

First record of *Zenion hololepis* (Zenionidae) in Portuguese continental waters: the northernmost occurrence in the eastern Atlantic

ROGELIA MARTINS¹, FILIPE O. COSTA², ALBERTO G. MURTA¹, MIGUEL CARNEIRO¹ AND MONICA LANDI²
¹INRB, IP/IPIMAR, Avenida Brasília 1400-06 Lisboa, Portugal, ²CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

The occurrence of Zenion hololepis in Portuguese continental waters is reported for the first time. A total of five specimens were collected in two separate fisheries research surveys, in 2008 and 2009. Analyses of morphometric and meristic characteristics confirmed all five specimens as Z. hololepis, while DNA barcode sequence data delivered a genus-level assignment. The records here described constitute a new extended northern limit for the distribution of Z. hololepis in the eastern Atlantic and the first DNA barcode data for this species.

Keywords: *Zenion hololepis*, Dwarf dory, north-eastern Atlantic, DNA barcode

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The species *Zenion hololepis* (Goode & Bean, 1896) belongs to the relatively small family Zenionidae, which comprises only three genera and seven species (Heemstra, 1986), although the number of species and genera within this family is not consensual (see discussion in Heemstra, 1986). Members of this family are typically found over mud or sand–mud bottom and usually at depths between 180 and 700 m (Heemstra, 1986). Information about the species *Z. hololepis* is relatively scarce, both in dedicated databases such as FishBase (www.fishbase.org) and in the scientific literature. This species seems to have a wide distribution (Figure 1), including the western and north-western Atlantic (Scott & Scott, 1988; Claro, 1994) and the western central Pacific (Heemstra, 1999). FishBase indicates the occurrence of *Z. hololepis* also in the western Indian Ocean and South China Sea (Froese & Pauly, 2010). In the eastern Atlantic, *Z. hololepis* occurs from Cape Timiris (Mauritania) to South Africa (Quéro, 1981).

On the course of two separate fisheries research campaigns of IPIMAR (Portuguese fisheries research agency) five uncommon specimens, subsequently identified as *Z. hololepis*, were collected. The specimens were caught off the south-west coast of Portugal (Figure 1), by the research vessel 'Noruega', in September 2008 (collection of two specimens at the geographical coordinates: 38°0.2'N 09°7.2'W 37°57.4'N 09°08.6'W) and June 2009 (sampling of three specimens at the geographical coordinates: 37°57.4'N 09°08.6'W 37°21.40'N 09°10.2'W and 37°24.9'N 09°09.6'W). Fish were captured using a bottom trawl operating at a depth between 340 and 361 m. The specimens were carefully examined for diagnostic morphological features, following description

provided by Heemstra (1986). According to this author, *Z. hololepis* displays an elongate body and a head length 2.3 to 2.7 times the standard length, the latter one being the measurement from the most anterior tip of the body to the posterior end of the vertebral column. The upper jaw is extremely protractible. The dorsal fin has 6 to 7 spines and 25–28 rays, with the front edge of the second spine distinctly serrate. Pectoral fins show 15–17 rays, while the pelvic fins are characterized by serrate spine and 6 branched rays. The main characteristic that distinguishes this species from the congeneric *Zenion leptolepis* is the anal fin, with 23–28 rays in the former species, and 28–32 rays in the latter one.

The specimens identified as *Zenion hololepis* (Figure 2) were photographed and preserved in 70% ethanol for storage at the National Museum of Natural History in Lisbon. Table 1 reports the range of meristic and morphometric characteristics and body proportions of the *Z. hololepis* specimens under consideration. Among the five specimens, the total length ranged from 79 to 134 mm and their total weight from 18.9 to 51.5 g.

To further characterize the specimens and substantiate the taxonomic identification, we complemented our observations using genetic tags for species identification, i.e. DNA barcodes (Hebert *et al.*, 2003). Namely, we analysed the sequence variation of a 652 base pair (bp) fragment of the mitochondrial DNA gene cytochrome *c* oxidase I (5'-COI).

A sample of muscle tissue of each individual was collected and preserved in 96% ethanol for molecular analyses. Total genomic DNA was extracted from white muscle tissue, and DNA barcode regions amplified and sequenced following published protocols (Ivanova *et al.*, 2007; Rock *et al.*, 2008).

After editing the bidirectional sequences, we obtained five 652 bp DNA barcodes, which were deposited in the GenBank® with the following accession numbers:

Corresponding author:

R. Martins

Email: rmartins@ipimar.pt

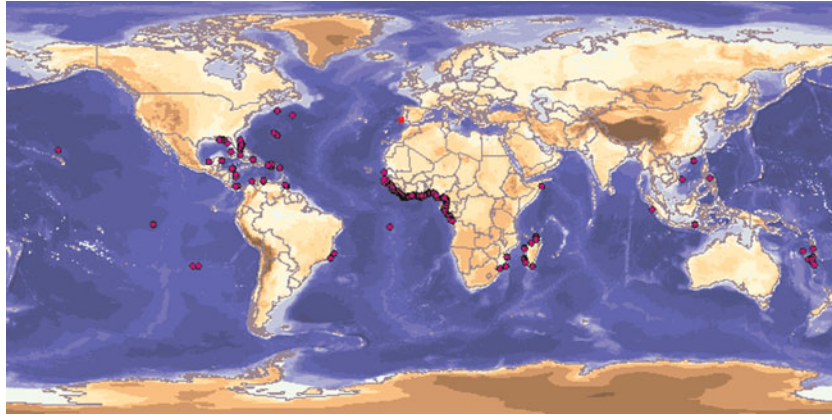


Fig. 1. AquaMaps for *Zenion hololepis* (Kaschner *et al.*, 2007), upon modification. Dots represent the occurrence of the species as reported in FishBase (www.fishbase.org). Squares show the locations where *Z. hololepis* specimens were caught along the Portuguese coasts.

JF718831–JF718835. Details of the respective PCR primers, sequences and trace files are publicly available on the Barcode of Life Data Systems (BOLD; www.barcodinglife.org, under the project titled ‘First record of *Zenion hololepis* in Portuguese continental waters [MLFPZ]’. Among all five specimens, DNA barcodes differed at most by two nucleotides, and consequently the average conspecific divergence was very low [0.2% Kimura-2-parameter (K2P)]. We submitted *Z. hololepis* DNA barcodes to GenBank’s BLAST® (<http://www.ncbi.nlm.nih.gov/BLAST>) and to BOLD’s (Ratnasingham & Hebert, 2007) identification engine, in search for matching sequences. No complete matches were found, but the nearest match (90.7% identity) belonged to a homologous COI sequence of the congeneric species *Zenion japonicum* (GenBank® Accession AP004434). *Zenion japonicum* is the only species of *Zenion* for which there is any genetic data available in GenBank® and BOLD. Comparison of the aligned nucleotide sequences of the two *Zenion* species revealed an average divergence of 10.1% (K2P), which is within the range found in DNA barcodes of congeneric fish species (Ward *et al.*, 2005). Hence, this nucleotide genetic distance provides evidence of the existence of at least two different species of the genus *Zenion*. Additionally, the corresponding amino acid sequences of the two *Zenion* species were identical, confirming their close evolutionary relatedness. The sequences here reported are the first published DNA barcodes for *Z. hololepis*, providing reference genetic tags for comparison with specimens of *Zenion* spp. collected elsewhere in the future.

There are no records in the literature of the occurrence of *Z. hololepis* in the north-eastern Atlantic Ocean, nor in the IPIMAR’s fisheries surveys database, which spans several decades of regular research campaigns along Portuguese waters.



Fig. 2. One of the five specimens of *Zenion hololepis* (134 mm total length and 51.49 g) caught in Portuguese continental waters (north-eastern Atlantic).

There have been various recent reports on expansions of the northern limit of warm water species towards the temperate north-eastern Atlantic, including new occurrences along the Portuguese coast (Azevedo *et al.*, 2004; Lima *et al.*, 2007; Bañón & Sande, 2008; Borges *et al.*, 2010).

Table 1. Range of meristic characteristics (number), morphometric characteristics (mm) and body proportions of *Zenion hololepis* specimens caught off the Portuguese continental coast.

Characteristics	Absolute	%L _S	%L _H
<i>Meristic</i>			
Dorsal fin rays	VI + 28		
Anal fin rays	I + 26		
Pectoral fin rays	15–17		
Pelvic fin rays	I + 6		
<i>Morphometric</i>			
Total length	79–134		
Standard length	60–110		
Head length	23.8–39.2	35.7–41.8	
Pre-orbital length	6.6–15.3		27.7–39.1
Post-orbital length	5.6–7.6		19.4–23.6
Eye diameter	11.5–16.2		38.6–48.3
Interorbital length	6.8–11.8		25.9–30.1
Body depth/height	23.3–45.8	37.6–43.2	
Pre-dorsal length	27.2–44.4	40.3–45.2	
Dorsal base length	32.5–59.4	53.2–56.1	
Pre-anal length	39.8–64.5	58.6–70.2	
Anal base length	21.6–36.6	33.3–35.9	
Caudal length	16.9–28.6	23.4–28.9	

L_S, percentage of standard length; L_H, percentage of head length. Roman and Arabic numerals represent hard and soft fin rays, respectively.

Based on current evidence, it is not possible to conclude if this new observation of *Z. hololepis* in areas previously unrecorded is a fortuitous observation or a more permanent expansion of its northern distribution range. However, the fact that this species was captured on two independent occasions separated by 9 months favours the latter possibility.

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Correspondence should be addressed to:

R. Martins
 INRB, IP/IPIMAR
 Avenida Brasília 1400-06
 Lisboa
 Portugal
 Tel.: +351 21 302 7000; fax: +351 21 301 5948;
 email: rmartins@ipimar.pt