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BRIEF COMMUNICATION

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Genetic validation of the unexpected presence of a tropical tuna, bigeye tuna (*Thunnus obesus*), in the Mediterranean

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Abstract

Bigeye tuna (*Thunnus obesus*, Lowe, 1839) is one of the eight recognized species of the genus *Thunnus*. It is considered a tropical species distributed in the Atlantic, Pacific and Indian Oceans. To date, no validated presence of this species has been reported inside the Mediterranean Sea. This study, however, confirms, for the first time, the presence of three young individuals of this species within the Mediterranean Sea.

KEYWORDS

bigeye tuna, Mediterranean, tropical species, vagrant

Bigeye tuna (*Thunnus obesus*, Lowe, 1839) is one of the eight recognized species of the genus *Thunnus* (Collette *et al*., 2001; Díaz-Arce *et al*., 2016) with an extensive fishery associated with all of them (Collette *et al*., 2011). One of the most targeted tuna species is the bigeye tuna. It is the second most captured tuna species worldwide, only exceeded by the yellowfin tuna (*Thunnus albacares*), accounting for *c*. 45% of total tuna catches (www.iccat.int). In response to intense fishery pressure, the International Commission for the Conservation of Atlantic tuna (ICCAT) classified the Atlantic stock as overfished (ICCAT, 2018). Accordingly, the IUCN catalogued this species as vulnerable with a decreasing population trend (Collette *et al*., 2015). A precise knowledge of the biological features of this species, including their range of distribution, is essential for proper management of the species (Link *et al*., 2011).

In the Mediterranean, only two species of the genus *Thunnus* are recognized: Albacore (*Thunnus alalunga*) and Atlantic bluefin tuna (*Thunnus thynnus*); all other species of the genus *Thunnus*, including bigeye tuna, are absent from this sea (Collette & Nauen, 1983). Bigeye tuna presents a pan oceanic distribution with a prevalence of tropical/warm waters, with adult individuals found in the Atlantic waters close to the Mediterranean Sea (Chassot *et al*., 2010; Collette & Nauen, 1983; Reygondeau *et al*., 2012). The abiotic and biotic preferences of this species (Arrizabalaga *et al*., 2015) could explain the migratory pattern that hampers the entrance of this species into the Mediterranean.

In this study, the authors genetically confirmed for the first time the presence of bigeye tuna in the Mediterranean Sea. Three individuals of bigeye tuna in the Alboran Sea (Western Mediterranean) were caught in a fishing tournament targeting large pelagic fish of the recreational fishery in Benalmádena, Spain. Table 1 presents the description of these three individuals. All three individuals could be considered young-of-the-year with a fork length below 60 cm (Duarte-Neto *et al*., 2012; Hallier *et al*., 2005). Because of the similarity between young bigeye and albacore specimens, some confusion in species identification can occur. Young bigeye tunas have relative long pectoral fins (Collette & Nauen, 1983). One meristic feature that is commonly used to discriminate among tuna species is the number of gill rakers on the first arch (Collette *et al*., 2001; Collette & Nauen, 1983). Nonetheless, the differences between bigeye and albacore in the number of gill rakers are very subtle and cannot be used to discriminate between these two species (Collette & Nauen, 1983). In this case, the three individuals presented a number of gill rakers in the range between 26 and 29, which overlaps the range of both species.

To confirm the species identification of these individuals, the authors conducted a genetic analysis of these three individuals to confirm their species. The methodology for tuna species identification was based on the one described in Viñas and Tudela (2009) using the mitochondrial control region (mtDNA CR) as genetic marker. This analysis included representative sequences (up to 33 distinctive mtDNA CR sequences) of all eight recognized tuna species. According

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TABLE 1 Summary of the biological characteristics of the three *Thunnus obesus* captured within the Mediterranean

				Blast E-value[a]		
Code	**Date**	**Total weight (kg)**	**Fork length (cm)**	**mtDNA CR**	**Tmo-4c4**	**rhod**
FR103	22/08/2015	5.70	62	2e-170	1e-112	1e-154
FR104	22/08/2015	5.05	59.8	1e-157	6e-139	6e-177
FR105	22/08/2015	5.45	62.5	1e-135	4e-156	5e-172

Note. Last columns, Blast E-values for the best hit to the GenBank sequence database for the mitochondrial mtDNA-CR, nuclear Tmo-4c4 and rhod genetic markers. In all cases, the best hit was a sequence of *T. obesus*.

[a] E-value of the best Blast match in the GenBank database. Sequences with best Blast in the GenBank corresponded to *T. obesus* mtDNA CR sequences *DQ126519* and *DQ126397*; Tmo-4c4 *DQ388106*; and Rhod *DQ080371*.

to these authors, this marker has a greater power of resolution in discriminating tuna species than the traditional marker used for DNA barcoding. In addition, two nuclear molecular makers, titin-like protein (Tmo-4c4) (Orrell *et al*., 2006) and rhodopsin (rhod) (Zanzi & Martinsohn, 2017), were also sequenced for all three individuals. Sequences were submitted to GenBank with accession numbers MW507299–MW507301 and MZ567166–MZ567171.

The phylogenetic tree analysis of the mtDNA CR sequences unambiguously grouped the sequences of the three dubious specimens within the cluster of bigeye tuna (see Figure 1). This cluster is well supported by a bootstrap value of 84%. In addition, BLAST (Altschul *et al*., 1990) results for the mtDNA CR, and both nuclear markers (Table 1) confirmed that all sequences corresponded to bigeye tuna. In all cases and for all markers, the best BLAST hit in GenBank data was a sequence of *T. obesus* with an E-value lower than 1e-112.

It should be noted, however, that species classification using mtDNA genetic markers can also be misleading because of the incomplete lineage sorting (Campbell *et al*., 2014) or mtDNA introgression among closely related species (Ardura *et al*., 2013). For instance, it is known that mtDNA introgression occurs between species of the same genus in the Scombridae family (Alvarado Bremer *et al*., 2005). Nonetheless, the unambiguous identification of bigeye using both mitochondrial and nuclear markers discards the hypothesis of hybridization or mtDNA introgression with other species of the same genus.

Following the recommendations of Bello *et al*. (2014) for reporting the first record of a species, the authors of this study extensively searched in the available catalogues and checklists (Coll *et al*., 2010; Evans *et al*., 2015; Evans *et al*., 2020; Golani *et al*., 2002; Kousteni *et al*., 2019; Zenetos & Galanidi, 2020). This search also included the collective article of New Mediterranean Biodiversity Records (from 2011 to 2020; see Supporting Information S1) and Fishbase (Froese & Pauly, 2019). No documented presence of bigeye tuna in the Mediterranean has been found. It should be mentioned that the ICCAT's fishery database (www.iccat.int) reports minimal catches of this species in the Mediterranean. Nonetheless, these reports need to be taken with caution because they come from fisheries data, and therefore, they are not morphologically or genetically validated. Thus, to the best of the authors' knowledge, the present report represents the first confirmed presence of bigeye tuna in Mediterranean waters.

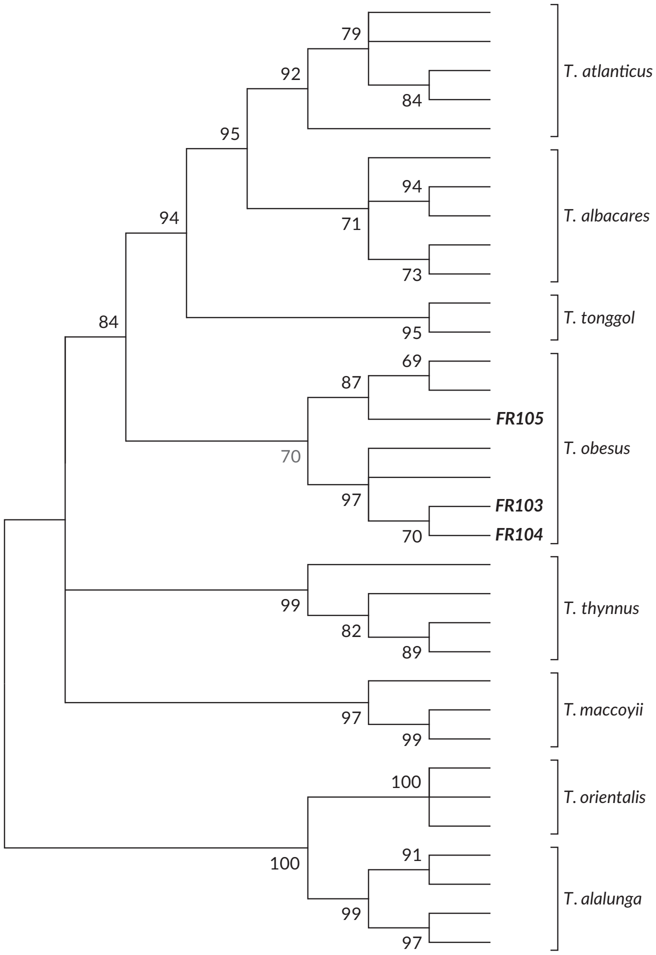

FIGURE 1 Mitochondrial DNA control region phylogenetic tree. Phylogenetic tree using the 33 mitochondrial control region sequences (mtDNA CR) representing the eight recognized tuna species from the study of Viñas and Tudela (2009). The nodes labelled as FR103, RF104 and FR105 correspond to the sequences of the three putative bigeye specimens. Tree is rooted at midpoint. Numbers above the nodes represent bootstrap support equal of above 70% after 5000 replicates

Finding three specimens of a new tuna species in the Mediterranean is a remarkable record. It is known that young individuals of bigeye tuna are usually found forming mixed schools with albacore and skipjack tuna (*Katsuwonus pelamis*) (Fonteneau *et al*., 2005; Scutt

Phillips *et al*., 2017). Considering that these individuals were caught with skipjack tuna, another tropical species, then it can be hypothesized that the three specimens entered into the Mediterranean following a skipjack tuna or even an albacore school. These individuals can be classified as vagrants because they conform with the definition proposed by Evans *et al*. (2020), as individuals outside their native distribution by natural migration. This is considering that bigeye tuna adults are found in the Atlantic in the same latitudinal range as the Mediterranean Sea, but not within this sea.

The presence of this species in the Mediterranean may be favoured by climate change and increase in water temperature in the Mediterranean. Recently, Ollé *et al*. (2019) also hypothesized that the presence of another Scombridae fish within the Mediterranean, frigate tuna (*Auxis thazard*), was probably related to the increase in Mediterranean water temperature in the last decades (Vargas-Yáñez *et al*., 2021). The climate change hypothesis can also be invoked to explain the recent appearance of another tropical Scombridae in the central Mediterranean such as wahoo, *Acanthocybium solandri* (Evans *et al*., 2020; Romeo *et al*., 2005). Therefore, these individuals may be evidence of another species entering the Mediterranean Sea as a consequence of changing oceanographic conditions (Coll *et al*., 2012; Marbà *et al*., 2015). Nonetheless, the hypothesis of the increase in Mediterranean water temperature may be challenged by the ICCAT data: some of the records of the presence of bigeye tuna in the Mediterranean date back to early 1980s. However, as mentioned before, these records must be used with caution because they are not morphologically validated.

In summary, the most plausible hypothesis of finding the three young-of-the-year individuals of bigeye within the Mediterranean is that they are vagrants that mistakenly followed the schools of skipjack and/or albacore. A possible implication of the individuals detected being young is that this introduction of bigeye tuna in the Mediterranean has been occurring repeatedly with the possibility of an increase in its occurrence because of climate change.

ETHICAL STATEMENT

The fish analysed in this study have been obtained from a recreational fishery. Thus, the authors did not have to follow a validated protocol to euthanize these individuals because they had been already killed before the analysis. The approval of any ethical committee was not necessary for the analysis and assessment of fish from this source.

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SUPPORTING INFORMATION

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