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Application of otolith shape analysis to species separation in silversides (*Odontesthes,* Atherinopsidae) from South America and the South Atlantic Ocean

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ABSTRACT

Argentina has one of the largest numbers of species of the genus *Odontesthes* that coexist in the world (*Odontesthes argentinensis*, *O. platensis, O. smitti, O. nigricans, O. incisa, O. bonariensis* and *O. hatcheri*). These species have an important ecological role in the trophic webs in the region and in commercial fisheries. However, due to the number of species of the genus *Odontesthes*, the identification of otoliths in feces and stomach contents of ichthyophages is difficult, as well as in fisheries where the different species are commercialized under the same common name. The aim of this study is the use of otolith shape outline as a morphometric tool for the identification of seven silverside species that coexist in marine and freshwater environments of Argentina. Otolith sagittae of 387 silverside specimens were processed using ShapeR package (R statistical software). The Wavelet coefficients (WCs) and Fourier descriptors (FDs) obtained were analyzed using multivariate methods. Results show that the percentage of correctly classified silversides considering WCs, FDs and WCs and FDs together were 71.83 %, 78.81 %, and 88.63 % respectively. There are significant differences among the otolith shape outline of these species (PERMANOVA test, $p < 0.0001$). *O. platensis, O. argentinensis, O. smitti* and *O. bonariensis* otoliths have elongated shapes, while those of *O. nigricans* and *O. incisa* are rounded. Shape differences in the otolith's antero-posterior axis (*rostrum-antirostrum*) and posterior end are the more important for species differentiation. Results could contribute to the identification of these species of silversides in dietary studies of ichthyophages and for the identification of the species marketed jointly under the name of silverside on which specific fishing measures could be carried out.

1. Introduction

The genus *Odontesthes* is a group of South American fishes that belong to the family Atherinopsidae in the order Atheriniformes, these fishes are commonly known as silversides. The order is a monophyletic group, consisting of six families and 49 genera of generally small, and medium-sized fishes (Dyer and Chernoff, 1996). Species identification and delimitation has been a challenge for South American silversides (Bemvenuti, 2006), mainly because several species have very similar morphology and have a well-recorded phenotypic plasticity (Tombari et al., 2005; Crichigno et al., 2013; Llompart et al., 2013; Vettorazzi et al., 2020). This plasticity allows them to adapt to various freshwater, brackish or marine environments (Campanella et al., 2015; González-Castro et al., 2022; Hughes et al., 2020). Because of this, the systematic of the group change drastically over the last 30 years with subfamilies and genera reassigned several times in the classification of the Atheriniformes (Dyer, 2006; Helfman et al., 2009; Betancur-R et al., 2013; Nelson et al., 2016). However, advances in molecular and morphological techniques have allowed a better understanding of the phylogenetic relationships and diversity of this group of fishes (Campanella et al., 2015; Hughes et al., 2020). In the Southern part of South America and the Southwest Atlantic Ocean (SWAO) the only

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family present is Atherinopsidae, represented by the genus *Odontesthes* Evermann and Kendall, 1909 (Dyer, 2006; Gonzalez-Castro et al., 2016; Hughes et al., 2020).

The *Odontesthes* genus has a great ecological and fishery importance (Baigún and Delfino, 2003; Colautti et al., 2015), both in the Pampean and in Patagonian ecoregions (*sensus* Arana et al., 2017) and in the SWAO (Llompart et al., 2013; Guidi et al., 2021). Ecologically they play a fundamental role in aquatic ecosystems, acting as key links in food webs. As consumers, silversides mainly consume zooplankton, small crustaceans, and fish (Piedras and Pouey, 2005; Thompson et al., 2022), and are part of the diet of other fish species, birds and marine mammals (Norbis and Galli, 2004; Riccialdelli et al., 2010; Magnone et al., 2015), thus contributing to energy flow and ecosystem dynamics. Furthermore, in coastal marine ecosystems, in the inland waters of Patagonia and central Argentina, silversides are one of the most abundant fishes (Baigún and Delfino, 2003; Colautti et al., 2015; Soricetti et al., 2020; Baigún et al., 2022). As a fishery resource, silversides (*Odontesthes* spp.) are one of the main target species of artisanal and recreational fisheries in Argentina (Dyer, 2006; Cousseau and Perrotta, 2013; Gonzalez-Castro et al., 2016; Guidi et al., 2021). They represent a food resource of great nutritional and economic value for coastal (Llompart et al., 2013; Guidi et al., 2021) and inland populations (Baigún and Delfino, 2003) and their exploitation provides important sources of income and employment, making them a key component of the regional economy. Silversides are of economic importance to both the local market and for export to Eurasia (MAGyP, 2025). However, silverside fisheries in sympatric zones are generally multispecific, with various species, often including juveniles and adults, being landed and commercialized indistinctly under the common name " silversides". Despite this, most of the less abundant or cryptic species lack specific management measures, underscoring the importance of developing accurate tools for species discrimination, such as otolith-based identification, to support both sustainable fishery practices and ecological studies.

In SWAO the species of silversides present are *Odontesthes argentinensis* (Valenciennes, 1835); *O. incisa* (Jenyns, 1841); *O. smitti* (Lahille, 1929); *O. platensis* (Berg, 1895) and *O. nigricans* (Richardson, 1848), all are costal species, all are predators zooplactivorous and are cached in artisanal and recreational fisheries in all its distribution, *O. incisa* is a small fish aprox 150 mm (Maximum Total Length) and is distributed in the north and central parts of SWAO; *O. nigricans* is a medium size specie (250 mm Maximum Total Length) and is distributed in the central and south SWAO, and *O argentinensis*; *O. smitti*; *O. platensis* are medium to large species (> 300 mm Maximum Total Length). *O. smitti* is the only known species that makes great migrations from the south to the north part of SWAO (Tierra del Fuego to Buenos Aires province).

While in freshwater in Southern part of South America are *O. bonariensis* (Valenciennes, 1835); *O. hatcheri* (Eigenmann, 1909); *O. humensis* (de Buen, 1953); *O. perugiae* (Evermann and Kendall, 1906), and *O. retropinisis* (de Buen, 1953). The most abundant species in freshwater are *O. bonariensis* and *O. hatcheri.* Both species are medium to large size species (> 250 mm Maximum Total Length) and are common in recreational fisheries, *O. bonariensis* is distributed in the north and central Argentina and was stocked in Colorado and Negro river in the north Patagonia and *O. hatcheri* is abundant in all Patagonia rivers. Many of these species are very abundant in the ecosystems they inhabit, and most of them are of high ecological and social relevance. However, several of the silverside species are sympatric in a wide range of their distributions (Cuello, 2020; Hughes et al., 2020), and due to their morphological similarities it is really difficult to establish which species is more affected by commercial and artisanal fisheries or by predators (Bemvenuti, 2006; Tombari et al., 2010) and therefore implement specific fisheries management measures.

Otoliths are calcium carbonate structures in the inner ear of teleost fish that aid in balance and hearing (Popper and Lu, 2000). They are species-specific and have been used for chemical analysis, species identification (especially among cryptic or similar sympatric species), age, growth estimation, stock assessment and fisheries management (Campana, 2023; Muñoz-Lechuga et al., 2023). Tuset et al. (2008) unified terminology of otoliths characterization and described otoliths of 348 species from the Atlantic and Mediterranean, while Volpedo et al. (2017) described otoliths from 155 argentinean freshwater and marine species. Both books provide comprehensive atlas for species identification. Otolith morphology, morphometry, and chemical composition can reveal information about fish populations and their environments (Avigliano and Volpedo, 2013; Avigliano et al., 2018; Biolé et al., 2019). Avigliano et al. (2012) studied the otoliths of freshwater silverside *Odontesthes bonariensis* from Lake Chasicó, Argentina, and suggested that changes in water chemistry due to changes in lake surface area were associated with alterations in otolith morphology and morphometry. Morawicki et al. (2022) using the morphometry of otolith were able to discriminate two different marine fish stocks for *O. argentinensis* from the north and from central coast of Argentina. With the development of different shape analyses tools and software, otolith shape has been used for the identification of several fish species (Ponton, 2006; Tuset et al., 2006; Avigliano et al., 2018; He et al., 2018; Morales et al., 2023). These studies suggested new possibilities in fields as paleoichthyology, trophic relations of predators and systematic where the identification of fishes could be made only by otoliths.

The ShapeR package for the software R facilitates automated extraction and analysis of otolith contours using Fourier or Wavelet transforms (Libungan and Pálsson, 2015). Morphological and morphometric characteristics of otoliths can be used for species identification and ecological studies (Tuset et al., 2008). The Shape R package has been employed in the last years in various studies to analyze otolith morphology (Berg et al., 2018; Soeth et al., 2019; Assis et al., 2020; D'Iglio et al., 2021; Morawicki et al., 2022; Schroeder et al., 2022; Park et al., 2023). Additionally, studies on little tunny utilized shape indices to differentiate between spatial units based on sagittal otolith shape (Muñoz-Lechuga et al., 2023). Furthermore, investigations on European sardines demonstrated the effectiveness of both Elliptic Fourier descriptor and Discrete Wavelet descriptor in reconstructing population structures and connectivity patterns, showcasing the utility of otolith shape analysis in understanding migration patterns and population dynamics. These studies collectively highlight the significance of otolith shape analysis in elucidating fish population dynamics, species identification, and ecological interactions (Rodríguez Mendoza, 2006).

The otolith shape has a high degree of intraspecific variation, which can be used to discriminate among different species (Härkönen, 1986; Smale et al., 1995; Volpedo and Echeverría, 2000; Assis, 2003; Campana, 2005; Tuset et al., 2008; Volpedo et al., 2017). In this context, the aim of this study is to analyze the otoliths shape of seven highly similar species of the genus *Odontesthes* that inhabit several freshwater environments in Buenos Aires and Rio Negro Provinces (Argentina, South America) and in coastal marine environments of the Southwestern Atlantic Ocean. This analyze could contribute to the identification of silverside species both in studies of trophic ecology of ichthyophages (dolphins, whales, penguins, sea lions among others) of the SWAO and Southern Ocean; and could generate a tool that complements the traditional identification of silverside species using external morphological characters, which is generally very difficult for workers in the fishing and in environmental sectors and decision-makers in fisheries management and development.

2. Materials and methods

2.1. Study area and species

Specimens of silverside were obtained from continental environments (shallow lakes and rivers) of Buenos Aires and Rio Negro Provinces (Argentina); Paraná Delta, Gómez, Chasicó and Chascomús lakes; Negro river and from coastal marine environments of the Southwestern Atlantic Ocean of Argentina; San Blás Bay; El Condor, Playas Doradas, and Mar del Plata (Fig. 1). A total of seven silverside species were analyzed [*Odontesthes bonariensis* (OB), *O. argentinensis* (OA), *O. smitti* (OS), *O. nigricans* (ON), *O. incisa* (OI), *O. hatchery* (OH), and *O. platensis* (OP); Table 1]. OB specimens of silverside were obtained from continental environments (shallow lakes and rivers) of Buenos Aires Provinces, the Paraná Delta, Gómez, Chasicó and Chascomús lakes. OH from the Negro river (Argentina). The marine species OA; OP; OS were obtained from coastal environments of the Southwestern Atlantic Ocean of Argentina (SWAO); San Blás Bay, El Condor, Playas Dorada. OI was catch in Mar del Plata in the North of SWAO (Fig. 1). All the sampling areas were chosen because they are the typical habitat of each species. Discrimination between different stocks in the Argentinean Sea was determined for OA (Biolé et al., 2019; Levy et al., 2019; Morawicki et al., 2022), so for this silverside species.We analyses specimens belonging only to the Northern Patagonian Stock (*sensus* Morawicki et al., 2022).

2.2. Sampling

Collections were conducted between October 2018 and March 2023. The fishing gears used were gillnets with 30, 40, 50 and 70 mm mesh opening and 20 m long trawls nets. A total of 387 silverside specimens were captured, which were transported to the laboratory for measurement (Total length = TL, mm). Identification to species level was done following the taxonomic keys proposed by Mancini et al. (2016) and Dyer (2006). Distribution of specimens among location and species, together with measurements is shown in Table 1. Specimens were frozen (-18 °C) and stored in the laboratory for further analysis. Only adult specimens were selected for otoliths analysis.

Sagittae otoliths were extracted and cleaned with Milli-Q water with a resistivity 18.2 mΩ cm (Merck Millipore) and dried. The left otolith of each individual was photographed in dark background (Fig. 2) with a Leica® MC170HD camera mounted to a Schonfeld® binocular microscope and the Leica Application Suite software (LAS version 4.5).

2.3. Shape analysis

The contours of otoliths were analyzed using the ShapeR package (Libungan and Pálsson, 2015) of the R statistical software (R Core Team, 2021). Using the "detect outline" function (threshold = 0.15), the contours of all otoliths were extracted. The "smootout" function was used to remove pixel noise around the contours that could interfere with the analyses. The detected outlines were controlled using the function (write.outline.w.org = TRUE) and were not manually modified.

Fourier descriptors (FDs) and Wavelets coefficients (WCs) were extracted using the function "generate Shape Coefficients". The deviation of the reconstructed contour of each otolith was compared with the original contour to determine the number of FDs and WCs needed for the analysis; with 45 FDs and 64 WCs an accuracy rate higher than 98.5 % was obtained. After this, coefficients that showed a high correlation with fish size ($p < 0.05$) were removed from the analysis (Longmore, 2010). Of the 45 FDs and 64 WCs used by the ShapeR package to describe the contours, only 18 FDs and 40 WCs remain and were used for classification.

Table 1
Sampling locations. Mean ± Standard Deviation values of Total Length (TL). N: sample number.

Species	Place	Location.	N	tot	TL (mm)
Odontesthes bonariensis	Chascomús Lake	35°35'48"S 58°01'17"W	15	60	289,33 ± 61,33
	Chasicó Lake	38°37'22"S 63°04'34"W	15		288,89 ± 36,98
	Paraná Delta	34°11'46"S 58°19'56"W	15		396,27 ± 85,86
	Gomez Lake	34°39'47"S 61°01'29"W	15		275,53 ± 13,93
O. argentinensis	El Cóndor Beach	41°03'07"S 62°49'17"W	15	109	239,8 ± 17,61
	San Blás Bay	40°32'06"S 62°18'41"W	64		268,55 ± 45,01
	San Antonio Este	40°49'06"S 64°45'14"W	30		279,93 ± 45,81
O. smitti	El Cóndor Beach	41°03'07"S 62°49'17"W	81	81	256,52 ± 38,91
O. nigricans	Playas Doradas	41°37'00"S 65°01'11"W	32	44	100,25 ± 18,45
	San Blas Bay	40°32'06"S 62°18'41"W	12		131,33 ± 10,3
O. incisa	Mar del Plata	38°02'19"S 57°31'10"W	10	37	74,4 ± 9,87
	Playas Doradas	41°37'00"S 65°01'11"W	27		92,3 ± 11,26
O. hatcheri	Negro River	40°42'49"S 63°17'52"W	23	23	158,17 ± 71,08
O. platensis	El Cóndor Beach	41°03'07"S 62°49'17"W	8	33	376,01 ± 34,03
	San Blás Bay	40°32'06"S 62°18'41"W	25		328,04 ± 67,13

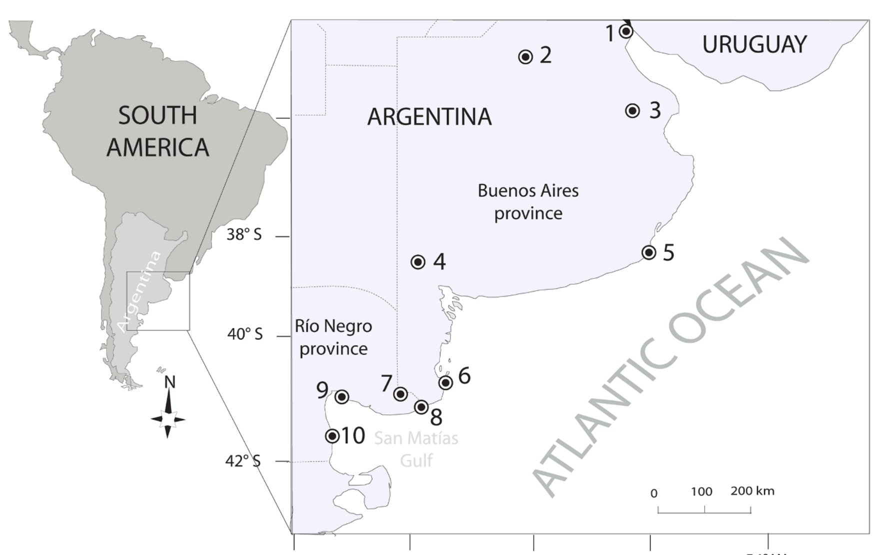

Fig. 1. Sampling sites. Ref.: 1-Paraná Delta; 2-Gómez lake; 3-Chascomús lake; 4-Chasicó lake; 5- Mar del Plata coast; 6-San Blás Bay: 7-Negro River; 8- El Cóndor beach; 9-San Antonio Este coast; 10- Playas Doradas.

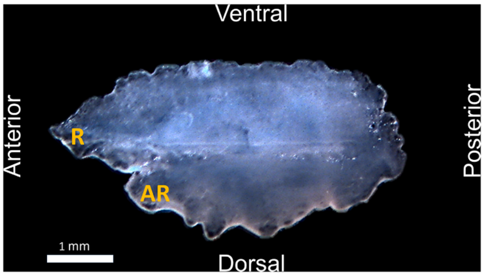

Fig. 2. Left *sagitta* otolith of *Odontesthes platensis*. Features variables. AR: Anti rostrum. R: Rostrum.

The average shape of the otoliths of each species, reconstructed using WCs, were plotted together in order to visually assess the differences. To determine which part of the otolith contributes more to the differentiation among species, the average shapes were plotted and their standard deviations were plotted against the otolith angle using the gplot package (Warnes et al., 2014). To quantify the differences among populations, the proportion of variation among groups (the intraclass correlation, ICC), was calculated along the outline of the otolith.

2.4. Multivariate analysis

A linear discriminant analysis (LDA) and PERMANOVA were performed with PAST software (Hammer, 2001) using as variable the FDs, the WCs and both together (FDs + WCs). The classification success was measured among the different groups and evaluated using a jacknifed confusion matrix. Comparison among the different groups was assessed using a permutational multivariate analysis of variance (Anderson, 2014) based on the Bray-Curtis dissimilarity measures (4999 random permutations).

3. Results

3.1. Mean shape features

Otolith contours of the different fish species from the different studied localities presented differences in mean shape (Fig. 3). The morphometry of the otoliths presented variations in the rostrum-antirostrum, the dorsal margin and the posterior end (Fig. 3). The variations detected for WCs (Fig. 3) were supported by the ICC plot (Fig. 4) for these regions on the otolith outline, showing peaks at 320–60° (dorsal margin), and 160–200° (rostrum-antirrostrum).

The otoliths of *O. platensis, O. argentinensis, O. hatcheri, O. smitti* and *O. bonariensis* have elongated shapes, while those of *O. nigricans* and *O. incisa* are rounded. The development of the face in these species is variable, which generates variations in the antero-posterior axis (Fig. 3). The greatest variations in shape are observed in the antero-posterior axis of the otolith (rostrum and antirostrum) and in the posterior end.

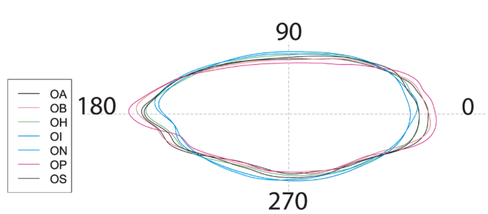

Fig. 3. Mean shape of otolith shapes of silverside from seven species of the genus *Odontesthes*. OA: *Odontesthes argentinensis*; OB: *O. bonariensis*; OH: *O. hatcheri*; OI: *O. incisa*; ON: *O. nigricans*; OP: *O. platensis*; OS: *O. smitti*.

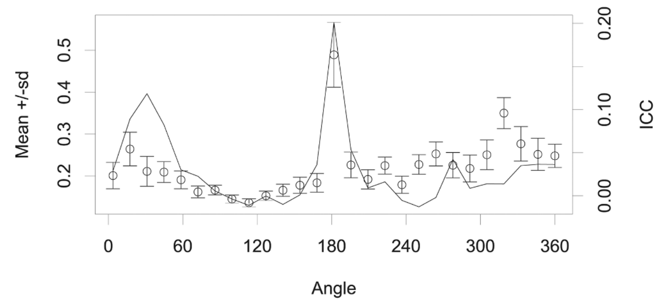

Fig. 4. Mean and Standard deviation (SD) of the wavelet coefficients representing shape for all combined otoliths and the proportion of variance among silverside of different localities or interclass correlation (ICC, black solid line). The central axis shows the angle in degrees (°) based on polar coordinates where the centroid of the otolith is the central point of the polar coordinates.

3.2. Multivariate analysis

The results of the Linear Discriminant Analysis showed different percentages of errors in the classification of individuals, depending on the variables used in the analysis (Table 2).

Using FDs as a variable resulted in a slightly higher percentage of correctly classified individuals than using WCs (78.81 % and 71.83 % respectively) (Table 2a,b), however using FDs + WCs combined resulted in the highest percentage of correctly classified individuals (88.63 %) (Table 2 c). Species with the higher classification problems were generally OB, OH and OS, which can also be seen in Fig. 5a–c where these species do not form well-defined clusters. The PERMANOVA, performed using both variables (FDs + WCs) showed significant differences between all species in this study (pseudo-f: $p < 0.0001$).

4. Discussion

All the silverside species studied here were well separated based on otolith morphometry. In the LDA, OI and OP were consistently shown separately from the other species, which formed a distinct group. In multispecies silverside fisheries, misidentification between species particularly between juvenile OA and adult OI, or between adult OA and

Table 2
Cross-classification table of Linear Discriminant Analysis. OA: *Odontesthes argentinensis*; OB: *O. bonariensis*; OH: *O. hatcheri*; OI: *O. incisa*; ON: *O. nigricans*; OP: *O. platensis*; OS: *O. smitti*.

Species	OA	OB	OH	OI	ON	OP	OS	% correctly classified
a) Fourier descriptors (FDs)								
OA	85	5	5	0	2	3	9	**78.81**
OB	6	40	7	1	0	1	5	
OH	2	3	14	0	0	2	2	
OI	0	0	0	37	0	0	0	
ON	0	0	1	0	41	2	0	
OP	0	3	2	0	0	24	4	
OS	5	7	1	0	2	2	64	
b) Wavelets coefficients (WCs)								
OA	73	12	10	4	1	1	8	**71.83**
OB	5	47	4	1	0	0	3	
OH	4	1	15	0	0	0	3	
OI	1	0	0	25	9	0	2	
ON	0	0	0	9	34	0	1	
OP	2	1	0	0	0	28	2	
OS	8	1	7	5	2	2	56	
c) FDs + WCs								
OA	95	4	4	0	1	0	5	**88.63**
OB	2	52	5	0	0	0	1	
OH	2	2	19	0	0	0	0	
OI	0	0	0	37	0	0	0	
ON	0	0	0	0	44	0	0	
OP	1	1	2	0	0	29	0	
OS	7	2	1	0	3	1	67	

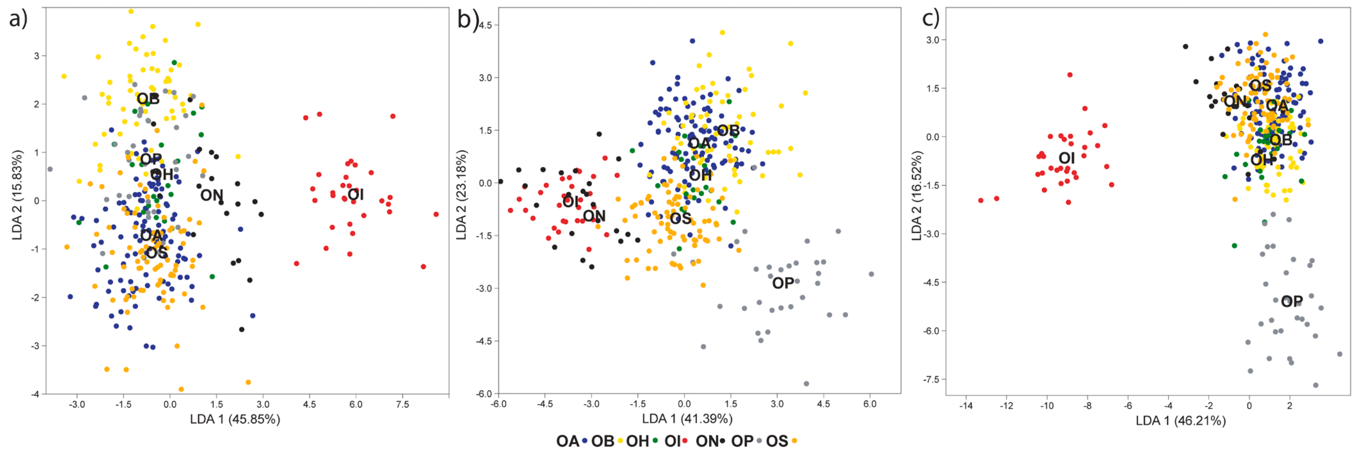

Fig. 5. Linear discriminant analysis (a) Fourier descriptors (FDs); (b) Wavelets coefficients (WCs); (c) FDs + WCs. The centroid of each study area is shown as a bold letters. OA: *Odontesthes argentinensis*; OB: *O. bonariensis*; OH: *O. hatcheri*; OI: *O. incisa*; ON: *O. nigricans*; OP: *O. platensis*; OS: *O. smitti*.

OP can lead to inaccurate assessments of catch composition and stock status, because these species are sympatric and morphologically similar. Although the species were distinguishable in our analysis using adult individuals, morphological similarities often cause confusion in practical fishing scenarios in the application of conservation measures. Therefore, the ability to differentiate species based on otolith morphometry provides a reliable tool to validate landings data, monitor species-specific exploitation rates, and support the implementation of more effective, sustainable management strategies.

It is very important to highlight that there is a great difference in the otolith shape between OP and OA. These two species are captured together by fisheries in several areas of the Argentinian coast and are visually easy to confuse. In this sense, the difficulty in identifying OP using external morphological characters led to consider this species as a synonym of OA. What is observed in relation to the shape of the otolith is that both species are very different, moreover OP is very different from all the species in the Argentine Sea. Similar results were obtained by González-Castro et al. (2019) following a genetic approach, which reinforces the independence of OP as a real species.

The otoliths of species that showed the greatest classification challenges were generally OB, OH, and OS. This may reflect higher levels of morphological variability, potentially driven by evolutionary, environmental, and anthropogenic factors. In the case of OB, for example, individuals were sampled from Chasicó, Chascomús, and Gómez Pampean lakes. The silversides of these water bodies may have increased variability due to historical introductions of OB individuals from the Chascomús Hydrobiological Station (Cuello, 2020), followed by interbreeding with local stocks. In this study we do not explicitly test geographic differentiation within OB. Factors such as the introduction of farmed specimens to the studied water bodies (Colautti et al., 2015) and the natural hydrological dynamics of the Pampas lakes. Fish populations alternate between isolation during periods of drought and connectivity among a isolate water bodies during flooding (Volpedo and Fernandez Cirelli, 2013); could be reflected in the morphological heterogeneity of otoliths (Avigliano et al., 2012). These factors could explain the greater dispersion of individuals observed in multivariate analyses, which complicates the classification of this species. In addition, these dynamics are affected by management practices and anthropochory.

In relation to OH, the number of samples is lower (23 specimens) and all come from Río Negro, which could affect the percentage of well-classified individuals.

The higher intraspecific dissimilarities found in the morphometry of the OS otoliths suggested the presence of different populations of this silverside species in the study area; all the specimens has been collected in the coastal area of El Condor beach, which is at the OS migration route. OS specimens migrate from different sectors of Patagonian coast to one place in the north (Mar del Plata beach; 57° 33’ 27.14"W; 38° 0′ 8.21"S) (Carballo et al., 2012). It could be suggested that during migration, different populations of OS move together as a group of migratory fishes. Moreover, the presence of a relevant rostrum in OS otoliths is typical of swimming fish species (Volpedo and Echeverría, 2003) and fish species with a wide distribution that makes large movements (Carballo et al., 2012). ON and OI are the two smallerspecies in the genus, the adult size for OI rarely exceeds 150 mm (Cousseau and Perrotta, 2013). Little is known about the ecology of both species, ON is very common on the southern coast (coast from Patagonia to Tierra del Fuego) (Cocito et al., 2019) and OI is frequent from Mar del Plata to northern Patagonia, therefore both species overlap their distribution in the northern part of Patagonia. Both species have round-shaped otoliths although they could be distinguished morphometrically and morphologically (Tombari et al., 2010).

Growth-related shifts often referred to as growth stanzas, can influence otolith morphology. To address this potential bias, intraspecific comparisons must be conducted using specimens individuals at comparable life stages. Although the species examined in this study differ in body size range (from small-sized species like OI to medium-to-large species such as OA and OP), all specimens of the 7 silverside species analyzed in this study were adults. The sizes analyzed were larger than the size of first sexual maturity defined in the literature (Conover and Present, 1990; Froese and Binohlan, 2000; Tombari et al., 2005, 2010; Dománico and Freyre, 2008; Llompart et al., 2012, 2013; Lattuca et al., 2015; Navoa et al., 2024). Therefore, the observed differences in otolith shape are not attributable to ontogenetic variation or body size differences among species.

For the genus *Odontesthes* the morphometry of the otoliths presented modifications in the rostrum-antirostrum, the dorsal margin and the posterior end. These morphological characteristics, which were partially observed by Tombari et al. (2010), allow the identification of the species. But further studies must be made to understand the different otoliths morphologies highlighted in this study and how it could be related to the life history of the different species, mainly because for several silversides little is known about their life histories or use habitat.

Fish otoliths play a crucial role in studying bird and marine mammal diets and paleontological research (Fitch and Brownell, 1968; Jobling and Breiby, 1986; Stolarski et al., 2023, Mion et al., 2024). As a species-specific structure, it allows the identification of prey species consumed by marine predators (Jobling and Breiby, 1986; Bowen, 2000). Silversides are one of the most common neritic fishes in SWAO (Llompart et al., 2013; Morawicki et al., 2022; Thompson et al., 2022), being a key part of the food chain, so the knowledge of the otoliths shape and ability of classification and separation of the different species of *Odontesthes* could be an important and useful tool in the studies of diet of predators in SWAO. Otolith analysis has revealed diverse prey species and foraging depths for various cetaceans, providing insights into their feeding habits (Fitch and Brownell, 1968). On the other hand, the results obtained in this work can also be applied to archaeological studies on the diets of native peoples, subsistence practices and the ecological relationships between humans and their prey (Scartascini and Volpedo, 2013; Furlani et al., 2007; Favier-Dubois and Kokot, 2011; Gómez Otero and Svoboda, 2022).

5. Conclusion

Despite the similarity in the contours of the otoliths between the different species of silverside, the application of DFs and WCs together allowed to discriminate the species studied with a high precision. This suggested that the simultaneous application of different methodologies allows obtained more reliable results in cryptic species such as the ones studied. On the other hand, the results found could have a direct impact on the studies of the trophic ecology of ichthyophagous, the management and application of fisheries management and conservation measures and on archaeological studies associated with ancient peoples.

CRediT authorship contribution statement

Gustavo A. Thompson: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Catalina Guidi:** Writing – review & editing, Methodology, Data curation. **Patricio Solimano:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Santiago Morawicki:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Volpedo Alejandra Vanina:** Writing – review & editing, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper: We did not receive any support from any additional organization If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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