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New data reinforcing the taxonomic status of *Lepidion eques* as synonym of *Lepidion lepidion* (Teleostei, Gadiformes)

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ABSTRACT

The Mediterranean morid codling *Lepidion lepidion* is thought to be endemic, yet its taxonomic distinctiveness from the morphologically similar and more wide-ranging Atlantic *Lepidion eques* is unresolved, and has been controversial since the beginning of the twentieth century. Despite the abundant taxonomic literature questioning the interspecific relationship between these taxa, their current status remains unchanged. To elucidate the differentiation of the specimens identified as *L. lepidion* and *L. eques* collected across much of their geographic ranges, the sequence divergence of the cytochrome oxidase I "DNA barcode" gene of the mitochondrial genome was evaluated. A network analysis indicates that the most observed haplotypes are common to both species throughout their Mediterranean and North Atlantic distribution areas. This molecular evidence suggests the absence of biogeographical barriers and is insufficient to support the different species designations, giving *L. eques* the taxonomic status of junior synonym of *L. lepidion*.

1. Introduction

The Mediterranean *Lepidion lepidion* (Risso, 1810) and the Atlantic *Lepidion eques* (Günther, 1887) are two codling fish species included in the family Moridae. Their taxonomic relationship has been controversial since the beginning of the twentieth century. The first known species was the former, described in one specimen from Nice, France, in the northwestern Mediterranean Sea (Risso, 1810). Afterwards, *Lepidion eques* was described in the northeastern Atlantic, following the examination of specimens captured in the Faroe Channel; a larger eye and shorter head appearing to be the key distinctive traits (Günther, 1887). However, the taxonomic similarity between these two species was soon pointed out by several authors (Collett, 1905; Roule, 1919; Norman, 1935; Grey, 1956; Raimbault, 1963). A more recent and comprehensive revision of the morphology of these two *Lepidion* species concluded that both could be considered to have a sub specific rather than a specific

Abbreviations: COI, cytochrome c oxidase I.

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relationship, although it would be unwise to make *L. eques* a synonym of *L. lepidion* (Templeman, 1970). This criterion remained unchanged until recently, when COI DNA sequencing along with morphological and meristic analysis of a number of *Lepidion* specimens from the eastern and western coasts of the Iberian Peninsula found no specific differences between the Mediterranean *Lepidion lepidion* and the Atlantic *Lepidion eques*, suggesting that the latter is a junior synonym of the former (Bañón et al., 2013). The aim of this investigation was to carry on a molecular analysis on specimens captured in their distribution areas in order to confirm the synonymy status of these two *Lepidion* species.

2. Materials and methods

2.1. Sample collection and specimen identification

Between 2007 and 2015, 72 specimens, 20 identified as *L. lepidion* and 52 as *L. eques*, were collected by commercial and research vessels at diverse locations (Fig. 1). The specimens of *L. lepidion* have a western Mediterranean origin, at the Balearic basin, off Barcelona. The L. *eques* specimens were captured in several locations in the eastern Atlantic, such as the Galicia Bank seamount, in the northwestern Spanish waters, (26 samples), Avilés Canyon, in the Bay of Biscay, (10 samples) and Porcupine Sea Bight, in the southwest of Ireland, (13 samples). Three specimens have a northwestern Atlantic origin and were captured in Canadian waters. The COI barcode of one sample from Greenland was mined from BOLD (GLF150-14). The specimens were immediately frozen on board and, once in the laboratory, muscle samples were removed from thawed individuals and stored in 95% ethanol. The DNA barcodes included in this study are deposited in GenBank under accession numbers JX437971–JX437986, JX437989–JX437998, KT351924–KT351953 and KT989513–KT989528.

2.2. DNA extraction, PCR amplification and sequencing

DNA was extracted by means of the E.Z.N.A Tissue DNA Kit from OMEGA bio-tek. The standard 5′ barcoding region of COI (ca. 650 bp) was amplified using the primers cocktail COI-3 (Ivanova et al., 2007), with Thermo Scientific Phire Green Hot Start II PCR Master Mix and reaction conditions as follows: 98 °C for 30 s followed by 35 cycles of 98 °C for 5 s, annealing at 52 °C for 5 s and 72 °C for 10 s, with a final extension at 72 °C for 1 min. COI amplicon bands were visualised on 1.2% agarose gels (Seakem LEAgarose), stained with ethidium bromide and purified with ExoSAP-IT (Affymetrix) following manufacturer's instructions. DNA sequencing reactions were carried out in the direct and reverse senses using the M13F (-21) and M13R (-27) primers. The resulting products were resolved in an ABI 3130 Genetic Analyzer (Applied Biosystems).

2.3. DNA analysis

Each consensus sequence was obtained after assembling the direct and reverse traces with SEQSCAPE v2.5 (Applied Biosystems, Foster City, CA), and aligned to each other employing MEGA 5.0 (Tamura et al., 2011). The list of haplotypes and their frequencies were calculated using DNAspV5 (Librado and Rozas, 2009). Pairwise distances among haplotypes were estimated using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). These distances were employed in the software HapStar 0.5 (Teacher and Griffiths, 2011) with the "Minimum Spanning Network" option for the graphic representation of the haplotype network.

3. Results

A total of 73 DNA sequences were used to produce a COI alignment 656 bp in length, including 32 polymorphic sites from which eight were parsimony informative. A total of 29 different haplotypes were found and 23 of them were detected only once. Three of the haplotypes were observed in 10 or more individuals and comprised 58% of all the sequences present in the dataset.

A COI haplotype network (Fig. 1) showed a star-like pattern with a central haplotype representing 14% of the sampled individuals from the three areas (Mediterranean Sea, North East Atlantic and North West Atlantic). This haplotype was connected with the other two most common, only one mutation away. All other haplotypes were separated from these main three, most of them only one or two mutations apart. There was no clear geographic structure in the network because these common COI haplotypes were shared among all the represented areas. Furthermore, four of the six haplotypes represented by more than one individual were shared between at least two different areas.

4. Discussion

The North Atlantic codling *Lepidion eques* is considered a valid species with a North Atlantic distribution in two of the most consulted fish faunas (Cohen, 1986; Cohen et al., 1990). The same result is obtained when the main two electronic repositories, The Catalog of Fishes (Eschmeyer et al., 2016) and FishBase (Froese and Pauly, 2016), are searched for using this species name as query. The latter considers the Mediterranean codling *Lepidion lepidion* a separated species with a Mediterranean distribution while the former also gives it an eastern Atlantic distribution.