



Non-destructive tissue sampling and the use of PCR-RFLPs in two edible sea cucumbers from the north-eastern Atlantic, *Holothuria mammata* Grube, 1840 and *H. sanctori* Delle Chiaje, 1823 (Echinodermata: Holothuroidea)

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Abstract

Historically, the harvest and consumption of sea cucumbers have been largely restricted to Asian countries. Recently, however, the western world has witnessed a rapid growth of holothurian-related industries, and *Holothuria mammata* Grube, 1840 and *H. sanctori* Delle Chiaje, 1823 are now being commercially harvested in European and North African countries. The identification of holothurian species tends to be difficult and time-consuming, and in many cases needs experienced taxonomists. We suggest a simple non-destructive DNA sampling, using tube feet or oral tentacles from these two species. We also describe a simple and fast method of diagnose based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using restriction nuclease *Sau3AI* on 16S rRNA fragments. The use of RFLPs to rapidly and inexpensively identify species with no need for taxonomical or genetic expertise could prove a valuable asset for conservation and fishery purposes targeting commercially harvested animals such as the holothurians in the north-east Atlantic and the Mediterranean Sea.

Keywords: *Non-destructive DNA sampling, PCR-RFLP, Holothuria mammata, Holothuria sanctori, north-eastern Atlantic*

Introduction

The sea cucumbers, class Holothuroidea, one of the five extant classes of the exclusively marine phylum Echinodermata (Pawson 2007), are a conspicuous presence throughout all oceans and seas, at all latitudes and depths, occurring in virtually any marine habitat, from corals to rocky, sandy, muddy and mangrove habitats (Purcell et al. 2012). Roughly 1400 extant species are known worldwide (Pawson 2007) but only 66 are commercially harvested, comprising predominantly species belonging to the order Aspidochirotida from tropical shallow waters (Purcell 2010). The

processed products (cooked or dried), also known as “beche-de-mer”, “trepang” or “haishen”, are chiefly exported to Asian markets (Purcell et al. 2012). Possibly due to the over-exploitation of the typical valuable holothurians and in response to the growing demand for sea cucumber products (Purcell 2010), several Atlanto–Mediterranean holothurians are now being increasingly harvested, such as *Holothuria mammata* Grube, 1840 and *H. sanctori* Delle Chiaje, 1823 (see González-Wangüemert et al. 2016). This could pose a potential threat to many important ecosystems (e.g. seagrass beds) as many of the targeted species are viewed as key species, contributing to the recycling of

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