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Recent findings 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

Recent findings 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).* 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. In this study, we compile information on the global occurrences of these two deep–see species, and they are uncommonly observed in these shallow waters of the Iberian Peninsula. It highlights the importance of conducting further research to understand the full range of species inhabiting these shallow waters. The presence of these species can provide insights into the environmental conditions and specific habitats within the Gulf of Cádiz, as these fish are often associated with particular depth ranges and oceanographic features.

Key words: Teleost, COI, Barcoding, Iberian Peninsula

Resumen

Hallazgos recientes de las especies de peces de aguas profundas Tetragonurus cuvieri *Risso, 1810 y* Notacanthus bonaparte *Risso, 1840 (Chordata, Teleostei) en el suroeste de Europa (golfo de Cádiz, SO de España).* Dos ejemplares de los peces de aguas profundas *Tetragonurus cuvieri* Risso, 1810 y *Notacanthus bonaparte* Risso, 1840 fueron capturados en el golfo de Cádiz en enero de 2023 a una profundidad de 14,15 m y en marzo de 2023 a una profundidad de 532 m, respectivamente. La identidad de los ejemplares se verificó mediante criterios morfológicos y genéticos. En este estudio recopilamos información sobre las apariciones de estas dos especies de aguas profundas en todo el mundo, cuya

observación es infrecuente en aguas poco profundas de la península Ibérica. Ponemos de relieve la importancia de efectuar más investigaciones para conocer toda la gama de especies que habitan estas aguas poco profundas, puesto que su presencia puede proporcionar información sobre las condiciones ambientales y los hábitats específicos existentes en el golfo de Cádiz ya que estos peces se asocian a menudo a rangos de profundidad y características oceanográficas particulares.

Palabras clave: Teleósteos, COI, Clasificación taxonómica mediante códigos de barras, Península Ibérica

Resum

Troballes recents de les espècies de peixos d'aigües profundes Tetragonurus cuvieri *Risso, 1810 i* Notacanthus bonaparte *Risso, 1840 (Chordata, Teleostei) al sud–oest d'Europa (golf de Cadis, SO d'Espanya).* Dos exemplars dels peixos d'aigües profundes *Tetragonurus cuvieri* Risso, 1810 i *Notacanthus bonaparte* Risso, 1840 van ser capturats al golf de Cadis al gener de 2023 a una profunditat de 14,15 m i al març de 2023 a una profunditat de 532 m, respectivament. La identitat dels exemplars es va verificar mitjançant criteris morfològics i genètics. En aquest estudi recopilem informació sobre les aparicions d'aquestes dues espècies d'aigües profundes a tot el món, l'observació de les quals és infreqüent en aigües poc profundes de la península Ibèrica. Posem en relleu la importància de portar a terme més recerques per conèixer tota la gamma d'espècies que habiten aquestes aigües poc profundes, perquè la seva presència pot proporcionar informació sobre les condicions ambientals i els hàbitats específics existents al golf de Cadis atès que aquests peixos s'associen sovint a rangs de profunditat i característiques oceanogràfiques particulars.

Paraules clau: Teleostis, COI, Classificació taxonòmica mitjançant codis de barres, Península Ibèrica

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Introduction

The deep–sea fish *Tetragonurus cuvieri Risso, 1810*, commonly known as smalleye squaretail, is a meso–bathypelagic fish that have been reported throughout almost all the seas of the planet (fig. 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). Many mesopelagic species usually develop in epipelagic waters, being this the area where they perform most of their activities, such as feeding, reproduction

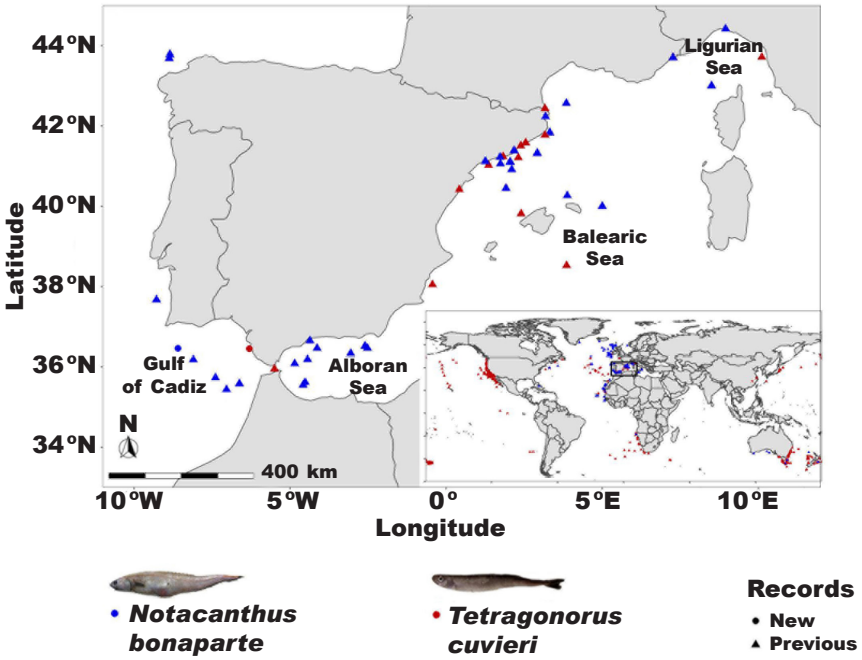

Fig. 1. Worldwide distribution (inset) and records of the *Tetragonorus cuvieri* and *Notacanthus bonaparte* in Atlantic-Mediterranean waters. Red triangles and the red dot indicate previous location and the recent record reported here of *Tetragonorus cuvieri*. Blue triangles and the blue dot indicate previous records and the recent record reported here of *Notacanthus bonaparte* on the Iberian Peninsula.

Fig. 1. Distribución mundial (recuadro) y registros de Tetragonorus cuvieri *y* Notacanthus bonaparte *en aguas atlántico-mediterráneas. Los triángulos rojos y el punto rojo indican las localizaciones anteriores y el registro reciente aquí descrito de* Tetragonorus cuvieri. *Los triángulos azules y el punto azul indican los registros anteriores y el registro reciente comunicado aquí de* Notacanthus bonaparte *en la península Ibérica.*

and development. They then migrate to greater depths as their swimming ability increases to adapt to their later adult life in the mesopelagic zone, after which most species begin their daily vertical migration, being able to cross density gradients such as the thermocline and halocline that generally inhibit mixing by physical processes (Roe and Badcock, 1984; Klevjer et al., 2016; Marohn et al., 2021). In the case of the here analyzed species 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).

The demersal fish *Notacanthus bonaparte* Risso, 1840, 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*, *Notacanthus chemnitzii* Bloch,

1788, *Notacanthus indicus* Lloyd, 1909, *Notacanthus sexspinis* Richardson, 1846 and *Notacanthus spinosus* Garman, 1899 (Konhamkakkada et al., 2023). Despite the high effort generally associated with deep–sea exploration, little is known about the biology of this species group (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 (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 2,200 m within the Mediterranean Sea (Macpherson, 1981; Coggan et al., 1998; Moranta et al., 1998; Rodríguez–Romeu et al., 2016; Barros–García et al., 2020).

The objective of this work is to document and verify the capture of two deep–sea fish specimens, *T. cuvieri* and *N. bonaparte*, in the Gulf of Cadiz. The study aims to provide information on the global occurrences of these species, particularly their uncommon observation in the shallow waters of the Iberian Peninsula. The presence of these species in the Gulf of Cadiz is investigated to gain insights into the environmental conditions and specific habitats within the region. This research highlights the importance of conducting further studies to comprehensively understand the range of these species inhabiting these shallow waters.

Material and methods

The identity of both specimens was verified using both morphological (Whitehead, 1986) 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 95 ºC, 40 cycles of 30s at 95 ºC, 45s at 45 ºC, and 45s at 72 ºC, and finally 5 min at 72 ºC. 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 and 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.

Results and Discussion

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.

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 (fig. 1). However, records are much more abundant on the central and eastern areas of the basin, specially within the Italian waters (Demestre and Roig, 1982; Ragonese and 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 (Morris, 2023; Quesada–Lara and 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 and 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 and Ramirez–Llodra, 2018; Guerrero et al., 2023), the Catalan (Chagnoux, 2023; Chic et al., 2023; FishBase, 2023; Guerrero et al., 2023; Quesada–Lara and Agulló–Villaronga, 2023) and Balearic Seas (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 (Olivas–González et al., 2023), as well as on southern Portuguese waters (European Bioinformatics Institute and 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 512 m (fig. 1).

Conclusion

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.

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