

# Occurrence of *Isarachnanthus* (Cnidaria: Anthozoa: Ceriantharia) at Ascension Island: a test of hypothesis

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*A theory that an ancient population of Isarachnanthus from the eastern Atlantic originated the current species is discussed. Based on analysis of DNA barcoding and cnidome it was possible to identify some specimens from Ascension Island. These specimens were identified as Isarachnanthus maderensis, since the divergence of DNA barcoding was very small and the cnidome data agreed with published data. This material permitted an enhanced discussion about the origin of this population.*

**Keywords:** biogeography, ocean currents, taxonomy, DNA barcoding

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## INTRODUCTION

Ceriantharia taxonomy has been an issue since early studies in the 19th Century (e.g. Haimé, 1854). Many of the characters show clear phenotypic plasticity, and almost nothing is known of this variation within this species (see van Beneden, 1924). Ceriantharia is a subclass within the class Anthozoa (Stampar *et al.*, 2014). The use of DNA barcoding (5' end of mtDNA cytochrome c oxidase I (COI)) methods in this class would be virtually impossible with the traditional approaches due to the low variability of mitochondrial DNA between species (Huang *et al.*, 2008). However this profile is completely different in Ceriantharia compared to other class members. Mitochondrial DNA shows similar substitution rates to those found in other animal groups (e.g. Medusozoa and/or Bilaterian groups), thus the use of classical methods of DNA bar coding is quite possible (see more in Stampar *et al.*, 2012, 2014).

*Isarachnanthus* Carlgren, 1924 is a genus of tube forming anemones within the order Ceriantharia which extend their tentacles only during the night (Stampar *et al.*, 2012). Unlike many anthozoan taxa, some ceriantharian species have larval stages with long planktonic lifespans. These planktonic forms confounded earlier researchers who classified them as a group of jellyfish that belong to the other subphylum, Medusozoa (see Rodriguez *et al.*, 2011). The genus *Isarachnanthus* consists of four species: *I. bandanensis* (Carlgren, 1924); *I. maderensis* (Johnson, 1861); *I. nocturnus* (den Hartog, 1977); and *I. panamensis* (Carlgren, 1924). A hypothesis about the origin of Pacific and West Atlantic species was raised by Stampar *et al.* (2012). The hypothesis stated that the ancestral species originated from the east

coast of the Atlantic. This could be verified with the unique occurrence of the species *Isarachnanthus maderensis* on the coast of Africa. However, the sub-Saharan African coast still remains a large gap in information, and large expeditions have only sampled in some parts of that region (e.g. Zibrowius & Gili, 1990). We had access to some specimens of *Isarachnanthus* obtained from the oceanic Ascension Island (Figure 1). This record is important because it is an area with no information about Ceriantharia, and the island is under the influence of African ocean currents (see Peterson & Stramma, 1991). The individuals were analysed for specific identification and the results discussed in relation to the data available from recent literature (Stampar *et al.*, 2012).

## MATERIALS AND METHODS

Ascension Island is an isolated volcanic island in equatorial waters of the South Atlantic Ocean, around 1600 km from the coast of Africa and 2250 km from the coast of South America. Two specimens of *Isarachnanthus* were collected around the island in September 2011 (MZUSP 001947/001948 and GENBANK KJ469802/KJ46980, respectively). The two specimens were preserved in formalin, but tentacle samples were preserved in ethanol. To study the specimens we followed the methods described by Stampar *et al.* (2012, 2014). The barcoding region (COI) was sequenced (535–639 bp) and then compared with data provided by the same authors. Kimura's two-parameter model of base substitution was used to calculate genetic distances in MEGA5 software (Tamura *et al.*, 2011). The maximum likelihood phylogenetic analysis was conducted via RAxML (500 replicates) (random accelerated maximum likelihood analysis) with general time reversible model and gamma rate heterogeneity (GTR + GAMMA) (Stamatakis *et al.*, 2008). To evaluate nodal support and especially to detect if

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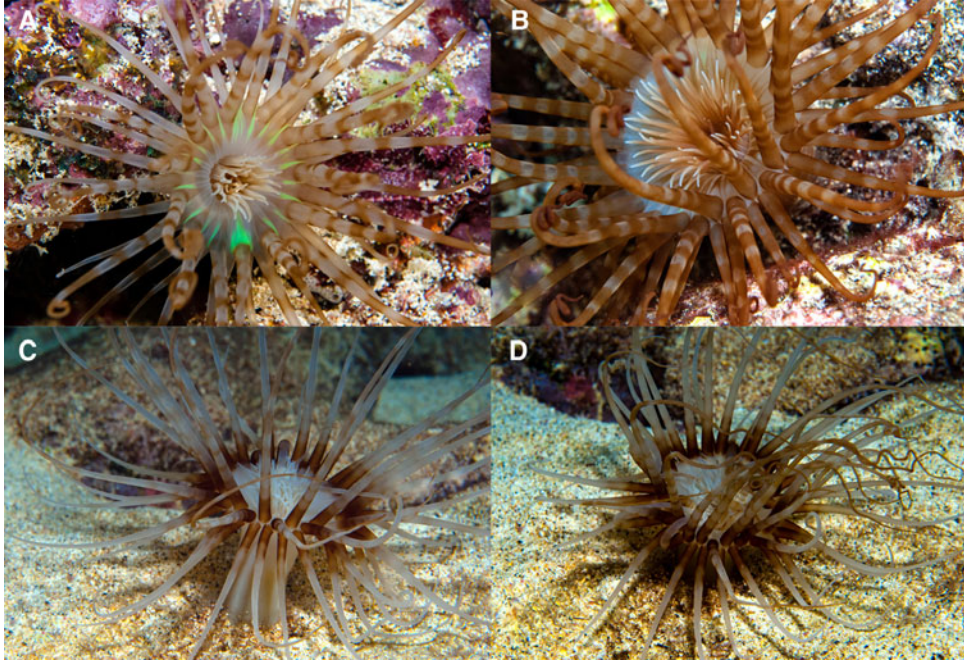


Fig. 1. *In situ* photographs of specimens of *Isarachnanthus maderensis* from Ascension Island: (A, B) oral view; (C, D) side view (images: Shallow Marine Surveys Group).

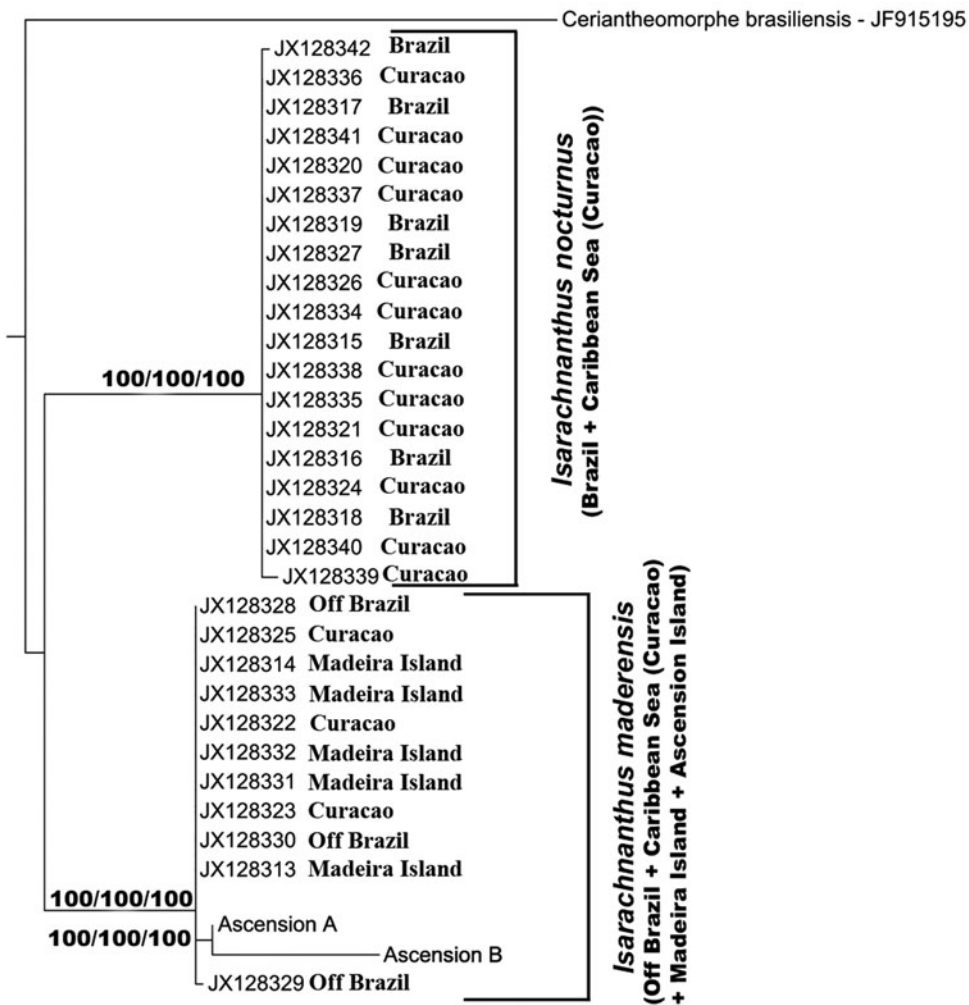


Fig. 2. Phylogenetic reconstruction (maximum likelihood) of the analysed specimens of *Isarachnanthus* using the mitochondrial marker COI. Numbers on the branches represent the estimated values of maximum likelihood (bootstrap)/SH-aLRT/aLRT. Ascension A and B are the specimens used for the analysis.

**Table 1.** Cnidome of the studied specimens of *Isarachnanthus maderensis* from Ascension Island (N = 2 specimens × 30 cnidae).

<i>Isarachnanthus maderensis</i> (N = 2 × 30)	
Marginal tentacles	
Atrich	53.5 (51.3–56) × 7.4 (6.3–8.1)
Microb. B-mastigophore 1	15.10 (11–17.1) × 3.4 (3–3.6)
Microb. B-mastigophore 2	103.1 (99.7–112) × 25.5 (24–28.8)
Microb. B-mastigophore 2	52.20 (50–58.5) × 7.5 (7.2–7.8)
Microb. P-mastigophore	38.12 (36–42) × 4.44 (4.2–5.4)
Labial tentacles	
Atrich	46.7 (43.2–54) × 7.14 (6.6–8.2)
Microb. B-mastigophore 1	37.7 (35.5–39) × 5.5 (5.4–6.1)
Microb. B-mastigophore 2	75.09 (72–79.2) × 15.8 (14.3–16.9)
Microb. B-mastigophore 2	53.58 (48–59.4) × 8.52 (7.2–9.6)
Microb. P-mastigophore	33.66 (30–38.4) × 5.64 (5.4–6)
Stomodeum	
Atrich	48.10 (43.2–52.8) × 7.08 (6.6–7.8)
Microb. B-mastigophore 1	38.56 (36–41.2) × 5.70 (5.4–6.65)
Microb. B-mastigophore 2	74.15 (73–77.8) × 15 (14.2–16)
Microb. B-mastigophore 2	53.5 (49–57) × 8.52 (7.2–9.6)
Microb. P-mastigophore	33.5 (31–37) × 5.5 (4.6–6)
Column	
Atrich b	35.5 (34–40) × 6.2 (5.1–7.2)
Pticocysts	42.50 (36–54) × 11.25 (9.6–12.6)
Microb. B-mastigophore 1	22.8 (20.4–25.2) × 5.82 (5.4–6.6)
Microb. B-mastigophore 2	91.2 (83–96) × 22.25 (18.9–25.2)
Microb. B-mastigophore 2	78.3 (72–82) × 14.16 (12–15.6)
Microb. P-mastigophore	22.8 (20.4–25.2) × 5.82 (5.4–6.6)
M-mesenteries (double cord)	
Atrich	36.24 (31.2–42) × 6.06 (6–6.6)
Microb. B-mastigophore 2	87.06 (83.4–92.4) × 23.82 (21.6–27)
Microb. B-mastigophore 2	77.94 (72–84) × 14.04 (12.6–16.2)
Microb. B-mastigophore 2	18.6 (16.8–21) × 6.9 (6.6–7.8)
Microb. P-mastigophore	40.14 (37.2–42.6) × 6.18 (6–6.6)
B-mesenteries (simple cord)	
Microb. B-mastigophore 1	20.80 (18.4–24) × 6.5 (6–6.9)
Microb. B-mastigophore 2	18.10 (16.1–19.6) × 6.6 (6–7.9)
Acontiods	
Atrich	41.23 (39–47.1) × 7.8 (7.2–9)
Microb. B-mastigophore 1	18.62 (14.4–21) × 4.62 (3.6–5.4)
Microb. B-mastigophore 1	19.20 (17.8–21) × 7.44 (7.2–8.4)
Microb. B-mastigophore 2	85.80 (81–92.4) × 25.5 (24–27.6)

support values were positively/negatively biased, parametric (aLRT) and non-parametric (SH-aLRT) tests were applied (Anisimova & Gascuel, 2006; Anisimova *et al.*, 2011). Bootstrap values were computed on RAxML v.7.3.2 (500 pseudoreplicates, same parameters as the original phylogenetic analysis) and other statistical tests were calculated using PhyML v.3.0.1 (Guindon *et al.*, 2010; Anisimova *et al.*, 2011). At the same time the measurements of cnidae were compared with those of specimens from type locations of *I. maderensis* and *I. nocturnus* (as presented by Stampar *et al.*, 2012).

## RESULTS

Based on our analyses (morphology, cnidae and barcode) the specimens from Ascension Island belong to the species *Isarachnanthus maderensis* (Figure 1). The barcoding data show that the K2P divergence is 0.02–0.07% between specimens from Ascension Island and Madeira Island + Caribbean Sea + off Brazil (Rocas Atoll) but 9–9.6% in comparison with Brazilian + Caribbean Sea specimens of *Isarachnanthus nocturnus*. Maximum likelihood analyses shows that specimens from

Ascension Island are grouped with other specimens of *Isarachnanthus maderensis* with very consistent support values (Figure 2).

The cnidome data from Ascension Island specimens (Table 1) also confirm the similarities with specimens from Madeira Island and off Brazil (Rocas Atoll). The measurements of cnidae capsules of Ascension Island specimens are in the same range as *Isarachnanthus maderensis*. Furthermore, the most relevant data are the presence of three types of microbasic b-mastigophores on the stomodeum and the column, and the presence of microbasic p-mastigophores on the column. This pattern was only observed in specimens of *Isarachnanthus maderensis* (see data from Stampar *et al.*, 2012).

## DISCUSSION

The finding of *Isarachnanthus maderensis* specimens on Ascension Island provides good evidence in support of the hypothesis that the species *Isarachnanthus nocturnus* is restricted to the west coast of the Atlantic Ocean, with *Isarachnanthus maderensis* only occurring on the east coast in the South Atlantic Ocean, as proposed by Stampar *et al.*



Fig. 3. Distribution pattern/area of *Isarachnanthus maderensis* in Atlantic Ocean (A).

(2012). *Isarachnanthus* specimens that are driven from the African coast by the South Equatorial Current must be exclusively 'maderensis' species (Figure 3). Ascension Island lies exactly under the influence of the South Equatorial Current (Dunbar, 1984) and probably receives a large amount of larvae derived from African coasts (McCartney *et al.*, 2000). The genus addressed in this study presents planktonic larvae that can be carried by sea currents (Rodriguez *et al.*, 2011). Unfortunately there are no data on the life cycle of this genus, except on the existence of a planktonic larva.

This study highlights the importance of investigating species distribution, especially considering remote islands. Geographical records on somewhat isolated places are necessary for a better understanding of evolutionary processes, particularly in testing theories already proposed, and in the subsequent understanding of marine populations. Furthermore, this study highlighted that the use of DNA barcoding in Ceriantharia, proposed by Stampar *et al.* (2012, 2014) is completely applicable and should be adopted by researchers.

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