to other prey. We suggest a stable isotope approach in the future to obtain a more representative snapshot of the trophic interactions in this system.

There is relatively little precise information on the trophic preferences of the species in our study and the inclusion of one newly described species (*H. piorskii*) means that inferences are restricted to similar species in other locations. Species of the genus *Hyphessobrycon* Durbin 1908 are usually classified as generalists with an insectivorous tendency (Benone et al., 2020), whereas *Hemigrammus* Gill 1858 tend to be insectivorous (Graciolli et al., 2003). The notable resemblance in dietary patterns between *H. piorskii* and *H.* cf. *ocellifer* can be attributed to their overlap within a shared morphospace. Conversely, *A.* cf. *bimaculatus* showed a distinct preference for seeds and plant material over insects. The substantial inclusion of plant-based material in the diet of *H.* cf. *ocellifer* may potentially be correlated with their morphological attributes, suggesting a digestive system adapted to process plant-based sustenance. We believe that if internal measurements had been included, it is expected that the two species would show differences in gut length, a trait associated with the digestion of plant matter (Nagelkerke et al., 2018).

*Nannostomus beckfordi* and *Characidium* sp. separated from the rest of the assemblage in morphospace, and were characterized by caudal peduncle depth, and increased gill raker distance. These traits are indicative of micro-predators sit-and-wait predation strategy (Bower and Piller, 2015). The gut contents corroborate this inference, as both species consumed relatively high proportions of insect larvae and zooplankton compared to the other species. Although the two species diverged in the niche as there was more algal matter in *N. beckfordi* gut contents compared to *Characidium* sp. which was dominated by insect larvae. However, due to the digestion process, the diet analysis remains only a snapshot of diet composition over time and reflects the opportunistic generalist feeding behavior of the assemblage. The trophic capacities of species of the genera *Nannostomus* Günther 1872 and *Characidium* Reinhardt 1867 are both concordant with those in Silva (1993).

Our findings strongly support our hypothesis that the functional morphology of Characiform species plays a pivotal role in determining their trophic niches within the Mata de Itamacaoca. The results revealed that the Characiform assemblage of the Mata de Itamacaoca is overwhelmingly represented by generalist insectivores that share trophic profiles. We provide dietary and functional morphology data from an underrepresented freshwater ecoregion, which improves our understanding of the mechanisms of coexistence in diverse and phylogenetically related fish assemblages. The persistence of so many functionally similar species may be facilitated by the integrity of the riparian vegetation of the protected area, as well as by the ecological stability of generalist strategies in dynamic habitats (Mazzoni et al., 2012; Roldi et al., 2014). In the case of the more morphologically extreme group of this assemblage, *N. beckfordi* and *Characidium* sp., there was weak evidence of niche divergence towards lower trophic resources by *N. beckfordi* and *Characidium* sp., despite the functional morphology being suitable for higher trophic profiles. Trophic plasticity related to functional morphological traits may facilitate resilience to future environmental changes and may be further regulated by the partitioning of habitat and position in the water column (Baldasso et al., 2019). Predictive functional morphology may have a higher discriminatory power when comparing different ichthyofaunal families rather than within the family due to their close similarities. Hence, the natural history information acquired through this research can significantly enhance our understanding of how species function in their ecosystems. Furthermore, these findings can serve as vital input for formulating more effective conservation strategies.

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## Supplementary Material

Supplementary material accompanies this paper.

Supplementary Material 1

Supplementary Material 2

This material is available as part of the online article from https://doi.org/10.1590/1519-6984.279881