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New records of the deep-sea fish species *Tetragonurus cuvieri* (Risso, 1810) and *Notacanthus bonaparte* (Risso, 1840) (Chordata: Teleostei) in Southwestern Europe (Gulf of Cádiz, SW Spain)

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Abstract

Two specimens of the deep-sea fish *Tetragonurus cuvieri* (Risso, 1810) and *Notacanthus bonaparte* (Risso, 1840) were captured in the Gulf of Cadiz on January 2023 at a depth of 14.15 m and on March 2023 at a depth of 532 m, respectively. The identity of the specimens was verified using both morphological and genetic criteria. They are uncommonly observed in these waters of the Iberian Peninsula, having being spotted *T. cuvieri* for the first time in this area.

KEYWORDS

Deep-sea fish, teleost, COI, barcoding, Iberian Peninsula.

The sighting of abyssal species is not frequent in coastal waters, however, occasionally, one of these deep-sea organisms is reported by fishermen or bathers. This is the case of two teleost fishes recently spotted in of the Gulf of Cádiz: *Tetragonurus cuvieri* (Risso, 1810) and *Notacanthus bonaparte* (Risso, 1840).

The deep-sea fish *T. cuvieri*, commonly known as smalleye squaretail, is a meso-bathypelagic fish that have been reported throughout almost all the seas of the planet (Figure 1), being found on the waters of the Pacific, Indian and Atlantic Oceans and the Mediterranean Sea (Ibanez, 1975; Carnevale et al., 2021; Ayas et al., 2022). The genus *Tetragonurus* (Risso, 1810) (squaretails) is the sole representative of the Tetragonuridae (Risso, 1827), a small group of scombriform teleosts. Along with *T. cuvieri*, two other species are included: *Tetragonorus atlanticus* (Lowe, 1810) and *Tetragonurus pacificus* (Abe, 1953) (Fricke et al., 2022). Information on the ecology of this species is scarce, but studies indicate that fish belonging to the *Tetragonurus* group tend to inhabit surface waters during their youth, while adults are meso-bathypelagic organisms that live in solitude and migrate to the surface during night (Tononaka, 1957; Ayas et al., 2022). It's considered that their diet consists mainly of gelatinous zooplankton, having been registered specialized morphological and behavioral adaptations to live in association with tunicates (Janssen & Harbison, 1981; Ayas et al., 2022).

The demersal fish *N. bonaparte*, known as the shortfin spiny eel, can be found in the North Atlantic Ocean and the Mediterranean Sea (Stefanescu et al., 1992; Poulsen et al., 2018; Barros-García et al., 2020). The genus *Notacanthus* (Bloch, 1788) belongs to the Notacanthidae (Rafinesque, 1810) and comprises six species: *Notacanthus abotti* (Fowler, 1934), *Notacanthus bonaparte* (Risso, 1940), *Notacanthus chemnitzii* (Bloch, 1788), *Notacanthus indicus* (Lloyd, 1909), *Notacanthus sexspinis* (Richardson, 1846) and *Notacanthus spinosus* (Garman, 1899) (Konhamkakkada et al., 2023). Little is known about the biology of this species group due to the high effort generally associated with deep-sea exploration (Barros-García et al., 2020). Biological studies carried out to date comprise only information about reproduction and diet from the Northeast Atlantic and the western Mediterranean Sea (e.g. Coggan et al., 1998; Fernandez-Arcaya et al., 2013; Rodríguez-Romeu et al., 2016; Barros-García et al., 2020). These studies reveal that *N. bonaparte* inhabits depths between 200 and 2200 m within the Mediterranean Sea (Moranta et al., 1998; Rodríguez-Romeu et al., 2016; Barros-García et al., 2020) and feeds on small benthic invertebrates and nektonic crustaceans (Coggan et al., 1998; Macpherson, 1981; Barros-García et al., 2020).

Regarding the distribution of *T. cuvieri* on the Iberian coasts, it has only been recorded so far in Mediterranean waters, where its habitat comprehends practically the entire basin, having been found on the eastern, central and western Mediterranean (Figure 1). However, records are much more abundant on the central and eastern areas of the basin, specially within the Italian waters (Demestre & Roig, 1982; Ragonese & Giusto, 2003; Psomadakis et al., 2006; Carnevale et al., 2021; Tsagarakis et al., 2021; Ayas et al., 2022). Thus, records on western areas, especially along the coast of the Iberian Peninsula are scarce, with sightings limited to the Spanish Mediterranean coast (Harvard University & Morris, 2023; Quesada Lara & Agulló Villaronga, 2023), as well as in the waters of the Strait of Gibraltar near the Moroccan coast (Grant et al., 2022). The specimen of *T. cuvieri* captured by bottom trawl is a female of 370 mm total length captured on 31-01-2023 by the minor arts vessel "Miguel y María" (36º 45' 54.3" N, 6º 30' 57.2" W) using a fish net at a depth of 14.4 m.

As for *N. bonaparte*, its distribution is restricted to the Northeast Atlantic off Faroe Islands to Mauritania and Western Mediterranean Sea (Froese & Pauly, 2016). It has been more frequently recorded between the central and the western coasts of the Mediterranean basin. That is, specially over the Italian waters (Tecchio & Ramirez-Llodra, 2018; Guerrero et al., 2023), the Catalan (Chic et al., 2023; Fishbase, 2023; Guerrero et al., 2023; MNHN & Chagnoux, 2023; Quesada Lara & Agulló Villaronga, 2023) and Balearic Sea (Chic et al., 2023), but also have been registered over the Alboran Sea near the Iberian Coast (Olivas González et al., 2023). Fewer records of *N. bonaparte* were spotted on Iberian Atlantic waters near the Strait of Gibraltar (Casañas Machín, 2023; Olivas González et al., 2023), as well as on southern Portuguese waters (European Bioinformatics Institute & GBIF Helpdesk, 2023; The International Barcode of Life Consortium, 2023). In this case, the shortfin spiny eel was captured in the Gulf of Cádiz; it is a male of 214 mm length captured by the trawler "Nuevo Amanecer Uno" (36º 46' 42.53" N, 08º 59' 10.22" W) on 23-03-2023 at a depth of 512m (Figure 1).

The identity of both specimens was verified using both morphological (Whitehead,1984) and genetic criteria. The specimens from Gulf of Cadiz were deposited in the Marine Fauna Collection of the Spanish Institute of Oceanography (CFM-IEOMA, Malaga, Spain) under the catalog numbers CFM-IEOMA-7795 for *T. cuvieri* and CFM-IEOMA-7796 for *N. bonaparte*. The barcode cytochrome c oxidase subunit I (COI) was used to verify the morphological identification of the specimens studied here. Total genomic DNA was extracted from muscle tissue of each species *T. cuvieri* and *N. bonaparte*, following a modified Chelex 10% protocol by Estoup et al. (1996). Target mitochondrial DNA from the COI gene was amplified with polymerase chain reaction (PCR) using the following cycling conditions: 2 min at 95ºC, 40 cycles of 30s at 95ºC, 45s at 45ºC, and 45s at 72ºC, and finally 5 min at 72º C. Primers COH6 (5´- TAD ACT TCD GGR TGD CCA AAR AAY CA -3´) and COL6b (5´- ACA AAT CAT AAA GAT ATY GG -3´) (Schubart & Huber, 2006) allowed the amplification of a maximum of 670 bp of COI. PCR products were sent to Stab Vida company to be purified and then bidirectionally sequenced. Sequences were edited using the viewer software Chromas Lite 2.6.4 (Technelysium Pty Ltd, 2017) and aligned with BioEdit Sequence Alignment Editor 7.2.6.1 (Hall, 1999).

With the final COI sequences obtained, a BLAST search was executed on the NCBI webpage (http://www.ncbi.nlm.nih.gov/genbank/), and a search was made in the official Barcode of Life database (BOLD) (http://v3.boldsystems.org/index.php/IDS_OpenIdEngine) to obtain the best matching sequences. Identifications were considered as positive when comparative sequences showed similarity values greater than 99%, with differences in 1–12 mutations. The COI sequence obtained for *T. cuvieri* (OR349736) has a 99.85% similarity (1 mutation) respect to the sequence EU148349 by Zhang et al. (unpublished) from a specimen collected at Northern mid Atlantic, and 99.7 to 99.1% similarity (2-6 mutations) respect to other 15 sequences deposited in Genbank from specimens collected in Southwest Indian Ocean, Eastern North Pacific, Japan, Australia, and New Zealand. In the case of *N. bonaparte,* its sequence (OR349737) has a 99,7% similarity (2 mutations) respect to the COI sequence included in NC_047186 (complete mitogenome) reared by Barros-García et al. (2020) from a specimen collected at North of the Iberian Peninsula.

Therefore, this work provides documented records of a specimen of *T. cuvieri* and *N. bonaparte* in the Gulf of Cádiz, this being the first occasion in which *T. cuvieri* has been found further southwest in the Iberian Peninsula.

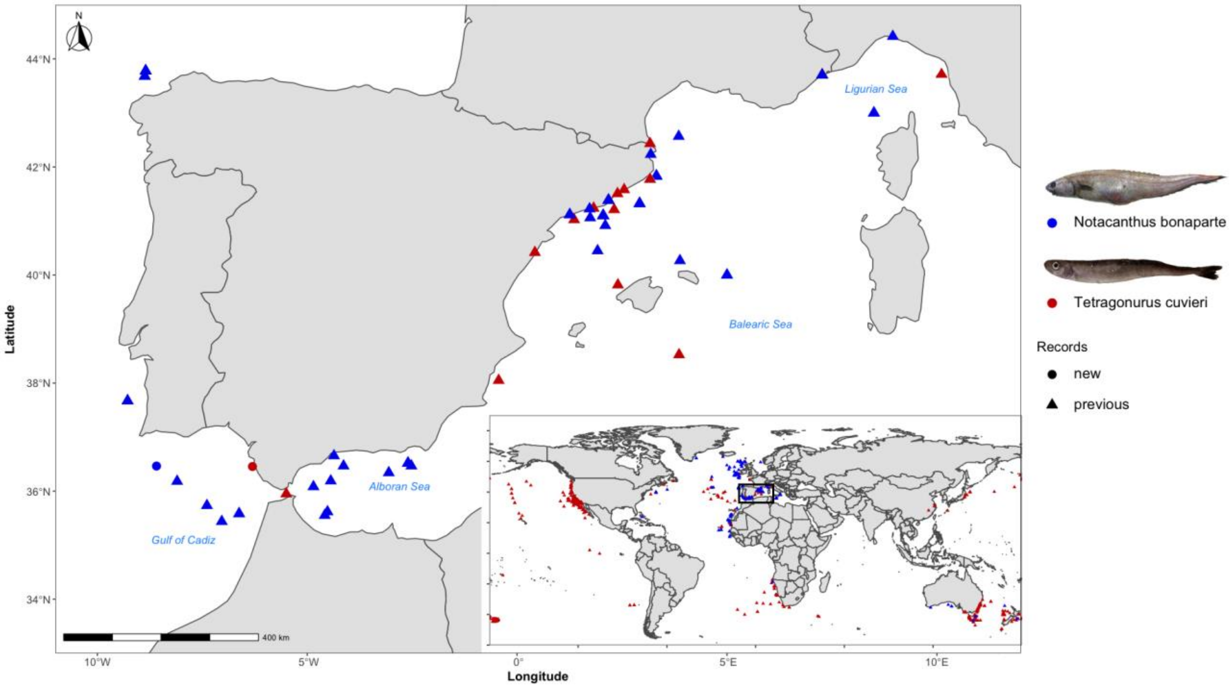

Figure 1: Records of the *Tetragonorus cuvieri* and *Notacanthus Bonaparte* in Atlantic-Mediterranean waters. Red triangles and the red dot indicate previous location and the recent record reported here of *Tetragonorus cuvieri*. Blue triangles and the blue dot indicate previous records and the recent record reported here of *Notacanthus Bonaparte* on the Iberian Peninsula.

AUTHOR CONTRIBUTIONS

All authors were involved in the conceptualisation of the study and in writing and editing the manuscript. All authors agreed to submission of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare. All co-authors have seen and agree with the contents of this manuscript.

ETHICAL STATEMENT

In this study no experiments with animals were performed. Specimens were collected as part of fishery-dependent surveys. Therefore, all examinations were made upon deceased animals that were captured during legal commercial fishing operations.

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