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ARTICLE

Synthesis of studies on Comparative brain morphology in Freshwater Fishes in Brazil: a 25-Year Review

Pedro Henrique Marinho-Nunes[1,3]; Gabriel Samora Chacra Amui[1,4]; Jaqueline Fernandes Gomes[2,5]; Alesandra Soares da Silva[2,6] & Thiago Nilton Alves Pereira[1,7]

[1] Universidade Federal do Tocantins, Programa de Pós-Graduação em Biodiversidade, Ecologia e Conservação. Porto Nacional, TO, Brasil.
[2] Universidade Federal do Tocantins, Curso de Ciências Biológicas. Porto Nacional, TO, Brasil.
[3] ORCID: 0000-0002-1652-8737. E-mail: pedromarino2001@gmail.com (corresponding author)
[4] ORCID: 0009-0002-5818-0588. E-mail: gabriel.samora@mail.uft.edu.br
[5] ORCID: 0000-0002-7302-966X. E-mail: jaqueline.fernandes@mail.uft.edu.br
[6] ORCID: 0009-0001-1037-1986. E-mail: soares.alesandra@mail.uft.edu.br
[7] ORCID: 0000-0001-6682-7471. E-mail: thiago.na@mail.uft.edu.br

Abstract. The Central Nervous System (CNS) of fishes in Brazil represents an anatomical domain that remains largely underexplored, despite its significant potential to advance phylogenetic, ecological, and behavioral studies. This review synthesizes 25 years (1999-2024) of literature on freshwater fish brain morphology in Brazil, identifying major advancements and persistent knowledge gaps. A total of 16 studies were identified, with a pronounced focus on Siluriformes (62.5%), while other orders outside the Characiphysi clade remain poorly studied. These descriptive and comparative investigations have provided valuable systematic insights, revealed taxonomic characters, and elucidated behavioral and ecological patterns. Despite these contributions, the field faces notable methodological and conceptual challenges. The lack of standardized protocols for brain extraction and analysis hinders consistency and comparability among studies. Furthermore, advanced techniques, such as non-invasive imaging methods, remain underutilized. Critical aspects such as population-level variation in CNS morphology and its responses to environmental pressures have also been largely overlooked. This review underscores the immense potential of comparative brain anatomy to enhance our understanding of biodiversity and evolutionary adaptations in the rich Brazilian freshwater ichthyofauna.

Keywords. Actinopterygii; Central Nervous System; Encephalon; Neotropical; Neuroanatomy.

INTRODUCTION

Neotropical rivers harbor a remarkable diversity of fish, with approximately 6.000 species currently described across the major river basins in this region (Reis *et al.,* 2016; Dagosta & de Pinna, 2019; Albert *et al.,* 2020). However, the full true extent of this diversity remains elusive, as the actual number of species in the region is underestimated (Mora *et al.,* 2011). The majority of this diversity is concentrated within Teleostei, particularly in Otophysi, which encompasses approximately 70% of Neotropical freshwater fish species (Malabarba & Malabarba, 2020). Over the past decades, systematic and taxonomic studies of fish have advanced considerably, with hundreds of species described annually in the region, estimating over 9.000 neotropical fish species (Reis *et al.,* 2016). These new taxa are largely supported by anatomical data, basically on osteology, myology, and molecular biology (Datovo & Vari, 2014). Despite some progress in recent decades, anatomical studies in fish remain limited, particularly concerning the Central Nervous System (CNS). Recent research has focused on understanding CNS patterns in non-tetrapod Osteichthyes and Chondrichthyes, though studies on ecomorphology and behavior are more prevalent (*e.g.,* Huber *et al.,* 1997; Kotrschal *et al.,* 2017; Shumway, 2010; Van Staaden *et al.,* 1995; Yopak *et al.,* 2007; Yopak & Montgomery, 2008, Yopak *et al.,* 2019), they are still limited when compared to other anatomical approaches. These studies (*e.g.,* Abraão & Shibatta, 2015; Angulo & Langeani, 2017; Datovo & Vari, 2014; Pupo & Brito, 2018) highlight the lack of alternative methods for testing phylogenetic hypotheses and developing proposals. The CNS presents a promising alternative to fill this gap, offering new perspectives for evolutionary and systematic research.

The Central Nervous System (CNS) is an anatomical complex composed of the encephalon, which includes the *Telencephalon, Diencephalon,*

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Mesencephalon, Metencephalon, and *Myelencephalon,* together with the brainstem (primarily comprising the *Mesencephalon, Metencephalon,* and *Myelencephalon*) and the spinal cord (Striedter, 2005). It is responsible for receiving sensory information from the external and internal environments, integrating this information, and responding according to the organism's physiological needs (Butler & Hodos, 2005). The most widely accepted hypothesis regarding the origin of the CNS suggests that neurons migrated from more peripheral and diffused regions of the body toward the cranial region, where they became concentrated and organized. In this area, they grouped based on their morphological, functional, and histochemical affinities, ultimately giving rise to centralized neural structures (Northcutt, 1984). This topographic shift from a diffuse nerve net to a centralized arrangement in the head region marks a crucial step in the evolution of the vertebrate Central Nervous System (Nieuwenhuys *et al.,* 1998; Northcutt, 1984). These groupings can develop differently across taxa according to the evolutionary pressure of the environment, where variations in the shapes and sizes of these structures allow for systematic evolutionary analysis (*e.g.,* Pereira & Castro, 2016; Rosa *et al.,* 2021) and ecological inferences (*e.g.,* Huber *et al.,* 1997; Pollen *et al.,* 2007).

In Brazil, freshwater fishes are represented by highly diverse taxa occupying a wide range of habitats (Reis *et al.,* 2016; Dagosta & de Pinna, 2019; Albert *et al.,* 2020). This diversity is reflected in the complexity of their CNS, which must be highly specialized to process and integrate environmental information (Northcutt, 1984). The Brazilian River basins exhibit considerable taxonomic and phylogenetic fish diversity, coupled with varied habitats, suggesting the existence of an unexplored neuroanatomical knowledge associated with this diversity. In this review, we synthesize comparative fish brain anatomy studies in Brazil conducted over the past 25 years, with a particular focus on non-tetrapod freshwater Osteichthyes and Chondrichthyes. While marine fish lineages represent significant components of global fish biodiversity, this analysis is limited to freshwater taxa due to the scope of available studies and data within the specified timeframe. This approach provides a more accurate representation of the current research landscape in Brazil, while underscoring the need for broader investigations in the future.

MATERIAL AND METHODS

Here, we conducted a systematic review with both quantitative and qualitative approaches (Canuto & Oliveira, 2020; Codina, 2020). Our objective was to identify and analyze studies on comparative brain morphology in freshwater non-tetrapod Osteichthyes and Elasmobranchii fishes conducted in Brazil over the past 25 years (1999-2024). Literature searches were performed using the Web of Science, SciELO Citation Index, and Google Scholar platforms. To optimize the retrieval of relevant publications, we combined search terms using Boolean operators, such as brain morphology + fish, brain anatomy + fish, fish brain, encephalon + fish, brain + Actinopterygii, brain + Teleostei, gross brain morphology + fishes, neuroanatomy + fish, innervation + fish, brain + Elasmobranchii, brain + Chondrichthyes. This strategy enabled a more comprehensive search, encompassing studies that may not have identical terminology. Additionally, the Lattes curricula of key researchers in the field were reviewed to identify complementary studies. All data analyses and graphs were generated using RStudio software, version 4.3.1 (2023-06-16 ucrt) (RStudio Team, 2020).

RESULTS

Sixteen studies published in journals between 1999 and 2024 were identified (Table 1). Among non-tetrapod Osteichthyes, the order with the highest number of studies involving brain morphology was Siluriformes, accounting for 62.5% of the works, followed by Characiformes and Cichliformes, each with 12.5%, and Gymnotiformes with 6.2% (Fig. 1). Additionally, one study was identified for Elasmobranchii, specifically in the order Myliobatiformes (Potamotrygonidae), also representing 6.2% of the total. Other fish orders occurring in Brazil still lack published records focused on the central nervous system. Among the families with formal descriptions of brain anatomy, Loricariidae stands out (Fig. 2), with the brain of the subfamilies Loricariinae (Angulo & Langeani, 2017), Hypoptopomatinae and Neoplecostominae (Rosa *et al.,* 2021), and Hypostominae being formally described (Chamon *et al.,* 2018). The other families are underrepresented, with descriptions of less inclusive taxa, except for Bryconidae, in which the brain of *Brycon orbignyanus* was described and phylogenetic inferences were made using neuroanatomical characters for Characiformes (Pereira

Table 1. List of families examined by order and their respective publications

Order	Family	Author(s)
Myliobatiformes	Potamotrygonidae	Fontenelle & de Carvalho, 2016
Characiformes	Bryconidae	Pereira & Castro, 2016
	Characidae	Abrahão *et al.,* 2019
Siluriformes	Callichthyidae	Pupo & Brito, 2018
	Callichthyidae	Espíndola *et al.,* 2018
	Loricariidae	Angulo & Langeani, 2017
	Loricariidae	Chamon *et al.,* 2018
	Loricariidae	Rosa *et al.,* 2021
	Heptapteridae	Abrahão *et al.,* 2018a
	Pseudopimelodidae	Abrahão & Shibatta, 2015
	Pseudopimelodidae	Abrahão *et al.,* 2018b
	Pseudopimelodidae	Abrahão *et al.,* 2021
	Trichomycteridae	Rizzato & Bichuette, 2024
Gymnotiformes	Apteronotidae	Albert, 2001
	Gymnotidae	Albert, 2001
	Hypopomidae	Albert, 2001
	Rhamphichthyidae	Albert, 2001
	Sternopygidae	Albert, 2001
Cichliformes	Cichlidae	Oliveira & Graça, 2020
	Cichlidae	Oliveira & Graça, 2024

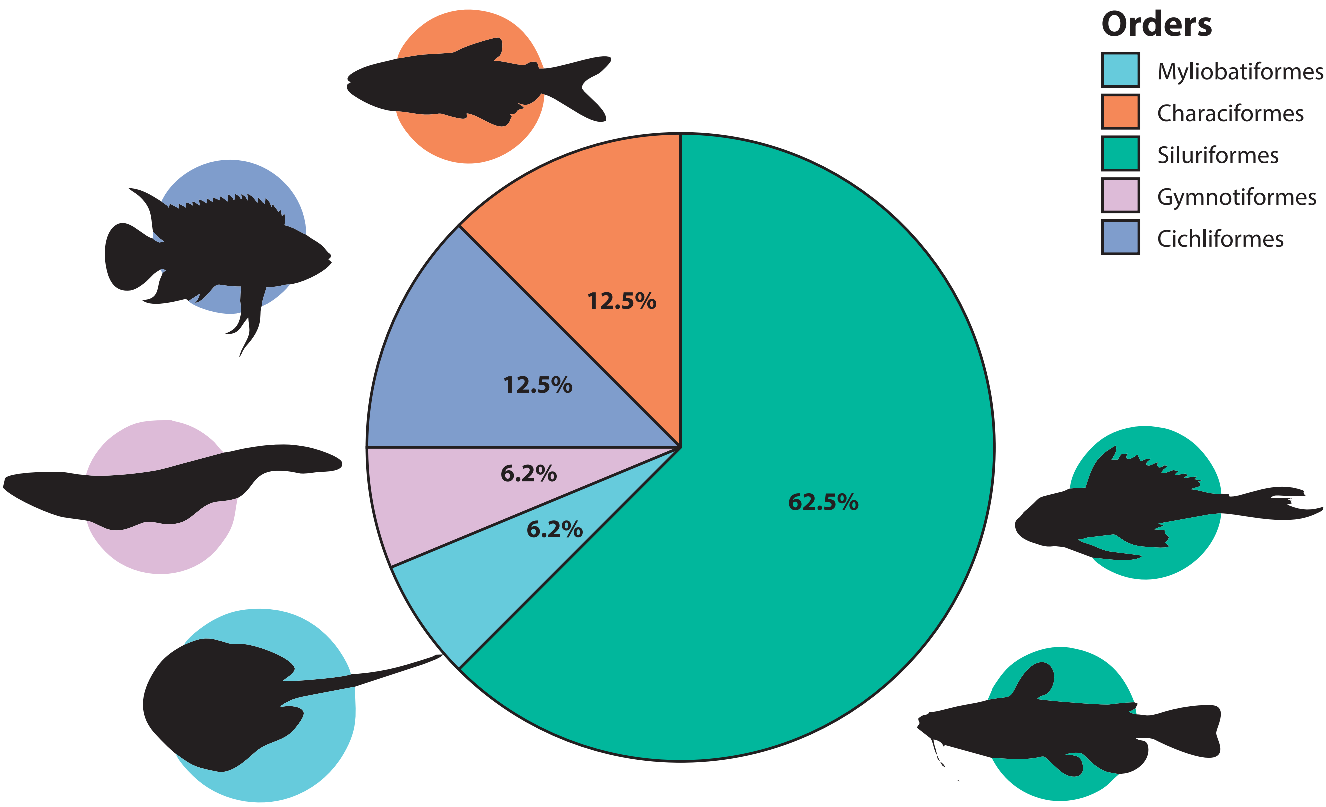

Figure 1. Number of studies on brain morphology descriptions for orders within Brazilian freshwater fish.

& Castro, 2016). Additionally, the brain morphology of the freshwater stingray family Potamotrygonidae (Myliobatiformes) was described by Fontenelle & de Carvalho (2016).

Although the first study in our review was published in 2001 (Albert, 2001), there was a significant gap in publications, lasting 14 years, from 2001 to 2015 (Abrahão & Shibatta, 2015). From 2015 onwards, one article was published per year (Abrahão & Shibatta, 2015; Pereira & Castro, 2016; Angulo & Langeani, 2017). However, in 2018, there was a surge in publications, with five studies published, all focusing on Siluriformes (Chamon *et al.,* 2018; Espíndola *et al.,* 2018; Abrahão *et al.,* 2018a; Abrahão *et al.,* 2018b). After 2018, the number of articles published per year decreased, with only one in 2019 (Abrahão *et al.,* 2019), two in 2020 (Oliveira & Graça, 2020; Rosa *et al.,* 2021), none between 2022 and 2023, and two articles in 2024 (Oliveira & Graça, 2024; Rizzato & Bichuette, 2024) (Fig. 3).

Historical context

The starting point for the study of comparative brain morphology in Brazilian fishes was the paper "Species diversity and phylogenetic systematics of American knifefishes (Gymnotiformes, Teleostei)" (Albert, 2001). In this work, 46 characters from the Nervous System were identified and applied to all families within the order (*e.g.,* Apteronotidae, Sternopygidae, Gymnotidae, Hypopomidae, and Rhamphichthyidae). The integration of non-osteological morphological characters was not common until the early 21st century, with 74% of the characters used in systematic analyses being osteological, and the CNS being used as a source in only 1% of the studies in the world (Datovo & Vari, 2014). Albert (2001) proposed 16 synapomorphies for Gymnotiformes, two of which were derived from the CNS: an expanded dorsal telencephalic area (character 101) and the absence of the accessory optical system (character 107). Additionally, neuroanatomical characters were also used to support other taxa within Gymnotiformes, with the expanded cerebellar lateral valve (character 125) being exclusive to the family Gymnotidae.

Between 2001 and 2014, there were no advancements on the topic (Fig. 3), with no publications during this period. It was only in 2015 that a comparative brain anatomy study of fishes was published (Abrahão & Shibatta, 2015), with the highest peak occurring in 2018, when four studies were published (Fig. 3).

Abrahão & Shibatta (2015) described the brain of *Pseudopimelodus bufonius* and conducted a comparative analysis with its congeners *P. mangurus* and *P. charus,* where they observed low intraspecific variation in brain anatomy, as well as phylogenetic correlations and intraspecific singularities. According to the authors, the brain morphology of *P. bufonius* supports the ecomorphological insights into the species' feeding habits (Shibatta, 2003), where predators tend to have a more developed *Corpus cerebellaris* and *Tectum opticum,* while the *Lobus facialis* and *Lobus vagi,* which are larger in detritivores fish, are more prominent (Kotrschal *et al.,* 1998).

Fontenelle & de Carvalho (2016) analyzed brain morphology, encephalic proportions, and the position of cranial nerves in 11 species representing the four genera of the family Potamotrygonidae, aiming to identify phylo-

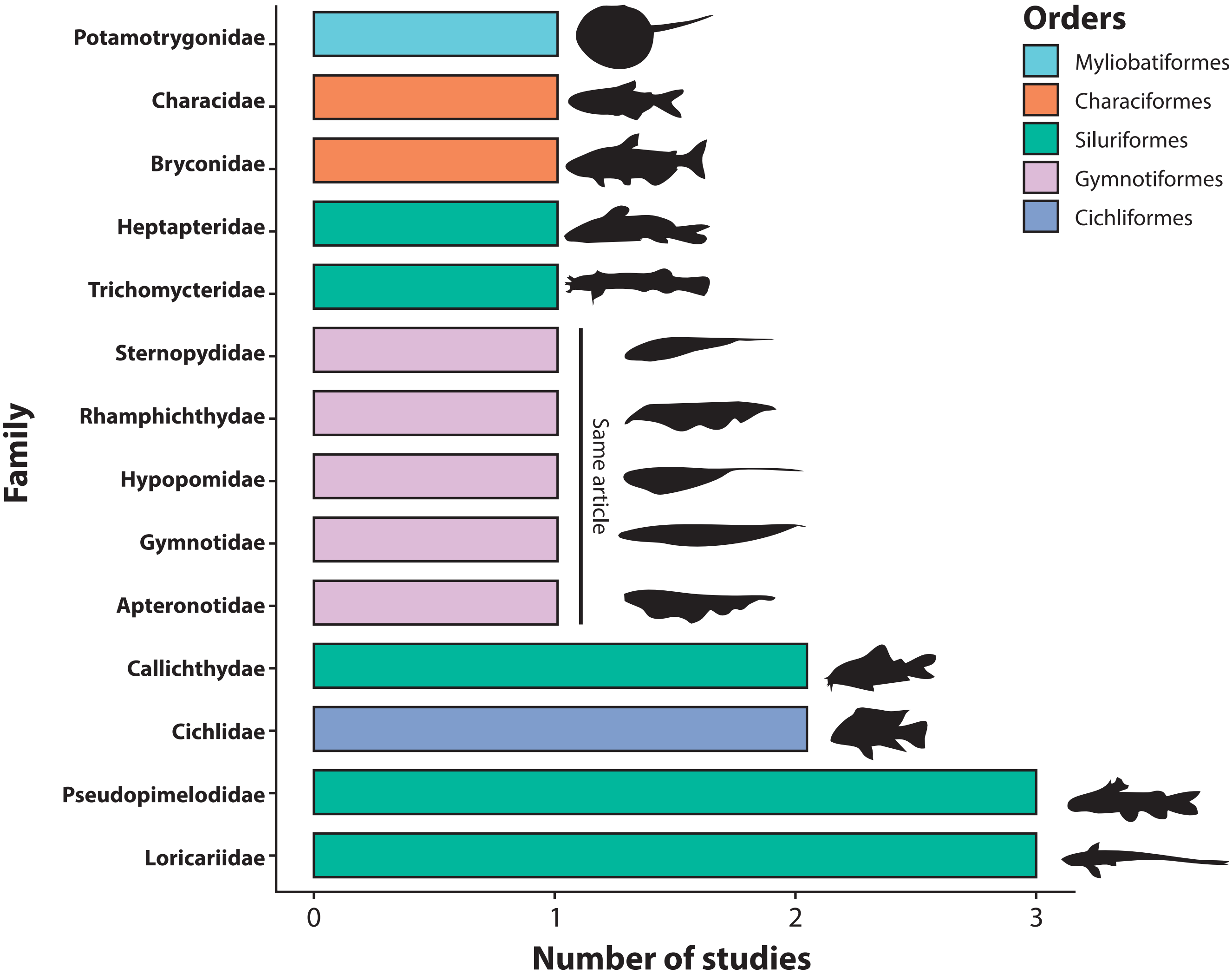

Figure 2. Number of studies on brain morphology descriptions for Family within Brazilian freshwater fish.

genetically informative neuroanatomical characters. The study also included comparisons with species from other families (such as Dasyatidae and Gymnuridae) to provide a broader phylogenetic framework. The authors found that the genera *Paratrygon* and *Heliotrygon* form a sister group, as do *Potamotrygon* and *Plesiotrygon,* based on shared features such as the orientation of the olfactory tracts, the shape and subdivision of the *Metencephalon,* and the trajectory of cranial nerves. The differences identified between these groups support previously proposed phylogenetic hypotheses based on morphological and molecular data, and suggest that brain mor-

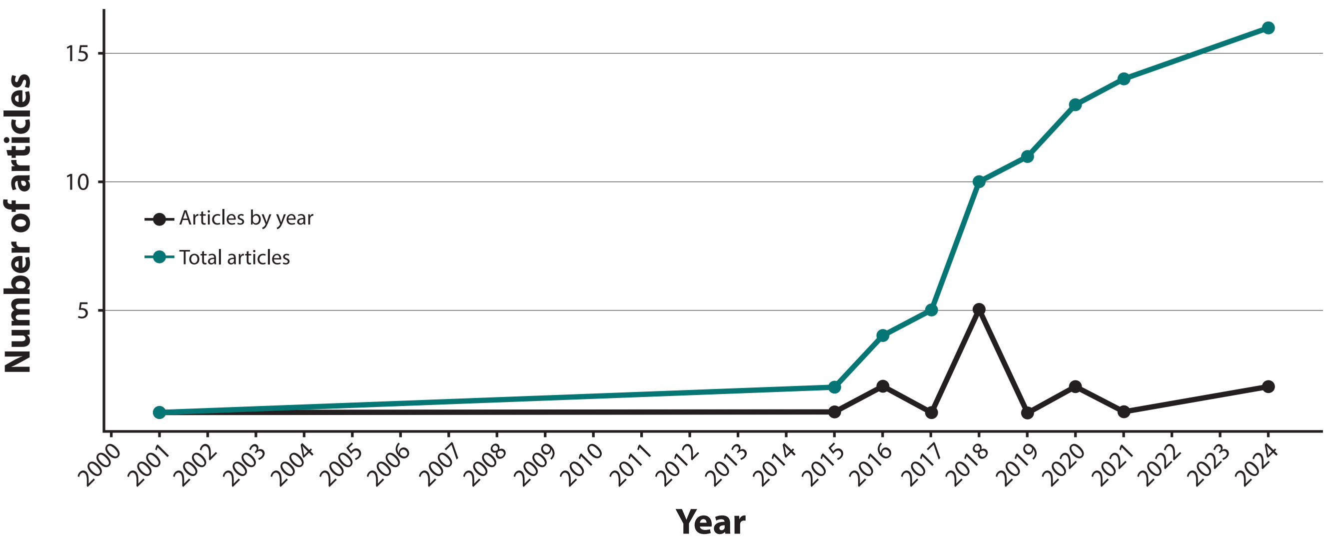

Figure 3. Cumulative number and Frequency of articles on comparative brain morphology of Brazilian freshwater fish published annually between 1999 and 2024.

phology is a promising source of informative characters for systematic studies in elasmobranchs. Furthermore, the study suggests that the observed encephalic variations are not attributable to ecological factors (*e.g.,* habitat or behavior), but rather reflect a shared evolutionary history.

Pereira & Castro (2016) described the archetype of gross brain morphology in Characiformes and tried to use encephalic characters by polarizing/rooting them in *Cyprinus carpio.* Additionally, the evolution of these characters was studied in representatives of Otophysi (Characiformes, Siluriformes, and Gymnotiformes). The species *Brycon orbignyanus* was chosen for description due to its basal position within Characiformes (Melo *et al.,* 2021). Furthermore, six synapomorphies were proposed that support the monophyly of Characiformes, as follows: (1) the shape of the *Area postrema* as an inverted triangle, narrowing in the anteroposterior direction; (2) the width of the *Rhombencephalon* not exceeding the width of the *Mesencephalon,* in both dorsal and ventral views; (3) a small and underdeveloped *Lobus vagi;* (4) an anatomically reduced *Lobus facialis,* barely perceptible, with a small oval structure at the base of the cerebellar body when visible; (5) a rounded and vertically elongated *Corpus cerebellaris;* and (6) a horizontally elongated *Tectum opticum,* in contact with the anterior margin of the cerebellar body.

Until 2017, published studies involving the macro brain anatomy of fishes had systematic objectives, with the exception of Abrahão & Shibatta (2015), who briefly addressed the relationship between ecology and brain anatomy. Angulo & Langeani (2017), in a study with *Rineloricaria heteroptera,* initiated an approach that had been scarcely discussed among Neotropical fishes. In addition to describing the brain anatomy of *R. heteroptera,* quantitative data were gathered through measurements of brain substructures for intraspecific comparisons, including both ontogenetic and sexual variations. Regarding ontogeny, juvenile individuals have a more developed *Tectum opticum* relative to the total brain size, suggesting that in the early stages, vision is a more important attribute, likely for detecting the presence of potential predators. In adults, the most well-developed structures are the *Bulbus olfactorius* and the *Medulla oblongata.*

In 2018, it was the most significant year in terms of published studies on the CNS of fishes in Brazil, with four papers published, all focused on Siluriformes, but from four different families: Pseudopimelodidae (Abrahão *et al.,* 2018b), Callichthyidae (Pupo & Britto, 2018), which included phylogenetic aspects of the analyzed families, Heptapteridae (Abrahão *et al.,* 2018a), with a descriptive focus, and Loricariidae (Chamon *et al.,* 2018), as part of the description of a new species of the genus *Spectracanthicus.*

The neuroanatomical characters of the Pseudopimelodidae family were examined by Abrahão *et al.* (2018b). All seven genera within the family were investigated in the analysis, and the monophyly of Pseudopimelodidae was supported, as corroborated by Lundberg *et al.* (1991), Shibatta & Vari (2017), and molecular studies (Sullivan *et al.,* 2013). The authors also proposed possible synapomorphies for Pseudopimelodidae, derived characters that distinguish this taxon from Pimelodidae and Heptapteridae, as well as the absence of a conspicuous protuberance in the anterior portion of the *Lobus vagi.* In Pseudopimelodidae, the *Lobus vagi* has anterior and posterior portions of the same width in the dorsal view, without the notable protuberance found in other families of Pimelodoidea. Additionally, through the shape and position of the brain structures, particularly the *Corpus cerebellaris,* it was possible to establish the phylogenetic relationships among the genera of Pseudopimelodidae, corroborating the arrangement proposed by Shibatta & Vari (2017).

Among the studies published with the aim of providing new characters derived from the brain morphology of fishes in Brazil, Pupo & Britto (2018) described the macro brain anatomy of the Callichthyidae family, comparing it with other members of the Loricarioidea superfamily. Callichthyidae is composed of two distinct and monophyletic lineages, Callichthyinae and Corydoradinae, both supported by morphological (Reis, 1998) and molecular data (Shimabukuro-Dias *et al.,* 2004). Despite the monophyly of the family and its subfamilies, the phylogenetic relationships within these taxa were, at the time of the study, uncertain or poorly understood. The phylogenetic position of *Aspidoras* and *Corydoras* was difficult to resolve based on traditional morphological and molecular studies. Additionally, Pupo & Britto (2018) proposed seven exclusive conditions in the brain of Callichthyidae: "(1) nasal organ with a circular shape in the dorsal view; (2) olfactory organ with fewer than 15 lamellae; (3) volume of the nasal organ lamellae increasing from medial to distal; (4) sessile positioning of the *Bulbus olfactorius;* (5) spherical central portion of the *Corpus cerebellaris;* (6) *Lobus facialis* detached from the medial margin of the lateral walls of the fourth ventricle; and (7) swelling of the *Lobus vagi.*" They also recovered the two subfamilies as monophyletic, with exclusive characters for Callichthyinae (*e.g.,* lamellae of the nasal organs flattened dorsoventrally; short *Telencephalon* with a curved lateral edge) and Corydoradinae (*e.g.,* distal area of the nasal organ lamellae detached from the floor of the nasal chamber; *Telencephalon* with straight and approximately rectangular edges).

In addition, systematic revisions for more inclusive groups (orders, families, and subfamilies) mentioned above, three species descriptions in particular provided characterization of the brain morphology of new taxa. *Spectracanthicus javae* Chamon *et al.,* 2018, *Corydoras benattii* Espíndola *et al.,* 2018, and *Tyttobrycon shibattai* Abrahão *et al.,* 2019 are three species described in 2018, where the authors, in addition to using traditional morphological characters, such as osteology and coloration, focused on providing neuroanatomical data of the new taxa to strengthen the hypotheses regarding these taxa.

Also in 2018, the brain anatomy of the species *Rhamdia quelen* was described and compared with seven other species from six genera within the family

Heptapteridae (Abrahão *et al.,* 2018a). In a second 2018 study, the same authors observed interspecific variations in all species analyzed, as well as low intraspecific variation (Abrahão *et al.,* 2018b). They also identified shared characters with other members of the Pimelodoidea superfamily, further supporting their phylogenetic relationships, as noted in Abrahão *et al.* (2018a). In addition to the neuroanatomical characters shared by the superfamily, new potential synapomorphies from this anatomical complex were proposed, found exclusively in Heptapteridae, including: (1) the position of the *Hypophysis* in the posterior area of the *Hypothalamus* (*vs.* anchored at the midpoint of the *Hypothalamus* in non-Heptapterid Pimelodoidea), and (2) the presence of a lateral subdivision in the *Lobus facialis* (*vs.* positioned anterolaterally in non-Heptapterid Pimelodoidea).

The first studies on the encephalic morphology of Cichlids were conducted with species from African lakes (Huber *et al.,* 1997; Pollen *et al.,* 2007; Reddon *et al.,* 2009; Shumway, 2010; Van Staaden *et al.,* 1995). Despite being a family with a widely studied CNS in the Ethiopian region, the first description of a Neotropical cichlid brain was only published in 2020 (Oliveira & Graça, 2020). The aim of this first study was to test the intraspecific variation in the encephalic morphology of *Geophagus sveni* Lucinda *et al.,* 2010, looking for possible sexual dimorphisms within the species, considering evolutionary, ecological, and behavioral aspects, as well as investigating potential relationships between the volume proportion of structures and behavioral habits. Oliveira & Graça (2020) found no significant differences between males and females of *Geophagus sveni*. However, they were able to correlate the volumes of the structures with the species' ecological habits. The *Tectum opticum* was the largest structure in the brain in terms of volume, likely due to the importance of vision in social behaviors, such as brood guarding and territorial defense. Additionally, the authors pointed out that the *Lobus vagi* is more developed compared to other teleosts, possibly as an adaptation to the specialized pharyngeal apparatus for suction feeding, a dietary habit in *Geophagus sveni* and other species of *Geophagini*. The same authors who characterized the brain of *Geophagus sveni* published a new study in 2024 (Oliveira & Graça, 2024), describing the brain morphology of the Geophagini tribe (*sensu* Ilves *et al.,* 2018), addressing evolutionary aspects such as behavior and phylogenetic relationships. The study identified a high development of the *Tectum opticum,* with a large volume relative to the brain, as synapomorphies for the Geophagini clade. This suggests that vision, facilitating functions such as color recognition, brood guarding, territorial defense, and foraging, is crucial, as highlighted in previous studies (Schneider *et al.,* 2020). In contrast, the olfactory system, less developed in cichlids, indicates a reduced reliance on smell in favor of vision for most behavioral activities.

Another study on the family Pseudopimelodidae was conducted by Abrahão *et al.* (2021), focusing on the brain of *Microglanis garavelloi*. The study highlighted significant morphological changes throughout development, such as the progressive increase in the number of olfactory lamellae, which ranged from 18-23 in immature individuals to 35-42 in adults. The authors also reported patterns of sexual dimorphism associated with brain growth rates. Although no significant differences were found in total brain volume between sexes, growth rates varied: males exhibited faster development in brain regions associated with social integration and motor control, including the *Tectum opticum, Diencephalon* (except the *Hypophysis*), and *Cerebellum*. These differences may be associated with early sexual maturation in males and the specific behavioral demands linked to this life stage.

Studies involving brain adaptations of subterranean fish are truly scarce; however, Rizzato & Bichuette (2024) described the brain morphology of species from the genus *Ituglanis*. The study compared the brain anatomy and the main sensory organs between subterranean and epigean species of the genus. The subterranean species had a slightly smaller *Tectum opticum,* especially in individuals with reduced or malformed eyes, and a slightly larger *Corpus cerebellaris,* covering a larger area of the *Diencephalon*. The *Bulbus olfactorius,* although similar to those of Epigean species, exhibited a smaller number of olfactory lamellae. The subterranean species also showed a slight enlargement of the swim bladder, and the semicircular canals of the inner ear compared to the epigean species, reflecting adaptations to subterranean life conditions. However, it is important to note that the authors discuss that despite the slightly smaller size of the *Tectum opticum,* few differences were found in the relative size of most structures in the main sensory organs, indicating that the brain morphology of this group is strongly associated with the evolutionary history of the family, which shares similar ecological aspects.

DISCUSSION

Most of the studies published so far have been on the order Siluriformes (62.5%), creating a large gap in the literature regarding other orders found in Brazil. One explanation for this is the background of the researchers working in the field. Victor Abrahão, Oscar Shibatta, and Fábio Pupo, the most frequent researchers in this area, already had experience with the studied groups, such as Callichthyidae, Loricariidae, and Pseudopimelodidae (Abrahão & Pupo, 2014; Shibatta, 2003; Shibatta & Vari, 2017), which facilitated the initiation of studies on these groups. In general, the studies have been concentrated on Characiphysi, and although there are also contributions involving Cichliformes and the freshwater Elasmobranch family Potamotrygonidae, the number of studies remains very limited when compared to the vast diversity of freshwater fishes found in Brazil.

Despite the first work, considered the starting point of Comparative Brain Morphology of fish in Brazil (Albert, 2001), the studies published in journals have a 14-year gap (Abrahão & Shibatta, 2015), making it an area that was neglected for a significant period. It is noteworthy that the studies only resumed after 2009, during the academic training of these researchers, with the published works being the result of dissertations and theses.

Despite considerable progress in recent years, the fish Central Nervous System remains a largely unexplored area in Brazil. The lack of studies involving a greater diversity of groups makes it difficult to conduct new research due to the absence of clear methodologies. To date, there are only three dissection protocols for neurocranial studies on the brain: one for Characiformes (Pereira & Castro, 2016), one for Siluriformes (Abrahão & Pupo, 2014), and one for Elasmobranchii (Soares *et al.,* 2025). Other studies published with different taxa (*e.g.,* Oliveira & Graça, 2020 with Cichliformes) adapted the dissection protocols for different groups. Furthermore, Esguícero & Bockmann (2022) developed a method for staining nerves in vertebrates, including fish, as part of the protocol. While this method primarily targets the Peripheral Nervous System, it remains significant for comparative morphological studies. The brain extraction is an invasive process, compromising a large part of the neurocranium of the specimen, therefore, there is a lack of methods and techniques that minimize material damage, enabling research with specimens from biological collections to reduce the potential harm.

The brain morphology has proven to be an important tool for systematics, interspecific analyses have revealed low intraspecific variation in the brains of the studied fish species (*e.g.,* Abrahão *et al.,* 2018a; Abrahão *et al.,* 2018b; Oliveira & Graça, 2020), and when differences are found, they are related to the volume of structures (*e.g.,* Angulo & Langeani, 2017; Abrahão *et al.,* 2021), usually associated with sexual dimorphism, where males and females perform different cognitive roles or exhibit different sexual maturation times or distinct volume differences between different developmental stages. Therefore, the central nervous system (CNS) has been neglected as a source of characters for systematic studies.

Considering that the CNS is a complex anatomical system closely related to the environment and the behavioral habits of organisms, there is still a considerable deficit in this approach, especially regarding population-level ecomorphological differences. There are records in the literature of different isolated populations exhibiting significantly distinct volumes related to environmental variables such as biotic and abiotic factors (Kotrschal *et al.,* 2017; Liu *et al.,* 2021), however, this has not yet been properly explored in Brazil. Differences in volume and development rate were found in the brain of *Cichla piquiti* when comparing native and non-native populations, likely attributed to predation pressure and competition between the environments. This suggests that, perhaps, this is an approach that should be further investigated (Marinho-Nunes, unpublished data).

CONCLUSION

The analysis of brain morphology in Brazilian freshwater fish, although still in its early stages, has shown significant potential to enhance the understanding of phylogenetic and ecological relationships within the Neotropical ichthyofauna. Despite the predominance of studies focused on Siluriformes, the gap in research on other orders highlights the need to diversify efforts to include a broader range of freshwater fish taxa, both Actinopterygii and Potamotrygonidae (Elasmobranchii). The continuation and expansion of these studies are essential to reveal the true neuroanatomical diversity of fish and its evolutionary impact, providing new insights into the adaptation and specialization of species in different environments. Advancements in this field could significantly contribute to a more accurate understanding of the morphological and functional diversity of fish inhabiting Brazil's river basins.

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