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Otolith shape analysis as a tool for species identification and management of cryptic congeners in the northern Benguela ocean warming hotspot

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ABSTRACT

Adaptive management is critical to maintaining resilient fisheries in our rapidly changing ocean environments. However, establishing appropriate and cost-effective monitoring programmes that adequately capture the responses of fisheries to climate change have been elusive. The coastal waters of central and northern Namibia are a global hotspot of ocean warming, with several responses by fish impacting its coastal fisheries. One documented change is a poleward distributional shift of the west coast dusky kob, *Argyrosomus coronus* into Namibia, where it has begun to hybridize with the congeneric *A. inodorus.* With considerably different life histories, it is critical that managers can differentiate between these species and the hybrids to appropriately manage the most important recreational and commercial linefish species (bycatch and biomass) in Namibia. In this paper, we used otolith shape analysis, Linear Discriminant Analyses (LDA) to show that otolith shape can be used to distinguish between species and their putative hybrids, identified based on mitochondrial DNA and nuclear microsatellite data. A total of 342 individuals from northern and central Namibia (northern Benguela) between 18.5 and 22.7°S were genetically identified, with a subsample of 217 paired with otolith shape analysis. Otolith shape analyses with LDA and leave-one-out cross validation showed successful species identification at 96.3 % accuracy, and improved accuracy of hybrid identification compared to using body morphology by 50 %. The Fourier descriptors provided excellent classification accuracy for separating *A. coronus* from *A. inodorus* (and putative hybrids, but poorer classification accuracy for separating the hybrids from *A. inodorus* (4/8). We thus recommend using otolith shape analysis for distinguishing the two species but suggest that genetic identification is still required to monitor hybridization. Monitoring for adaptive management should therefore include routine otolith collection and species classification to improve stock assessment and promote the development of appropriate management strategies for this important fishery in the northern Benguela.

1. Introduction

With climate change rapidly altering marine ecosystems and fisheries throughout the world, our adaptation strategies are critical for determining the efficacy of the conservation and management of living marine resources (Bryndum-Buchholz et al., 2021). In their global

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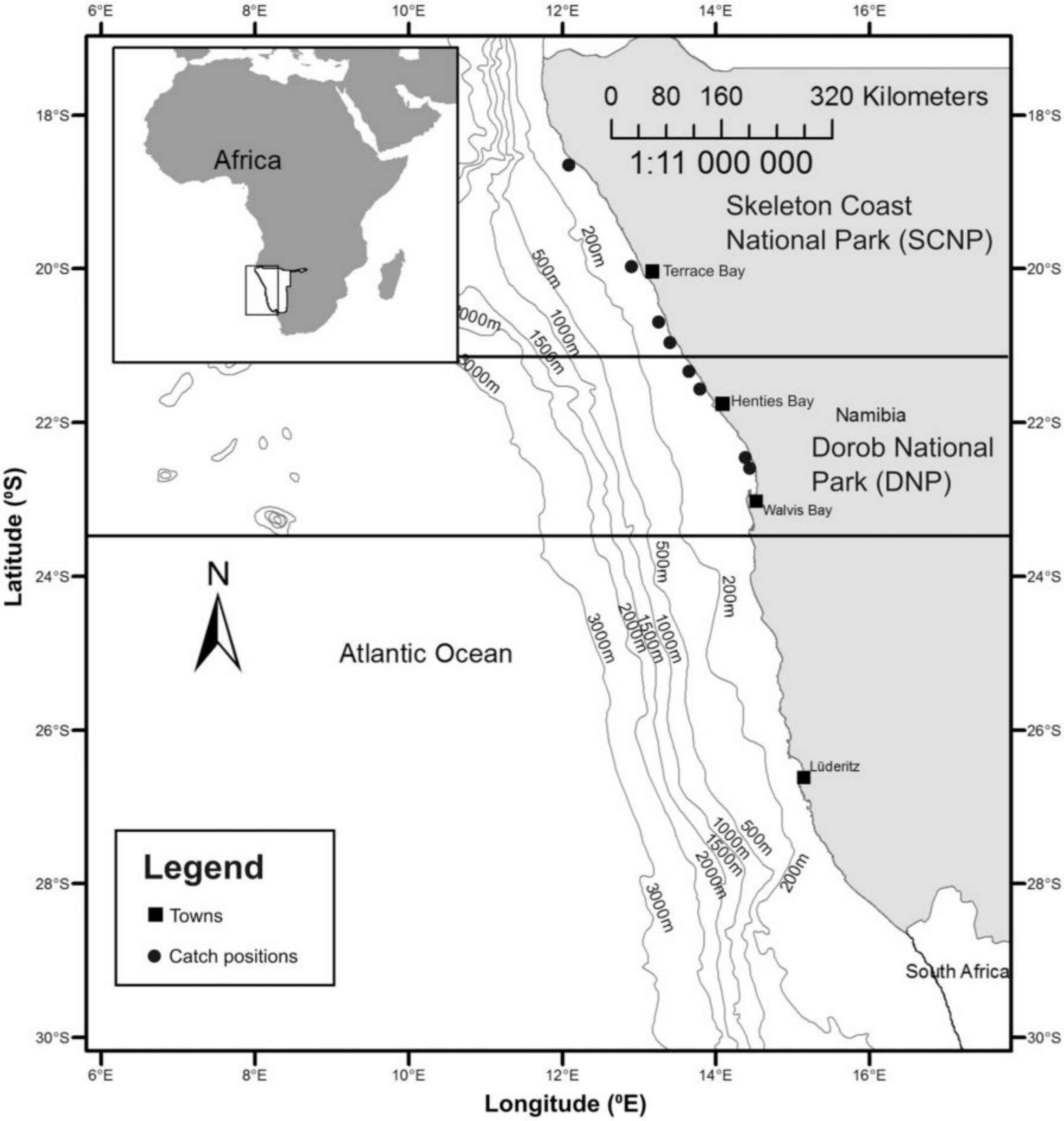

Fig. 1. Map of the sampling area of *Argyrosomus* spp. collected by hook-and-line angling between January 2018 and July 2019.

systematic literature review of climate change adaptation in fisheries, Galappaththi et al. (2022) identified three types of responses, namely coping mechanisms, adaptive strategies, and management changes. Management changes included the incorporation of adaptive management and adaptive planning into governance systems and are fundamentally important for maintaining resilient fishery systems in a rapidly changing climate.

Although several adaptive management responses, such as changing harvest allocations to align with shifting fish distributions (Pinsky et al., 2018) or switching targets to harvest newly established species (Malpica-Cruz et al., 2021) have been suggested, most countries have been slow to implement these processes into their fisheries governance practices (Bryndum-Buchholz et al., 2021). One of the reasons for this has been the lack of appropriate monitoring programs that can detect changes and inform adaptive strategies (Hobday and Evans, 2013). Several types of data have been used for monitoring the signals of climate change impacts in fisheries. These include fisheries-dependent data, such as catch data and conventional tagging data, and fisheries-independent data, such as surveys, electronic tagging and genetic data (Hobday and Evans, 2013). However, often traditional sampling approaches are too simple to account for the complexity associated with the observed changes and alternative methods must be explored. Therefore, building a body of knowledge on alternative, cost-effective monitoring strategies to inform adaptive management is an essential step towards resilient fisheries.

The northern Namibian and southern Angolan coastal waters are a hotspot for ocean warming with average sea surface temperatures increasing at 0.6 °C per decade (Hobday and Pecl, 2014; Potts et al., 2014a). The rapid warming in this region has resulted in a range of responses by the coastal fisheries species in the region, including distributional shifts (Potts et al., 2014a), changes in reproductive scope (Potts et al., 2014b), feeding patterns (Potts et al., 2016) and even hybridization (Potts et al., 2014a). Together, these responses are likely to result in a complex suite of ecosystem changes, which may have considerable impacts on the coastal fisheries (e.g. Engelhard et al., 2024).

The dominant fishes captured in the Namibian economically and socially important shore-based line fishery are the “kob” (Kirchner et al., 2000), which actually comprises two species, namely silver kob, *Argyrosomus inodorus* and west coast dusky kob, *A. coronus* (Griffiths and Heemstra, 1995; Kirchner, 1998; Potts et al., 2010). Although the species are morphologically similar and difficult to distinguish, they have markedly different life histories, with *A. inodorus* growing slower and maturing at a smaller size (Kirchner and Voges, 1999; Potts et al., 2010; Jagger, 2024). After early studies described the "kob" catch of *A. coronus* as incidental (< 10 %, van der Bank and Kirchner, 1997), it was decided to ignore the presence of *A. coronus*, pool the two species in monitoring and to assess and manage the "kob" fishery using the life history information of *A. inodorus*. Updated assessments and management measures are, however, essential for this important stock (Gusha et al., 2024).

Potts et al. (2014a) documented a warming-driven, poleward shift by *A. coronus* from Angolan waters in Namibia. They also presented genetic evidence to suggest that the proportion of *A. coronus* in the Namibian "kob" fishery was increasing. Pringle et al. (2023) compared the thermal physiology of the two "kob" species and suggested that the warming coastal waters of northern and central Namibia will increasingly favor the warm water congener (*A. coronus*) over *A. inodorus*. In addition, there is evidence to suggest that the two "kob" species have begun to hybridize (Potts et al., 2014a; Henriques et al., 2018). The consequences of the hybridization are unknown, however, Jagger (2024) found that hybrids had similar growth rates to *A. coronus*, while Pringle et al. (2023) conducted a thermal physiology study and suggested that hybrids may be more resilient to warmer temperatures (Pringle et al., 2023). While these findings may suggest that hybridization may be a powerful mechanism for *A. inodorus* to adapt the rapidly changing climate, this complex situation must be well managed to prevent rapid overexploitation, particularly of *A. coronus* by the fishery.

Potts et al. (2014a) called for the implementation of adaptive management strategies to prevent rapid overexploitation of *A. coronus* by Namibia's coastal fisheries, particularly because the present management measures are based on the life history characteristics of *A. inodorus*. However, to do this, it is critical to develop an accurate method to monitor the species composition of the "kob" fishery in Namibia, without a cost-prohibitive genetic monitoring program (Waldman, 2007; Leguá et al., 2013). Several alternative and cheaper methods have been used to classify cryptic marine fishes. These include morphometric and meristic analyses; the use of parasites as biological tags (e.g. MacKenzie and Abaunza, 1998); externally and internally attached artificial tags (e.g. Kirchner and Holtzhausen, 2001); and studies of biological parameters in relation to life cycles (e.g. Wilhelm et al., 2015). In terms of morphometric and meristic analyses, the use of otolith shape analysis for stock identification has been successfully applied in the field of fisheries science for temporal and geographical discrimination of stocks (Campana and Casselman, 1993; De Vries et al., 2002; Jónsdóttir et al., 2006; Zhang et al., 2017; Smoliński et al., 2020), and requires less expertise, than, for example, parasite identification (e.g. Amakali et al., 2022).

Griffiths and Heemstra (1995) described the diagnostic characters of *Argyrosomus* spp. around southern Africa and stated that silver kob had a distinct otolith morphology compared to five other species of the genus, *A. regius*, *A. japonicus*, *A. hololepidotus A. thorpei*, and *A. coronus*. They also described ostium-cauda distance (OCD) as a proportion of otolith height (OH) on the otoliths (see Fig. 2) as diagnostic characters between the six species with a difference shown in relationships of otolith weight (OW), OH, otolith length and OCD against fish total length (TL) between *A. inodorus* and *A. japonicus*. The OCD of *A. inodorus* was described as large (0.37–0.63 of OH) in comparison with other species of the genus, including *A. coronus* (0.26–0.34 of OH).

The aim of this study was to test whether otolith shape can be used to distinguish between *A. inodorus*, *A. coronus* and their hybrids (F1, F2, or backcrosses) in order to inform the development of appropriate monitoring protocols of the presence of hybrids among the two populations, for adaptive management in the Namibian "kob" fishery.

Table 1

Number of *Argyrosomus inodorus*, *A. coronus* and their potential hybrids (determined by external features), used for genotyping and otolith shape analysis, caught in Namibia from the Skeleton Coast National Park (17°14'S to 21°11'S) to the Dorob National Park (21°11'S to 23°19'S) between January 2018 and July 2019. Fish length was measured as total length (TL) of the fish.

Samples with genotype and otolith for shape analysis	N	Fish length range (cm)	Fish length standard deviation (cm)	Latitude range (ºS)
A. inodorus	184	34–91	9.21	18.5–22.7
A. coronus	31	43–76	8.45	18.5–21.9
Potential hybrid (morphology looked "mixed")	2	50–51	9.37	18.5–20.8
Total	217	34–91	9.17	18.5–22.7

2. Materials and methods

The care and use of animals complied with Namibian animal welfare laws, guidelines and policies as approved by National Commission for Research Science and Technology (NCRST), Namibia authorization number: 20190204.; University of Namibia Decentralized Ethics Committee (Reference number: SNC0002 of University of Namibia, 2021) and the Rhodes University Animal Research Ethics Committee (RU-AREC), reference number: 2019–0174–271.

2.1. Sampling

The area of this study extended from the Skeleton Coast National Park (SCNP 17°14'S to 21°11'S) area in northern Namibia, to the Dorob National Park in central Namibia (DNP 21°11'S, 13°38'E to 23°19'S, 14°29'E) (Fig. 1). Genetic ID was collected for a total of 342 individuals, and Genetic ID paired with otoliths were collected for a total of 217 individuals (most of which had external features of *A. inodorus*) (Table 1; Jagger, 2024; Pringle et al., 2023). All samples were collected randomly and opportunistically from fish catches of recreational and commercial fishers between 17°S and 23°S along the Namibian coast (Table 1). No samples were collected from the southern region of Namibia as this is a restricted area. Most individuals were collected during the austral summer, from November to April, 2018 – 2019, characterized by the highest catch rate in the Namibian line fishery (Kirchner, 1998) and the spawning season for silver kob (Kirchner and Holtzhausen, 2001).

For each fish, the species (as per phenotype), total length (TL, to the nearest cm), sex and approximate catch location were recorded. The phenotype was recorded by the following features: *A. coronus* have a darker colouration when compared to *A. inodorus*. *A. coronus* are also wider at the pectoral fins than *A. inodorus*, and in general look more robust. In addition, internal features, e.g. swim bladder size, can be used to distinguish the phenotypes (Griffiths and Heemstra, 1995). A small piece of the pectoral fin was removed immediately after capture and preserved in 96–100 % ethanol for DNA analysis. Otoliths were removed, cleaned and stored dry in labelled paper envelopes.

2.2. Genetic based species and hybrid identification

Total DNA was extracted using a standard CTAB-chloroform/isoamyl alcohol method (Winnepenninckx et al., 1993). For mtDNA analyses, a fragment of the cytochrome oxidase I (COI) gene was amplified by PCR using the FF2d and FF1d primers described by Ivanova et al. (2007). Sequencing of mtDNA amplicons was performed using the FF2d primer with Big Dye technology and an ABI 3500 DNA analyser (Life Technologies). The mtDNA sequences were edited and aligned using BIOEDIT (Hall, 1999). A minimum spanning network was constructed in NETWORK (www.fluxus-engineering.com/sharenet.htm) mtDNA sequences were identified as belonging to either *A. inodorus* or *A. coronus*

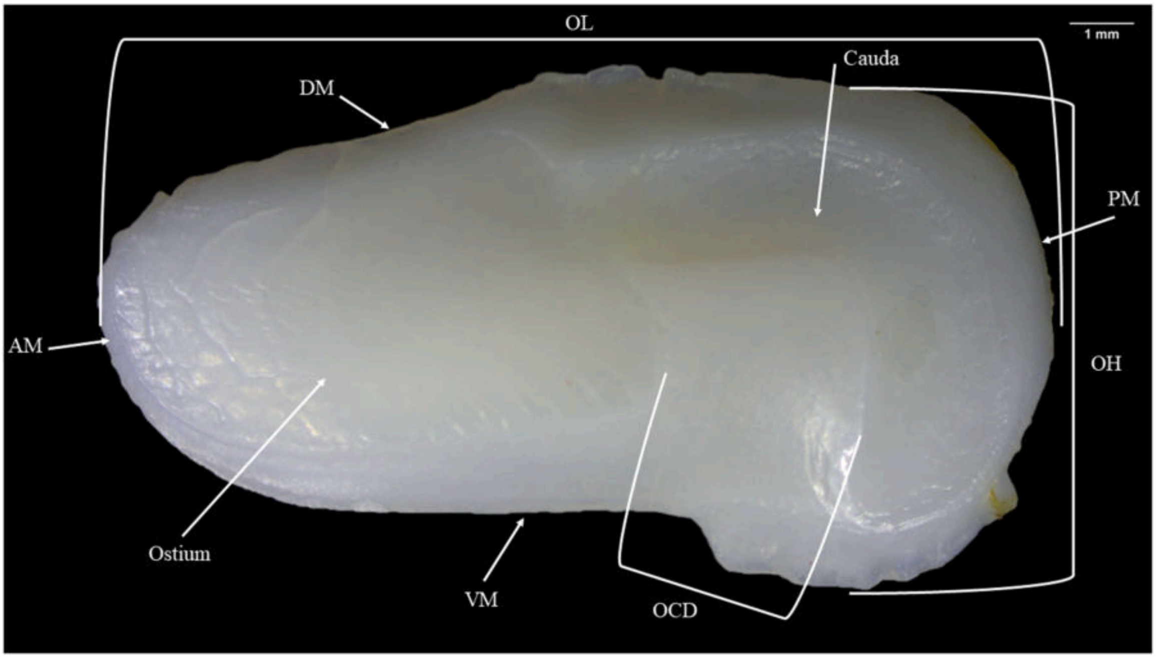

Fig. 2. Proximal view of the right sagittal otolith of a 59 cm (total length) female pure-bred *Argyrosomus inodorus* caught in northern Namibia Skeleton Coast National Park (see Fig. 1). Otolith terminology and measurements are shown on the figure: DM = Dorsal margin, VM = Ventral margin, PM = Posterior margin, AM = Anterior margin, OL = Otolith length, OH = Otolith height, OCD = Ostium to cauda distance.

by phylogenetic grouping alongside comparison with sequences from Mirimin et al. (2014). For nuclear analysis, individuals were genotyped at five microsatellite loci (UBA5, UBA40, UBA91, UBA853, UBA854) developed by Archangi et al. (2009) and shown to be useful for hybrid identification in *Argyrosomus* (Potts et al., 2014a) in an ABI 3500 analyser (Life Technologies) using PEAKSCANNER software (v 2.0) for genotype calling. Following the same parameters as Potts et al. (2014a) and Pringle et al. (2023) individual admixture proportion were investigated using both STRUCTURE (v 2.3.4; Pritchard et al., 2000) and NewHybrids (Anderson and Thompson, 2002). Individuals were only considered hybrids if identified as such by both STRUCTURE and NewHybrids. The power of the data to detect hybrids and potential backcrosses was assessed by running the STRUCTURE and NewHybrids analysis on simulated genotypes (F1, F2, backcross to *A. coronus* or backcross to *A. inodorus*) generated with HYBRIDLAB (v 1.0; Nielsen et al., 2006).

Assignment of individuals to species and hybrid categories was conducted by comparing morphological information with mitochondrial DNA (mtDNA) and nuclear microsatellite data. Hybrids were identified if there was an obvious mismatch between morphological and mtDNA haplotypes, between mtDNA haplotypes and microsatellite genotypes, or evidence of mixed ancestry in the microsatellite genotypes, as per Potts et al. (2014a) and Pringle et al. (2023). Individuals with congruence between morphological, mtDNA and microsatellite data were labelled as “pure” representatives of each species and used as the baseline for hybrid identification.

2.3. *Otolith shape analysis*

All undamaged right sagittal otoliths were photographed using a Zeiss Stemi 508 stereo microscope fitted with a Carl Zeiss Axiocam 208 colour camera. Otoliths were placed under reflected light against a black background, on the proximal side in the same orientation, with the dorsal margin facing up (Fig. 2). It was assumed that right and left otoliths were comparable (e.g. Cardinale et al., 2004; Libungan et al., 2015). Thus, if the right otolith was not available, the image of the left otolith was flipped. To improve clarity, the magnification (0.63x, 0.67x, 0.8x, or 1.0x) depended on the size of the otolith. Pixel-to-mm ratios were used for measurements at each magnification. The images were improved in contrast between otolith and background with GIMP (Natterer and Neumann, 2008).

For otolith shape analysis, the methods of Smoliński et al. (2020) were followed in R (R Core Team, 2023a). Images were first binarized using the package “imager” (Barthelme et al., 2021). The “Momocs” package (Bonhomme et al., 2014) was used to automatically extract outlines from the binary images in combination with the R packages: “tidyverse” (Wickham et al., 2019; Wickham, 2023), “foreach” (Folashade and Weston, 2022), “parallel” (R Core Team, 2023b) and “jpeg” (Urbanek, 2022). All data and R scripts used in this study are available at GitHub (https://github.com/margitwilhelm/kobOtolithShape).

Otolith outlines were quantified using elliptical Fourier analysis, with elliptical Fourier descriptors (Kuhl and Giardina, 1982). Here, the two-dimensional shape of the otolith is decomposed with a sum of harmonics, each described by four coefficients. The harmonics are successive, and with each additional harmonic the otolith outline is described in finer detail (increased precision). To define the approximate number of harmonics needed for optimal precision of shape reconstruction, the Fourier power (PF*n*) spectrum and cumulative harmonic Fourier power (PF*c*) were calculated as for all otoliths follows (Crampton, 1995):

$$\text{PFn} = \frac{a_n{}^2 + b_n{}^2 + c_n{}^2 + d_n{}^2}{2} \tag{1}$$

where a_n, b_n, c_n, and d_n are the four coefficients of the n^{th} harmonic and

$$PFc = \sum_1^n PF_n \tag{2}$$

The number of harmonics needed to reach 99.9 % cumulative power of 30 harmonics were then used to summarise otolith shapes (see Figure S1). The number of harmonics needed to achieve a 99.9 % cumulative Fourier power were 17, and therefore all analyses were continued with only using the first 17 harmonics, i.e. 68 descriptors.

Average otolith shapes were constructed for each taxa category (silver kob, west coast dusky kob or putative hybrids), after normalising the otolith shapes by size, orientation and starting point. The first three coefficients of the first harmonic were changed to $a_1 = 1$, $b_1 = c_1 = 0$, which reduced the number of Fourier coefficients used for further analyses by three, i.e. 65 Fourier descriptors. R packages used for the Fourier analysis and subsequent plotting of results (apart from those mentioned before) were: “broom” (Robinson et al., 2014), “dplyr” (Wickham et al., 2023a) and “ggplot2” (Wickham et al., 2023b).

In addition to the Fourier coefficients, for each otolith, the ostium-cauda distance (OCD) was measured in ImageJ (Rasband, 2023), and otolith length (OL), otolith height (OH) (Fig. 2), otolith area (OA) and otolith perimeter (OP) were extracted using the “ShapeR” package (Libungan and Pálsson, 2015). For comparisons with Griffiths and Heemstra (1995), OCD was also calculated as a proportion of otolith height (OCD-OH ratio) and as a linear function of fish total length and comparing differences in slope by species using Analysis of Covariance (ANCOVA). The following additional otolith shape indices were calculated from and in addition to OA = otolith area (mm^2), OL = otolith length (mm), OP = otolith perimeter (mm) and OH = otolith height (mm):

$$\text{Aspect ratio (AR)} = OL\ /\ OH \tag{3}$$

$$\text{Circularity (circ)} = OP^2\ /\ OA \tag{4}$$

$$\text{Ellipticity (ellip)} = (OL - OH)\ /\ (OL + OH) \tag{5}$$

$$\text{Form-factor (FF)} = 4\pi OA\ /\ OP^2 \tag{6}$$

$$\text{Rectangularity (rect)} = OA\ /\ (OL \text{ x } OH) \tag{7}$$

$$\text{Roundness (round)} = 4OA\ /\ (\pi OL^2) \tag{8}$$

2.4. *Statistical analyses*

Each Fourier coefficient and each shape index was tested for normality (separately for *A. inodorus* and *A. coronus*) using the Shapiro-Wilk test. Descriptors that showed significant deviation from normality (n = 25) were removed from further analyses. For the shape indices, otolith area was log-transformed to achieve normality and five others were removed because of their significant deviation from normality (see Supplementary Material Table S1b). Principal component analysis (PCA) was used to assess the overall variance in the shape of the otoliths by species and by sex. PCA was integrated with morphospaces using the R package “Momocs” (Bonhomme et al., 2014). ANCOVA was used to test for allometric effects of fish size on the remaining normally distributed Fourier coefficients and otolith shape indices, with fish total length (TL) as a covariate and species as a factor. Fourier coefficients that showed significant interaction between TL and species were removed. Those coefficients and those otolith shape indices that showed significant effect of TL (but no significant interaction) were corrected for TL with a common slope (for all species combined) with the R package “tidyr” (Wickham et al., 2023c).

Linear discriminant analysis (LDA) performed on the length-standardized data (Fourier descriptors plus otolith shape indices and Fourier descriptors alone) was used to discriminate between the three taxa. LDA was done using the “MASS” package (Venables and Ripley, 2002; Ripley, 2023) and the “psych” package (Revelle, 2023). Classification accuracy was estimated with leave-one-out cross-validation, LOOCV (where the class of each sample is predicted from all others) with LDA, which is usually more accurate for small sample sizes, but with higher variance than other cross-validation methods. This was done with the “LOOCV” function in the “caret” package (Kuhn, 2008).

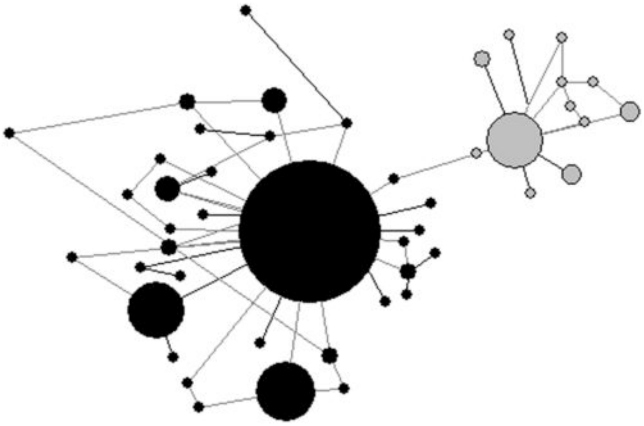

Fig. 3. mtDNA median joining network showing the separation of haplotypes assigned to *A. inodorus* (black) and *A. coronus* (grey) (n = 342, sampled in along the Namibian coast 17°S–23°S between January 2018 and July 2019). Disc size corresponds to overall abundance.

3. Results

3.1. *Species identification*

A 243 base pair (bp) COI fragment was obtained for 342 individuals revealing 46 haplotypes. Phylogenetic analysis of these haplotypes identified two distinct groups which corresponding to either silver kob (34 haplotypes) or west coast dusky kob (12 sequences) (Fig. 3). Simulation analyses of the microsatellite data to assess their ability to distinguish between species and hybrid categories (F1, F2 and back-crosses to parental species) indicated two things, namely, high power to distinguish pure individuals of both species from each other (100 %), and also from F1 and F2 hybrids (100 % and 98 %, respectively), but reduced power to distinguish backcrosses from pure individuals (86 % for backcrosses with silver kob and 90 % for backcrosses with west coast dusky kob for NewHybrids, but only 50 % and 10 %, respectively for STRUCTURE).

To account for this loss of accuracy, a conservative approach was adopted and we only considered individuals as hybrids if identified in both STRUCTURE and NewHybrids analyses. Applying this consensus approach to the 217 individuals included in otolith analysis reported 8 individuals as hybrids (Table 2). Only one individual, with phenotype and mitotype of west coast dusky kob, was identified as a putative F2 hybrid (Table 2). Most hybrids were caught in the northern region (75 %), with only 25 % caught in the central region (2/8, Table 2).

Of the 217 individuals included for otolith shape analysis, 180 were genetically identified as *A. inodorus*, 29 as *A. coronus* and 8 as putative hybrids. Out of the 31 individuals morpholocially identified as *A. coronus* (Table 1), this morphological identification was accurate for 87.1 % of *A. coronus* (27/31), with 9.2 % (3/31) genetically assigned to *A. inodorus* and 3.2 % (1/31) identified as an F2 hybrid. In total 95.1 % of the 184 individuals morphologically identified as *A. inodorus* (Table 1) were correctly identified (175/184), with 3.8 % identified as hybrids (7/184) and 2.4 % (2/184) as *A. coronous*. The overall accuracy of external identification was 93.1 %.

3.2. *Otolith shape analysis*

Of the 65 Fourier descriptors, 25 were removed because they significantly deviated from normality either for *A. inodorus* or *A. coronus* (Supplementary material Table S1a). One additional Fourier descriptor (B10) was removed because of its significant species-fish length interaction in the ANCOVA models ($p < 0.05$, Table S2a). In terms of the shape indices, OCD, OH, OL, OP and rectangularity were removed due to significant deviation from normality ($p < 0.05$, Table S1b). In addition,

Table 2
Each individual identified as hybrids by both STRUCTURE and NewHybrids analysis. Phenotype (identified by exterior features, e.g. colour and morphology), mtDNA COI sequence (mitotype) as either A. inodorus (AI) or A. coronus (AC), Latitude (Lat °S) and region of capture, total length (to the nearest cm), and sex are indicated for each individual. q is the probability of assignment to A. inodorus in STRUCTURE. NewHybrids assignment is either (F2), backcross with A. inodorus (b1) or A. inodorus (AI). The region captured was divided into North (SCNP, 17°14'S to 21°11'S) or central (Dorob National Park, 21°11'S to 23°19'S) regions. Sex was identified as either female (F), male (M), juvenile (J) or unidentified (U).

Individual	Phenotype	mtDNA	STRUCTURE (q)	NewHybrids assignment	Lat°S	Region	Total length (cm)	Sex
A220	AI	AI	0.760	b1	22.7	Central	67	M
A544 / AI_5	AI	AI	0.885	F2	20.8	North	41	M
A588	AI	AI	0.843	F2	18.5	North	50	F
A613	AI	AI	0.847	F2	20.8	North	45	F
A545 / AC_1	AC	AC	0.366	F2	20.8	North	51	J
A549 / AI_9	AI	AI	0.843	F2	20.8	North	52	M
A505	AI	AI	0.648	b1	21.2	Central	68	U
A476 / AI_25	AI	AI	0.583	F2	20.8	North	60	F

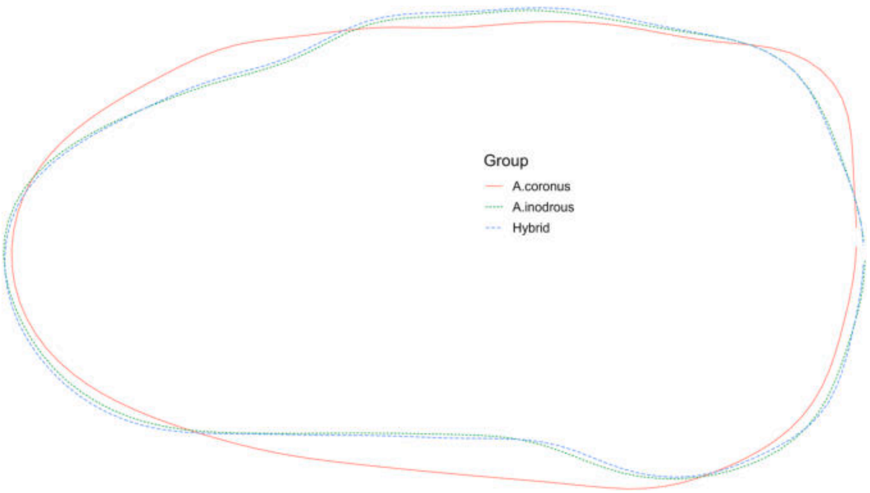

Fig. 4. Mean shape of the proximal view of the right sagittal otoliths of *A. coronus* (n = 29), *A. inodorus* (n = 180) and their putative hybrids (n = 8).

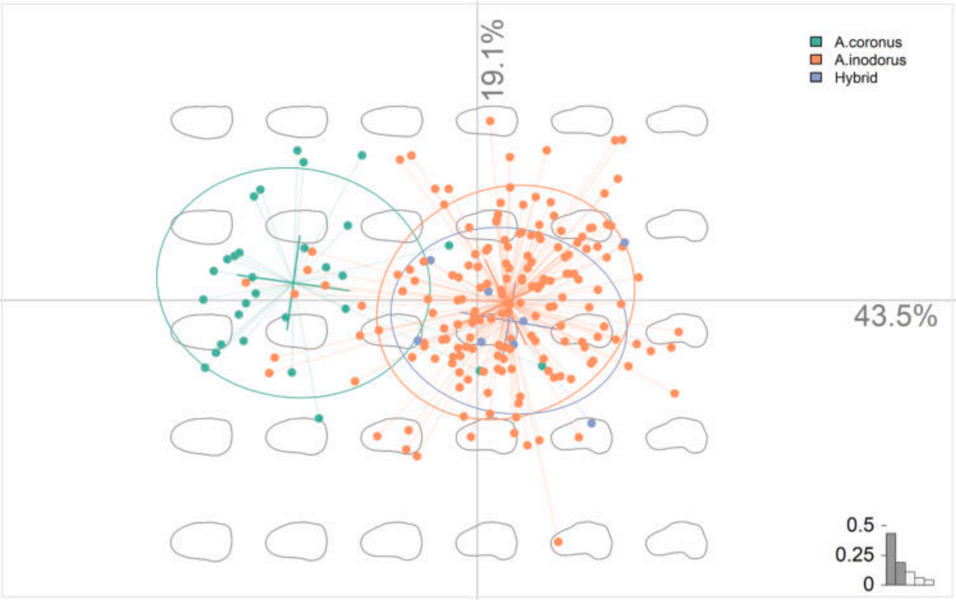

Fig. 5. Principal component analysis (PCA) conducted on the 65 Fourier coefficients of otolith shape for *Argyrosomus coronus* (n = 29), *A. inodorus* (n = 180) and their putative hybrids (n = 8). The levels of variance explained by the first two PCA axes are shown on the axes. The small otolith shapes plotted over the observations represents theoretical otolith shapes reconstructed based on the PCA scores.

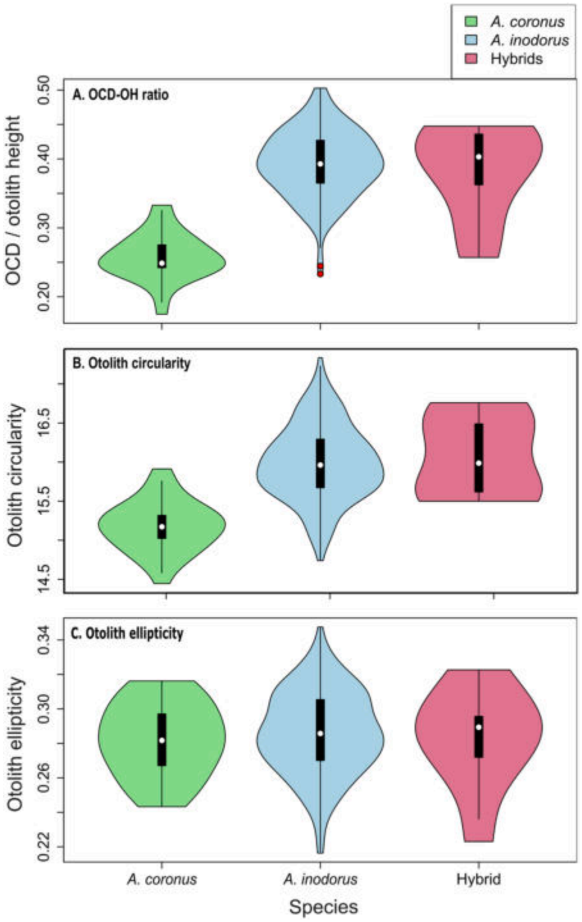

Fig. 6. Violin plots of values of A. Ostium-caudal distance to otolith height ratio (OCD-OH ratio), B. Otolith circularity and C. Otolith ellipticity by species *Argyrosomus coronus* (n = 29), *A. inodorus* (n = 180) and their putative hybrids (n = 8). White dots show the median, black bars show the interquartile range (IQR), black lines show the gates (1.5 x IQR), red dots show the outliers and the colour-shaded areas show a (smoothed) histrogram of the data density.

one shape index, log(OA), was removed from further analysis due to significant species-fish length interactions ($p < 0.05$, Table S2b). A total of 19 of the 39 remaining Fourier descriptors, and a total of 3 of the remaining 6 shape indices (OCD-OH ratio, form factor and circularity) were corrected for fish length effect using a common slope (see Tables S2 and S3). No significant collinearity was detected between each of the 39 remaining Fourier descriptors (Figure S3). When testing for collinearity, a further three shape indices were removed from the linear discriminant analysis, namely form-factor (FF) and roundness (round) due to their strong correlation with circularity; and aspect ratio (AR) due to its strong correlation with ellipticity (see Figure S4).

There was a clear difference in mean otolith shape between *A. coronus* and *A. inodorus*; although the latter did not differ much from the hybrids, regardless if they were F2 or backcrosses (Fig. 4). The difference in otolith shape between *A. coronus* and *A. inodorus* individuals were visually distinguishable, with *A. coronus* having a wider and evenly curved otolith, and *A. inodorus* having a prominent bulge on the ventral margin and narrowing towards the anterior margin. *A. coronus* has a straight posterior edge, while that of *A. inodorus* is more rounded (Fig. 4). The first two PCA axes explained 62.6 % of the overall variance in the otolith shapes of *Argyrosmus* spp. The otolith shapes of *A. coronus* were separated from the others mainly along the first PCA axis, and a strong overlap was observed between *A. inodorus* and the hybrids along both axes (Fig. 5). No differences in sex were visible in the overall otolith shapes (Figure S2).

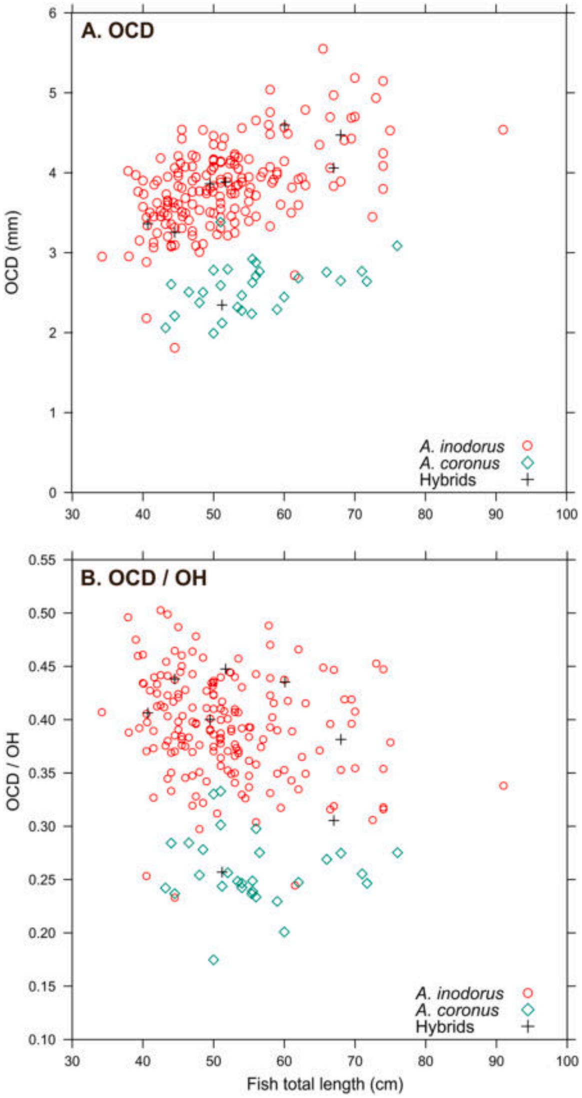

Fig. 7. A. Ostium-cauda distance (OCD) and B. OCD-otolith height ratio (OCD / OH) against fish total length of *Argyrosomus coronus* (n = 29), *A. inodorus* (n = 180) and their putative hybrids (n = 8) collected along the Namibian coast between 2018 and 2019.

In terms of otolith shape indices, fish length-standardised ostium cauda distance - otolith height ratio (OCD.OH) differed significantly (ANOVA, $F_{2,\,214} = 95.4$, $p < 0.0001$, Table S4) between species with a significantly lower OCD-OH ratio for *A. coronus* when compared with *A. inodorus* and the hybrids (Fig. 6A, Fig. 7). Significant differences by species were also observed for otolith circularity (ANOVA $F_{2,\,214} = 71.2$, $p < 0.0001$, Table S4), with otolith circularity significantly lower for *A. coronous* than for the other two taxa (Fig. 6B). Ellipticity showed no significant difference between the taxa (ANOVA $F_{2,\,214} = 45.6$, $p > 0.05$, Table S4, Fig. 6C).

OCD increased with fish length and OCD / OH appeared to slightly decrease with fish length (Fig. 7). OCD was about 2 mm higher for *A. inodorus* than for *A. coronus* (Fig. 7A) and OCD-OH ratio was about 15 % for *A. inodorus* than for *A. coronus* (Fig. 7B).

Linear discriminant analysis (LDA) of the 39 fish length-standardised Fourier coefficients that accounted for 99.9 % of the variance of otolith shape as well as 3 fish length-standardised otolith shape indices (circularity, OCD-OH ratioand ellipticity) showed separation between *A. coronus* and *A. inodorus* and the hybrids in LD1 and separation of 4 of the 8 hybrids and *A. inodorus* in LD2 (Fig. 8, Table S5). When using LOOCV to predict fish taxon from otolith shape (Table 3), the overall

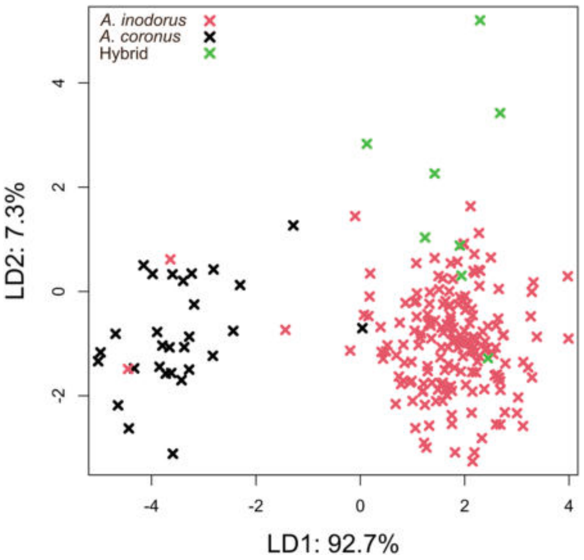

Fig. 8. Values of the first two linear discriminant functions (LD1 and LD2) of 65 Fourier descriptors and 3 shape indices showing species identification of each individual by *Argyrosomus inodorus*, *A. coronus* and their putative hybrids (N = 217) (See Table S5 for details).

classification accuracy was 96.3 %. Misclassification rate was small for the pure-bred species (2 % for *A. inodorus* and 3 % for *A. coronus*, Table 3). For the hybrids, on the other hand, the misclassification rate was 50 % (Table 3) although this was a 50 % improvement from external identification from which no hybrids could be identified correctly.

Hybrids were 50 % of the time misclassified to be *A. inodorus*. In terms of the shape analyses, the ease of distinguishing between the otoliths of *A. inodorus* and *A. coronus* was evident from the average shapes (Figs. 4 and 5). *A. coronus* otoliths were visually distinguishable, with a wider (higher) and more evenly curved otolith. In addition, the prominent bulge on the ventral margin, which is present on *A. inodorus* otoliths was absent on *A. coronus* otoliths. The posterior edge of *A. coronus* otoliths were also straight and angular compared with the more rounded edge of *A. inodorus*. This was supported by the otolith circularity index (mean uncorrected otolith circularity index for *A. inodorus* = 15.99 ± 0.476 SD and for *A. coronus* = 15.18 ± 0.323 SD, Fig. 6B, Table S1), which suggested that the shape of the otoliths of *A. coronus* was closer to a circle than those of *A. inodorus*. (A perfect circle would have a circularity index of 4 π (12.566)).

4. Discussion

The present study for the first time, tested the power of otolith shape analysis using Elliptical Fourier descriptors as well as shape indices as a tool to distinguish between *Argyrosomus inodorus*, *A. coronous* and their putative hybrids. The correct classification of the catch composition of “kob” and identification of hybrids is a fundamental component for the implementation of adaptive management in the rapidly changing coastal waters of Namibia. This study demonstrated that although Elliptical Fourier descriptors of otoliths provided a powerful and cost-effective method to distinguish between *A. inodorus* and *A. coronous*, these methods were able to correctly classify only 50 % of hybrids, due to the morphological similarity of their otoliths with *A. inodorus*. These findings suggest that while otolith shape analyses can be an appropriate adaptive management monitoring tool for species composition, further refinement or an alternative method may need to be developed to closely monitor patterns of hybridization.

To facilitate the adaptive management process in Namibia, we recommend that “kob” otoliths are routinely collected from the line fishery and classified. To ensure that the collection process is cost effective, we recommend that this be incorporated into the existing roving creel surveys of the recreational shore-based (recreational and subsistence) fishery and the point access surveys of the commercial fishery. This monitoring process would allow managers to estimate the relative abundance and demographic characteristics of each species separately and provide opportunities for stock assessment. Efficacy of the process would depend on the managerial response to these findings. At a simple level, if the species composition of the catch is assessed annually (for example during a month prior to the primary fishing season), the regulations could be adjusted on an annual basis to suite the life history of the dominant species. Ideally, the collection of annual species composition data in the line fishery catches would not only be used to determine the regulation for the year, but when combined with oceanographic information (e.g. *in situ* temperature) could facilitate prediction of the catch composition.

Our findings, that the otolith shape of *A. coronus* was different to *A. inodorus* support the original description of the two species (Griffiths and Heemstra, 1995). *A. coronus* also consistently showed a lower OCD and OCD-OH ratio than *A. inodorus* and the hybrids, supporting descriptions given by Griffiths and Heemstra (1995). However, otolith indices showed low discriminant power as a whole compared to Fourier coefficients; they were often collinear; and these measurements were often confounded by their fish size dependency. This supports the study of Tuset et al. (2021), who recommended using contour analyses rather than shape indices for fish stock discrimination. When comparing two different shape descriptors used for otolith contour analysis, Elliptical Fourier descriptors and Discrete Wavelet descriptors, Neves et al. (2023) showed a similar classification success between the two methods to classify stocks of the European sardine *Sardina pilchardus*. However, classification success depends on the number of descriptors used (Smoliński et al., 2020; Tuset et al., 2021). Fourier descriptors are widely used in otolith shape analysis studies (e.g. La Mesa et al., 2020; Ibañez et al., 2022), and are suitable for elliptical-like large-scale features (such as *Argyrosomus* otoliths), while wavelet analysis is better

Table 3
Cross-Validated (leave-one-out) prediction accuracy for fish identified to the reference (genotype) from their otolith shape (39 Fourier coefficients and 4 shape indices, Table S5) using Linear Discriminant Analysis (LDA). In the the first part of the table, entries are the number of fish correctly classified to the taxon according to molecular reference data. In the second part of the table entries are % per taxon.

	Reference: Assignment based on molecular data			
Prediction	***A. inodorus* (180)**	***A. coronus* (29)**	**Hybrid (8)**	**Total correct assigments**
A. inodorus	**177**	1	4	**177**
A. coronus	3	**28**	0	**28**
Hybrid	0	0	**4**	**4**
Total	**180**	**29**	**8**	**209**
% prediction of reference				
A. inodorus	**98**	3	50	
A. coronus	2	**97**	0	
Hybrid	0	0	**50**	
Total	**100**	**100**	**100**	**96.3**

suitable for capturing sharp features such as for example mollusk shells with large spines (e.g. *Bolinus brandaris* Tuset et al., 2020), John's snapper, *Lutjanus johnii* otoliths (Sadighzadeh et al., 2014), or *Dissostichus mawsoni* (Parisi-Baradad et al., 2005) otoliths. We therefore recommend that Fourier descriptors be used for monitoring species composition of *Argyrosomus* in Namibia.

Otolith contour analyses have been found useful for the discrimination of congeneric species in several contexts (e.g. Lord et al., 2012; Zhang et al., 2017; D'Iglio et al., 2021), including other *Argyrosomus* spp. (Ferguson et al., 2011; Barnes, 2015); to distinguish between other stocks in the region (e.g. Griffiths, 1996; Shoopala et al., 2021), that were previously genetically verified (Henriques et al., 2016); for population structure inference (e.g. Leguá et al., 2013; Libungan and Pálsson, 2015; Hüssy et al., 2016; Smoliński et al., 2020; Neves et al., 2023); or to distinguish between spawning groups (e.g. Jónsdóttir et al., 2006; Burke et al., 2008). Specifically, otolith contour analysis and the use of statistical classifiers has been used in combination with molecular (and morphological) analysis to distinguish between cryptic species (e.g. La Mesa et al., 2020; Ibañez et al., 2022; Riley et al., 2023). Some differentiation sometimes may be undetected on the COI gene (e.g. Ibañez et al., 2022) or morphology (e.g. La Mesa et al., 2020). This makes otolith shape analysis with statistical classifiers (in combination with molecular and other markers) particularly useful and relevant to distinguish between cryptic congeners such as *Argyrosomus* spp. in the northern Benguela. We thus recommend testing further use of integrating multiple approaches for species identification, for example combining genetics, otolith shape analysis and otolith chemistry (Ferguson et al., 2011; Barnes, 2015; Khan et al., 2021; Vaz-dos-Santos et al., 2023; Riley et al., 2023).

The collection of otoliths during monitoring also has other benefits as these structures can provide information of population age structure or growth rates (e.g. Kirchner and Voges, 1999), otolith chronologies (e.g. Wilhelm et al., 2020) or otolith chemistry. Otolith chemistry has historically been described to have better stock allocation success than microsatellites (e.g. Tanner et al., 2014), but depended on the number of microsatellites used (Barnes, 2015). More detailed genomic analyses (e.g. using more microsatellites), a combination of more classifiers (e.g. including otolith chemistry) and a larger sample size of otoliths may also give more success in classification of the hybrids.

Otolith shape analyses showed 50 % power to distinguish between pure-bred species and hybrids. In addition, information on the proportion of hybrids in this sample was valuable. In this study, we also showed that in a dataset of 217 fish, 8 (3.7 %) were found to be hybrids. This is similar to previously described levels of hybridization, both in 2009 (7/220 = 3.2 %, Potts et al., 2014a) and in 2019 (5/74 = 6.8 %, Pringle et al., 2023), suggesting that hybridization between these two species has remained low, but is still on-going. Other studies in marine fish species, where hybridization appears to be ongoing, have usually highlighted different hybridization rates in different areas (Pecoraro et al., 2024), e.g. for rockfishes *Sebastes vulpes* and *S. zonatus* (Muto et al., 2013) or anglerfishes *Lophius piscatorius* and *L. budegassa* (Aguirre-Sarabia et al., 2021). The stability in the proportion of hybrids in the present study is interesting, as one may have expected the proportion to grow over time, primarily because the mechanism (ocean warming) thought to be driving this process is continuing and since the hybrids are viable, backcrosses should become commonplace over time. It is however, possible that instead of a gradual linear increase in the proportion of hybrids over time, increases may assume a non-linear pattern, where stability is interspersed with rapid change (e.g. Muto et al., 2013), which follow conditions that are favourable for the recruitment of *A. coronus* in Namibia. Temporal stability in otolith shape is assumed to occur in this genus as has been shown for example, for Atlantic cod from 1933 to 2015 (Denechaud et al., 2020). However, temporal stability in otolith shape needs to be tested by comparing otolith shapes of *A. inodorus* and *A. coronus* by collection time, for example through genetic assignment of archived otoliths.

In terms of monitoring the morphological (general and otolith) similarity of *A. coronus*, *A. inodorus* and their putative hybrids, interestingly, the majority of molecularly identified hybrids had the general and otolith phenotype and mitotype of *A. inodorus*, with very few identified as *A. coronus*. From an ecomorphological perspective, this suggests that there may be a higher degree of ecological overlap between *A. inodorus* and the hybrids. This may be concerning for the long-term viability, particularly the rapidly warming Namibian coastal ocean appears to be increasingly unsuitable for *A. inodorus* (Pringle et al., 2023).

Differences in growth and/or metabolism between taxa, especially between *A. inodorus* and the hybrids (Pringle et al., 2023) did not appear to drive differentiation in otolith shape. Previous studies have shown both genetic and environmental influences to be responsible for determining otolith shape variations, including empirical studies (e.g. Cardinale et al., 2004; Burke et al., 2008; Vignon and Morat, 2010; Hüssy et al., 2016). For example, Vignon and Morat (2010), by introducing non-indigenous fish in different environments, showed that differences in otolith shape were determined both genetics as well as change in environmental conditions in the absence of genetic differences. The exact disentanglements of these causes are still not well understood (Vignon and Morat, 2010; Nazir and Khan, 2021). However, in the present study, fish were collected in the same environment, and so were exposed to similar environmental conditions for at least part of their life histories. Their environmental exposure throughout their life history was not known. This can be clarified by using otolith chemistry as well as by comparing otolith shapes by genotypes between different environments for the pure-bred species of *Argyrosomus*.

In this study, no otolith shape analysis by area was done since samples in this study were restricted to the northern part of the northern Benguela (north of Walvis Bay). The collection of samples south of Walvis Bay may be important to obtain a better understanding of the population structure of *Argyrosomus inodorus* and *A. coronus* within the northern Benguela. In South Africa, Griffiths (1996) showed that there were four types of otoliths in South African *A. inodorus* based on the appearance of their growth zones on the frontal sections. The author also showed that the relationship between OW and TL was the best metric to distinguish fishes caught in the South-Eastern Cape from those of the Southern and South-Western Cape of South Africa. Based on these findings, population structuring of *A. inodorus* and *A. coronus* including otolith shape should be studied in the future.

In conclusion, otolith shape analysis by contour analysis (Fourier descriptors) can be used to distinguish between otoliths of *A. coronus* and *A. inodorus* in Namibia, and improves identification of hybrids by 50 %. Thus, otoliths should be routinely randomly collected from catches and their subsequent shape analysis should be used to separate catches by pure-bred species vs. hybrid, with the addition of genetic sampling to monitor hybridization.

Declaration of Competing Interest

The authors have no competing commercial or other interests to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2024.107262.

Data availability

I have included a link to all data in the manuscript

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