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# DNA barcoding suggests hidden diversity within the genus *Zenopsis* (Zeiformes, Zeidae)

Florencia Matusevich[1], Valeria Gabbanelli[1], Gonzalo Vulcano[2], Natalia Plá[3], Victoria M. Lenain[1], Diego M. Vazquez[4], Juan M. Díaz de Astarloa[1], Ezequiel Mabragaña[1]

1 *Laboratorio de Biotaxonomía Morfológica y Molecular de Peces, Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata–CONICET, CC1260, Funes 3350, 7600 Mar del Plata, Argentina*

2 *Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, 7600 Mar del Plata, Argentina*

3 *Laboratorio de Virología, Área de Producción Animal, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Ruta 226 km 73,5, Balcarce, Buenos Aires B7620, Argentina*

4 *Instituto Nacional de Limnología (INALI), Universidad Nacional del Litoral (UNL), CONICET, Ruta Nacional 168 km 0, Ciudad de Santa Fe, Santa Fe S3001XAI, Argentina*

*https://zoobank.org/66BE7D3A-255C-47E5-8FB7-2AE4C4EFCFD1*

Corresponding author: Valeria Gabbanelli (vgabbanelli@mdp.edu.ar)

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## Abstract

Currently, the genus *Zenopsis*, also known as silver John Dory, comprises at least five valid species with a wide range of distribution. However, recent studies have proposed the existence of a new *Zenopsis* species inhabiting the Indian Ocean, and a preliminary search in the Barcode of Life Database reveals the presence of different barcode index numbers (BIN) for the nominal species *Zenopsis conchifer*. In the Southwest Atlantic Ocean (SWA), *Z. conchifer* is the only species reported so far. Therefore, the aim of this work was to evaluate, at the molecular level, the potential taxonomic diversity within the genus *Zenopsis* and to assess if the species occurring in the SWA corresponds with *Z. conchifer*. Using data available in worldwide genetic databases, a maximum likelihood tree, a BIN, and an automatic barcode gap discovery analysis were carried out. Additionally, specimens sampled from the SWA were morphologically compared with specimens from different parts of its distribution using available data. The specific identity at the molecular level of specimens occurring in the SWA was confirmed as *Z. conchifer*. The results of the molecular analysis highlight the existence of hidden specific diversity within the genus.

## Key Words

Barcode index number, distribution area, silver John Dory, Southwest Atlantic Ocean

## Introduction

Fishes of the genus *Zenopsis* Gill, 1862, also known as "silver John Dory," are a group of marine species characterized by a similar body plan with laterally flattened bodies (Swaby and Potts 1999), scales present only along the lateral line, and large bucklers along the bases of dorsal and anal fins, ventrally anterior to the pelvic fin, and between pelvic and anal fins (Tyler et al. 2003; Nakabo et al. 2006). Currently, the genus comprises five valid species: *Zenopsis conchifer* (Lowe, 1852), *Z. nebulosa* (Temminck & Schlegel, 1845), *Z. oblonga* Parin et al., 1997, *Z. stabilispinosa* Nakabo et al., 2006, and *Z. filamentosa* Kai & Tashiro, 2019. Additionally, a recent genetic study suggests the occurrence of a new *Zenopsis* species in the Eastern Indian Ocean (Kai and Tashiro 2019). These species are ecologically successful due to their ability to withstand episodic recruitment (Zidowitz et al. 2002) and are widely distributed around the world. *Zenopsis filamentosa*, *Z. stabilispinosa*, and *Z. nebulosa* are found in the Pacific Ocean around Asia and Oceania; *Z. nebulosa* is also present along the coast of America in the Pacific

Ocean. *Zenopsis oblonga* is present in the Eastern Pacific Ocean (Froese and Pauly 2021). *Zenopsis conchifer* is distributed in the Atlantic Ocean along the coasts of America, Europe, and Africa; in the Pacific Ocean off the coasts of Chile; and in the Indian Ocean (Maurin and Quéro 1982; Sáez and Lamilla 2017; Froese and Pauly 2021). *Zenopsis conchifer* has recently been reported along the coast of north-eastern Brazil (Malafaia et al. 2015) and for the first time in the Mediterranean Sea (Ragonese and Giusto 2007; Fernández et al. 2012; Pinto et al. 2023). Ragonese and Giusto (2007) suggested that this range expansion of *Z. conchifer* distribution could be explained by the tropicalization phenomenon, which states that the increase in sea temperature could be responsible for the increasing range of thermophilic species (Bombace 2001). However, there is no evidence of a self-sustaining population found in the Mediterranean Sea (Fernández et al. 2012).

In the last few decades, molecular taxonomy, specifically DNA barcoding, has emerged as a way to complement morphological taxonomy (Teletchea 2010). Barcoding is a methodology that includes the sequencing and analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene and is used for species identification (Hebert et al. 2003). It has been widely used to study fish biodiversity (Ward et al. 2005, 2008; Hubert et al. 2008; Mabragaña et al. 2011) and to assess the species composition of the fishery industry catches (e.g., Bineesh et al. 2016 Delpiani et al. 2020). The Barcode of Life Database System (BOLD) aims to be a worldwide reference library for species identification through the storage of COI sequences (BOLD; https://www.boldsystems.org) (Hebert et al. 2003). To do so, BOLD performs a barcode index number (BIN) analysis, which clusters the sequences as taxonomic operational units that generally agree with nominal species. A preliminary search in the BOLD database reveals the presence of more than one BIN for the nominal species *Z. conchifer*, suggesting that further analysis into the diversity of the genus is needed. In this sense, the aim of this work is to evaluate the potential taxonomic diversity within the genus *Zenopsis* based on the analysis of DNA barcoding and to assess, at the molecular level, if the species occurring in the Southwest Atlantic Ocean (SWA) corresponds with *Z. conchifer*.

## Materials and methods

Forty-five COI sequences available in BOLD (https://www.boldsystems.org) for *Z. stabilispinosa* (n=1), *Z. conchifer* (n=17; one submitted previously by the authors), *Z. nebulosa* (n=15), and samples identified exclusively at the genus level, named *Zenopsis* (n=11), *Zeus faber* (n=1), and seventeen sequences obtained in Kai and Tashiro (2019) from the International Nucleotide Sequence Database Collaboration (INSDC; https://www.insdc.org) of specimens of *Z. stabilispinosa* (n=1), *Z. conchifer* (n=3), *Z. nebulosa* (n=5), *Zenopsis* sp. (n=4), and *Z. filamentosa* (n=4) were downloaded. Sequence data are shown in Suppl. material 1. Additionally, two specimens of *Z. conchifer* from the Argentine Sea were sequenced; however, one of these sequences had low quality and was excluded from the analysis (the sequence was uploaded to NCBI at https://www.ncbi.nlm.nih.gov). Sequences of *Z. oblonga* were not available in either database.

The DNA extraction was carried out in the Argentine International Barcode of Life Reference Laboratory (IIMyC, CONICET, Mar del Plata, Argentina) from muscle tissue preserved in 70% ethanol. DNA extraction, polymerase chain reaction (PCR), and sequencing of the 5′ region of the COI gene were performed following standard DNA barcoding protocols (Ivanova et al. 2006) coupled with primers and primer cocktails specifically designed for fishes (Ward et al. 2005; Ivanova et al. 2006) for base positions 6474–7126 of the *Danio rerio* mitochondrial genome. PCR reaction mixtures and the reaction profile were carried out following Díaz de Astarloa et al. (2008). The PCR products were purified and sequenced at the Canadian Centre for DNA Barcoding in Ontario. The full set of sequences was aligned using MEGA 11 (Tamura et al. 2021; Stecher et al. 2020). A maximum likelihood (ML) tree was reconstructed with a bootstrap of 1000 replications. The HKY model was chosen for cluster analysis as it was determined to be the best-fit model under the Akaike information criterion.

Sequences were also analyzed using the BIN analysis provided by the BOLD platform, and species limits were explored using the automatic barcode gap discovery method (ABGD) (Puillandre et al. 2012). The ABGD sorts sequences into putative species based on the barcode gap distance. This analysis was run with the default settings (P min=0.001, P max= 0.1, steps=10, X relative gap width=1.5, Nb bins=20) and K2P distance on the ABGD web server (https://bioinfo.mnhn.fr/abi/public/abgd/). Additionally, within-group and between-group mean distance analyses were carried out using MEGA 11 (Stecher et al. 2020; Tamura et al. 2021) with the K2P model (Kimura 1980) and the default parameters. Two sets of groups were defined and analyzed. (1) Grouped by species name: *Z. nebulosa*, *Z. filamentosa*, *Z. stabilispinosa*, *Z. conchifer*, *Zenopsis*, and *Zenopsis* sp. (Kai and Tashiro 2019), and (2) grouped by ABGD groups (as shown in Fig. 2).

Ten specimens of *Zenopsis* cf. *conchifer*, collected in the Argentinean Sea (Fig. 1A), were used for the morphometric analysis. Two specimens are housed in the collection of the Instituto de Investigaciones Marinas y Costeras, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata-CONICET (IIMyC) (n=2, UNMDP 4500, UNMDP 4881), and five in the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) (n=5; catalogue number 76), both found in Mar del Plata, Argentina. Three fresh specimens were obtained from fish markets and included in the analysis; afterwards, they were accessed in the IIMYC collection (UNMDP 5159, UNMDP 5160, and UNMDP 5161). Counts, measurements, and terminology were used following Kai and Tashiro (2019) and are summarized in Fig. 1B. Measurements were standardized to the standard length and compared with available data on *Z. conchifer* from different areas of its distribution: North Western Africa (NW Africa) (Kai and Tashiro 2019), the Mediterranean Sea (Ragonese and Giusto 2007), and North Eastern Brazil (NE Brasil)

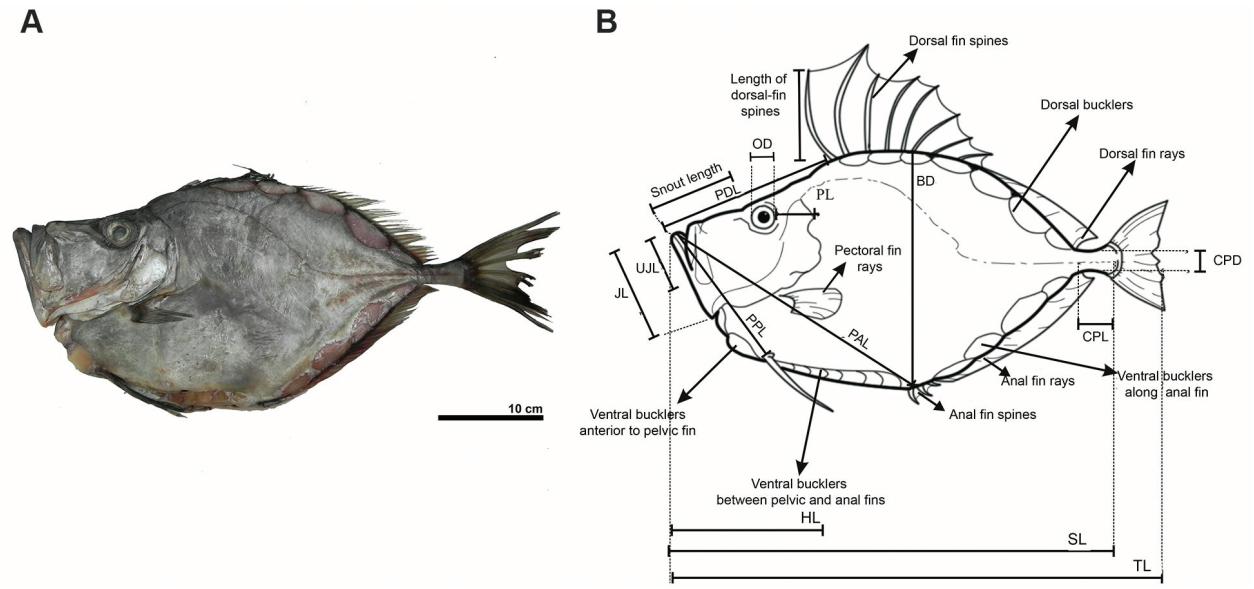


**Figure 1. A.** *Zenopsis* cf. *conchifer* specimen from the Argentine Sea; **B.** Measurements taken on specimens of *Zenopsis* cf. *conchifer*. **TL**—total length. **SL**—standard length. **BD**—body depth. **CPD**—caudal peduncle depth. **CPL**—caudal peduncle length. **HL**—head length. **JL**—jaw length. **OD**—orbit diameter. **PAL**—preanal length. **PDL**—predorsal length. **PL**—postorbital length. **PPL**—prepelvic length. **UJL**—upper jaw length. (Illustration carried out by the co-author, Vulcano, G.).

(Malafaia et al. 2015). Due to the small number of specimens studied, the analysis was exclusively descriptive.

## Results

The ML tree (Fig. 2) showed that the COI sequences of the different *Zenopsis* species were grouped into six different clusters; these clusters corresponded to: *Z. nebulosa*, *Z. stabilispinosa*, a cluster with sequences of *Z. filamentosa* and specimens identified exclusively at the genus level, named *Zenopsis*; two clusters with sequences identified as *Z. conchifer*; and one cluster with sequences of *Z. conchifer* and *Zenopsis* sp. (Kai & Tashiro, 2019). The three clusters that included sequences identified as *Z. conchifer* did not form a single clade in the ML tree.

In the BOLD database, six different BINs for the *Zenopsis* genus were found. However, these sequences were recorded as four nominal species: *Z. conchifer*, *Z. nebulosa*, *Z. filamentosa*, and *Z. stabilispinosa*.

Sequences identified as *Z. conchifer* were grouped in three different clusters in the ML tree (Fig. 2) that corresponded to the three different BINs (BOLD:AAC3708; BOLD:ADK0258; BOLD:AAZ3127). Although the three BINs are assigned to *Z. conchifer* in BOLD, the specimens are from different sample locations (Fig. 2; Suppl. material 1). Sequences identified as *Zenopsis* sp. by Kai and Tashiro (2019) were grouped with sequences from BIN BOLD:ADK0258. Sequences for *Z. nebulosa* and *Z. stabilispinosa* were included in a singular BIN for each species (BOLD:AAB79893, BOLD:ACH5427, respectively), in accordance with the clusters found in the ML (Fig. 2). All four sequences of *Z. filamentosa* from Kai and Tashiro (2019) clustered with sequences of BIN BOLD:AEB2424. Within this BIN, a single sequence was registered as *Z. filamentosa*, and the remaining sequences were identified exclusively at the genus level and named *Zenopsis*. Thus, BIN BOLD:AEB2424 was determined to correspond with *Z. filamentosa*.

The analysis of ABGD resulted in eight initial partitions. In six partitions (P= 1.00 e-3, 1.67 e-3, 2.78 e-3, 4.64 e-3, 7.74 e-3, and 1.29 e-2), seven candidate species were found, which grouped consistently with the clusters found in the ML tree (Fig. 2). The other two partitions (P= 2.15 e-2, 3.5 e-2) presented two candidate species, which corresponded with one group with sequences of all *Zenopsis* species together, separated from a group with the sequence of the outgroup, *Zeus faber*.

In the first distance analysis, which grouped sequences by species, *Z. conchifer* presented 3% (SE=0) mean within-group distance and *Z. nebulosa* 1% (SE=0), while the other species showed a distance value close to 0%. The between-group distance analysis (Table 1) ranged from 0.08% to 20.33%. The lower distance of 0.08% was found between *Z. filamentosa* and sequences exclusively identified at genus level in BOLD (named as *Zenopsis*), and the highest distances (ranging between 18.23 and 20.33%) were found between each *Zenopsis* species and the outgroup *Zeus faber*. Within the genus *Zenopsis*, the highest distance value was found between *Z. stabilispinosa* and *Zenopsis* sp. (6.9%). However, when analyzed by ABGD groups, group 4 (containing sequences of BIN BOLD:AAB7893) presented a 1% (SE=0) mean within-group distance, while the mean distance for the rest of the groups was close to 0. The between-group distance ranged from 2.66% to 20.33% (Table 2). The highest values corresponded with the comparisons between each *Zenopsis* group and the outgroup. Within the genus, the lowest value was found between group 4 and group 5 (BINs BOLD:AAB7893 and BOLD:AAZ3127,

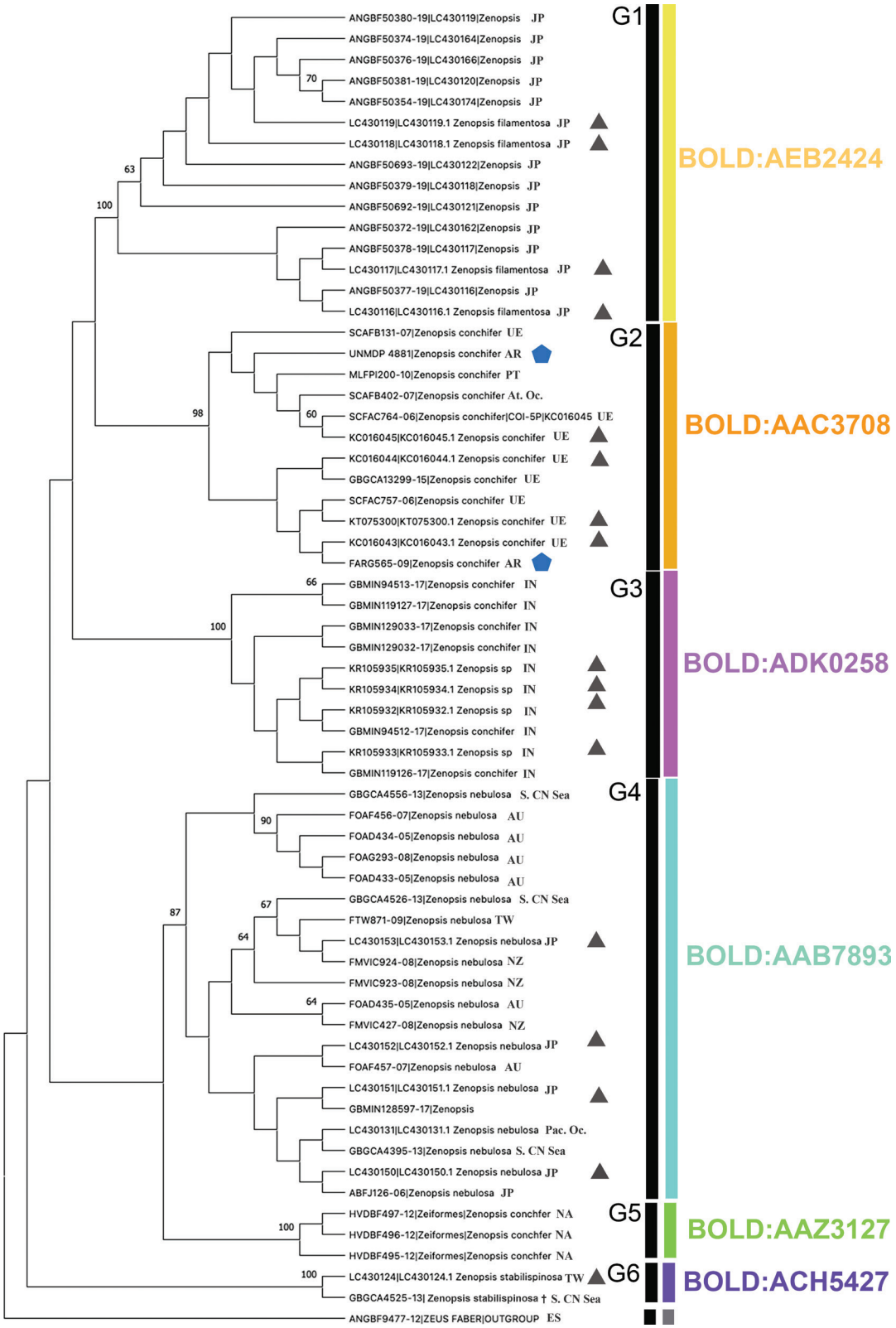


**Figure 2.** Maximum likelihood tree for the *Zenopsis* species analyzed. The tree was based on the Cytochrome Oxidase Subunit I gene and reconstructed with MEGA 11. Sequences from Kai and Tashiro (2019) are indicated with a grey triangle. Sequences obtained by the authors are indicated with a blue pentagon. All remaining sequences were obtained from BOLD. Color bars represent different clusters resulting from ML analysis with 1000 bootstraps. Black bars represent groups resulting from ABGD analysis. Country abbreviations were made following ISO 3166 Alpha-2 and Alpha-3. AR—Argentina. AU—Autralia. IN—India. JP—Japan. NA—Namibia. NZ—New Zeland. PT—Portugal. ES-Spain. TW—Taiwan. US—United States. At. Oc.—Atlantic Ocean. Pac. Oc.—Pacific Ocean. S. CN Sea—South China Sea. † Sequence GCA4525-13, originally identified as *Z. nebulosa* in BOLD, is actually a misidentification and corresponds to *Z. stabilispinosa*, as previously determined by Kai and Tashiro (2019).

respectively), and the highest one between group 3 and group 6 (BINs BOLD:ADK0258 and BOLD:ACH5427).

Regarding species distribution, BINs identified in BOLD as *Z. conchifer* contained specimens collected in different locations. BIN BOLD:AAC3708 contained specimens from the Atlantic Ocean, including the United States, Argentina, and Portugal (near the type locality) (Fig. 2; Suppl. material 1). BIN BOLD:ADK0258 contained specimens from the Indian Ocean (Fig. 2; Suppl. material 1). Samples included in BIN BOLD:AAZ3127 came from Namibia (Fig. 2; Suppl. material 1); also, private sequences within this BIN (therefore not included in this study) were sampled in Cape Verde, Guinea-Bissau, and Morocco. Sequences of *Z. nebulosa* were collected from Australia, New Zealand, Taiwan, Japan, the South China Sea, and the Pacific Ocean (Fig. 2; Suppl. material 1). All the sequences of *Z. filamentosa* were collected from Japan (Fig. 2; Suppl. material 1). *Zenopsis stabilispinosa* was collected in Taiwan and the South China Sea (Fig. 2; Suppl. material 1).

A summary of the morphological measurements found in a bibliographic revision (Ragonese and Giusto 2007; Malafaia et al. 2015; Kai and Tashiro 2019) is shown in Table 3, and the full set of measurements is available in Suppl. material 2. To avoid errors based on differences associated with size or stage (i.e., juveniles, immature adults, mature adults), the *Z. conchifer* specimens revised for this study were compared exclusively with those analyzed by Kai and Tashiro (2019), considering that the standard length (SL) range was similar in both studies (141–339 mm for specimens from the Argentine Sea and 140–173.4 mm for specimens from Kai and Tashiro 2019). Slight differences between them were found in some measurements (Table 3). Specimens of *Z. conchifer* captured in NW Africa (Kai and Tashiro 2019) presented a longer orbital diameter (9.5–10.6 times SL) than those found in the Argentine Sea (5.84–9.87 times SL). Specimens from NW Africa (Kai and Tashiro 2019) showed a shorter snout (15.2–16.1 times SL vs. 18.6–21.5 times SL) and a longer postorbital length (12.9–14.1 times SL vs. 7.7–11.9 times SL) than those from the Argentine Sea. The specimens collected from the Argentine Sea had a smaller number of bucklers at the base of the anal fin than the specimens from other parts of the world (4 vs. 5–6).

**Table 1.** Between-groups mean distance (and standard error). Sequences were grouped by species names: *Z. nebulosa*, *Z. filamentosa*, *Z. stabilispinosa*, *Z. conchifer*, *Zenopsis*, and *Zenopsis* sp. (Kai and Tashiro 2019).

| | ***Z. conchifer*** | ***Z. nebulosa*** | ***Z. stabilispinosa*** | ***Z. filamentosa*** | ***Zenopsis* sp.** | ***Zenopsis*** | ***Zeus faber*** |
|---|---|---|---|---|---|---|---|
| *Z. conchifer* | | 0.04 (0.006) | 0.06 (0.009) | 0.04 (0.007) | 0.03 (0.005) | 0.04 (0.007) | 0.2 (0.024) |
| *Z. nebulosa* | | | 0.05 (0.009) | 0.05 (0.009) | 0.04 (0.008) | 0.05 (0.009) | 0.18 (0.024) |
| *Z. stabilispinosa* | | | | 0.06 (0.01) | 0.07 (0.011) | 0.06 (0–01) | 0.19 (0.024) |
| *Z. filamentosa* | | | | | 0.05 (0.01) | 0.0008 (0.0009) | 0.19 (0.024) |
| *Zenopsis* sp. | | | | | | 0.05 (0.01) | 0.2 (0.256) |
| *Zenopsis* | | | | | | | 0.19 (0.024) |

**Table 2.** Between-groups mean distance (and standard error). Sequences were grouped by ABGD analysis (as shown in Fig. 2).

| | **G1** | **G2** | **G3** | **G4** | **G5** | **G6** | ***Zeus faber*** |
|---|---|---|---|---|---|---|---|
| G1 | | 0.04 (0.008) | 0.05 (0.01) | 0.05 (0.009) | 0.04 (0.009) | 0.06 (0.01) | 0.19 (0.024) |
| G2 | | | 0.04 (0.009) | 0.04 (0.008) | 0.04 (0.008) | 0.06 (0.01) | 0.2 (0.025) |
| G3 | | | | 0.04 (0.007) | 0.03 (0.007) | 0.07 (0.011) | 0.2 (0.025) |
| G4 | | | | | 0.03 (0.006) | 0.05 (0.009) | 0.18 (0.024) |
| G5 | | | | | | 0.05 (0.009) | 0.19 (0.024) |
| G6 | | | | | | | 0.19 (0.024) |

**Table 3.** Comparison of morphological and meristic data for *Zenopsis conchifer* analyzed in this study and information taken from available bibliography (Ragonese and Giusto 2007; Malafaia et al. 2015; Kai and Tashiro 2019), including different areas of its distribution. NW: North Western; NE: North Eastern. Standard length is in mm. All remaining measurements are standardized with regard to the standard length. The range and mean between parenthesis are given for each measurement as a percentage of the standard length.

| | **Argentine Sea (Present Study) (N=10)** | **NW Africa (Kai and Tashiro 2019) (n=5)** | **Mediterranean Sea (Ragonese and Giusto 2007) (n=1)** | **NE Brasil (Malafaia et al. 2015) (n=1)** |
|---|---|---|---|---|
| Standard Length (SL; mm) | 141–395 | 140.0–173.4 | 550 | 525 |
| Snout length | 18.6–21.5 (19.9) | 15.2–16.1 (15.7) | | 16.2 |
| Orbit diameter | 5.84–9.87 (8.57) | 9.5–10.6 (10.1) | 6.4 | 6.3 |
| Postorbital length | 7.7–11.9 (10.3) | 12.9–14.1 (13.5) | 16.5 | 11.42 |
| Body depth | 49.1–62.2 (56.0) | 26.3–62.2 (59.5) | 50.9 | 48.6 |
| Upper jaw length | 13.9–17.8 (16.0) | 16.3–17.8 (17.4) | 14.9 | 15.4 |
| Dorsal bucklers | 5–7 | 1–2 + 5 | 8 | 7 |
| Ventral bucklers anterior to pelvic fin | 2 | | 2 | 2 |
| Ventral bucklers between pelvic and anal fins | 6–8 | 5 | 7 | 7–8 |
| Ventral bucklers along anal fin | 4 | 5–6 | 5 | 5 |

## Discussion

The molecular analysis, based on COI, suggests that the genus *Zenopsis* is more specious than previously assumed. Furthermore, information about the known distribution range (Maurin and Quéro 1982; Froese and Pauly 2021; Fricke et al. 2023) was added based on the location of the specimens analyzed here. This information aids in the determination of possible distribution areas for each potential species found in the ML tree.

In the ML tree, for each cluster, a BIN number from the BOLD database could be associated. These clusters also corresponded with the seven groups found in the ABGD analysis (Fig. 2). For *Z. nebulosa*, *Z. filamentosa*, and *Z. stabilispinosa*, a single cluster and BIN were found. For *Z. conchifer*, three clusters corresponding to three different BINs were found, and these three clusters did not form a single clade in the ML tree. BINs usually correspond with a nominal species, and sequences of one species tend to cluster together in a ML tree. Additionally, the within-group mean distance analysis showed that if all three *Z. conchifer* BINs are grouped together, the mean distance is 3%, the highest found for any species analyzed in this study. However, if these sequences are separated into the three groups determined by ML, BIN, and ABGD analysis, the mean within-group distance of each group decreases by close to 0%. Thus, these results suggest the existence of hidden diversity within this species.

BIN BOLD:AAC3708 contained *Z. conchifer* from the Atlantic Ocean, including those of waters off Portugal, close to the type locality of *Z. conchifer* (Lowe, 1852) (Fig. 2; Suppl. material 1). The sequence of the samples from the Argentine Sea is grouped within this cluster. This leads us to the assumption that the molecular identity of the species inhabiting these waters corresponds to *Z. conchifer*.

On the other hand, the sequences of BIN BOLD:ADK0258 corresponded with specimens from the Indian Ocean and clustered together with *Zenopsis* sp., which was proposed as a possible new species by Kai and Tashiro (2019). Our results showed that sequences from this area did not cluster with specimens collected in the type locality; therefore, we hypothesize that *Z. conchifer* does not occur in the Indian Ocean and that the captures recorded in this area correspond to the new species, *Zenopsis* sp., proposed by Kai and Tashiro (2019).

Finally, our results suggest the existence of another new species from Namibia, Cape Verde, Guinea-Bissau, and Morocco, corresponding to BIN BOLD:AAZ3127, from now on named *Zenopsis* sp. 1. These sequences were previously identified as *Z. conchifer* but clustered independently from this species, and they were closely related to the sequences from *Z. nebulosa*. *Zenopsis conchifer* is also found in other regions of the west coast of Africa; thus, it is possible that in Namibia, Cape Verde, Guinea-Bissau, and Marocco, there are two sympatric *Zenopsis* species.

The current distribution of *Z. conchifer* includes the Atlantic Ocean along the coasts of America, Europe, and Africa, the Pacific Ocean off the coasts of Chile, and the Indian Ocean (Maurin and Quéro 1982; Sáez and Lamilla 2017; Froese and Pauly 2021). However, the specimens available from the eastern coast of Africa were identified solely based on morphological and meristic analysis, and no genetic data is available. Therefore, the occurrence of this species in this area should be confirmed through genetic and morphological studies. Additionally, all the specimens analyzed here that were collected in India clustered together with the sequences of the new species proposed by Kai and Tashiro (2019) (Fig. 2). In this sense, it is probable that *Z. conchifer* is not found in the Indian Ocean.

We observed some morphological differences between specimens of *Z. conchifer* from different localities. These differences could be the result of population variability, allometric growth, or a small sample size. Further morphological studies based on a larger number of specimens from each locality are needed to establish potential causes for these differences.

This work highlights the importance of the existence of worldwide reference libraries (e.g., the BOLD system and INSDC) to carry out large-scale studies, especially for wide-range distribution species like *Zenopsis conchifer*. Using the information available in these databases, the species identity at the molecular level of *Zenopsis* specimens found in the Southwest Atlantic Ocean was confirmed. Additionally, the results shown here suggest that the genus *Zenopsis* has a higher specific diversity than previously stated and could be used as a starting point for future taxonomic and genetic studies.

## Author contributions

Funding acquisition: JMDA and EM. Conceptualization and methodology: EM, VG, and FM. Investigation: FM, NP, GV, and VG. Formal analysis: FM, NP, GV, VG, and DMV. Illustration: GV. Writing–original draft: FM. Writing–review and editing: VML and FM. All authors read and contributed critically to the drafts and gave final approval for publication.

## Data availability

All sequence data generated during this study have been deposited at the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov) together with associated metadata under BioProject OR750557.

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# Supplementary material 1

## Sequences data of the cytochrome oxidase subunit I mitochondrial gene

Authors: Florencia Matusevich, Valeria Gabbanelli, Gonzalo Vulcano, Natalia Plá, Victoria M. Lenain, Diego M. Vazquez, Juan M. Díaz de Astarloa, Ezequiel Mabragaña

Data type: xls

Explanation note: Sequences data of the cytochrome oxidase subunit I mitochondrial gene, used in the present paper, correspond to *Zenopsis* species and *Zeus faber* (used as an outgroup). INSDC: International Nucleotide Sequence Database Collaboration; BOLD: barcode of life datasystem. Footnotes: † Sequence GCA4525-13, originally identified as *Z. nebulosa* in BOLD, is actually a misidentification and corresponds to *Z. stabilispinosa*, as previously determined by Kai and Tashiro (2019). ‡ Near the type locality (Lowe 1852). § Sequenced for this study. | Sequences originally named *Z. conchifer*, identified in the present study as a potential new species, *Zenopsis* sp. 1.



Link: https://doi.org/10.3897/zse.100.122293.suppl1

# Supplementary material 2

## Comparison of all morphological and meristic data of specimens of *Zenopsis conchifer*

Authors: Florencia Matusevich, Valeria Gabbanelli, Gonzalo Vulcano, Natalia Plá, Victoria M. Lenain, Diego M. Vazquez, Juan M. Díaz de Astarloa, Ezequiel Mabragaña

Data type: xls

Explanation note: Comparison of all morphological and meristic data of specimens of *Zenopsis conchifer* analyzed in this study and information taken from available bibliography (Ragonese and Giusto 2007; Malafaia et al. 2015; Kai and Tashiro 2019), including different areas of its distribution. NW: North Western; NE: North East. Standard length is presented as mm. All remaining measurements are standardized with regard to the standard length. The range and mean between parenthesis are given for each measurement as a percentage of the standard length.



Link: https://doi.org/10.3897/zse.100.122293.suppl2