Neotropical Ichthyology

Original article

https://doi.org/10.1590/1982-0224-2020-0149

Discrimination of species and populations of the genus *Cichla* (Cichliformes: Cichlidae) in rivers of the Amazon basin using otolithic morphometry

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The genus *Cichla* is a highly diverse group, with 16 species already described. Externally, some species are very similar and discriminating between them may be very difficult. Nevertheless, discrimination of fish stocks is essential for management purposes. Morphometric analyses of otoliths have been successfully used to distinguish species and fish stocks, especially in marine environments. This study evaluated whether sagittal otolith shape can be used to discriminate among the species *Cichla temensis*, *C. monoculus*, and *C. orinocensis*, as well as within populations of *C. temensis* in rivers of the Amazon. Shape indices and Fourier coefficients were used to describe the shape of the otoliths. Among the groups of species, the morphology of the sagittal otolith of *C. temensis* was totally distinct from the species *C. monoculus* and *C. orinocensis*. While among populations of *C. temensis*, individuals from the Negro and Jatapú Rivers were different, regardless of the methods used. These results confirm the ability to differentiate species and populations by using the morphology of otoliths. However, more research is needed to verify the role of genetic versus environmental and biotic effects, and thus be able to explain the discrimination observed in otoliths.

Keywords: Amazonian rivers, Fourier analysis, Otolith, Peacock bass, Shape indices.

Submitted March 2, 2021
Accepted September 15, 2021
by Matt Kolmann
Epub December 13, 2021

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 19, no. 4, Maringá 2021

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O gênero *Cichla* é bastante diverso, com 16 espécies descritas. Algumas espécies são externamente muito similares e sua discriminação pode ser bastante difícil. Ao mesmo tempo, a discriminação de estoques pesqueiros é essencial para propostas de manejo. Análises morfométricas em otólitos têm sido utilizadas com sucesso para a distinção de espécies e estoques pesqueiros, principalmente em ambientes marinhos. Este estudo avaliou se o formato do otólito sagittal pode ser utilizado para discriminar entre espécies *Cichla temensis*, *C. monoculus* e *C. orinocensis*, bem como dentro de populações de *C. temensis* em diferentes rios amazônicos. Índices de forma e coeficientes de Fourier foram utilizados para descrever a forma dos otólitos. Dentre as espécies, a morfologia do otólito sagittal do *C. temensis* mostrou ser totalmente distinta das espécies *C. monoculus* e *C. orinocensis*. Enquanto no grupo das populações de *C. temensis*, os indivíduos dos rios Negro e Jatapú mostraram-se diferentes independente dos métodos utilizados. Esses resultados confirmam a capacidade de diferenciação de espécies e populações através da morfologia dos otólitos. No entanto, são necessárias mais pesquisas para verificar o papel dos efeitos genéticos em comparação aos efeitos ambientais e bióticos para explicar a discriminação observada nos otólitos.

Palavras-chave: Análise de Fourier, Índices de forma, Otólito, Rios amazônicos, Tucunaré.

INTRODUCTION

Species of the genus *Cichla* Bloch & Schneider, 1801, popularly known as peacock bass, are widely distributed in the rivers of the Amazon basin (Kullander, Ferreira, 2006; Willis *et al*., 2012). This genus includes medium to large piscivorous species, which are ecologically important given their involvement in the processes of trophic structuring and nutrient cycling in aquatic ecosystems (Jepsen *et al*., 1997; Winemiller, 2001). They also contribute significantly to commercial, subsistence, and sport fisheries (Freitas, Rivas, 2006; Inomata, Freitas, 2015). Currently, 16 species of the genus *Cichla* are known and these have been described in a traditional manner from their meristic and morphological characteristics, as well as using DNA sequencing for species delimitation (Kullander, Ferreira, 2006; Sabaj *et al*., 2020).

Historically, the genus *Cichla* has been subject to contradictory opinions about its taxonomy (Stiassny, 1987). Recent studies show that there are disagreements about the precise identification of some of the peacock bass species (*Cichla* spp.), as a result of hybridization (for example between *Cichla monoculus* Spix & Agassiz, 1831 and *C. temensis* Humboldt, 1821 (Andrade *et al*., 2001; Willis *et al*., 2007, 2012). In addition, morphological variation and differences in intraspecific coloring patterns lead to erroneous identification of some species, in detriment to research and management activities (Winemiller, 2001; Reiss *et al*., 2012).

Studies based on the morphological characteristics of otoliths have been successfully used to distinguish among species, populations, and even fish stocks, especially in marine environments (Tuset *et al*., 2016; Rashidabadi *et al*., 2020). The species-specific shape of otoliths and their lower variability compared to other morphological structures of fishes are the main reason for this (Campana, Casselman, 1993). However, although the shape of the otolith is specific to each species, intraspecific variation may occur according to geography, environmental factors, and differential patterns of growth among populations (DeVries *et al*., 2002; Tuset *et al*., 2003; Mérigot *et al*., 2007; Cañás *et al*., 2012; Jemaa *et al*., 2015).

Different techniques have been applied for analyzing otolith shape (Ponton, 2006), and including some methods based on linear measurements, such as otolith nucleus size and shape variability (Postuma, 1974), otolith increment dynamics (Torres *et al*., 1996) and relationships between fish size and otolith radius (Zabel *et al*., 2010). However, biological information that is useful for taxon discrimination require an inherent multivariate approach. This stimulated the development of techniques for examining the shape of the otolith as a whole (Cadrin, Friedland, 1999).

Elliptical Fourier analysis (Kuhl, Giardina, 1982) is one technique that can be used to quantify otolith shape differences among species. The Fourier series describes shape (silhouettes) by means of descriptors called harmonics, representing the relative contribution of the empirical shape of an object by its elongation and triangularity (Bird *et al*., 1986). In addition, harmonics define several shape parameters which, when combined, provide an image close to reality. Another approach is based on the use of shape indices (roundness, rectangularity, ellipticity, circularity, shape factor), which can be used to characterize the shape of objects (Tuset *et al*., 2003).

Some studies that have used EFA and shape indices in combination have obtained results that are more accurate and complete for describing the shape of otoliths (Campana, Casselman, 1993; Stransky, MacLellan, 2005; Duarte-Neto *et al*., 2008). EFA provides a quick and objective response, while using the shape indices has the advantage of its simple calculations for presenting the growth patterns of otoliths (Tuset *et al*., 2003).

Otoliths are most often used to perform analysis on growth (Holley *et al*., 2008; Campos *et al*., 2015) and patterns of movement with otolithic microchemistry (Garcez *et al*., 2014; Sousa *et al*., 2016). Nevertheless, studies of otolithic morphometry have been shown to be efficient for distinction among species in marine and freshwater environments (Avigliano *et al*., 2018), identification of natal nurseries (Avigliano *et al*., 2017) and discrimination between populations (Afanasyev *et al*., 2017; Vasconcelos *et al*., 2018). However, these studies are still incipient, especially in the Amazon basin (Costa *et al*., 2018).

Although more invasive than other approaches, such as the use of genetic markers, the use of otoliths to discriminate populations or species could be useful due its lower cost in comparison with genetic techniques and when the catch of fish is essential. This study evaluated the application of a combination of otolith shape analysis techniques for discriminating among species and populations of *Cichla* from different Amazonian River basins. Two hypotheses were tested: 1) that the shape of the otolith differs between *C. temensis*, *C. monoculus*, and *C. orinocensis* Humboldt, 1821, and 2) there is intraspecific variation in the shape of the otolith in *C. temensis* from the blackwater rivers of the Amazon basin. We hoped, therefore, that the results might contribute to the establishment of strategies for the management of peacock bass stocks that are exploited by fisheries in the Amazon region.

MATERIAL AND METHODS

Study area. *Cichla* species were sampled in three rivers of the Amazon basin: the middle Negro River, in the reservoir generated by the Balbina Hydroelectric Dam along the Uatumã River, and the Jatapú River (Fig. 1). These rivers have their headwaters located in the Guiana Shield and are classified as blackwater rivers, given their tannin-stained color, acid pH, and very low net primary productivity (Sioli, 1984). These rivers host a great abundance and diversity of fishes, which in turn support an important commercial and subsistence fisheries (Santos, Oliveira Jr, 1999; Freitas, Rivas, 2006).

Data collection. The sampling took place between October 2011 and November 2018 during the low water period. The specimens of *Cichla temensis*, *C. monoculus*, and *C. orinocensis* were collected through experimental fisheries using gear such as reels and gill nets. Immediately after the capture, the specimens were euthanized using the spinal cord incision method and the sagittae otoliths were extracted from the auricular cavity using surgical equipment (scalpel and forceps). After extraction, the otoliths were washed with distilled water to eliminate the remaining tissue of the macula and vestibule. Sagittae otoliths were selected for being the most used in comparative taxonomy work, due to the large size and relative ease of access to the structures (Nolf, 1985).

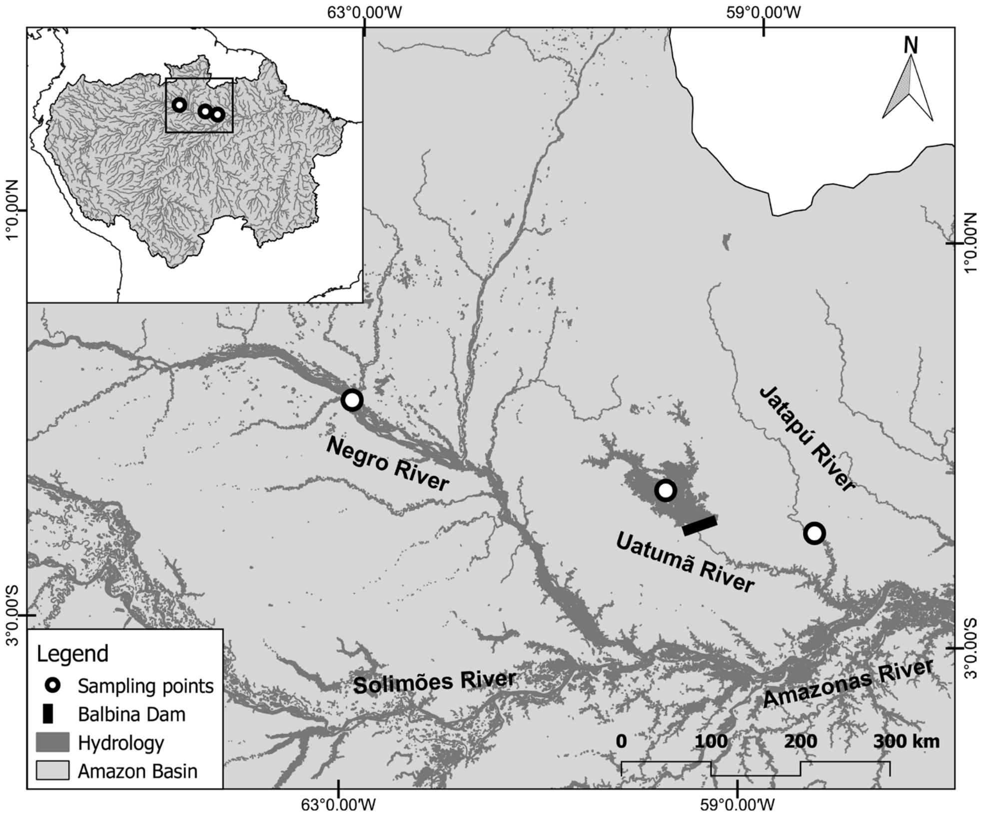

FIGURE 1 | Location of sampling points in the Negro, Uatumã and Jatapú rivers in the Amazon basin.

Subsequently, the otoliths were dried and stored in Eppendorf tubes, labeled and sent to the Laboratório de Ecologia Pesqueira, Universidade Federal do Amazonas (UFAM), where preparations were made for photographic analysis. Voucher specimens were deposited in the fish collection of the Instituto Nacional de Pesquisas da Amazônia (*Cichla monoculus* INPA 52111; *C. orinocensis* INPA 43012; *C. temensis*, INPA 35563; Tab. **S1**).

Image acquisition. For the photographic analysis, the right otoliths of each species were selected. They were placed with the lateral face facing downwards, with the sulcus upwards and the rostrum pointing to the left (Fig. 2). Two-dimensional orthogonal digital images of the otoliths were captured using a USB digital camera (Olympus, SC30) with 10x magnification coupled to a magnifying glass (Meiji Techno EMZ-13TR). High contrast digital images were obtained using reflected light with a dark background, producing bright two-dimensional objects.

Shape indices. The following morphometric variables of the otoliths were recorded using the ImageJ image processor (Rasband, 1997): otolith length (OL), otolith width (OW) and otolith perimeter (OP) in millimeters, in addition to the otolith area (OA), in square millimeters. These measurements were used to calculate the shape indices, as recommended by Tuset *et al.* (2003) (Tab. 1).

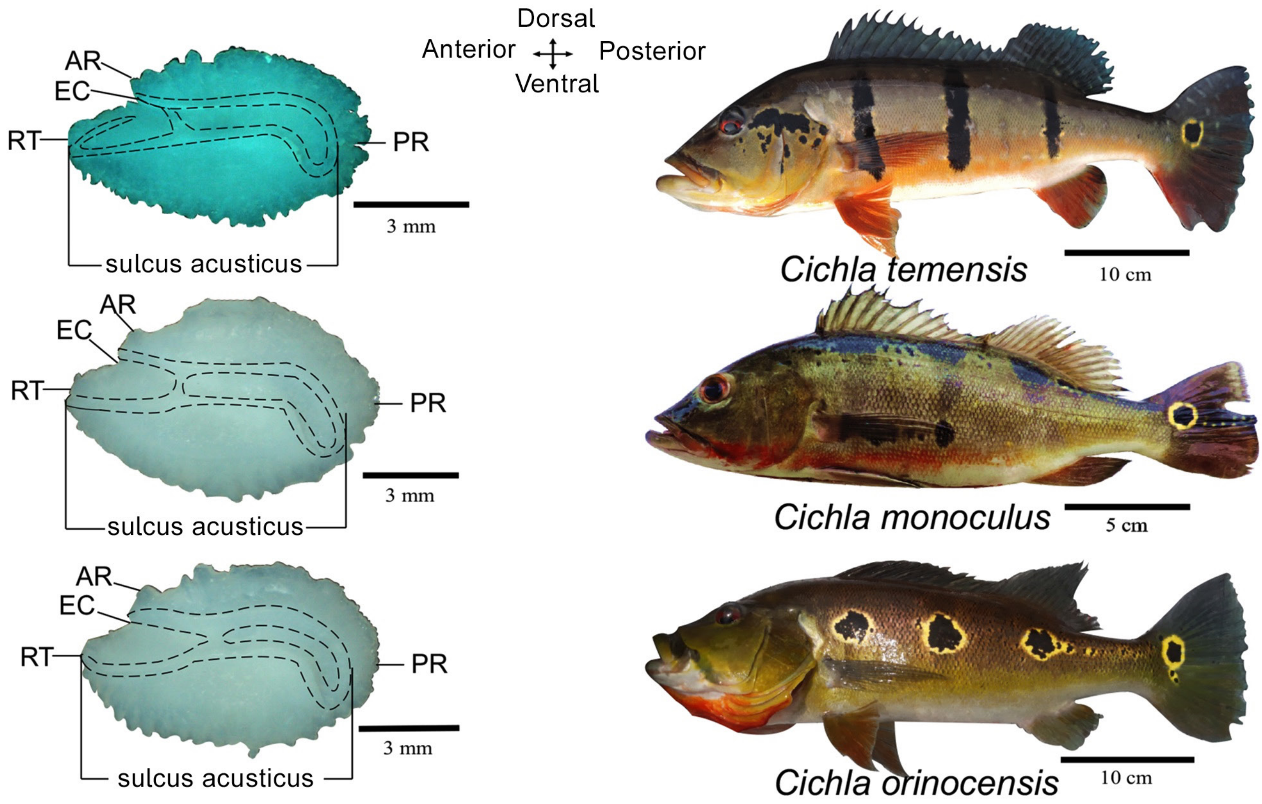

FIGURE 2 | Labelled right-side sagittae otoliths in mesial view of the species *Cichla temensis*, *C. monoculus*, and *C. orinocensis*, according to Gomiero, Braga (2007). RT: rostrum; PR: postrostrum; EC: excisura; AR: antirostrum.

TABLE 1 | Shape indices of otoliths calculated from morphometric measurements. OA: otolith area (mm^2), OP: otolith perimeter (mm), OL: otolith length (mm) and OW: otolith width (mm).

Shape indices	Equation
Form factor	$(4\varpi \times OA / OP^2)$
Roundness	$(4OA) \times (\varpi \times OL^2)$
Circularity	(OP^2 / OA)
Rectangularity	$(OA / OL \times OW)$
Ellipticity	$(OL - OW / OL + OW)$

The shape factor estimates the irregularity of the otolith area, and assumes values of 1.0 when it is a perfect circle, and <1.0 when it is irregular. Roundness and circularity provide information about their similarity to a perfect circle, when the values are closer to 1 and 12.57, respectively (Russ, 1990). The rectangularity describes the variations in length and width in relation to the area, and 1.0 corresponds to the perfect square. Ellipticity indicates whether changes in axes are proportional (Russ, 1990).

Elliptical Fourier analysis. Fourier coefficients were calculated using the program SHAPE v. 1.3 (Iwata, Ukai, 2002). This program quantitatively evaluates biological shapes, based on elliptical Fourier descriptors (EFDs). The Chain Coder program was used to convert the black-white image into a binary image in order to extract its contour. The demarcation of the shape occurs through the "chain coding" algorithm, which represents an object as a closed two-dimensional curve, and applies a combination of harmonically related sine and cosine functions consisting of four (a, b, c and d) Fourier coefficients (FCs) (Kuhl, Giardina, 1982). In the present study, we calculated 20 harmonics for each otolith, thus generating 80 FCs per individual. The program standardized the size and orientation, and provided constant values for the first three FCs; these being: a1 = 1, b1 = 0 c1 = 0. Each individual was therefore represented by 77 unique FCs (Iwata, Ukai, 2002). Finally, the normalized coefficients of the EFDs are stored in files for other statistical analyses.

Statistical analysis. Individuals collected in the middle Negro River were used to test the hypothesis of differences in the shapes of otoliths among the species of *Cichla temensis*, *C. monoculus*, and *C. orinocensis*. Otoliths from individuals of *C. temensis* collected in the Negro, Uatumã and Jatapú rivers were used to test the hypothesis of otoliths shape differences among populations.

Mean and standard deviation were calculated for each of the morphometric variables for each species and each population. Ordinary least squares (OLS) regression analysis was performed for the shape indices and the otolith length within each species and within populations in order to evaluate the existence of an allometric effect. When the regression coefficient presented a significant value, the morphometric parameter was corrected using the equation proposed by Cardinale *et al.* (2004):

$$V_{aj} = V_i - b.OL$$

In which, V_{aj} is the adjusted variable, V_i is the analyzed variable, OL is the length of the otolith and its inclination within the group (b). The length of the otolith was chosen to remove the effect on the calculated indices, instead of the length of the fish, since this variable is not affected during the preservation, shrinkage or distortion process (Campana, Casselman, 1993). In addition, the length of the otolith and the fish usually have good correlation (Mereles *et al*., 2020).

Due to the high dimensionality of the descriptors (77 per individual), two independent principal component analyses (PCA) were applied to the FCs matrices generated among the species *C. temensis*, *C. monoculus*, and *C. orinocensis*, and within populations of *C. temensis*, in order to reduce these to a smaller number of dimensions with decreasing importance for explaining the existing variation, without losing the information from the shapes. To detect the significant eigenvalues, we plotted the percentage of the total expected variation of eigenvalues versus the proportion of expected variance estimated by the "Broken-Stick" method (MacArthur, 1957). Significant principal components (PCs) of the Fourier shape characteristics were used as variables in later analyses.

One-way MANOVA using Pillai statistics were applied to test the hypotheses of no differences among groups of the three species (*C. temensis*, *C. monoculus*, *C. orinocensis*) and the three populations of *C. temensis* (middle Negro, Uatumã and Jatapú Rivers). The package Candisc (Friendly, Fox, 2017) was used to perform a canonical discriminant analysis, allowing for separation between the groups to be graphically verified, and aiding in explaining variation between canonical axes. The successful classification into groups was tested by jack-knife cross-validation, using the package MASS (Ripley, 2011).

The assumption of multi-homogeneity of variances within the groups (Anderson, 2006) were tested for each model using Betadisper function in the package Vegan, on a matrix of Euclidean distance (Oksanen *et al*., 2016). When necessary, outliers were detected based on Mahalanobis distances and then removed to adjust the models using the package mvOutlier (Filzmoser *et al*., 2014). All statistical tests and graphical representations were performed using R software (R Development Core Team, 2020 http://www.r-project.org). The value of $p < 0.05$ was considered statistically significant for the analyses.

RESULTS

A total of 168 otolith samples were analyzed, 50 of which were *C. monoculus*, 36 *C. orinocensis* and 82 *C. temensis*. Of this total, only the 127 samples obtained from individuals caught in the middle Negro River were used in the identification of the three species, to avoid a potential effect of distinct sites, and 82 were used for the population analysis of *C. temensis* (Tab. 2).

Species discrimination. Five significant principal components (PCs; Fig. **S2**) derived from the Fourier descriptor matrix for the species group (*C. temensis*, *C. monoculus*, and *C. orinocensis*), explaining 79.48 % of the total variation were used to differentiate

TABLE 2 | Mean (± standard deviation) of the shapes indices estimated from the morphometric parameters measured on otoliths of three *Cichla* species; N: number of individuals sampled.

Species	N	Form factor	Roundness	Circularity	Rectangularity	Ellipticity
C. monoculus (Negro River)	50	0.53 ± 0.04	0.61 ± 0.07	23.67 ± 2.01	0.73 ± 0.08	0.21 ± 0.03
C. orinocensis (Negro River)	36	0.53 ± 0.06	0.53 ± 0.03	23.95 ± 3.71	0.70 ± 0.02	0.25 ± 0.02
C. temensis (Negro River)	41	0.38 ± 0.06	0.53 ± 0.03	33.58 ± 5.42	0.69 ± 0.02	0.24 ± 0.02
C. temensis (Uatumã River)	15	0.46 ± 0.07	1.52 ± 0.09	27.98 ± 4.28	1.39 ± 1.75	-0.21 ± 0.52
C. temensis (Jatapú River)	26	0.35 ± 0.06	1.49 ± .010	36.91 ± 6.42	0.34 ± 0.13	-0.56 ± 6.42

the three species. When visualizing the variation in shape (mean ± SD) explained by the significant principal component axes, PC1 was determined to be variation along the postero-dorsal and antero-ventral margins; PC2 as variation along the anterior and posterior regions; PC3 along the dorsal and ventral margins; PC4 in the excisura; PC5 at the rostrum (Fig. 3).

Significant differences were observed in the shape indices and the main components (PCs) of the species *C. temensis*, *C. monoculus* and *C. orinocensis* (MANOVA, Pillai = 1.30, $F_{(2,\ 170)} = 32.05$, $p < 0.001$; and Pillai = 0.87, $F_{(2,\ 192)} = 14.971$, $p < 0.001$) respectively.

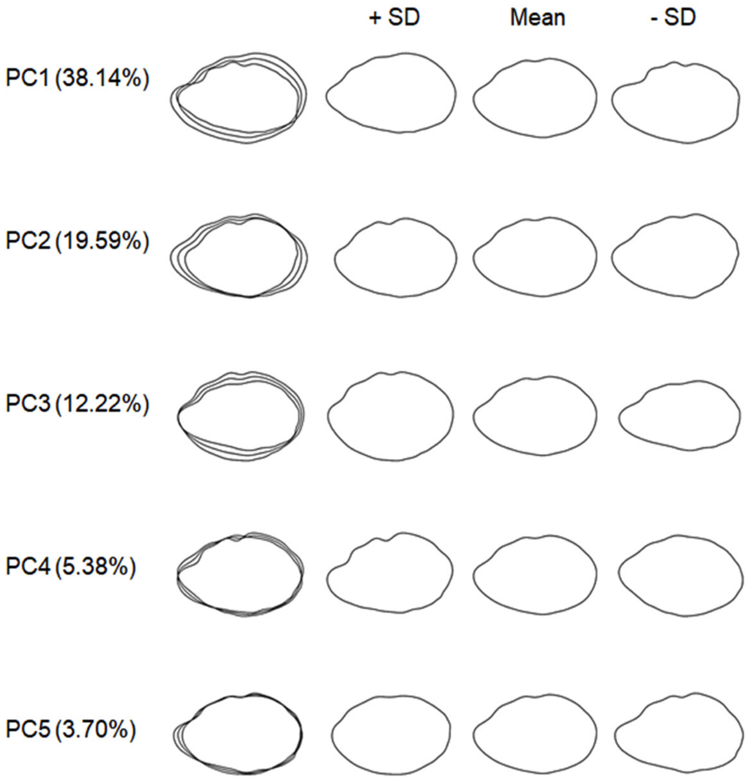

FIGURE 3 | Variation in shape (mean ± standard deviation – SD) in the sagittae of *Cichla* species explained by the first five principal components (PCs).

Canonical discriminant analysis of shape indices and elliptical Fourier descriptors provided visualization of the distinctions between the three studied species (Figs. 4A–B). For both methods, the species *C. temensis* was readily distinguished from its congeneric, especially in axis 1 of the CDA, with the values of the shape and PC indices explaining 93.20 % and 91.90 % of the total variations, respectively. Axis 2 of the CDA, for the same attributes, contributed to the distinction between *C. monoculus* and *C. orinocensis* in a smaller proportion (Figs. 4A–B). The shape index variables that most contributed to the differences found in the first discriminant function were ellipticity and circularity, and were associated with the species *C. temensis*; and shape factor, rectangularity and roundness, was related to the species *C. orinocensis* and *C. monoculus*. In the analyses using Fourier descriptors, only the variable PC4 was associated with the individuals of *C. temensis* and the other variables (PC1, PC2, PC3 and PC5) were more intensely related to the individuals of the species *C. orinocensis* and *C. monoculus*. The results discriminated between the three species with an overall cross-validation rating of 82.41 % for shape indices and 76.47 % for elliptical Fourier descriptors.

***Cichla temensis* population discrimination.** For the Fourier matrix of *C. temensis* populations, six PCs were determined to be significant (Fig. **S3**), and these explain 79.83 % of the total variation. The mean variation of the shape explained by the first six PCs showed variations in the anterior ventral and posterior dorsal regions, in the anterior and posterior region and excision of the otoliths, evidenced mainly by PCs 1, 2, 3 and 4. PCs 5 and 6 did not show a clear variation associated with the shape indices (Fig. 5).

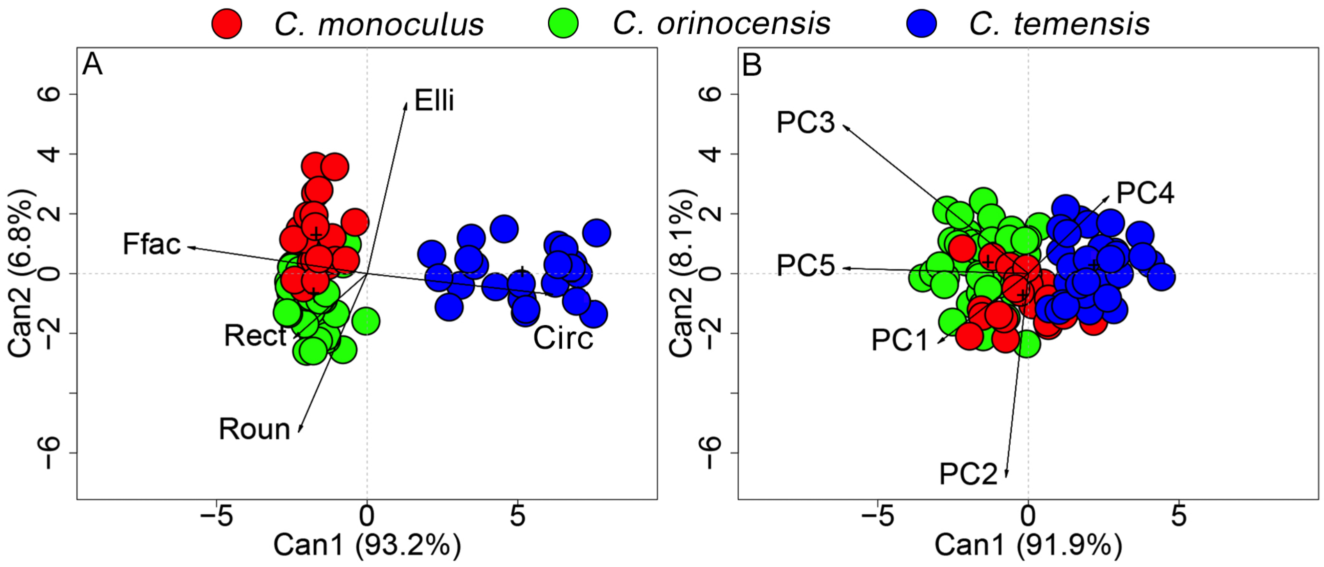

FIGURE 4 | Canonical discriminant analysis based on (**A**) shape indices and (**B**) elliptic Fourier coefficients for the species *Cichla temensis*, *C. monoculus*, and *C. orinocensis*. The vectors indicate the direction and intensity of the influence of the estimated characteristics: Roundness (Roun), Rectangularity (Rect), Ellipticity (Elli), Circularity (Circ), shape factor (Ffac); PC1 to PC5 correspond to the significant scores of the PCA performed on the Fourier matrix.

The shapes indices and Fourier descriptors also allowed to discriminate the three geographic populations of *C. temensis* from the Negro, Jatapú and Uatumã Rivers (MANOVA, Pillai = 1.41, $F_{(2,\ 152)} = 37.08$, $p < 0.001$ and Pillai = 0.90, $F_{(2,\ 134)} = 9.19$, $p < 0.001$, respectively). However, canonical discriminant analysis showed different patterns between the shape indices and Fourier descriptors.

The first discriminant function using shape indices explained 98.40 % of the variation, and distinguished the individuals of the Negro River from the other localities, while function 2 explained only 1.60 % of this total, and showed an overlap of the individuals of the Jatapú and Uatumã Rivers (Fig. 6A). The ellipticity and the shape factor explained most of the variation in the first discriminant function, and was associated with the population of *C. temensis* of Negro River, while the circularity and roundness were the indices associated with the populations of the Uatumã and Jatapú Rivers.

In contrast, Fourier descriptors distinguished the individuals of the Jatapú River in the first canonical function (95.70 %), and the second discriminant function (4.30 %) distinguished the populations of the Negro and Uatumã Rivers (Fig. 6B). The variable PC6 was the only one that was associated with the population of the Jatapú River in the first discriminant function, while the variables PC1, PC3, PC4, and PC5 contributed to a greater explanation of the populations of the Negro and Uatumã Rivers. The overall cross-validation rating rate for populations was 91.46 % for shape indices and 78.37 % for elliptical Fourier descriptors.

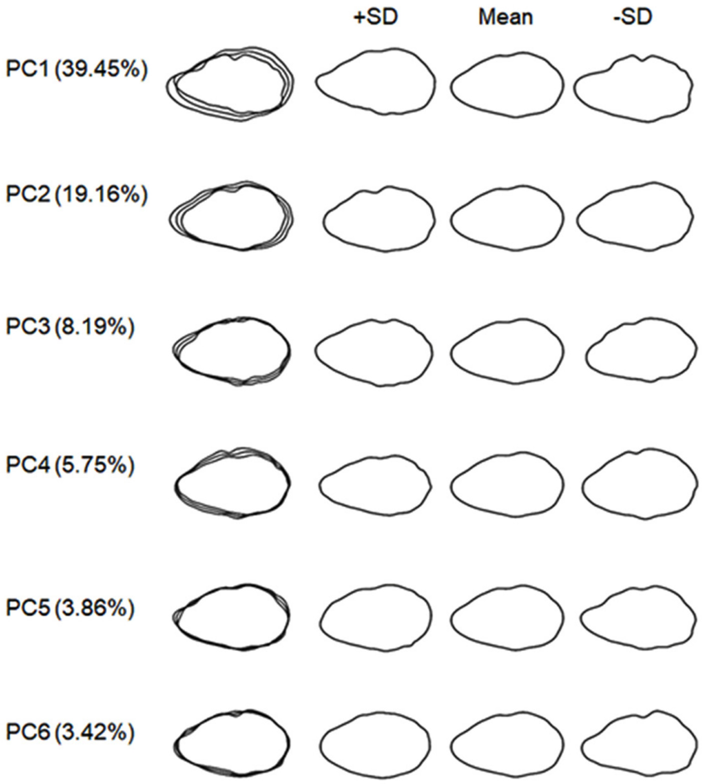

FIGURE 5 | Variation in shape (mean ± standard deviation – SD) in sagittae of *C. temensis* populations explained by the first five principal components (PCs).

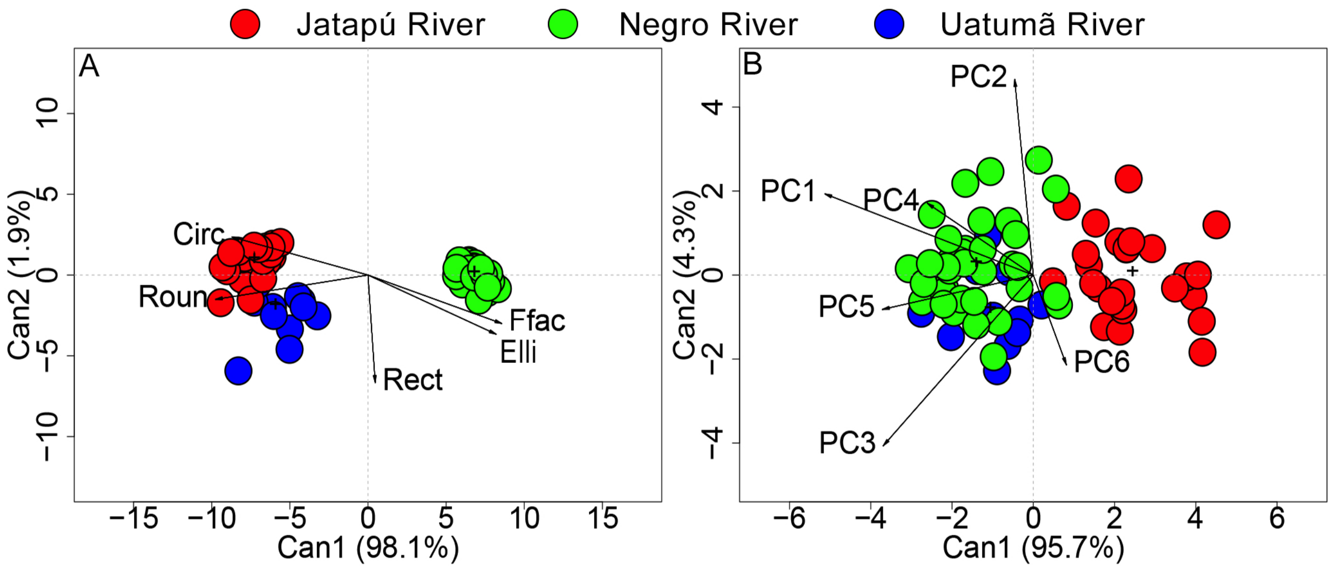

FIGURE 6 | Canonical discriminant analyses performed on shape indices (**A**) and elliptic Fourier coefficients (**B**) measured in populations of *Cichla temensis* from the Negro, Jatapú and Uatumã rivers. The vectors indicate the direction of increase in the various measured characteristics: Roundness (Roun), Rectangularity (Rect), Ellipticity (Elli), Circularity (Circ), form factor (Ffac); PC1 to PC6 correspond to the significant scores of the PCA performed on the Fourier matrix.

DISCUSSION

The approaches employed here, using elliptical Fourier analysis (EFA) and shape indices, demonstrated that these analyses can distinguish among *Cichla* species and *C. temensis* populations through the shape of their otoliths, with estimates of cross-validation higher than 75 % for both analytical approaches (Friedland, Reddin, 1994). However, the results showed that the ability to detect differences among species and populations was lower when using Fourier descriptors than when using shape indices. The possible reason for this result may be associated with the regular shape of the sagittae otoliths of the studied species, since the shape index has greater efficiency in the analysis of regular shapes (Agüera, Brophy, 2011), while the EFA can efficiently capture information from more complex structures (Lestrel, 1997).

From a methodological point of view, EFA is considered to be more powerful and has greater potential for capturing all the shape variations and small-scale individual differences in the otolith silhouette (Mérigot *et al*., 2007). However, its biological interpretation is more complex than traditional techniques (Stransky, MacLellan, 2005). On the other hand, form indices have the advantage of being easy to calculate when compared to the Fourier series (Tuset *et al*., 2003). Recently, a study of 42 species showed that wavelet transform presented better results than otolith shape indices and the authors of the study did not recommend the use of shape indices for the identification of species (Tuset *et al*., 2021). However, the high value obtained for the cross-validation using shape indices in our study shows that this may not be a general pattern.

The reconstruction of the outlines of the sagittae using FCs indicates that the changes in the shape of the *Cichla* species otoliths depend mainly on the dorsoventral extension and, consequently, on the extension of the anterior and posterior axis. The differences observed in the shape of the otoliths of the species (*C. temensis*, *C. monoculus*, and *C. orinocensis*) were expected, considering that the shape of the otoliths is, in general, species-specific (Campana, Casselman, 1993).

The morphology of otoliths is influenced by several factors that are generally difficult to interpret, since they can be generated by a variety of processes and interactions occurring throughout the life history of fish, such as ontogenetic, adaptive, biogeographic and phylogenetic processes (McLachlan, Ladle, 2011; Tuset *et al*., 2016). Vignon and Morat (2010) stated that genetic and environmental factors play a substantial role in determining the shape of the otolith. More specifically, the environment induces a general change in shape and genetics locally affect the shape of the otolith. Some authors have included biological and behavioral attributes of fish, such as activities related to swimming, feeding, and reproduction as determinants for the observed variation in otolith structure (Aguirre, Lombarte, 1999; Lychakov, Rebane, 2000; Mérigot *et al*., 2007).

The results of the present study suggest that the shape of otoliths can be explained by phylogeny, corroborating with the findings of Willis *et al.* (2007), who studied the phylogenetic relationships between species of the genus *Cichla*, and showed that *C. temensis* has a clade of specific haplotypes, which distinguishes it from the species *C. orinocensis* and *C. monoculus*. On the other hand, the species *C. orinocensis* and *C. monoculus* were allocated in the same clade (sub-clade B1), and showed similarities in their haplotypes, which is a pattern that may be related to similarities in the evolutionary lineage attributed to these species. According to Jepsen *et al*. (1997), this pattern may be related to the ecology of the species, *C. temensis* prefers habitats that are deeper and it is found in lakes and the main channel of the rivers, while *C. monoculus* and *C. orinocensis* prefer shallow, slow-moving water. Other studies also support the hypothesis that *C. monoculus* and *C. orinocensis* are sister species to the exclusion of *C. temensis*, forming a genetic group that is distinct from either species (Farias *et al*., 1999, 2000, 2001; Renno *et al*., 2006).

Other studies that have analyzed the morphology of otoliths have also been able to discriminate among congeneric species in marine and freshwater environments. Avigliano *et al.* (2018) observed differences in the shape of otoliths among three sympatric species of the genus *Astyanax* Baird & Girard, 1854 in streams of the Atlantic Forest (Argentina), and concluded that these results may help for future taxonomic and phylogenetic studies. Similarly, He *et al.* (2018) successfully discriminated among three species within the genus *Scomber* Linnaeus, 1758 from China, Norway and Japan, and found that otolith shape analysis can be a complementary approach to morphological and genotypic analysis in order to distinguish among fish species. In general, these studies confirm that the use of the analyses of otolith shape can be used as a natural marker for the identification of species of fish inhabiting a diverse array of environments.

Among individuals of the same species, variations in the shape of the otolith can be directly attributed to local characteristics (Mérigot *et al*., 2007). The populations of *C. temensis* analyzed in the present study showed differences in the shape of otoliths among the rivers sampled, although divergences were noted between the methods used. The shape indices more clearly discriminated the populations according to their place of origin, showing that the individuals of the Negro River have an otolith form that is different from those of the populations of the Uatumã and Jatapú Rivers. However, Fourier descriptors indicated that the population of the Jatapú River was the most distinguished in the data matrix, and showed an overlap between the populations of the Negro and Uatumã Rivers. These ambiguities suggest that the population of *C. temensis* of the Uatumã River does not have a specific form of otolith. In turn, it was seen that the otolith shape of *C. temensis* populations from the Negro and Jatap Rivers are distinct, regardless of the method used.

Pérez, Fabré (2013) associated otolith shape variation from the Orinoco River *Pseudoplatystoma metaense* to differences in growth rate, life cycle, and habitat occupation among populations of these fishes. Although the rivers sampled in the present study have the same type of water (blackwaters) and similar limnological characteristics (acid waters pH ≤ 4, low conductivity ≤ 8 μS cm^{-1}, high transparency, between 1.3–2.9 m) (Junk, 1979; Sioli, 1984), these fishes are subjected to different environmental conditions. The Negro and Jatapú Rivers are generally considered to be intact (ignoring fishing pressure), with few alterations from anthropogenic actions. On the other hand, the sampling area of the Uatumã River is directly influenced by the Balbina hydroelectric plant. In modified aquatic environments, biotic interactions such as space competition, feeding and reproduction can occur in different ways (Silva *et al*., 2008), thus directly influences the metabolism of fishes, and which in turn affects the growth of otoliths and their shape (Allemand *et al*., 2007).

In a study based on genetic divergences, Willis *et al.* (2015) demonstrated that *C. temensis* populations are spatially structured in the rivers of the Amazon, with little gene exchange between localities, which corroborates the results found in the present study. The authors suggested that the geographical distance among populations, coupled with the non-migratory nature of *Cichla*, contribute to the genetic differentiation among localities. This spatial pattern indicates that the management of this species needs to be based on local stocks. However, the lack of basic data on the stocks of most neotropical fishes that are harvested is still a major obstacle to the development of effective and sustainable management of these resources (Willis *et al*., 2015).

Therefore, the sagittal otolith shape descriptors (EFA and shape index) used in this study are appropriate techniques for differentiation of species and geographic population in cichlids, which in turn provides an instrument for managing inland fishery resources.

Furthermore, the combined use of morphometric analyses with the microchemistry of otoliths and genetic markers can be a potentially useful tool for studying the distribution of fishes in freshwater environments (Avigliano *et al*., 2014). However, additional studies are needed to investigate the influence of genetic factors and their interactions with environmental and biotic factors to affect the shape of otoliths among different species and populations.

ACKNOWLEDGMENTS

We would like to thank the Programa de Pós-Graduação em Ciência Animal e Recursos Pesqueiros (PPGCARP) for the opportunity to carry out this study, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Finance Code 001), for the grant of a scholarship and to the Universidade Federal do Amazonas (UFAM) for conceding their laboratory facilities to us in order to perform the analyses.

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AUTHORS' CONTRIBUTION

Marcos de Almeida Mereles: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing-original draft.

Raniere Garcez Costa Sousa: Visualization, Writing-review and editing.

Lorenzo Soriano Antonaccio Barroco: Visualization, Writing-review and editing.
Caroline Pereira de Campos: Visualization, Writing-review and editing.
Marc Pouilly: Validation, Visualization, Writing-review and editing.
Carlos Edwar de Carvalho Freitas: Resources, Supervision, Validation, Writing-review and editing.

ETHICAL STATEMENTS

All the collection procedures for biological material were carried out with the approval of the Animal Use Ethics Committee (CEUA) of the Universidade Federal do Amazonas under the approval number 046/2018, and followed the recommendations of the Brazilian Guide for Good Practices in Euthanasia in Animals – Concepts and Recommended Procedures (2012). Sistema de Autroização e Informação em Biodiversidade – SISBIO license protocols 25606–2 and 51293–2.

COMPETING INTERESTS

The authors declare no competing interests.

HOW TO CITE THIS ARTICLE

- **Mereles MA, Sousa RGC, Barroco LSA, Campos CP, Pouilly M, Freitas CEC.** Discrimination of species and populations of the genus *Cichla* (Cichliformes: Cichlidae) in rivers of the Amazon basin using otolithic morphometry. Neotrop Ichthyol. 2020; 19(4):e200149. https://doi.org/10.1590/1982-0224-2020-0149

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