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First record of *Phymactis papillosa* (Lesson, 1830), a Pacific south sea anemone in European shores

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Abstract

Since 2017, a sea anemone species has been registered in central Portugal with a morphology comparable to *Phymactis papillosa*, from the Pacific shore of south and central America. In this paper, we have collected two individuals from Cascais, Portugal, extracted their DNA and amplified and sequenced the internal transcribed spacers I and II, markers frequently used in Cnidaria barcoding. The sequences obtained were compared with the ones available on GenBank, using the Basic Local Alignment Search Tool (BLAST) on the NCBI website. This procedure allowed the identification of *P. papillosa*. This is the first confirmed record of *P. papillosa* in European coasts. Citizen science data suggest occurrences in the Alentejo coast (south-west Portugal) and Mediterranean coast around Murcia, although no genetic or morphological confirmations of these records have been made. The potential effects of this species introduction are discussed.

Introduction

Phymactis papillosa (Lesson, 1830) is an abundant, widely distributed anemone of the Pacific coast of Central and South America (Chile, Peru, Mexico and Sea of Cortez) (Figure 1). This species is the most common sea anemone of the coastline in Chile, occurring in the intertidal and subtidal up to 16 m depth. It is most abundant in the inferior part of the intertidal, where it can occur at aggregations of one to more than 100 individuals. It can reach the mean high tide level (Haussermann, 2004). The size of the individuals appears to be vertically distributed, the smallest at higher levels of the intertidal and the biggest specimens at its lower levels (Stotz, 1979).

Phymactis papillosa is a middle-sized anemone with a red, brown, green or blue tentacle and column colour with a pedal disc size up to 100 mm. The colour of the up to 400 tentacles is paler than the colour of the column, that is covered with distinctive non-adhesive vesicles. It has a variable number of acrorhagi (Haussermann, 2004). It reproduces sexually and asexually by longitudinal fission (Uribe *et al*., 2013).

Phymactis papillosa was initially considered a synonymy of *P. clematis* (Drayton in Dana, 1846), which was thought to occur in the Pacific and Atlantic Ocean, off the coast of Argentina (Haussermann, 2004). However, the morphological analysis made by Haussermann (2004) on the Pacific populations considered *P. clematis* as a synonymy of *P. papillosa*. More recently Gomes *et al*. (2012) studied the Atlantic populations using morphological and genetic analysis. They used allozymes and sequenced a fragment of the nuclear internal transcribed spacer I, 5.8 rDNA and internal transcribed spacer II. Their genetic results were compatible using both kinds of genetic markers and supported the distinction between Pacific *P. papillosa* and the Atlantic populations. This study revealed that the latter population is a distinct species, belonging to a different genus (*Bunodosoma*).

In 2017, an unusual sea anemone was recorded in the region of Cascais, Portugal by the MARE–ISPA monitoring intertidal team. Since then, the abundance of this anemone has been increasing in this region, with green, red and brown individuals recorded. The increased abundance, with individuals of various size classes, and the occurrence of different morphotypes, suggest that this species reproduces locally and has established local populations. The spread of invasive, non-indigenous species has been recognized as a growing problem in marine habitats, with their early detection being one of the highest priorities (Xiong and Zhan 2016), as invasive species can have multiple impacts such as displacement of native species, and changes in community structure and in food webs (Molnar *et al*., 2008).

In this paper we used genetic methods and external morphology analysis to identify this anemone.

Materials and methods

Two green sea anemones at 5-m distance from each other were collected at Bafureira beach, Cascais (38.6921°N 9.3659°W). Sea anemones were anaesthetized with menthol crystals, a pedal disc clip was conserved in 70% ethanol and individuals in a 4% formaldehyde in salt

Fig. 1. Distribution of *Phymactis papillosa* (Source: www.gbif.org/pt/species/2256771).

water. Total DNA was obtained using REDExtract-N-Amp kit (Sigma-Aldrich) from ~2 mg of ethanol conserved tissue, following the manufacturer's instructions. Total concentration of DNA was measured in Nanodrop. Amplifications were conducted in 20 µl total-reaction volume with 10 µl of REDExtract-N-amp PCR reaction mix (Sigma–Aldrich), 0.8 µl of each primer (10 µM), 4.4 µl of sigma-water, and 4 µl of template DNA. Ribosomal internal transcribed spacers I and II (ITS1-rDNA and ITS2-rDNA) were amplified using the primers 18SF (5′-TCA TTT AGA GGA AGT AAA AGT CG- 3′) and 28SR (5′-GTT AGT TTC TTT TCC TCC GCT T – 3′) (Lôbo-Hajdu *et al*., 2004). The amplicon is a single sequence of 689 bp, which includes a partial 18S ribosomal DNA sequence, complete ITS-I, 5.8S ribosomal DNA and ITS-II sequences and a partial 28S sequence. PCR thermal cycling protocol was 94°C for 2 min, 40 cycles of [94°C 30 s, 51.5°C 45 s, 72°C 1 min], 72°C 10 min. DNA purification with magnetic beads and forward and reverse sequencing (Sanger method) were performed in Stabvida (www.stabvida.com). Sequences were inspected and edited using CodonCode Aligner (CodonCode Corporation, www.codoncode.com). Edited sequences were compared with sequences from known specimens on the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

The sequences obtained from the two individuals were identical (GenBank accession numbers OM574908, OM574909). BLAST results showed 99.85% identity with *P. papillosa* with a high coverage (97%). This sequence corresponds to an individual collected in Coquimbo, Chile by Gomes *et al.* (2012) (GenBank accession number JN118564.1). The second most identical species is *Phymactys clematis* with 99.19% identity but only 71% coverage (GenBank accession number KY789425.1) and the third *Bunodossoma caissarum* Corrêa in Belém, 1987, a species from Brazil, with 88.59% identity and 100% coverage (GenBank accession number JN118560.1).

The individuals collected in this study were both green (Figure 2). Their external morphology corresponds to the one described by Haussermann (2004) for *P. papillosa* (Table 1): the individuals had ~3 cm pedal disc diameter, the column was covered with rounded non-adhesive vesicles, the tentacles and oral disc, with a prominent mouth opening, were slightly paler than the column. The oral disc had numerous short tentacles in its margin, and acrorhagi were present in the fosse (Figure 2).

Discussion

Globally, invasive species have transformed marine habitats and have been described as the second leading cause of species extinction (Molnar *et al*., 2008; Bellard *et al*., 2016). Habitat displacement of native species and changes in the structure of communities and in the food chain are among the impacts they can cause (Molnar *et al*., 2008). Although only a small fraction of introduced marine species manage to proliferate and invade new habitats, their impact can be quite detrimental (Mack *et al*., 2000). Any knowledge regarding the invasive species is of extreme interest since it may be possible to predict their behaviour and expansion.

Many anemone species tolerate hard conditions and potentially can proliferate rapidly by asexual reproduction, making this group well-adapted for introduction. Although active dispersal by sea anemones is limited to crawling or floating of adults or short-range dispersal of gametes and larvae, passive transport by rafting or by human mechanisms enables long-distance (transoceanic and interoceanic) dispersal. There are several documented sea-anemone invasions associated with mariculture. Additionally, commercial shipping and cruise liners can also promote invasions by hull fouling, transport in ballast water, and attachment in sea-chests, which probably have been underestimated (Glon *et al*., 2020). Given the distance between the native distribution of *P. papillosa* and Europe, the passive dispersal of adult individuals attached to vessels seems the more probable vector although the exact vector and point of arrival of this species into Europe is not known.

After transport and establishment, an invasive species must be capable of spreading (Glon *et al*., 2020). The behaviour and

Fig. 2. Left: *Phymactis papillosa* recorded at intertidal shore of Sintra, Portugal (date: 21 July 2021) (coordinates: 38°51′33.55″N 9°26′56.53″W); Right: *Phymactis papillosa* amplified photo to highlight column external morphology.

interaction with native species are crucial at this point. In fact, agonistic behaviours with other anemones have already been reported for the species *P. papillosa*, namely with an invasive species *Anemonia alicemartinae*, revealing *P. papillosa* as a stronger competitor, even when it is at a disadvantage in number of individuals (Brante *et al.*, 2019). Furthermore, the different phenotypes that this species presents seem to be related to aggressiveness. Green anemones are generally more aggressive in interspecific interactions (Brante *et al.*, 2019). It is necessary to consider the aggressive behaviour characteristic of the species described in this study, since its interaction with native species can lead to disturbances in the ecosystem, such as changes in habitat occupation. The knowledge on this topic is extremely relevant since interactions of this anemone with native marine species in Europe are not described. Thus, further studies are needed to infer the effect of the rapid increase in the abundance of *P. papillosa* in the European marine ecosystems.

Data available from citizen science platforms (iNaturalist www.inaturalist.org/taxa/195551-Phymactis-papillosa and reflected in www.gbif.org/pt/species/2256771) suggest occurrences in Alentejo coast (south-west Portugal) and Mediterranean coast around Murcia (Spain) (although there is no scientific validation of the identity of the species for each record), but the exact distribution of *Phymactis papillosa* in European shores is yet to be understood.

In this study, DNA barcoding was used to identify a non-native sea anemone species. This method, widely used in other taxonomic groups, has a hindered application in Cnidaria, due to the difficulties in selecting the appropriate marker, as the conventional COI (cytochrome oxidase I) has a very slow rate of evolution in some groups (e.g. Hellberg, 2006), but not in all (e.g. Ortman *et al.*, 2010). In fact, Shearer & Coffroth (2008) have shown that levels of intraspecific variability and interspecific divergence overlap in scleractinean corals, precluding the use of this molecular marker in barcoding. McFadden *et al.* (2011) found the same pattern in Octocorallia. The inclusion of additional molecular markers increased the success of identification of morphospecies, but it seems to have also a limited application (e.g. 28S ribosomal nuclear DNA – McFadden *et al.*, 2014, Quattrini *et al.*, 2019; Internal transcribed spacer II – Oliverio *et al.*, 2008). Recently Gong *et al.* (2018) recommended the use of nuclear DNA (ITS, 18S rDNA and 28S rDNA) for barcoding in Cnidaria. However, the success of this approach is dependent on complete and reliable DNA barcode reference libraries (Paz & Rinkevich, 2021). *Phymactis papillosa*, identified in this study by molecular barcoding, has only one available sequence on GenBank (ITS I and ITS-II), which stresses the need to compile DNA sequences from organisms identified by experts, to enable an effective use of DNA barcoding in this group.

Table 1. Description of *Phymactis papillosa*, after Häussermann (2004)

Characters	Description
Column	Densely covered with round non-adhesive vesicles of uniform colour; column rarely visible between vesicles. Column red, green, blue or brown.
Acrorhagi	Single acrorhagus on oral side of some marginal vesicles; sometimes absent in juveniles.
Tentacles	Short, conical, and very numerous (until 400). Length about half diameter of oral disc. Can be slightly transparent.
Body size	Up to 100 mm pedal disc diameter.
Oral disc	Circular to slightly lobed in large individuals. Mouth opening prominent. Young individuals with dense white radial lines on the centre of the oral disc, mouth opening dark. Oral disc paler than the column, usually the same colour.
Pedal disc	Diameter slightly greater than or equal to that of column; robust, slightly lobed, strongly adhesive to the substratum.
Habitat	Intertidal and shallow subtidal. Individuals found alone or in aggregation.

Author contributions. AMP and OM collected the samples and designed this study; AMP and MMS performed the laboratory work; AMP conducted data analysis; all authors wrote the manuscript.

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Conflict of interest. The authors declare none.

Data availability. Genetic sequences are available in GenBank (accession numbers OM574908, OM574909). Voucher specimens used in this study are available upon request and stored at ISPA – Instituto Universitário collections.

References

Bellard C, Cassey P and Blackburn TM (2016) Alien species as a driver of recent extinctions. *Biology Letters* **12**, 1–4.

Brante A, RIera R and Riquelme P (2019) Aggressive interactions between the invasive anemone *Anemonia alicemartinae* and the native anemone *Phymactis papillosa*. *Aquatic Biology* **28**, 127–136.

Glon H, Daly M, Carlton JT, Flenniken MM and Currimjee Z (2020) Mediators of invasions in the sea: life history strategies and dispersal vectors facilitating global sea anemone introductions. *Biological Invasions* **22**, 3195–3222.

Gomes PB, Schama R and Solé-Cava AM (2012) Molecular and morphological evidence that *Phymactis papillosa* from Argentina is, in fact, a new species of the genus *Bunodosoma* (Cnidaria: Actiniidae). *Journal of the Marine Biological Association of the United Kingdom* **92**, 895–910.

Gong S, Ding Y, Wang Y, Jiang G and Zu C (2018) Advances in DNA barcoding of toxic marine organisms. *International Journal of Molecular Sciences* **19**, 2931.

Häussermann V (2004) Re-description of *Phymactis papillosa* (Lesson, 1830) and *Phymanthea pluvia* (Drayton in Dana, 1846) (Cnidaria: Anthozoa), two common actiniid sea anemones from the south east Pacific with a discussion of related genera. *Zoologische Mededelingen* **78**, 345–381.

Hellberg ME (2006) No variation and slow synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology* **6**, 24.

Lôbo-Hajdu G, Guimarães AC, Mendes AM, Lamarão FRM, Vieiralves T, Mansure JJ and Albano RM (2004) Intragenic, intra- and interspecific variation in the rDNA ITS of Porifera revealed by PCR-single-strand conformation polymorphism (PCR-SSCP). *Bolletino dei Musei e degli Instituti Biologici dell'Universita di Genova* **68**, 413–423.

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M and Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**, 689–710.

McFadden CS, Benayahu Y, Pante E, Thoma AN, Nevarez A and France SC (2011) Limitations of mitochondrial gene barcoding in Octicirallia. *Molecular Ecology Resources* **11**, 19–31.

McFadden CS, Brown AS, Brayton C, Hunt CB and van Ofwegen LP (2014) Application of DNA barcoding in biodiversity studies of shallow-water octocorals: molecular proxies agree with morphological estimates of species richness in Palau. *Coral Reefs* **33**, 275–286.

Molnar JL, Gamboa RL, Revenga C and Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* **9**, 485–492.

Oliverio M, Barco A, Modica MV, Richter A and Mariottini P (2008) Ecological barcoding of corallicory by second transcribed spacer sequences: hosts of coralliophiline gastropods detected by the cnidarian DNA in their stomach. *Molecular Ecology Resources* **9**, 94–103.

Ortman BD, Bucklin A, Pagès F and Youngbluth M (2010) DNA barcoding the Medudozoa using mtCOI. *Deep Sea Research* **57**, 2148–2156.

Paz G and Rinkevich B (2021) Gap analysis of DNA barcoding in ERMS reference libraries for ascidians and cnidarians. *Environmental Sciences Europe* **33**, 4.

Quattrini AM, Wu T, Soong K, Jeng M-S, Benayahu Y and McFadden CS (2019) A next generation approach to species delimitation reveals the role of hybridization in a cryptic species complex of corals. *BMC Evolutionary Biology* **19**, 116.

Shearer TL and Coffroth MA (2008) DNA barcoding: barcoding of corals: limited by interspecific divergence, not intraspecific variation. *Molecular Ecology Resources* **8**, 247–255.

Stotz WB (1979) Functional morphology and zonation of three species of sea anemones from rocky shores in southern Chile. *Marine Biology* **50**, 181–188.

Uribe AR, Rubio RJ, Carbajal EP and Berrú PP (2013) Invertebrados marinos bentónicos del litoral de la Región Áncash, Perú. *Boletín Instituto del Mar del Perú* **28**, 136–293.

Xiong W and Zhan A (2016) Early detection of invasive species in marine exosystems using high-throughput sequencing: technical challenges and possible solutions. *Marine Biology* **163**, 139.