

New record of *Opisthoteuthis massyae* (Grimpe, 1920) from Canary Islands, Spain

(Mollusca, Octopoda, Opisthoteuthidae)

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A new record of the cirrate octopod *Opisthoteuthis massyae* (Grimpe, 1920) is described from the vicinity of Tenerife Island (Canary Island, Spain). The specimen was a male having arms of pair I markedly thicker and enlarged suckers on the proximal fields of all arms and the distal field of arms II, III and IV. This record was confirmed applying phylogenetic analysis based on the cytochrome oxidase 1 mitochondrial gene sequence (Cox-1). This is the first cirrate octopod reported from the Canary Islands and the third Cox-1 sequence obtained for this species.

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Introduction

The family of the flapjack octopus (Opisthoteuthidae) comprises most of the cirrate octopus knows. Among them, the genus *Opisthoteuthis* Verrill, 1883 is the most diverse, inhabiting in the deep waters in all oceans of the world (Villanueva et al. 2002). The relative scarcity of specimens collected and the delicate consistency of the species of this genus make it difficult to obtain well-preserved specimens for detailed comparison and this has contributed to the confusion in the taxonomy of the group (Collins & Villanueva 2006). Recent works, including revisions of the family and descriptions of new species by Villanueva et al. (2002), Collins (2005), Collins &

Villanueva (2006), Lu (2010) and Pardo-Gandarillas et al. (2021) have recognised a total of 20 nominal species. Among these works, only Pardo-Gandarillas et al. (2021) applied molecular taxonomy and phylogenetic analysis to this group.

Currently, six species to belong to this genus have been described in the Atlantic-Mediterranean region: *O. agassizi* Verrill, 1883; *O. grimaldii* Joubin, 1903; *O. massyae* (Grimpe, 1920); *O. calypso* Villanueva, Collins, Sánchez & Voss, 2002; *O. hardy* and *O. borealis* Collins, 2005. In the Macaronesia region (Azores, Madeira, Salvagens and Canary Islands) only few specimens of *O. grimaldii* and *O. massyae* have been recorded in Azores and Madeira islands. Herein, we describe a new specimen of *Opisthoteuthis* caught

in waters of Tenerife Island (Canary Islands) and provide information on the morphometry and molecular phylogeny based on the cytochrome oxidase 1 (Cox-1) mitochondrial gene sequence.

Material and methods

During a whale watching trip performed on 22 July 2021 in the southwest coast of Tenerife Island a well-preserved specimen of the deep-sea cirrate octopus of the genus *Opisthoteuthis* Verrill, 1883 was found dead floating at sea surface (28°02.918'N, 16°46.172'W). The specimen was frozen at -20 °C until its examination in the laboratory. It was measured, weighed and sexed before its morphological classification. In addition, this morphological identification was verified using a molecular approach. The DNA was extracted from a portion of arm tissue with Chelex-based technique (Chelex 100® resin Bio-rad), then a fragment of the cytochrome oxidase 1 (Cox-1) gene was amplified using modified Folmer primers (Yu et al. 2012) in order to secure amplification. Prior to molecular identification, sequence chromatograms were edited using the software Geneious 2014.7.1 (ver. 7.1.3 created by Biomatters, available from www.genious.com), then the ID engine in BOLD (The Barcode of Life Database) and Genbank BLAST tool were used for obtaining the sequences of *Opisthoteuthis* genus with maximum correlation (99–100%). With the whole set of available sequences, we create the final alignment using the method of Geneious alignment with the default parameters. Finally, a maximum likelihood (ML) tree was made with all available *Opisthoteuthis* genus sequences of Cox-1 using the IQ-Tree web-page application (Nguyen et al. 2015) using *Octopus vulgaris* as outgroup.

Results

The specimen showed a bright orange colour at the time of picking up (Fig. 1). The specimen was 101 mm of total length, 570 mm of mantle length and weighted a total of 70 gr. Interpupillary distance was 57 mm. The arms were subequal in length (arm pair I: 980 mm, arm pair II: 940 mm, arm pair III: 911 mm and arm pair IV: 940 mm). The arms of pair I were markedly thicker (130 mm) than the pairs II, III, IV (90–80 mm), which had a single row of suckers on arms. All arms showed 4–7 enlarged proximal suckers that starting from the fourth buccal sucker of each arm. In addition, a second field of 11–9 enlarged distal suckers were present near the margin of the interbrachial membrane in arms II, III and IV. The interbrachial membrane, well developed and equal in depth, reached two-thirds of the arm's length. Fins length was 162 mm. The specimen was classified as *Opisthoteuthis massyae* (Grimpe, 1920)

following Sánchez & Guerra (1989) and Villanueva et al. (2002), due to the presence of two fields of enlarged suckers in all arms and thicker arms I, considered as diagnostic characters of the species. These authors also consider these characters as a form of hectocotylization in males (Fig. 1A).

This morphological identification was verified by the molecular approach. The ID engine in BOLD provided a 100 % match with *Opisthoteuthis massyae*, and the Genbank blast tool also provides a correlation match between 99–100%. The ML phylogenetic tree on the Cox-1 sequence revealed that the Canarian specimen is clustered and shares the same haplotype as the specimens from United Kingdom (Genbank accession no. AY545187.1) and South Africa (BOLD Sequence ID GBCPH0203-06) (Fig. 2).

Discussion

This finding constitutes the first record of a cirrate octopus (suborder Cirrata) for the Canarian archipelago, as well as the third species of the genus for Spanish waters, which include the species *Opisthoteuthis calypso* Villanueva, Collins, Sánchez & Voss, 2002 and *Opisthoteuthis grimaldii* (Joubin, 1903) (Villanueva et al. 2002). Previous records of cirrate octopuses in the Macaronesian region (Azores, Madeira, Salvagens and Canary Islands) were the following: *Grimpototeuthis umbellata* (P. Fischer, 1884), *Opisthoteuthis massyae* and *O. grimaldii* (Joubin, 1903) all reported for Azores and *O. massyae* erroneously recorded as *O. agassizii* Verrill, 1883 for Madeira (Clarke & Lu 1995, Villanueva et al. 2002, Gomes-Pereira et al. 2016). *Opisthoteuthis massyae* has been considered as deep-sea benthic species which inhabits a depth range of 600 to 1500 m and is distributed from the west coast of the British Isles to the Namibian coast in the southeast Atlantic (Collins & Villanueva 2006). The absence of previous records of cirrate octopuses in the Canary Islands can be explained by the ban on trawling imposed since 2005 by the regional government, which limits the collection of deep benthic species for study. In this context, population trends of *O. massyae* worldwide are considered decreasing due to the extension of commercial fishing into deeper waters, especially bottom trawling that capture accidentally this species. The fishing mortality in addition to their extreme longevity, slow growth and low fecundity make this species be considered as vulnerable by the International Union for Conservation of Nature (IUCN) along with *O. calypso* (Lyons & Allcock 2014), while *O. mero* is catalogued as endangered and *O. chathamensis* as critically endangered (Pardo-Gandarillas et al. 2021). The ban on trawling in the Macaronesian archipelagos makes this region

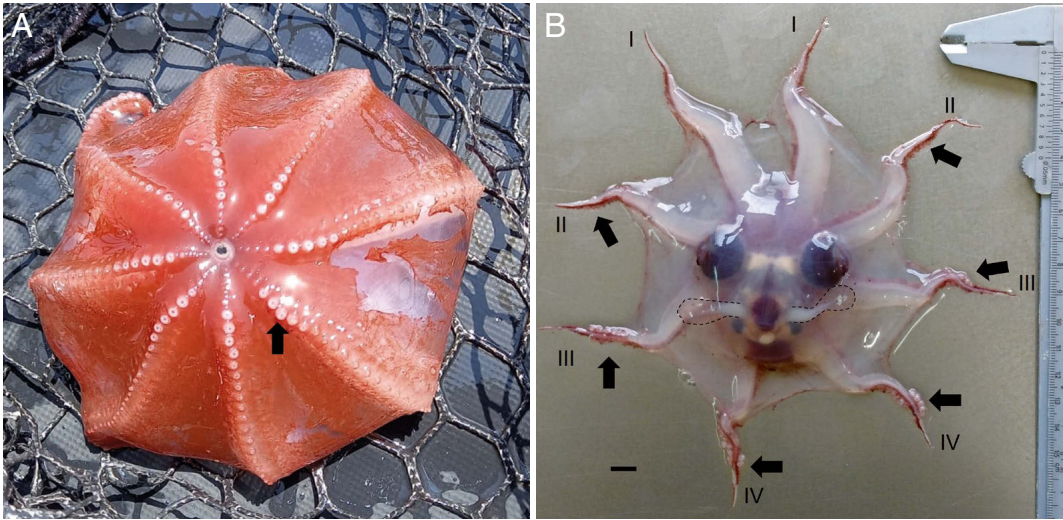


Fig. 1. Male of a Massy's flapjack octopus, *Opisthoteuthis massyae* (Grimpe, 1920), from Tenerife Island. **A.** Ventral view of fresh specimen at the time of picking up, arrow points to proximal enlarged suckers field (photo: S. D. Hernández); **B.** dorsal view of the specimen after thawed, arrows mark distal enlarged suckers fields on arm pairs II, III and IV. Dotted lines delimit the fins (photo: A. Escánez). Scale bar 10 mm.

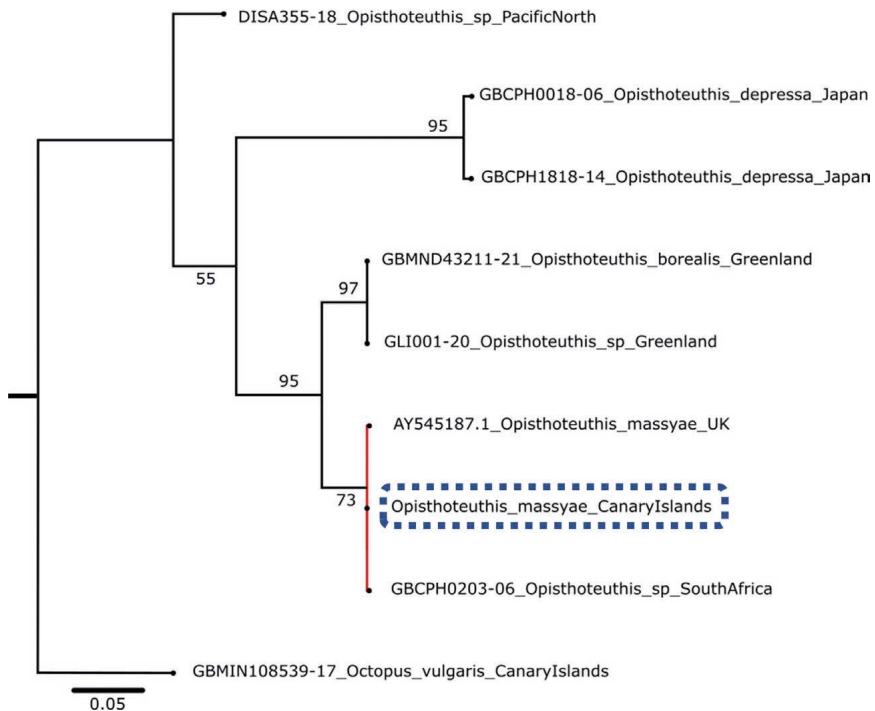


Fig. 2. Maximum likelihood tree with the available sequences of the genus from BOLD and Genbank databases using *Octopus vulgaris* Cuvier, 1797 as outgroup. The red branches provide the *O. massyae* clade, and marked in blue the sample of Tenerife. Bootstrap support of node branches is represented. The phylogenetic tree represents a sharing haplotype with South Africa and United Kingdom. The sequence is available in Genbank with accession number OL981225.

a refuge for deep-sea benthic species.

Until now, a total of 85 cephalopod species from 31 families were known from the Canary Islands (Escáñez et al. 2021), and with this new record the number increases up to 86 species and 32 families. In the last eight years, octopods species have accounted for 71.4% of the new cephalopod's records for the Canary Islands. Of them deep sea benthic species have been dominant with three species recorded *Pteroctopus tetracirrhus* (Delle Chiaje, 1830), *Haliphron atlanticus* Steenstrup, 1861 and *Opisthoteuthis massyae* (Grimpe, 1920) (Escáñez et al. 2019), while only two coastal species *Amphioctopus burryi* (Voss, 1950) and *Macrotritopus defilippi* (Vérany, 1851) have been reported in this period (Guerra et al. 2013). Therefore, further scientific investigations in the deep sea around the Macaronesian archipelagos will be necessary to complete the knowledge of the cephalopod diversity of this region.

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