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Millepora alcicornis (Hydrozoa: Capitata) at Ascension Island: confirmed identity based on morphological and molecular analyses

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The reef-building fire coral *Millepora alcicornis* is reported from Ascension Island (South Atlantic). This record, based on a recent expedition to Ascension, is the first in which the identification of a *Millepora* coral is supported by photographic evidence from the field and by morphological and molecular analyses of collected specimens. This finding is discussed in relation to earlier *Millepora* records from Ascension and the biogeographic range of this particular species in the Atlantic.

Keywords: fire coral, distribution range, isolation, morphology, molecular analysis, South Atlantic

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INTRODUCTION

Millepora is a hydrozoan genus of so-called 'fire corals', which are well known for inflicting painful stings to humans and for being important reef-builders because of their large calcareous skeletons (Lewis, 2006). The distribution of this genus is limited to 50 m depth in tropical seas (Lewis, 1989, 2006; Cairns *et al.*, 1999), with a clear distinction between species in the Atlantic (Amaral *et al.*, 2002, 2008) and the Indo-Pacific (Razak & Hoeksema, 2003). There are seven recognized species in the Atlantic Ocean, which can be distinguished by their growth form, pore sizes and pore patterns (Boschma, 1948; de Weerdt, 1984; Amaral *et al.*, 2008). *Millepora alcicornis* Linnaeus, 1758 is the only species recorded from across the Atlantic, while three other species are only found in the Caribbean (*M. complanata* Lamarck, 1816, *M. squarrosa* Lamarck, 1816 and *M. striata* Duchassaing & Michelotti, 1864) and three are endemic to Brazil (*M. braziliensis* Verrill, 1868, *M. laboreli* Amaral, 2008 and *M. nitida* Verrill, 1868).

The existence of *Millepora* at Ascension Island was first mentioned by Irving (2013), who stated that both *Millepora alcicornis* and *M. complanata* occur 'in small patches of open area of bedrock' from 8 to 15 m depth. Although he reported that specimens were collected during the Operation Origin Expedition in 1985, he did not mention their exact locality (Irving, 2013: table 1) and how they were identified. His observations took place over the course of numerous dives, down to a depth of 45 m, reported on in an earlier publication (Irving, 1989), which at the time did not indicate the presence of *Millepora*. So far, no photographic records were known of *Millepora* at Ascension and no published reports were available on museum specimens, despite the presence of a sample in the Natural History Museum in London (BMNH 1972.9.1.2) from English Bay, which was donated by the Ascension Historical Society and identified as *Millepora alcicornis* by Solene Whybrow (H. Zibrowius, personal communication).

As *Millepora* corals are notoriously difficult to identify (Boschma, 1948; Stearn & Riding, 1973; Amaral *et al.*, 2002; Clemente *et al.*, 2011; Brown & Edmunds, 2013), colonies were photographed and a sample for genetic analysis was taken during a recent expedition to Ascension Island. This evidence is used in the present study to supply conclusive information on the identity of this *Millepora* species as part of Ascension's shallow-water fauna.

MATERIALS AND METHODS

During an expedition to Ascension Island in August/September 2012, coral specimens were photographed *in situ* and collected by Dr Peter Wirtz during scuba-diving in a depth range of 0 to 25 m (Zibrowius *et al.*, 2014).

Material

RMNH Coel. 40167 from Red Rock, near English Bay (07°53.654′S 14°23.676′W), 8 m depth, 6 September 2012: 8 small fragments (~1 cm in size) in 96% ethanol. Additional material from Ascension was available for morphological study: BMNH 1972.9.1.2 from English Bay (07°53′S 14°23′W), 1971: 4 dry specimens.

DNA was extracted from specimens preserved in ethanol using the DNeasy Blood and Tissue kit (Qiagen). The 16S subunit of the ribosomal RNA was amplified by polymerase

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chain reaction using published primers (Cunningham & Buss, 1993) and sequenced on an ABI 3130XL gene analyser. The obtained sequences were compared with 16S sequences from *M. alcicornis* from the North and East Atlantic (Bermuda, Colombia, Florida, Panama and Cape Verde Islands) (N = 10), *M. braziliensis* (N = 10), *M. laboreli* (N = 9) and *M. nitida* (N = 10) (de Souza, 2013), and *Millepora* sp. fragments from the Canary Islands, Tenerife, Poris de Abona, 6 m, 26 September 2010, don. A. Brito, RMNH Coel. 39907 (N = 1). Pairwise genetic distance was calculated between the Ascension specimen and the 40 *Millepora* specimens using MEGA v5.2 in order to identify the Ascension specimens to the most similar species. The sequences have been deposited in GenBank (*M. alcicornis* Ascension Island MA KF871426; *M alcicornis* Tenerife MT1 KF871427).

Abbreviations: BMNH, The Natural History Museum, London, formerly British Museum (Natural History); RMNH, Naturalis Biodiversity Center, Leiden, formerly Rijksmuseum van Natuurlijke Historie.

RESULTS

Habitat

So far *Millepora* corals observed and sampled at Ascension (RMNH Coel 40167, Figure 1; BMNH 1972.9.1.2, Figure 2) were only found in English Bay and Red Rock at the north side of the island (for maps, see Price & John, 1980: figure 2; Irving, 2013: figure 16.1). The underwater landscape of English Bay has been described as an area consisting of massive outcrops of bedrock with terraces, cliffs, small caves and underhangs (Irving, 2013).

Morphology

The fragments collected in September 2012 consisted of tiny branch tips, which are unsuitable for identification based on pore patterns (De Weerdt, 1984). The coral colonies themselves, like the one from which the fragments were taken (Figure 1), are large encrusting plates with short, finger-like branches that may be laterally compressed at their tips, a growth pattern typical for *M. alcicornis* (Boschma, 1948; De Weerdt, 1984). A similar growth form is shown by *Millepora* specimens that were recently discovered at Tenerife (Clemente *et al.*, 2011; RMNH Coel. 39907). The BMNH specimens are 2–11 cm in length and show a low density of relatively small dactylopores (Figure 2), which is typical for *M. alcicornis* (see De Weerdt, 1984). Owing to their phenotypic variation, identification of milleporids based on morphological criteria is most reliable when a combination of characters is used (De Weerdt, 1984). Sizes of dactylopores (0.05–0.15 mm) and gastropores (0.15–0.25) in *M. alcicornis* are similar to those in *M. striata* but the density of dactylopores in the former (roughly 50–200 cm^{-2}) is much lower than in the latter (300–500 cm^{-2}) and similar to the density found in *M. complanata*, while the density of gastropores is less useful as a diagnostic character (De Weerdt, 1984). Specimens from Ascension (BMNH, Figure 2) show a low density (~150 cm^{-2}) of the small dactylopores (~0.10 mm), whereas its gastropores are twice as large in diameter (~0.20 mm), which agree with data known from *M. alcicornis* (De Weerdt, 1984). Ampullae, organs in which *Millepora* medusae are kept (see Lewis, 1991), were not present in the material from Ascension.

Molecular genetics

The average pairwise genetic distance between the *Millepora* specimen collected in Ascension Island and individuals of *M. alcicornis*, *M. braziliensis*, *M. nitida* and *M. laboreli* was found to be 0.0062 ± 0.0042, 0.0786 ± 0.0015, 0.0830 ± 0 and 0.0906 ± 0.0039 respectively, while for the *Millepora* specimen from Canary Islands the average pairwise distances

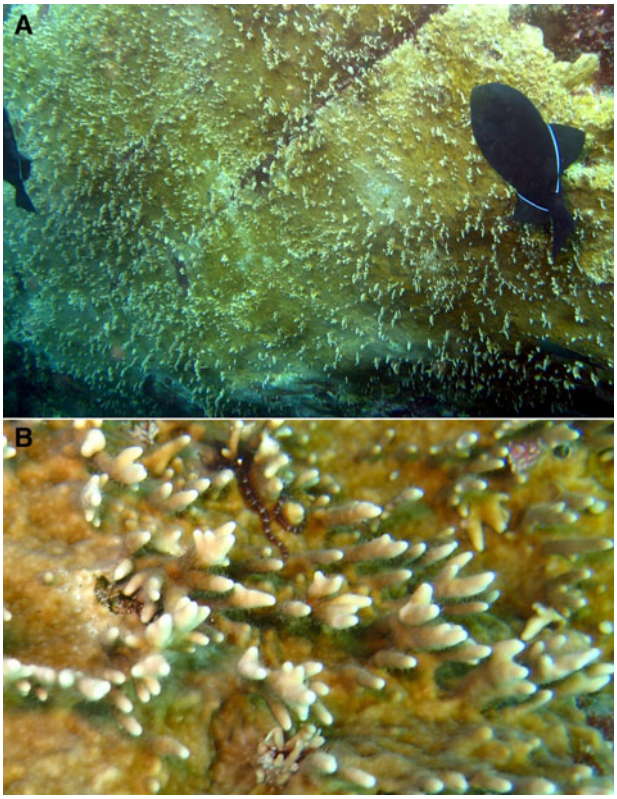

Fig. 1. *Millepora alcicornis* colony in about 8 m depth in English Bay, Ascension Island. (A) The black trigger fish (*Melichthys niger*) in the photo is about 25 cm long. (B) Close-up. Photos by P. Wirtz.

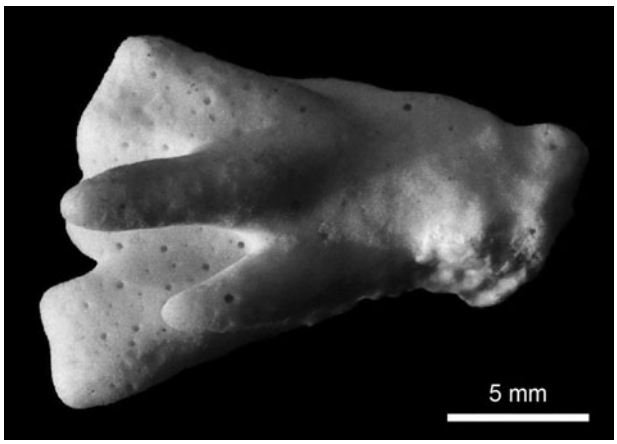

Fig. 2. Small branch of *Millepora alcicornis* from English Bay (BMNH 1972.9.1.2), showing regeneration at its base. The pores are relatively large in comparison to other Atlantic *Millepora* species. Photo by H. Zibrowius.

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were 0.0081 ± 0.0016, 0.0759 ± 0.0019, 0.0760 ± 0, 0.0870 ± 0.0022. The genetic distance between the *Millepora* specimens from Ascension and Canary Islands was 0.009. Genetic distance is approximately one order of magnitude lower for *M. alcicornis*, compared with the other three species for both specimens from Ascension and Canary Islands. The most similar sequences (one *M. alcicornis* individual from Cape Verde and one from Florida) had only one mutational difference from the Ascension specimen. The molecular data confirm the identification of the Ascension and Canary Island specimens as that of *M. alcicornis*.

Conclusion

Although morphological characters are very variable in *Millepora* species, based on a combination of branch form, pore density and poor size, the *Millepora* species at Ascension is most likely *M. alcicornis*. This finding is supported by molecular evidence.

DISCUSSION

Occurrence

This second published record of *Millepora* from Ascension Island, after Irving's (2013), is the first with evidence from the field. Both records are remarkably recent while the BMNH specimens were already collected in 1971, confirming that natural history collections may be important as sources for earlier species records and the reconstruction of previous fauna compositions (Rainbow, 2009; Luttikhuizen & Dekker, 2010; Hoeksema *et al.*, 2011; Hoeksema & Wirtz, 2013). An explanation for these scarce records may be that *Millepora* has a very restricted distribution at the northernmost tip of Ascension Island, or because most previous submarine sampling took place in shallow water, especially in the intertidal zone (compare Price & John, 1980; Reimer *et al.*, 2014; Zibrowius *et al.*, 2014). English Bay is among the most protected sites to wave exposure in Ascension (Irving, 2013). It is possible that hydrodynamic turbulence and wave action in other localities prevent the settlement of *Millepora* in shallow water.

Identity

Based on morphological and genetic analyses, all specimens in the present study were identified as *Millepora alcicornis*. The earlier record of *M. complanata* (Irving, 2013) could not be confirmed. In the field, specimens of both species may be hard to separate (Stearn & Riding, 1973; Brown & Edmunds, 2013). Evidence suggests that they may form a 'species complex' (Ruiz-Ramos *et al.*, 2014).

Morphology

The growth form of *Millepora alcicornis* at Ascension predominantly consists of large crusts with small branches. Although *M. alcicornis* is generally known to show much ecophenotypic variation, this encrusting shape is relatively most common at shallow depths and on vertical rock surfaces, where it helps to resist wave action (Stearn & Riding, 1973; De Weerdt, 1981; Edmunds, 1999).

Distribution range

Millepora alcicornis is the only *Millepora* species with an amphi-Atlantic distribution range. *Millepora* spp. are generally known as infrequent breeders and may apply fragmentation as an asexual reproduction strategy (Lewis, 1989, 2006; Edmunds, 1999), favouring short-distance dispersal, which may explain why most Atlantic *Millepora* species have limited, western Atlantic ranges or very local distributions, as shown by Brazilian endemics (Amaral *et al.*, 2008). In contrast, most Indo-Pacific *Millepora* species have wide ranges (Boschma, 1948; Razak & Hoeksema, 2003). It is unclear why *M. alcicornis* has such a wide range, including not only Ascension but also the Cape Verde Islands (Boekschoten & Best, 1988) and the Canary Islands (present results), from where it was reported as recently introduced (Clemente *et al.*, 2011). Although *M. alcicornis* has been recorded from very shallow depths (Lewis, 2006), it may be rare in the intertidal zone (Stearn & Riding, 1973). Since *Millepora* corals are able to settle as crusts on various substrates (De Weerdt, 1981; Lewis, 2006), species that would be able to survive well in shallow water could also be successful as rafters (Hoeksema *et al.*, 2012). Comparative studies on the reproduction, dispersal, settlement and phylogeography of the various *Millepora* species are required (cf. Nunes *et al.*, 2011) in order to clarify the occurrence of *M. alcicornis* as a single *Millepora* species at Ascension and islands in the eastern Atlantic.

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