

Three non-indigenous species from Madeira harbors, including a new species of *Parasmittina* (Bryozoa)

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Abstract During a study investigating the identity and abundance of fouling non-indigenous species in marinas from the Madeira Archipelago, three species of bryozoans were detected on experimental settlement plates. These three species are described and figured. *Celleporaria inaudita* was previously only known from Vanuatu (South Pacific Ocean) and Safaga Bay (Red Sea). *Parasmittina alba* was previously only known from the Brazilian coast. The origin of *Parasmittina multiaviculata* sp. nov. is unknown. Secondary introduction by anthropogenic vectors (via hull fouling of recreational vessels) seems the most plausible explanation for the presence of these species in the Madeira Islands.

Keywords Non-indigenous species · Biological Invasions · *Parasmittina* · New species · Atlantic Ocean · *Celleporaria*

Introduction

The introduction of non-indigenous species (NIS) into new ecosystems is now considered a major environmental and economic threat and, along with habitat destruction, a leading cause of biodiversity loss at a global scale (Nentwig 2007; Canning-Clode 2015). In the marine system, the most significant vector contributing to new biological invasions is commercial shipping, particularly through the unintentional transfer of large numbers of organisms in ballast water and hull fouling (Ruiz et al. 2000).

Every hard substratum in the marine environment (e.g., artificial structures such as ship hulls, piers, pontoons, pilings, seawalls, and buoys) is subject to a process known as biofouling — the accumulation of unwanted biological material at an interface (Dobretsov et al. 2014). The organisms forming this biofouling community may vary in size from microorganisms and propagules of sessile macroorganisms to complex multi-species and multidimensional assemblages including mobile associated fauna and large macroalgae (Wahl 1997; Dürr and Thomason 2010; Dobretsov et al. 2014). In fact, one of the most ancient vectors of introduction of NIS is the transfer of fouling species such as sponges, hydrozoans, barnacles, bryozoans, mollusks, tunicates and macroalgae on ship hulls (Carlton and Hodder 1995). In this context, several new records of these groups have been found globally, particularly in marinas and harbors (e.g., Piola and Johnston 2008; Canning-Clode et al. 2013a, b; Zabin et al. 2014).

The Madeira Archipelago is composed of two inhabited volcanic islands — Madeira and Porto Santo — and is located southwest of continental Europe and ~700 km off the

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Moroccan coast in the Atlantic Ocean. Historically, the main island of Madeira has offered an important port for re-fuel and rest stops for merchant and passenger ships between Europe and the Caribbean, and the American and African continents. Nowadays, most shipping can be attributed to tourist cruise ships and sailing yachts from different parts of the world (APRAM 2014). In recent years, the Madeira Archipelago has been the site of a comprehensive monitoring survey of marine NIS. As a result, several new species have been detected (e.g., Wirtz and Canning-Clode 2009; Canning-Clode et al. 2013a; Ramalhosa et al. 2014; Ramalhosa and Canning-Clode 2015) including previously un-described species (Souto et al. 2015). The knowledge about bryozoans from Madeira Island is far from complete, but several papers record approximately 150 species from this phylum in this area. The first papers about bryozoans from these islands date back to the end of the past century (Busk 1858a, b, 1859, 1860, 1861; Hincks 1880; Johnson 1897; Waters 1899; Norman 1909) but in recent years new records, including undescribed species and NIS, have been added (d'Hondt 1985; Alves and Cocito 2002; Berning and Kukliński 2008; Berning et al. 2008; Wirtz and Canning-Clode 2009; Berning 2012; Souto et al. 2014, 2015; Ramalhosa et al. 2016). In the present paper, we describe one new species and two new records of Bryozoa collected from artificial settlement plates as part of an ongoing monitoring survey of NIS conducted in several marinas in the Madeira Archipelago.

Material and methods

Since July 2013, we have been investigating the species composition and abundance of fouling assemblages in four marinas of the Archipelago: Calheta (32°43' N, 17°10' W), Funchal (32°38' N, 16°54' W), Caniçal (32°44' N, 16°42' W) and Porto Santo Island (33°3' N, 16°18' W) (Fig. 1) as part of an ongoing survey of NIS in Madeira. Following the methods used by Canning-Clode et al. (2013a) and most recently by Ramalhosa et al. (2014), ten polyvinylchloride (PVC) settlement plates (14 × 14 × 0.3 cm) were individually attached to a brick in a horizontal orientation facing downwards and hung at about 1 meter depth, from pontoons in all four marinas. Settlement plates were collected every 3 months (starting in July 2013; finishing in August 2014) for fouling species identification, a duration after which fully developed colonies could be observed. Fouling communities were first observed with the aid of a stereomicroscope (Leica Wild-M3 Heerbrugg), and digital photographs were taken of specimens from settlement plates using a Sony DSC-W55 camera. For the purpose of this analysis, only bryozoan samples were collected from the plate in the different marina sites, and preserved in 95 % ethanol. Bryozoan specimens were later examined with a stereomicroscopy Leica MZ12. Colony

fragments were dried, and scanning electron microscope (SEM) photographs of uncoated material were taken using an FEI Inspect S50 SEM at the University of Vienna, Austria. Some samples were treated in an ultrasound bath and bleached with sodium hypochlorite prior to SEM examination. The SEM was used with a back-scattered electron detector in low vacuum mode. Zooidal measurements were taken from the SEM images using the software ImageJ@ (<http://rsbweb.nih.gov/ij>). Specimens were deposited in the collections of the Museu Municipal de História Natural (MMF), in Funchal, Madeira Island, Portugal.

Systematics

Phylum Bryozoa Ehrenberg, 1831

Class Gymnolaemata Allman, 1856

Order Cheilostomata Busk, 1852

Family Lepraliellidae Vigneaux, 1947

Genus *Celleporaria* Lamouroux, 1821

Celleporaria inaudita Tilbrook, Hayward and Gordon, 2001

(Fig. 2, Table 1)

Celleporaria inaudita Tilbrook, Hayward and Gordon, 2001: 72, Figs 13D-F

Material examined: MMF44608 and MMF44609 from Funchal, MMF44610 from Calheta and MMF44611 from Caniçal.

Description

Irregular encrusting colony, multilaminar or developing nodules. Irregular autozooids with an irregular disposition. Frontal wall finely granular, with only a few rounded, marginal pores. Primary orifice slightly wider than long, anter semi-circular, poster with sides slightly concave and indented by two asymmetrical sinuses, separated by one cusp; two more cusps can appear in the lateral part of the sinus. Development and size of the orifice, the sinuses, and the cusps are very variable between autozooids, in some cases dependent on the degree of the calcification. Condyles lacking. No oral spines. Avicularia suboral on an umbo, present in some zooids, rostrum elliptical, with the distal portion denticulate. Large vicarious avicularia, cystid with rounded marginal pores similar to the ones present in the autozoid; rostrum raised, with rounded and serrated tip, sometimes externally cover by secondary calcification. Cross-bar complete, thin, without columella, palate with very large foramen. Ooecium non-cleithral, widely open; covered by granular secondary calcification.

Distribution

Known from Vanuatu (South Pacific Ocean) and Safaga Bay (Red Sea). This paper reports its first record for Madeira Island. To date, our monitoring surveys did not detect *Celleporaria inaudita* in Porto Santo Island.

Remarks

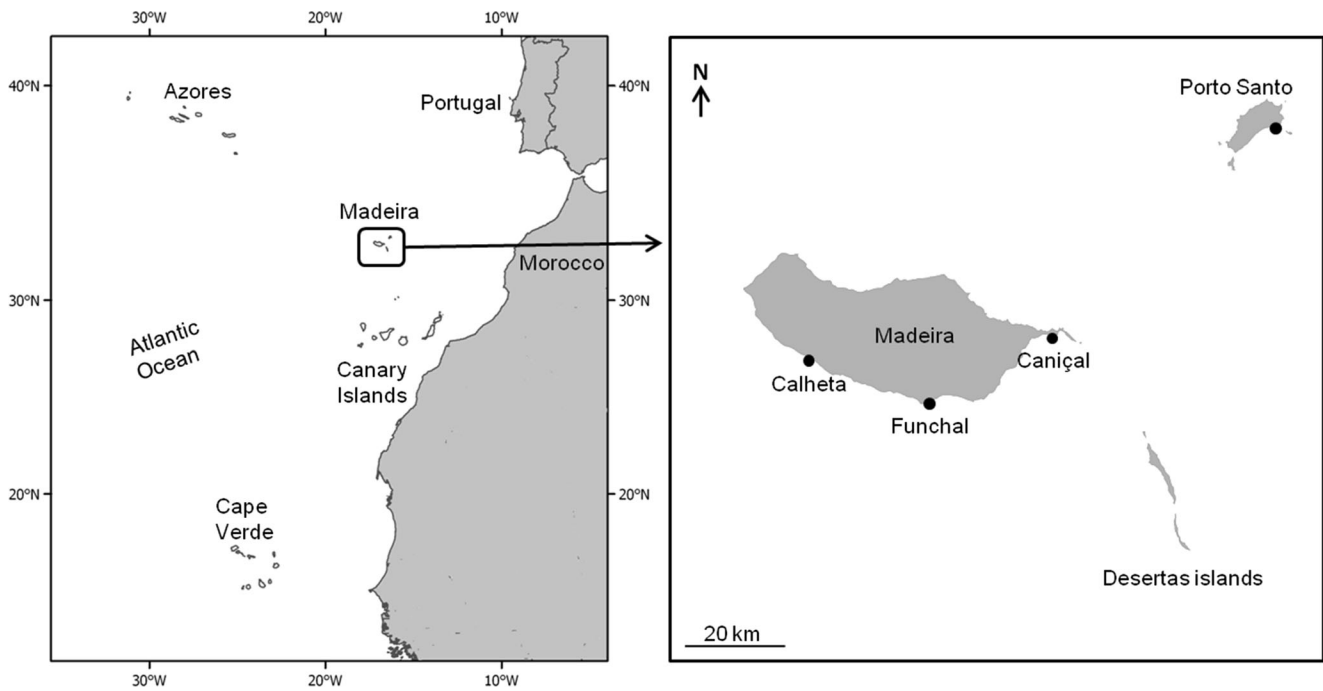


Fig. 1 Location of the four study sites where samples were collected in the Madeira archipelago

Celleporaria inaudita was described from Vanuatu as forming nodules on corals and anthropogenic structures, e.g., Port Vila harbor on Efate Island (Tilbrook et al. 2001). Ostrovsky et al. (2011) also included this species in a checklist of bryozoans from Safaga Bay (Red Sea); see figures in Ostrovsky et al. (2015). According to Tilbrook et al. (2001), *C. inaudita* is similar to *C. aperta*, but the latter species has oral spines and a lanceolate rostrum in the spatulate avicularium, which is absent in *C. inaudita*. The specimens collected in Madeira, as well as the specimens described by Tilbrook et al. (2001) and the Safaga Bay specimens, indicate that there is variability in several characters, not only between colonies, but also between zooids in the same colony. The morphology of the orifice is very variable, and although the presence of the two asymmetrical sinuses is constant, their morphology is very variable and the smaller sinus can be very reduced. Here we figured the denticulation of the rostrum of the spatulate avicularia, not visible in the Safaga Bay collection, was probably due to erosion of the specimens. Similarly, the lyrula is very variable and not a good taxonomic character (see, for example, Kuklinski et al. 2013; Berning et al. 2014). The morphology of the orifice in general, and the sinus in particular can be very variable in some species of *Celleporaria*, both between colonies and within the same colony; see the case of *Celleporaria brunnea* (Hincks, 1884) (Canning-Clode et al. 2013b; Harmelin 2014; Lodola et al. 2015). Therefore, orifice morphology is not a very useful taxonomic character for this genus. In 2006 and 2007, Canning-Clode et al. (2013a) collected specimens at Quinta do Lorde Marina that were identified as *Celleporaria cf. inaudita*; thus,

this new record confirms its identification, and its presence across the marinas of Madeira island.

Family Smittinidae Levensen, 1909

Genus *Parasmittina* Osburn, 1952

Parasmittina alba Ramalho, Muricy and Taylor, 2011 (Fig. 3, Table 2)

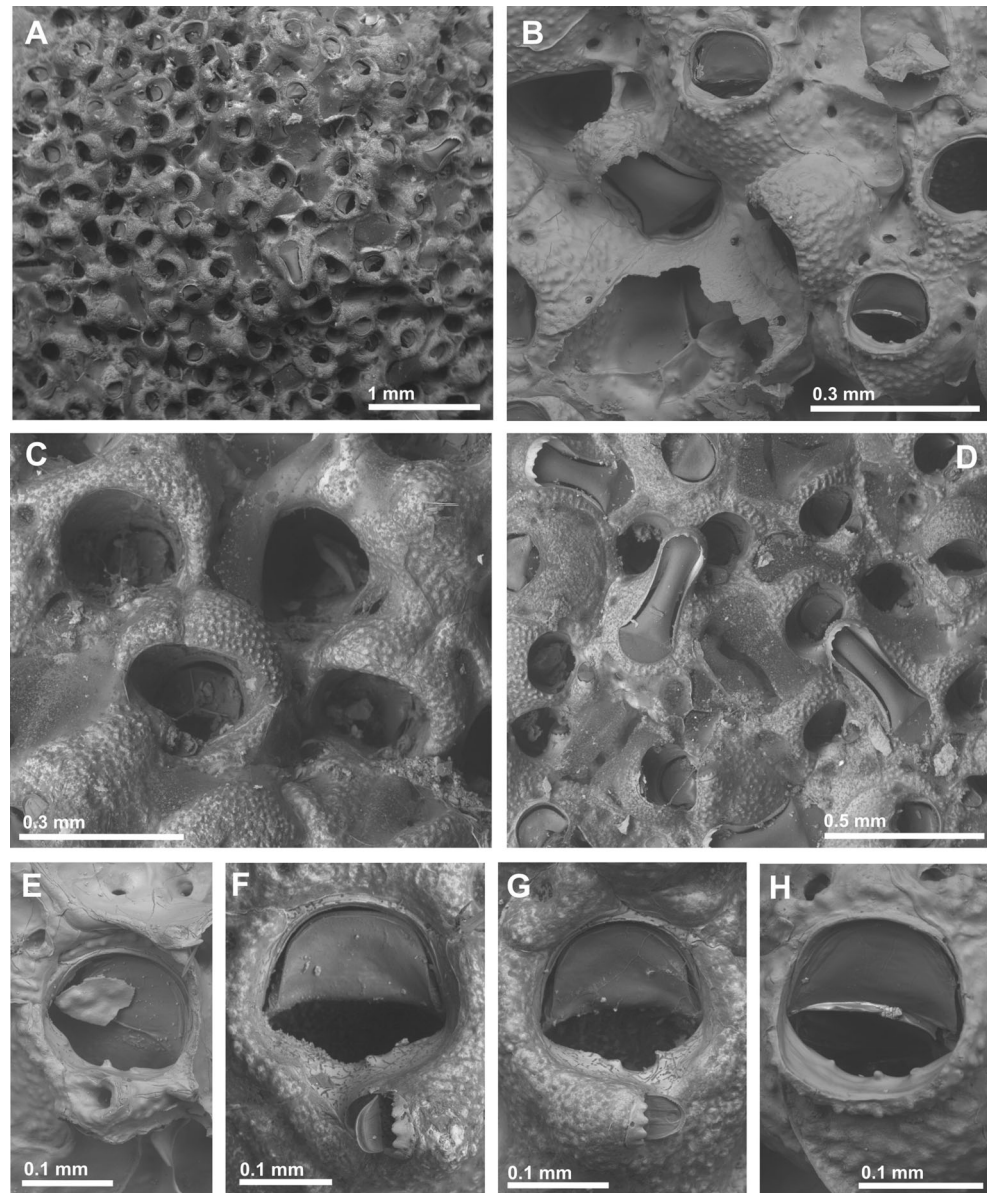
Parasmittina alba Ramalho, Muricy and Taylor, 2011: 769, Fig. 2.

Material examined: MMF44612 from Caniçal, MMF44613 and MMF44614 from Calheta, MMF44615 from Porto Santo.

Description

Colony encrusting, unilaminar or multilaminar, pale white in color. Autozooids mainly rectangular, in some cases hexagonal, longer than wide, disposed initially in linear series; linear pattern in old colonies lost. Frontal wall coarse, imperforate centrally, and with large marginal pores. Primary orifice wider than long, surrounded by a proximo-lateral peristome, incomplete proximally, with a proximal fissure usually quadrangular. Distal edge round, with two curved, thin, and pointed condyles, curved and directed to the lyrula. In ovicellate zooids, the distal margin of the orifice has two symmetrical notches that could be more or less developed. Wide lyrula in the proximal border. One or two spines in the distal margin of the orifice. Three kinds of adventitious avicularia. In most cases only one per zooid (although two in some cases), located latero-proximal to the orifice. Although, there are large areas in the colony without avicularia. Avicularia are small oval, very abundant, variable in size and form, generally longer than wide and with the distal margin rounded and wider than the

Fig. 2 *Celleporaria inaudita*. **a** General aspect of the colony surface, with vicarious avicularia. **b** Autozoid orifices, vicarious avicularia and oecia. **c** Ooecia in unbleached specimen. **d** Vicarious avicularia. **e, f, g, h** Morphological variability between orifices and suboral avicularia



proximal portion. Small triangular avicularia directed to the margin of the zooid, lightly raised, and with the margins slightly serrated. Large, spatulate avicularia not common, situated close to the orifice and directed proximally, rostrum

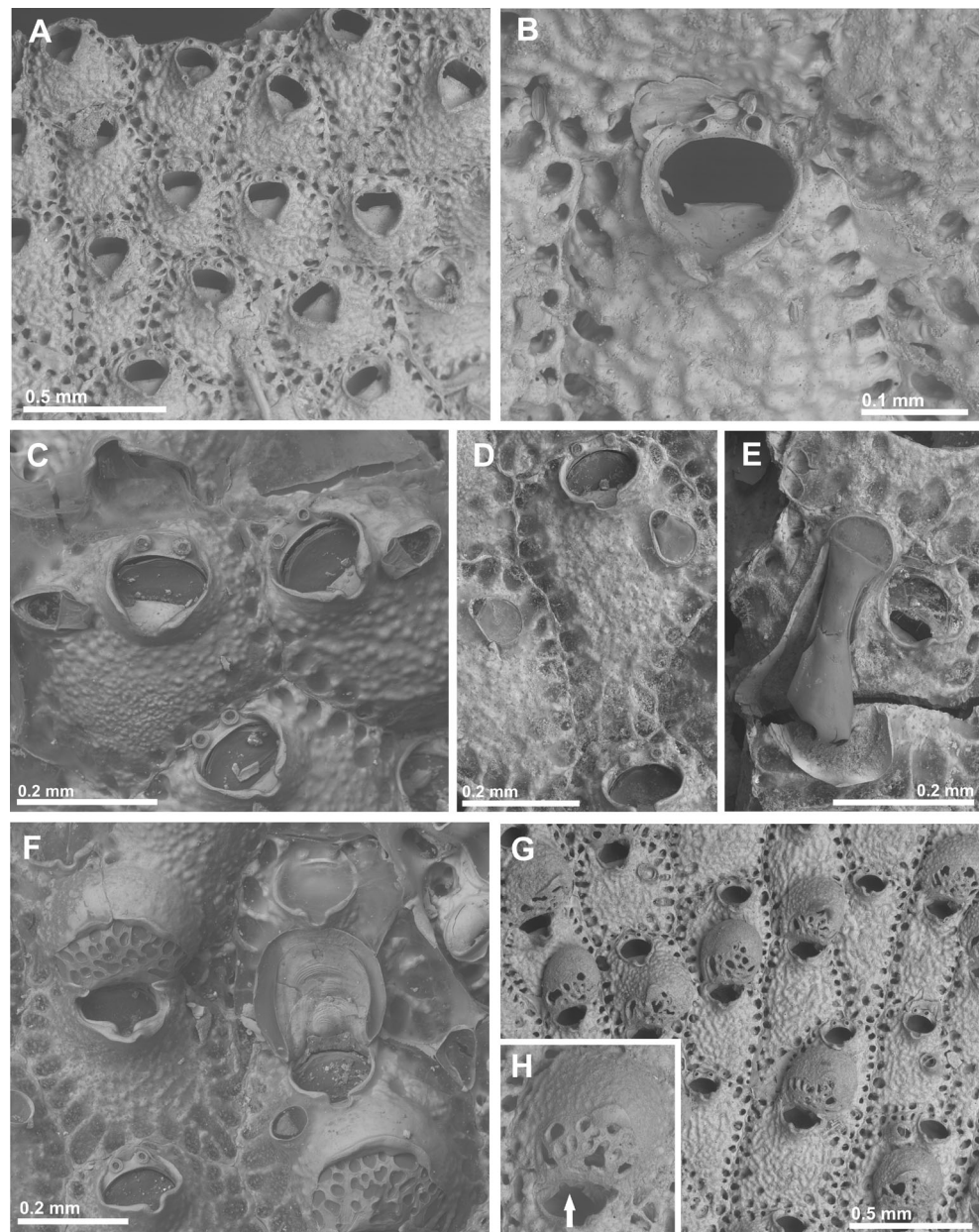
elongated and spatulate of variable morphology, covering almost the whole frontal wall of the zooid. Ovicell hyperstomial, oecium formed by the distal autozoid, globular, about as wide as long, ectooecium perforated by

Table 1 Measurements (in mm) of *Celleporaria inaudita*

	Mean	SD	Minimum	Maximum	<i>N</i>
Zooidal orifice length	0.147	0.0107	0.126	0.169	19
Zooidal orifice width	0.169	0.0168	0.147	0.204	19
Spatulate avicularia length	0.430	0.0148	0.411	0.448	6
Spatulate avicularia width	0.192	0.0110	0.175	0.204	6
Ooecium length	0.130	0.0186	0.094	0.175	11
Ooecium width	0.275	0.0191	0.228	0.299	11

SD standard deviation, *N* number of measurements

Fig. 3 *Parasmittina alba*. **a** General view of the colony margin. **b** Autozooid orifice, with condyles and the base of oral spines. **c** Autozooids with lateral small triangular avicularia. **d** Autozooids with latero-suboral oval avicularia. **e** Spatulate avicularia. **f, g** Ovicellate zooids with variability in the morphology of the oecia. **h** Detail of ovicell; *arrows* indicate symmetrical notches in the distal margin of the orifice



numerous, although variable in number, irregularly-shaped pseudopores. Secondary calcification very variable in development, covering the distal and lateral portions of the ovicell, the ectooecium visible only in the circular frontal-proximal area.

Distribution

Only known previously from Arraial do Cabo, Rio de Janeiro, Brazil. Now present in Porto Santo and Madeira Island (Calheta and Caniçal), Portugal.

Remarks

The specimens found in Madeira fully match the morphological characteristics given for *Parasmittina alba*. This species was described from Arraial do Cabo in Rio de Janeiro, Brazil (Ramalho et al. 2011)

and had not been recorded elsewhere until now. Some differences are noted between the original material and our specimens; mainly in the figured ovicells (Ramalho et al. 2011: Fig. 2b, h), which have large pores in the ectooecium, but are smaller in our specimens. This difference could be attributed to the variability of this structure and difference in level of calcification, as was observed by us between colonies and even between ovicells in the same colony (Fig. 3f, g, h). Madeira specimens have two symmetrical notches in the distal margin of the orifice in ovicellate zooids (Fig. 3h); this structure was also noted in *P. alba* from Arraial do Cabo (Fig. 2g and h in Ramalho et al. 2011), although comments about this

Table 2 Measurements (in mm) of *Parasmittina alba*

	Mean	SD	Minimum	Maximum	<i>N</i>
Zooidal length	0.488	0.0697	0.390	0.670	24
Zooidal width	0.340	0.0580	0.240	0.460	24
Primary orifice length	0.082	0.0056	0.076	0.101	20
Primary orifice width	0.125	0.0102	0.103	0.143	20
Oval avicularia length	0.092	0.0095	0.074	0.104	9
Oval avicularia width	0.056	0.0037	0.051	0.063	9
Pointed avicularia length	0.078	0.0067	0.068	0.089	10
Pointed avicularia width	0.047	0.0050	0.037	0.054	10
Ooecium length	0.265	0.0177	0.235	0.297	20
Ooecium width	0.267	0.0184	0.233	0.300	20

SD standard deviation, *N* number of measurements

were not included in the original description. Only one avicularium per autozoid is indicated in the original description, while two triangular avicularia were observed in one ovicellate zoid, but this seems very infrequent. In addition, the adventitious avicularia were not seen in our specimens.

This species is very similar to *Parasmittina betamorphaea* Winston 2005, distributed in the Gulf of Mexico and Brazil. But according to Ramalho et al. (2011) *Parasmittina alba* is differentiated by the presence of more than one avicularium per zoid in *P. betamorphaea*, lacking the serrated rostrum in small avicularia, possessing the giant avicularia with a more rounded rostrum, and by the different positioning and shape of the pores in the ovicell. Much of the difference between these species, in the ovicell and avicularia morphology, is attributed to characters that are very variable between colonies or even between zooids in the same colony. In the figures of the original description of *P. betamorphaea*, it is not possible to see more than one avicularium per zoid. Both species, *P. alba* and *P. betamorphaea*, were described from only two colonies, which indicates that more specimens should be studied more carefully, particularly to examine the intra-colonial variability and to evaluate the possibility of the two species being synonyms.

***Parasmittina multiaviculata* sp. nov.**

(Fig. 4, Table 3)

Material examined: Holotype: MMF44616 from Porto Santo; Paratype: MMF44617 from Porto Santo.

Etymology: In reference to the numerous avicularia per zoid.

Description

Colony encrusting, unilaminar or multilaminar, initially translucent. Rectangular to hexagonal autozooids in the first layer and more irregular in the second layers. Frontal wall nodular, with marginal large pores. Orifice longer than wide, surrounded by a normally rectangular (but very variable in morphology, and more rounded in ovicellated zooids)

peristome, laterally more developed, proximally poorly developed and distally interrupted by one (in some cases two) distal spines. This peristome is not visible when the secondary calcification is more developed. Proximal border of the orifice with a variable lyrula, usually short and occupying from one quarter to two third of the proximal border. Condyles stout, sharply pointed. Avicularia monomorphic, oval (lightly pointed when is in direct contact with the margin of the zoid), very numerous in some parts of the colony, but always two or more per zoid. The avicularia occur on the margins of the zooids. Rostrum is slightly spatulate. Ovicell subimmersed, and becoming endozooidal because of the strong secondary calcification. Ooecium formed by the distal autozoid. Ectooecium almost flat frontally, with several irregularly-shaped pseudopores. The secondary calcification covers the distal, lateral and, in some cases, the proximal part of the ectooecium, leaving only a central area with exposed pseudopores. In some ovicells only the peripheral area of the secondary calcification is truly calcified, whereas the rest is membranous, in cleaned specimens showing the ectooecium with pseudopores underneath.

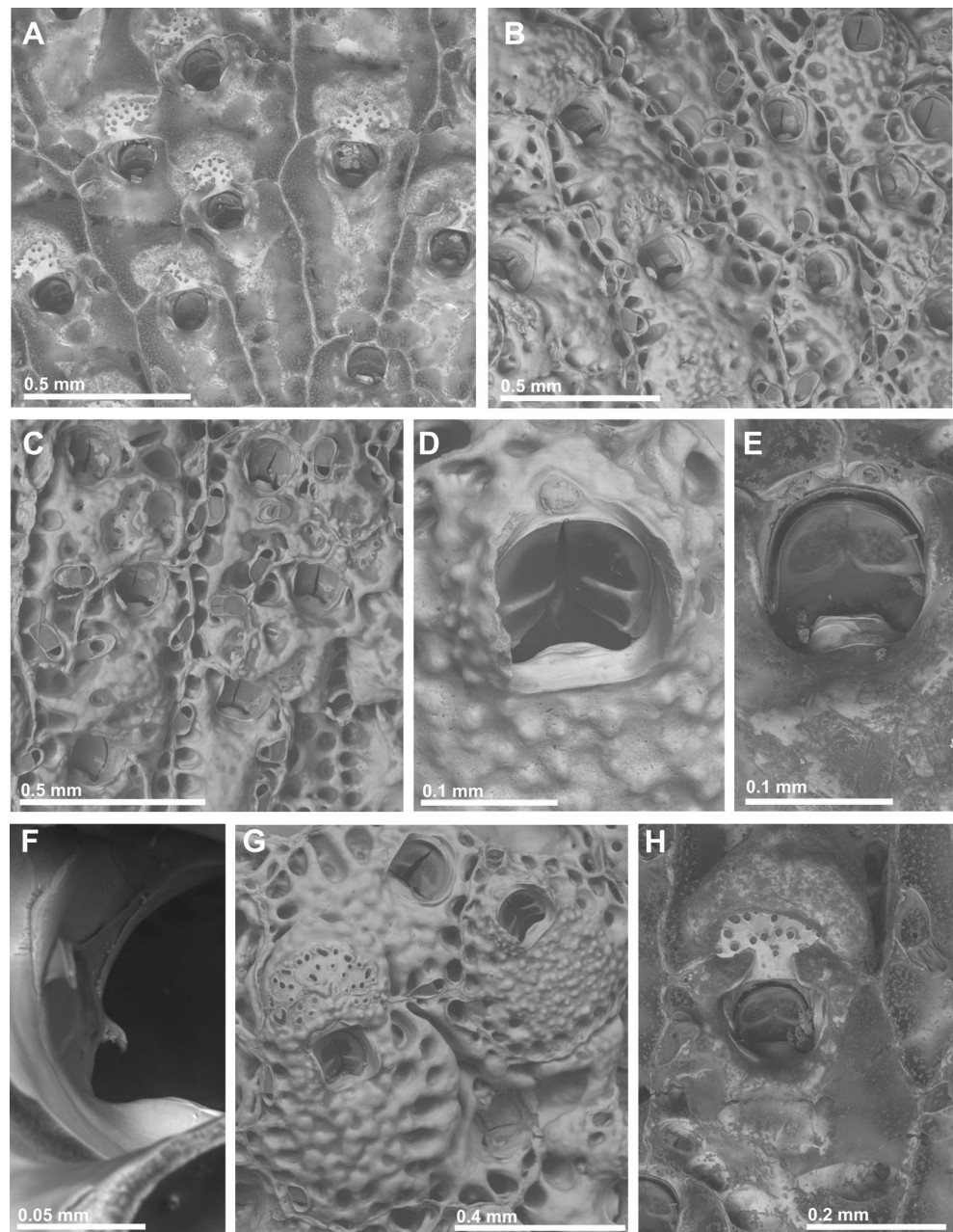
Distribution

Currently only known from Porto Santo Island, Madeira, Portugal.

Remarks

According to the literature, only two species of this genus were previously recorded in Madeira, *Parasmittina trispinosa* (Johnston, 1825) by Waters (1899) and Norman (1909) and *Parasmittina protecta* (Thornely, 1905) by Canning-Clode et al. (2013a). The two colonies reported in the current study show significant differences from these two species and from the previously described species, *P. alba*. According to Harmelin et al. (2009), *P. protecta* presents a peristome interrupted distally, and raised proximally; the orifice is broader than high, and the avicularia are infrequent and when present, they occur in two morphologies and are

Fig. 4 *Parasmittina multiaviculata* sp. nov. **a** General view of the autozooids and oecia in an unbleached specimen. **b** Bleached specimen, showing oecia and abundant frontal avicularia. **c** Ovicellate zooids and frontal avicularia. **d, e** Primary orifice with variations in the lyrula morphology. **f** Condile. **g** Bleached ovicellate and non ovicellate zooids. **h** Ovicellate zooid in unbleached specimen



large. According to the descriptions by Hayward and Ryland (1999) and Soule and Soule (2002), *P. trispinosa* presents an orbicular orifice with a big triangular avicularium lateral to the orifice, which is, in some cases, replaced by a smaller avicularium. Furthermore, the ovicells in *P. trispinosa* are characterized by having few, irregular large pores.

The genus *Parasmittina* is very speciose with currently 122 known species (Bock 2015a; WoRMS 2015), distributed around all oceans, but with a high number of species in the Pacific and Indian Oceans. Among this diversity, there are few species that have an orifice that is longer than wide, monomorphic avicularia, and one or two distal oral spines. Madeira

specimens are similar to *Parasmittina inalienata* Tilbrook 2006, described from the Solomon and Fiji Islands; but differ from that species by having a slightly bigger orifice with a wider lyrula, (Fig. 35D in Tilbrook 2006), less globular ovicells which are almost flat in the frontal plane, and by lacking a round peristome in the ovicellate zooids. The avicularia of *P. inalienata* seem to be numerous with a slightly spatulate rostrum which is always directed proximo-medially or medially, while in the Madeira specimens, the direction of the rostrum is disordered, without a clear pattern, and the rostrum in some cases is slightly pointed.

Most species of this genus (c. 99 species) seem to be natives from the Pacific and Indian Oceans (e.g.,

Table 3 Measurements (in mm) of *Parasmittina multiaviculata* sp. nov.

	Mean	SD	Minimum	Maximum	<i>N</i>
Zooidal length	0.576	0.0719	0.464	0.720	14
Zooidal width	0.366	0.0392	0.307	0.429	14
Primary orifice length	0.110	0.0076	0.093	0.124	14
Primary orifice width	0.081	0.0081	0.079	0.109	14
Avicularia length	0.103	0.0161	0.065	0.130	18
Avicularia width	0.050	0.0082	0.036	0.062	18
Ooecium length	0.218	0.0125	0.198	0.241	18
Ooecium width	0.275	0.0158	0.249	0.297	18

SD standard deviation, *N* number of measurements

Tilbrook et al. 2001; Tilbrook 2006; Soule and Soule 1973, 2002; Gordon 1984, 1989; Hayward 1988; Bock 2015a; Seo 2002; Hayward and Parker 1994), and are particularly abundant in coral reef habitats (Hayward and Parker 1994). Several other *Parasmittina* species identified or recorded from other oceans and seas are considered non-indigenous (e.g., Harmelin et al. 2009). The exclusive presence of *Parasmittina multiaviculata* sp. nov. on artificial plates deployed in harbors in Madeira, and the fact that there are no previous records of similar species in the area, suggests this could be a new introduction. The presence of this species in only one locality may indicate a recent introduction. Its morphological proximity to *P. inalienata* could indicate *Parasmittina multiaviculata* sp. nov. may have a Pacific origin.

Discussion

Knowledge of the Madeira marine invertebrate fauna is scarce and in the case of bryozoans, very limited (Busk 1858a, b, 1859, 1860, 1861; Hincks 1880; Johnson 1897; Waters 1899; Norman 1909). However, in recent years, monitoring surveys for non-indigenous species in the archipelago has resulted in new records and undescribed species in the islands (d'Hondt 1985; Alves and Cocito 2002; Berning and Kukliński 2008; Berning et al. 2008; Wirtz and Canning-Clode 2009; Berning 2012; Souto et al. 2014, 2015; Ramalhosa et al. 2016). Nevertheless, the lack of a comprehensive checklist of bryozoans in Madeira makes the detection and dating of new anthropogenic introductions a complex challenge. Due to the sessile mode of life of their adults and the estimated short-lived non-feeding pelagic phase of their larvae, the presence of the three species reported here in Madeiran waters is difficult to explain by natural dispersion methods, so anthropogenic vectors seem more reasonable. The presence in other substrates different from the settlement panels (natural or artificial) was not checked, so no inferences can be made regarding

the extent by which these species have established themselves in the natural environment.

The natural fauna of Madeira shows close affinities with the fauna of Lusitania, Mediterranean, and Mauritanian regions (Wirtz 1998); nevertheless, the three species of bryozoans found here show closer affinities with species from the Pacific (*C. inaudita*, *P. multiaviculata* sp. nov.) and the Caribbean (*Palba*). Several Brazilian and Caribbean species of macroinvertebrates were detected in recent years in Madeiran waters (e.g., Wirtz and Canning-Clode 2009; Canning-Clode et al. 2013a, b; Ramalhosa et al. 2014; Souto et al. 2015). *Beania maxilladentata* Ramalho, Muricy and Taylor 2008, a bryozoan only previously known from Brazil (Ramalho et al. 2008; Vieira et al. 2010), was also found in Madeiran waters (Souto et al. 2015), but in this case, only on natural substrates. In addition, several other species with West Atlantic distributions, mainly from the Gulf of Mexico, were also recently found in Madeira (Canning-Clode et al. 2013a; Ramalhosa et al. 2014) such as the bryozoans *Schizoporella pungens* (Canu and Bassler 1928), *Cradoscrupocellaria bertholetti* (Audouin 1826), *Amathia verticillata* (Delle Chiaje 1822), the ascidian *Distaplia corolla* Monniot F. 1974 and the sabellid polychaete *Branchiomma bairdi* (McIntosh 1885). Also a few species from the Indo-Pacific region were detected (Norman 1909; Canning-Clode et al. 2013a; Ramalhosa et al. 2014; Ramalhosa and Canning-Clode 2015), but most of these species appeared first in European waters (e.g., Cabezas et al. 2015; Arias et al. 2013; Cinar 2009; Giangrande et al. 2013; Garcia Raso 1981; Turon et al. 2005; Ryland et al. 2009, 2011). Nevertheless, none of the three species reported in the current paper were recorded in other areas close to Madeira. Of them, only *Celleporaria inaudita* was recently found in the Red Sea, after its original description in the Pacific Ocean. The direct introduction of these species from their original localities to Madeira seems not very plausible. It is possible that these species have a broader distribution than currently known, particularly in European and North African waters, and have been probably introduced to Madeira via hull fouling (recreational

and/or cruise vessels). Currently, most shipping can be attributed to tourist cruise ships and sailing yachts visiting for refuel and rest stops from different parts of the world (APRAM 2014). Furthermore, recent findings by Ramalhosa et al. (2016) have shown that dry dock inspections on local and foreign recreational vessels support the hypothesis from other studies, that emphasize hull fouling as the key introduction vector that contribute to the arrival and spread of NIS to neighbouring islands. Moreover, the genus *Parasmittina* is a very speciose genus with currently 122 known species (Bock 2015a; WoRMS 2015) and 17 fossil species. Similar diversity is present in the *Celleporaria* genus, with 90 species (Bock 2015b). This large number of species in these genera makes correct identification challenging, possibly resulting in some species being misidentified and lumped together. So records in other parts of the world of these species could go unnoticed.

Here, three species of bryozoans are added to the nine other non-indigenous bryozoan species known from the Madeira archipelago (Wirtz and Canning-Clode 2009; Canning-Clode et al. 2013a; Ramalhosa et al. 2016). Currently, the xenodiversity in Madeira Island is probably underestimated, and therefore this number is likely to increase in the next years.

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