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ARTICLE

DNA Barcoding revealing the occurrence of *Isarachnanthus* (Cnidaria; Anthozoa; Ceriantharia) in Cape Verde

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Abstract. The occurrence of *Isarachnanthus* Carlgren, 1924 (Cnidaria: Anthozoa: Ceriantharia) specimens in Cape Verde Islands is recorded. Identification of the tube anemone species *Isarachnanthus maderensis* (Johnson, 1861) was possible based on DNA Barcoding. A discussion on biogeographic patterns associated with ocean circulation and life cycle is presented.

Key-Words. Taxonomy; Biogeography; Marine Invertebrates.

INTRODUCTION

The genera *Arachnanthus* Carlgren, 1912 and *Isarachnanthus* Carlgren, 1924 (Cnidaria: Anthozoa: Ceriantharia) correspond to the only ones with described adult forms of the family Arachnactidae (den Hartog, 1977; Stampar *et al.*, 2016). This family is distinguished from the family Cerianthidae (most of the currently valid species; cf. Stampar *et al.*, 2016) mostly due to the presence of planktonic larvae with long prevalence in the water column (Stampar *et al.*, 2015). This peculiarity of the life cycle resulted in complex biogeographic patterns, since the dispersion based on this larval type seems to be quite effective (Stampar *et al.*, 2012, 2015). Recently, Stampar *et al.* (2012) demonstrated that the distribution pattern for the genus *Isarachnanthus* in the Atlantic maintains the gene flow due to marine currents. The finding of specimens at the Ascension Island (Stampar & Morandini, 2017) demonstrated that the scenario proposed seems correct. However, the data available for the Eastern Atlantic records come from very distant areas, Madeira Island and Ascension Island (more than 4,500 km away). Thus, records in between those extreme regions are quite important to further test the scenario proposed. Consequently, this study presents occurrence data of the genus at the Cape Verde islands and discuss the relevance of biogeographic patterns based on life cycle aspects.

MATERIAL AND METHODS

Two specimens of *Isarachnanthus* (Fig. 1) were sampled by SCUBA diving during night time in Santo Antão Island, Porto Novo harbour, nine meters depth, 11 November 2014 (1) and Tarrafal, Santiago Island, 27 October 2015 (2). Each animal was directly preserved in Ethanol 95%. These specimens were studied following the methods described by Stampar *et al.* (2012, 2014) and Rach *et al.* (2017). The barcoding region (COI) was sequenced using Folmer *et al.* (1994) primers (619 bp after assembly) (GenBank MK904564 and MK904565) and then compared with data of Stampar *et al.* (2012) and Stampar & Morandini (2017). The voucher is deposited at Museu de Zoologia (USP) – MZUSP (MZSP 8468). P-distance model of base substitution was used to calculate genetic distances in MEGA7 software (Kumar *et al.*, 2016). The maximum likelihood phylogenetic analysis was conducted via MEGA7 (500 replicates) with general time reversible model (GTR) (Kumar *et al.*, 2016).

RESULTS

The sequences obtained were compared with the dataset available for the genus (after Stampar *et al.*, 2012; Stampar & Morandini, 2017). The first

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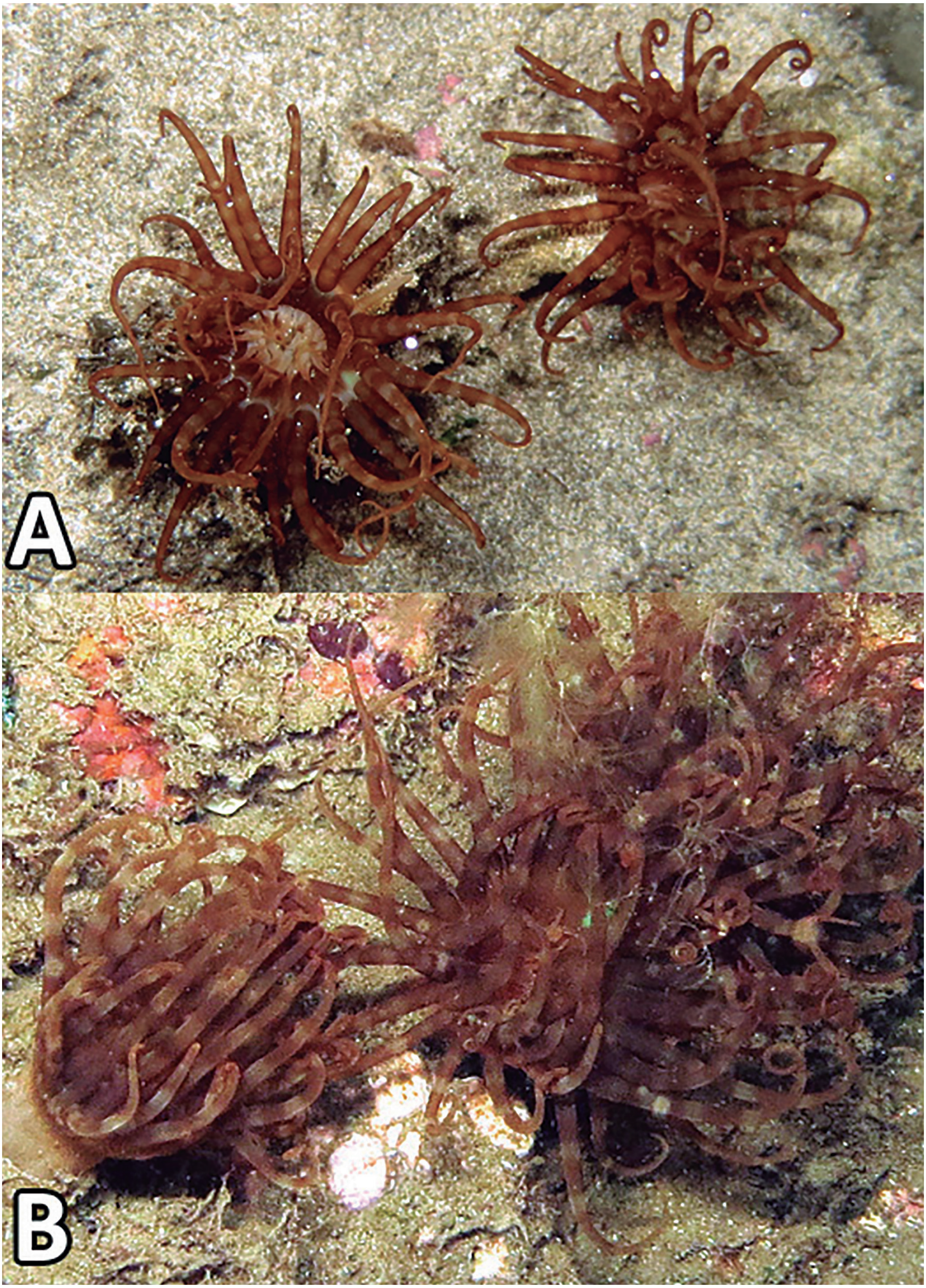

Figure 1. Specimens of *Isarachnanthus maderensis* (Johnson, 1861) in Santo Antão Island, Porto Novo harbor (A) and Tarrafal, Santiago Island (B). (Diameter around 3 cm). Photos: Peter Wirtz.

approach was a phylogenetic reconstruction by maximum likelihood, which indicated two monophyletic clades (Fig. 2). The specimens obtained from Cape Verde were clustered among the specimens delimited in the clade of the species *Isarachnanthus maderensis* (Johnson, 1861). The estimated genetic distance (p-value) indicates that there is no variation in the DNA Barcoding sector between the Cape Verde, Madeira and Curaçao specimens (Table 1). At the same time, the variation for the Ascension Island specimens was not constant, for one specimen there was practically no variation, but for the other the variation was almost 8%. The distance to the other species, *Isarachnanthus nocturnus* (Hartog, 1977), was always constant between 7 and 8%. At the end, the DNA Barcode results indicate that the Cape Verdean specimens are *Isarachnanthus maderensis.*

DISCUSSION

This first record of the genus *Isarachnanthus* in Cape Verde based on molecular data is very relevant because it presents information from an area closer to the coast of the African continent. Stampar & Morandini (2017) inferred that the coast line of the African continent should be inhabited only by the species *Isarachnanthus maderensis,* if the proposed evolutionary scenario is correct (Stampar *et al.,* 2012). In this way, the present data maintain the 2012's proposal valid and still throw some light on the possible reproductive dynamics in this region. Some records have indicated the occurrence of *Isarachnanthus nocturnus* in the Eastern Atlantic (*e.g.,* Bianchi *et al.,* 2000), but results presented in this study and in Stampar & Morandini (2017) indicate that this occurrence is not likely – further investigation on these specimens should be conducted both morphological and molecularly.

The distribution of the studied specimens from Cape Verde, Madeira and Curaçao (Fig. 3) are under the influence of the North Atlantic Gyre currents and this can be demonstrated by the absence of genetic distance between these individuals (Table 1). This same pattern can be recognized in the other species of *Isarachnanthus* on the coast of South America and the Caribbean, where

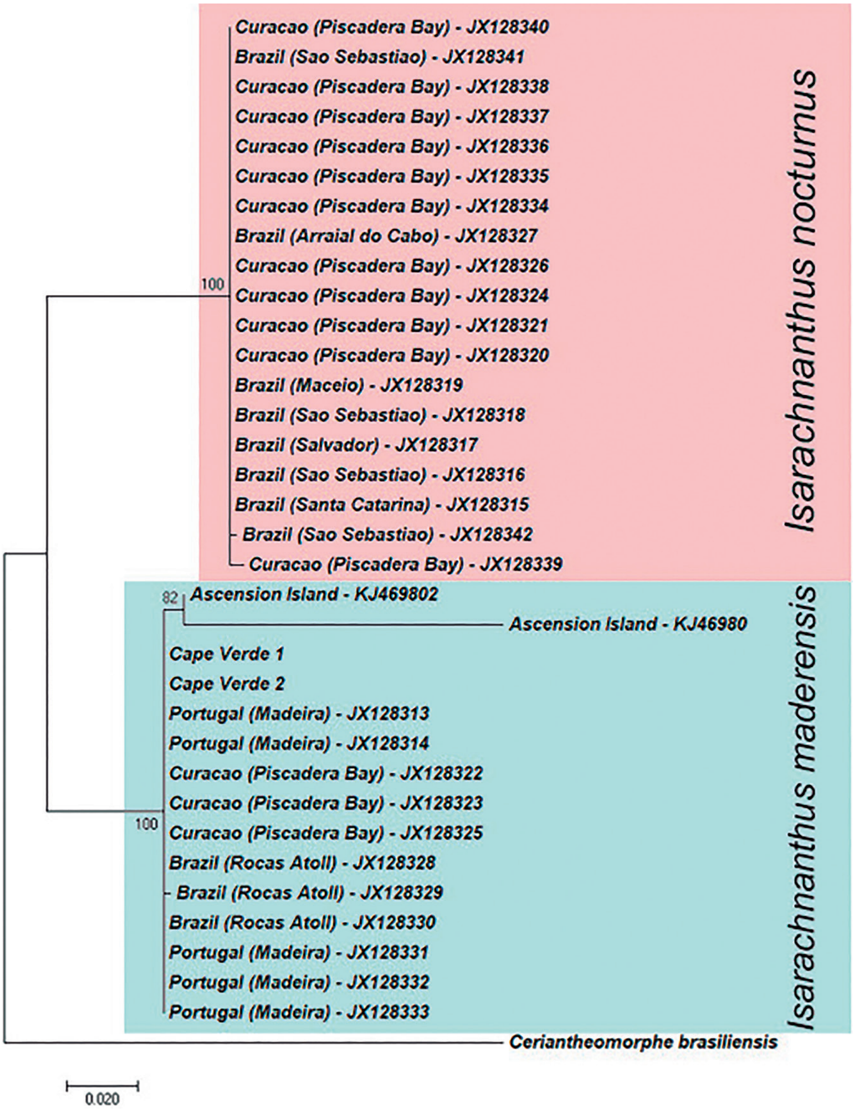

Figure 2. Evolutionary reconstruction of *Isarachnanthus* Carlgren, 1924 by Maximum Likelihood method based on the General Time Reversible model, likelihood (-1410.7055) with 523 positions in the final dataset.

Table 1. Estimates of evolutionary divergence (p-distance) between Barcode sequences (COI) of *Isarachnanthus* from the Atlantic Ocean.

	I. nocturnus	***I. maderensis*** **(Madeira)**	***I. maderensis*** **(Rocas Atoll)**	***I. maderensis*** **(Curaçao)**	***I. maderensis*** **(Ascension Is.)**
Cape Verde	0.07-0.08	0.0	0.0-0.002	0.0	0.006-0.08

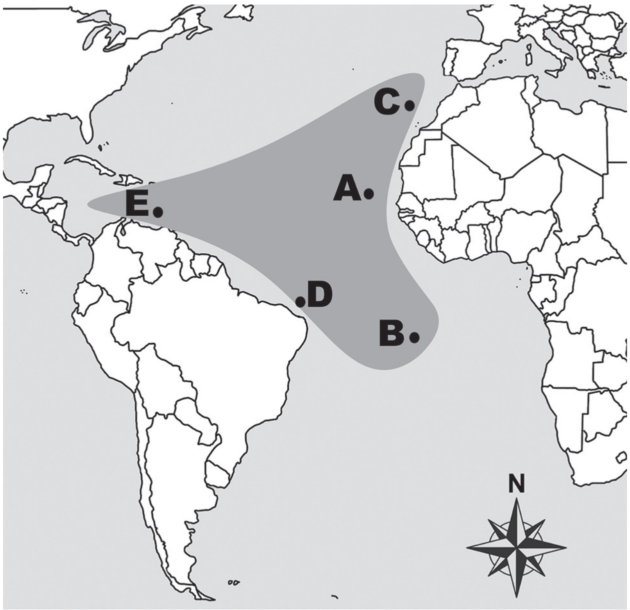

Figure 3. Area of occurrence of *Isarachnanthus maderensis* (Johnson, 1861), as follows: (A) Cape Verde (this study), (B) Ascension Island (Stampar & Morandini 2017), (C) Madeira Island, (D) Rocas Atoll and (E) Curaçao (Stampar *et al.,* 2012).

the specimens are under the influence of coastal currents from Argentina to the Caribbean Sea (Stampar *et al.,* 2012). Both scenarios are maintained by the presence of a long-lived planktonic larva (Stampar *et al.,* 2015) and the gene flow resulting from the action of these larvae is still very little known. Recently, an enormous phenotypic plasticity of the larvae has been discovered (Stampar *et al.,* 2017), which can also have influence on these patterns. Still in this approach, it is possible to recognize a distinct genetic pattern in relation to the studied specimens of *Isarachnanthus maderensis* from Ascension Island and Rocas Atoll (Table 1). This different pattern must be the result of a partial biogeographic isolation, since these two areas are mainly under influence of the South Atlantic Gyre currents. The influence of marine currents on genetic structuring in the Atlantic Ocean is already known, including fairly similar areas and patterns (*e.g.,* Muss *et al.,* 2001). However, the possible greater isolation of the population of the Ascension Island deserves better attention, because at some level this can result in complete isolation (and eventually leading to speciation) in future.

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