

First record of the sea cucumber *Parastichopus tremulus* (Gunnerus, 1767) (Echinodermata: Holothuroidea: Aspidochirotida) in the Mediterranean Sea (Alboran Sea, western Mediterranean)

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Abstract: The holothurian *Parastichopus tremulus*, a species only known from the north-eastern Atlantic, is reported for the first time from the Mediterranean Sea. Five individuals of the species were collected in the vicinity of the Seco de los Olivos sea mount in May 2017 from a bottom trawl sample carried out during the MEDITS trawl survey. Scanning Electron Microscope images of body wall ossicles are supplied to support species identification. Connectivity between Atlantic and Mediterranean populations of *P. tremulus* is suggested due to the existence of a nearby Atlantic population in the Gulf of Cádiz and the documented presence of abundant holothuroid larvae in the anticyclonic gyre which originates in the Atlantic current entering the Mediterranean, near the Strait of Gibraltar.

Résumé : Premier signalement du concombre de mer *Parastichopus tremulus* (Gunnerus, 1767) (Echinodermata : Aspidochirotida) en Méditerranée (Mer d'Alboran, Méditerranée occidentale). L'holothurie *Parastichopus tremulus*, une espèce connue seulement du nord-est Atlantique, est signalée pour la première fois en Mer Méditerranée. Cinq individus de l'espèce ont été récoltés près du mont sous-marin Seco de los Olivos en mai 2017 d'un échantillon de chalut de fond effectué pendant la campagne de recherche avec chalut MEDITS. Des photographies au microscope électronique à balayage d'osselets de la paroi du corps sont fournies pour valider l'identification de l'espèce. La connectivité entre les populations atlantiques et méditerranéennes de *P. tremulus* est suggérée en raison de la proximité d'une population atlantique dans le Golfe de Cádiz et de la présence documentée de larves d'holothurie abondantes dans le vortex anticyclonique originaire du courant atlantique entrant en Méditerranée, près du Déroit de Gibraltar.

Keywords: Echinoderm • Stichopodidae • Atlantic inflow • Taxonomy • Morphological examination

Introduction

The genus *Parastichopus* was established by Clark (1922) for the sea-cucumber *Parastichopus tremulus*. Nowadays, it includes only two species: *P. tremulus* (Gunnerus, 1767) and *P. regalis* (Cuvier, 1817). Both species are distributed in the north-eastern Atlantic Ocean. However, only *P. regalis* has been documented to occur in the Mediterranean Sea, where it is widespread distributed in shelf and upper slope bottoms from 5 to 800 m depth, and even represents a resource of local commercial importance (Mortensen, 1927; Tortonese, 1965; Smith et al., 2000; Massuti & Reñones, 2005; Tanti & Schembri, 2006; Abad et al., 2007; Aydin, 2008; Ramon et al., 2010).

On the contrary, the distribution of *P. tremulus* is poorly known and is currently based on disjunctive records from the Scandinavian Peninsula, where it is also present in the Fjords, to the Canary Islands, including Iceland, Faroe Islands, Ireland and Great Britain (Mortensen, 1927; Perez-Ruzafa et al., 1992; Samyn, 2013; Buhl-Mortensen & Buhl-Mortensen, 2014). This *Parastichopus* species has a wide bathymetric range, from 20 to 1900 m depth, in which it has shown preference for muddy or sandy-mud sediments (Mortensen, 1927; Buhl-Mortensen & Buhl-Mortensen, 2014; Robert et al., 2016). It is a benthic deposit-feeder that seems to feed selectively on faecal pellets from other benthic animals and on other sediment aggregates that are richer in organic matter than the surrounding sediments (Hauksson, 1979; Godbold et al., 2009).

Increasing exploration and studies on the composition and structure of benthic communities from coastal to deep-sea areas of the Mediterranean Sea has resulted in a better knowledge on the distribution of specific benthic species that were not expected to occur in this basin, e.g. the echinoderm *Luidia atlantidea* (Madsen, 1950) (Gallardo-Roldán et al., 2015). Regarding *Parastichopus*, OCEANA

(2011) explored the Seco de Palos seamount (western Mediterranean) using a Remote Operated Vehicle (ROV) and reported the image and the frequent presence of an holothuroid which was listed as *Parastichopus cf. tremulus* indicating the possible species identity or resemblance. Nevertheless, the species identity could not be confirmed since no anatomical nor molecular analyses were done.

This study reports the first substantiated record of *P. tremulus* in the Mediterranean Sea, by studying the morphological traits, including scanning electron microscopy of ossicles of individuals collected during the MEDITS bottom trawl survey in the Alboran Sea.

Material and methods

In May 2017, during the MEDITS bottom trawl survey (see Bertrand et al. (2002) for specifications on sampling scheme and gear used), five specimens of a *Parastichopus* species were collected from an otter trawl haul carried out at a depth of 640 m in the vicinity of Seco de los Olivos seamount (also known as Chella Bank) located in the Alboran Sea (western Mediterranean) (Fig. 1).

The external morphology (length, shape, color and presence of feet) of all individuals was studied. Two of them were dissected and their feeding tentacles were isolated and photographed using a stereomicroscope Leica M165 C. Moreover, a small cube-shaped piece of dorsal body wall was excised for isolation of ossicles. Following a similar methodology to that described in Hickman (1998), the piece of body wall was placed in a tube with 10% concentrated bleach (NaCl) until the body wall was dissolved. Then the tube was lightly centrifuged (3000 rpm during 3-5 minutes) and the supernatant was pipetted off and discarded. Precipitated ossicles were resuspended in distilled water, lightly centrifuged whereupon the supernatant was again discarded. In order to clean the

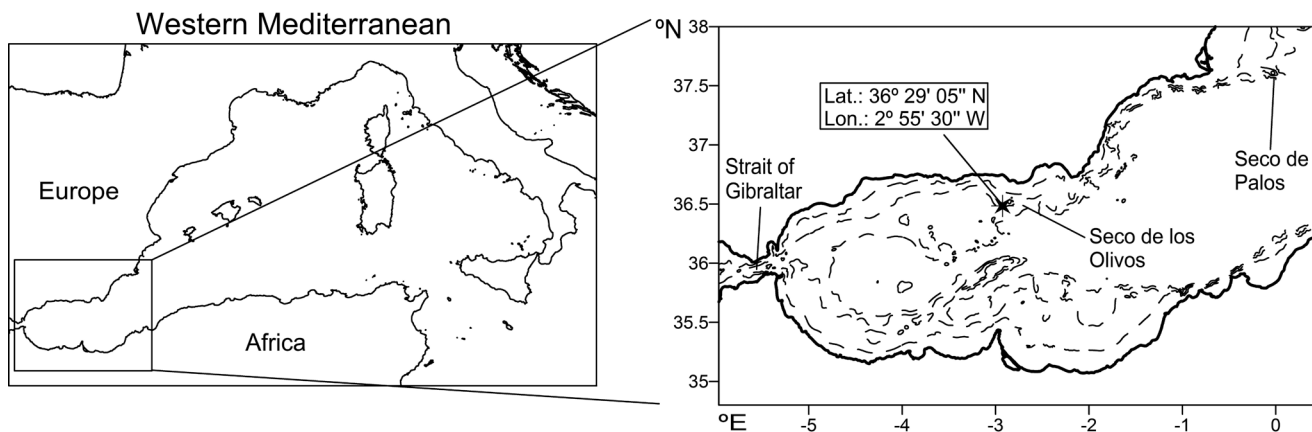


Figure 1. *Parastichopus tremulus*. Map of the study area (Alboran Sea, western Mediterranean), indicating with a star symbol the sampling station where the individuals were caught. Isobaths correspond to 200, 600 and 1000 m depth.

ossicles from any remaining tissue, a hydrogen peroxide digestion step was included. One milliliter of a 30% solution of hydrogen peroxide was added to the precipitated ossicles and the tube was gently shaken on an orbital shaker for several hours. After shaking, ossicles were let to settle for 15 minutes, rinsed several times with distilled water and finally preserved in absolute ethanol until they were prepared for scanning electron microscopy (SEM). Two aliquots of suspended ossicles were mounted onto copper conductive tape, air-dried, sputter-coated with gold and observed with a SEM HITACHI S-3400N. Two of the remaining specimens have been permanently fixed and preserved in 4% formalin, whereas the rest were preserved in 70% ethanol. All of them are kept in the Marine Fauna Collection (<http://www.ma.ieo.es/cfm/>) based at the Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, where they are labelled correlatively as CFM-IEOMA-6405 to CFM-IEOMA-6409.

Results

The size of the collected *Parastichopus tremulus* specimens ranged between 19.3 to 20.5 cm in length. Their fresh weight varied between 190 and 234 g. The collected individuals belong to the order Aspidochirotida due to the following characters: presence of tube feet and characteristic shield-shaped feeding tentacles (Fig. 2), and the absence of retractor muscles. They also belong to the Stichopodidae family due to the presence of gonads on both sides of the dorsal mesentery. We identified them as *P. tremulus* due to their cylindrical body shape with the ventral side slightly flattened; dorsal side was reddish in colour whereas the ventral side was white (Fig. 2). Body wall ossicles were very abundant with rods and tables as the most common ones, but also with star-shaped bodies

(Fig. 3). C-shaped rods and buttons absent. Tables presented disks with serrated edge, perforated by 14 to 21 peripheral and 4 central larger holes; disk diameters ranged from 96.5 to 118.7 μm and averaged 105.6 μm ($n = 21$); the spire was composed by four pillars connected by two beams and terminating with three levels of bifid spines. Rods were spinous, ranging from 107.9 to 307.4 μm and averaging 179.5 μm in length ($n = 30$), with variable shape but usually bearing two to three branches at the ends. Star-shaped bodies were highly ramified with thorn-like spines in the branches; their maximum diameters ranged from 98.3 to 140.9 μm and averaged 120.8 μm ($n = 6$). All these morphological traits are in total coincidence with the re-description of the species by Mortensen (1927).

Discussion

The present study represents the first confirmed record of *P. tremulus* in the Mediterranean Sea, specifically in the slope close to the seamount Seco de los Olivos, in the Alboran Sea. The presence of this species had recently been suggested from the Seco de Palos, another seamount located eastwards from our record, at a depth of around 550 m (OCEANA, 2011; Fig. 1). This coincidence in habitat characteristics, and the lack of similar records in other sampled areas of the Alboran Sea, may indicate the species distribution in the Mediterranean could be restricted to slope bottoms in the seamounts or their vicinity. This species, together with *P. regalis*, has generally been collected in sedimentary habitats from the nearby Gulf of Cádiz in the annual bottom trawl surveys ARSA (Campaña de evaluación demersales ARrastre SurAtlántica) carried out in that area for the assessment of the exploitation state of the bottom trawl fishery (Sobrino & Burgos, 2016). According to these surveys, in the Gulf of Cádiz, *P.*

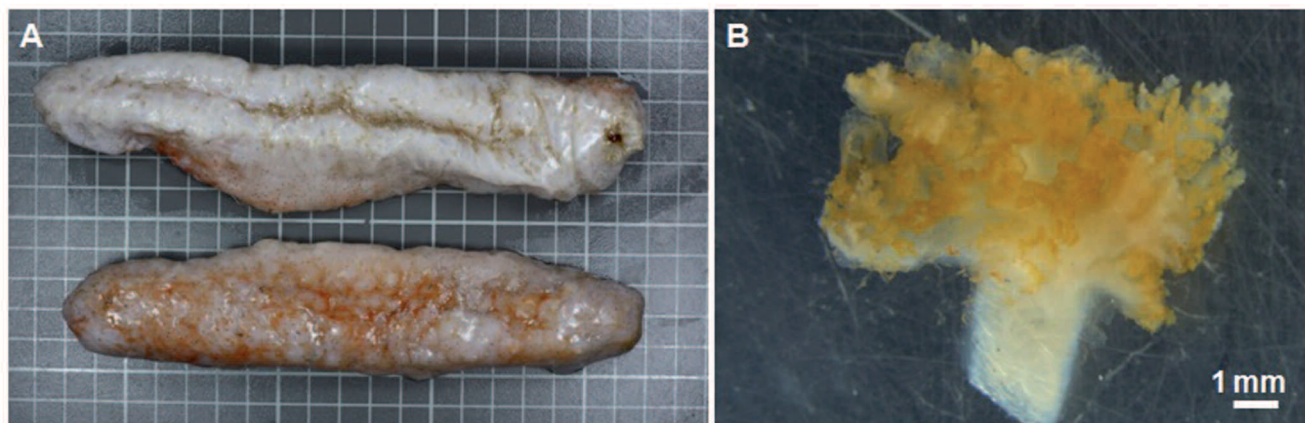


Figure 2. *Parastichopus tremulus*. Photographs of the individuals caught. **A.** Ventral and dorsal views (squares are 1x1 cm). **B.** Typical shield-shaped tentacle of the Holothuroidea, order Aspidochirotida.

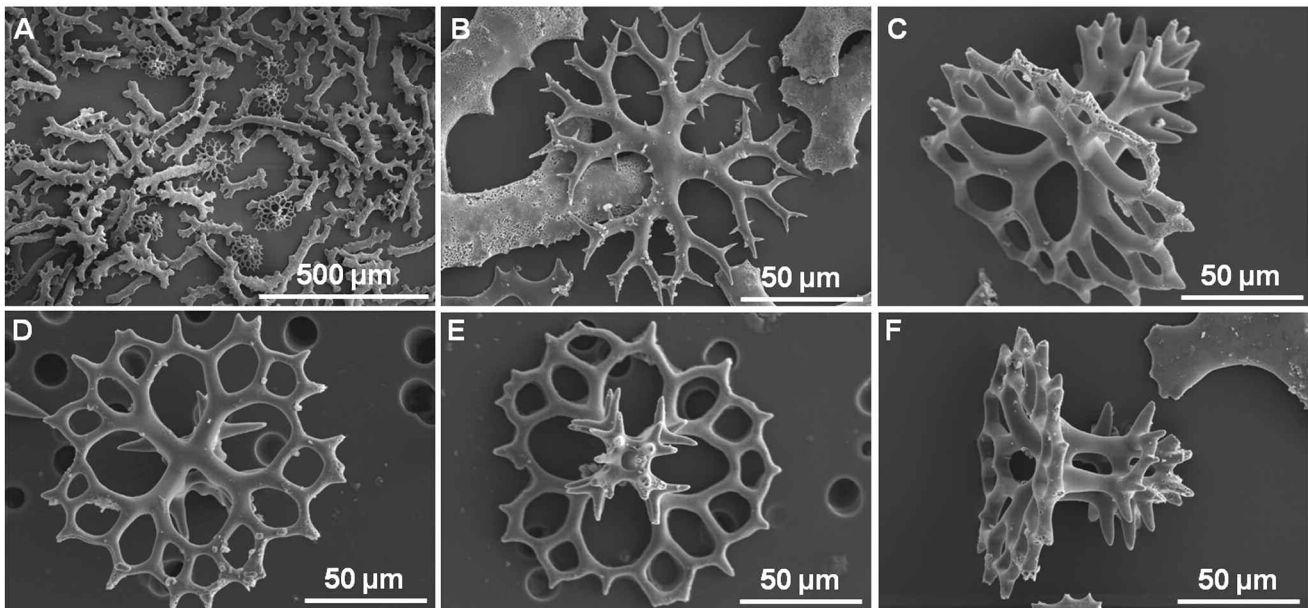


Figure 3. *Parastichopus tremulus*. Scanning electron microscope photographs of body wall ossicles. **A.** A wide field with most important ossicles, rods, star-shaped bodies, and tables, represented. **B.** Detailed photograph of a star-shaped body. **C-F.** Different perspectives of the characteristic tables.

tremulus is among the most important echinoderm species, in terms of both biomass and abundance, in the bathymetric stratum from 500 to 800 m depth, where the species showed average yields of around $1500 \text{ g} \times \text{h}^{-1}$ of trawling (Sobrinho & Burgos, 2016).

The Alboran Sea is an area highly influenced by Atlantic waters that flow through the Strait of Gibraltar into the Mediterranean in a layer from the surface to around 180 m depth, below which the Mediterranean water outflows in a countercurrent (Bryden & Stommel, 1982; Jia et al., 2007). In the last years, several species of different phyla with Atlantic affinity (either boreal or from subtropical west Africa) have been detected in different habitat types of the Alboran Sea (e.g. the crustacean *Pagurus mbizi* (Forest, 1955), the echinoderm *Luidia atlantidea* and the fish *Dipturus nidarosiensis* (Storm, 1881), recorded in García Raso et al. (2010), Gallardo-Roldán et al. (2015) and Ramírez-Amaro et al. (2017), respectively), showing the singularity of this area, located at the crossroads between the Mediterranean Sea and the Atlantic Ocean, that probably supports the highest biodiversity in European waters (Coll et al., 2010; García Raso et al., 2010; Templado, 2011). The presence of these Atlantic species has been related to the hydrodynamics of this basin (Bouchet & Taviani, 1992; Cebrian & Ballesteros, 2004). This could also be the case for the occurrence of *P. tremulus* in the Mediterranean Sea, a population that could be connected to nearby Atlantic populations through larval dispersal mediated by Atlantic superficial waters entering

the Mediterranean Sea. Pedrotti & Fenaux (1996) remarked that echinoderm larvae and postlarvae in the part of the Alboran Sea more influenced by Mediterranean waters were scarce in comparison with the area more influenced by Atlantic waters, where the abundance and species richness was higher. These authors detected holothuroid larvae in the eastern Alboran Sea mainly from 75 to 600 m depth in association with the anticyclonic gyre which originates in the Atlantic current near the Strait of Gibraltar. Hence, the population of *P. tremulus* in the Alboran Sea could exchange individuals with, or be sustained by, Atlantic populations that could act as a source of larvae, such as those from the Gulf of Cádiz, which could be transported inside the Mediterranean within the Atlantic superficial currents.

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