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The Seven-arm Octopus, *Haliphron atlanticus* Streenstrup, 1861 (Cephalopoda, Alloposidae), in the Fernando de Noronha archipelago, Brazil

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Abstract: The rare deep-sea octopus, *Haliphron atlanticus* is the only known species recognized within the genus. A fragment of *H. atlanticus* was found in the Fernando de Noronha archipelago, Brazil (South Atlantic). Both phylogenetic reconstruction and pairwise genetic divergence show that the specimen recorded in South Atlantic is closely related to individuals from North Pacific. However, there is a greater divergence among these specimens and a giant octopus from North Atlantic. This evidence suggests that *Haliphron* is not monospecific, with at least two species, both represented in the Atlantic Ocean.

Key words: giant octopus; Octopoda; molecular genetics; COI mitochondrial gene

The rare gelatinous Seven-arm Octopus, *Haliphron atlanticus* Streenstrup, 1861 is the only known species recognized within the genus *Haliphron* Steenstrup, 1861 (Alloposidae). This giant octopus is a benthopelagic species with a circumglobal distribution, reaching a total length up to 4 m (Alvariño & Hunter 1981; Young 1995; Finn 2014). It lives in a depth range from about 1200 m, over depths up to 6500 m (O'Shea 2004; Finn 2014). It is believed that this species lives in pelagic habitats during certain phases of its life cycle (mainly juveniles), and inhabits the seabed of the continental slope during other phases (Thore 1949; O'Shea 2004).

Notwithstanding, records of this species are rare and only few adults are known (O'Shea 2002). Furthermore, most specimens are incomplete, sometimes restricted to a single arm fragment, as the type material, limiting systematics accounts (O'Shea 2004). On the other hand, according to Alvariño & Hunter (1981), *H. atlanticus* may not be as rare as it seems, and, mainly adults, are probably adept at avoiding nets of all types.

The most remarkable morphological characters are the gelatinous and well-pigmented body; deep web between all arms; and a single series of suckers near the mouth, which become biserial closer to edge of the web. Males are smaller than females and have the third right arm hectocotylized, detachable, and developed in pouch in front of the eye; this gives the impression that it has only seven arms (Thore 1949; Willassen 1986; Young 1995; O'Shea 2004; Finn 2014).

Given the difficulty of obtaining data on deep-sea cephalopods, and consequently, the lack of information in the literature about this group, this study validates the occurrence of *H. atlanticus* in Southwest Atlantic. In addition, it aims to provide molecular data for future phylogenetic and phylogeographic studies involving this species.

A fresh fragment of *H. atlanticus* (arms and part of the mantle) was found in November 2015 floating in the water column to a depth of 4 m, near Rata Island, in the Fernando de Noronha Marine National Park. Rata Island is part of the Fernando de Noronha archipelago, in the Tropical Southwest Atlantic province, about 360 km off the northeastern coast of Brazil (03°48′53.05″ S, 032°24′14.13″ W) (Figure 1). The fragment was collected by hand (permit SISBIO 10706-5) and tissue samples were stored at 95% ethanol. Part of the animal was frozen and the tissue samples were deposited in the Collection of Invertebrates at the Universidade Federal do Rio Grande do Norte (CTR 4560).

Cytochrome oxidase subunit I gene (COI) amplicons were obtained using universal primers LCO and HCO

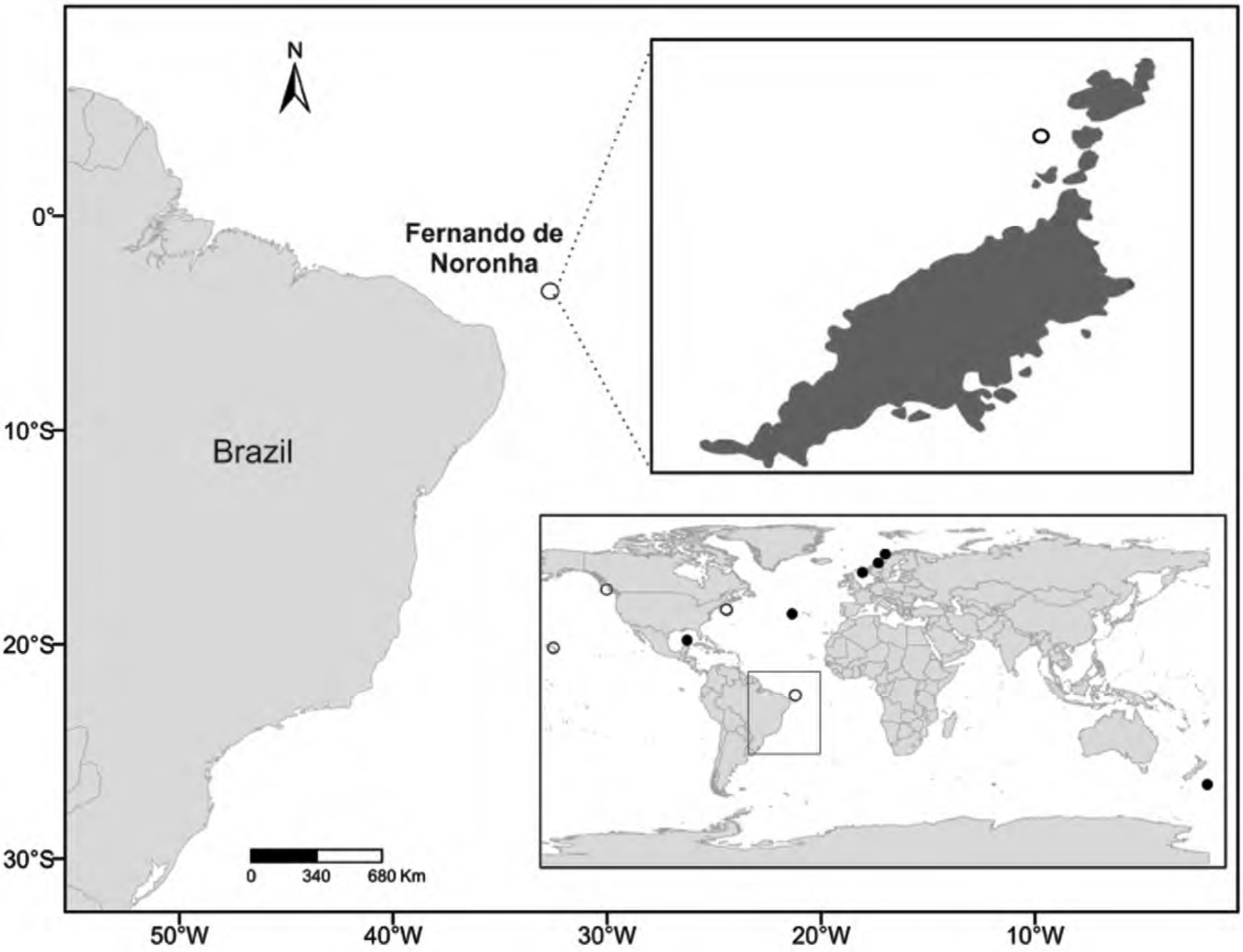

Figure 1. Geographical location of the Fernando de Noronha archipelago, where *Haliphron atlanticus* was recorded. The archipelago in detail is shown above, with white circle highlighting the sampling area of the giant octopus. In the global map, the dark circles indicate records of adults around the world. The open circles are the locations of samples used in the molecular analysis.

(Folmer et al. 1994). The PCR amplification reaction was conducted in a final volume of 25 μL containing 1 μL forward primer (10 mM), 1 μL reverse primer, 12.5 μL Taq DNA Polymerase Master Mix Ampliqon, 8.5 μL H_2O and 2 μL DNA. PCR cycle parameters used for COI were 3 min at 95°C for denaturation, followed by 40 cycles of 1 min at 94°C, 1 min at 45°C for annealing, 1.5 min at 72°C for extension and a final extension step of 4 min at 72°C. Finally, 25 μL of the PCR products were purified and sequenced by Macrogen Inc, Seoul, Korea.

The electropherograms were edited in Geneious 9.0.2 (http://www.geneious.com, Kearse et al. 2012) and sequences were aligned using MEGA 6 (Tamura et al. 2013). A molecular phylogenetic analysis (Bayesian Coalescent reconstruction) was carried out in BEAST 1.75 (Drummond et al. 2012). The substitution model GTR+G was used for COI, as suggested by jModeltest (Posada & Crandall 1998). An estimated rate of evolution for cephalopods (3.81 substitutions per site per billion years) was used in the Bayesian analyses (Strugnell et al. 2012). A total of 5×10^7 MCMC runs, saving of one every 5×10^3 runs was performed, 10,000 trees were saved and the first 15% removed as burnin. A consensus tree accessing the posteriori probability values of each clade was generated using TreeAnnotator 1.6.1 (Drummond et al. 2012).

A pairwise matrix of Kimura 2-parameter (K2P) was also performed in MEGA 6 (Tamura et al. 2013) to compare the genetic distances within and among other octopus species. COI sequences of some incirrate (cirrus absent) octopuses available in GenBank were also used in this study (Table 1).

The specimen was incomplete (Figure 2A) and the correct identification was only possible after molecular analysis (Table 1). However, some diagnostic features related to the gelatinous long arms and biserial arrangement of the distal suckers were observed (Figure 2B). Molecular analysis using 615 bp fragments of COI mitochondrial gene of 14 individuals belonging to 11 species (pelagic and benthonic octopods) corroborated the morphological identification (Table 1). The phylogenetic reconstruction showed that all sequences identified as *H. atlanticus* form a monophyletic and well-supported clade (Posterior Probability – PP = 1) (Figure 3). The specimens from North Pacific are more closely related to the Brazilian specimen than the other individual from the Atlantic Ocean (USA), which is the sister group of this clade. According to phylogenetic analysis, the sister species of *H. atlanticus* is the deep-sea *Tremoctopus*

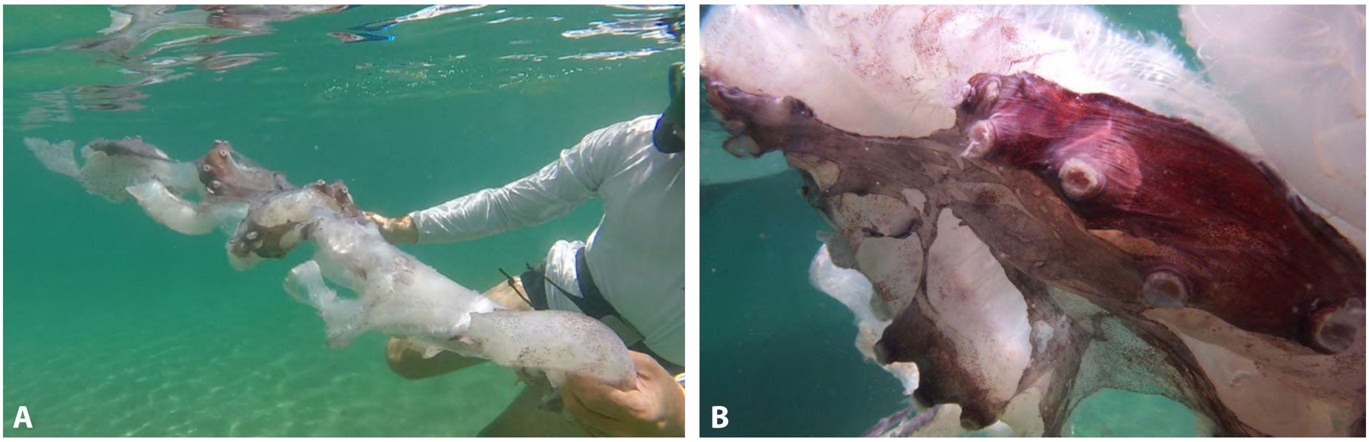

Figure 2. Collection of *Haliphron atlanticus* gelatinous body fragment (**A**) and detail of the biserial arrangement of distal suckers in the giant octopus arms (**B**). Photos: Liana Mendes (A) and Leonardo Veras (B).

Table 1. List of octopus specimens used in this study showing the sampling regions, accession number of DNA sequences, geographical coordinates and references. All sequences are obtained from GenBank, except the sequence with an asterisk (downloaded from BOLD database).

Species	Location	Accession Number	Latitude	Longitude	Reference
Haliphron atlanticus	Fernando de Noronha, Brazil	—	03°48′53.05″ S	032°24′14.13″ W	This study
Haliphron atlanticus	Hawaii, USA	AF377964	21°53′54.21″ N	156°07′35.0″ W	Carlini et al. 2001
Haliphron atlanticus	North Atlantic, USA	AY557516	39°50′39.00″ N	067°28′54.00″ W	Lindgren et al. 2004
Haliphron atlanticus	North Pacific, Canada	AAY1678*	50°32′11.96″ N	129°03′7.84″ W	Carlini et al. 2001
Tremoctopus violaceus	Key Biscayne, USA	AF377978	25°39′32.06″ N	080°06′02.02″ W	Carlini et al. 2001
Bathypolypus arcticus	Saquatucket Harbor, USA	AF000029	41°03′57.95″ N	068°50′52.86″ W	Carlini & Graves 1999
Ocythoe tuberculata	—	AY557519	—	—	Lindgren et al. 2004
Grimpella thaumastocheir	Pt. Victoria Jetty, Australia	HM104259	34°36′26.86″ S	137°02′29.78″ E	Strugnell et al. 2013
Pareledone albimaculata	South Shetland Islands, Antarctica	GQ843899	61°47′58.41″ S	056°50′04.96″ W	Allcock et al. 2011
Pareledone charcoti	Ross Sea, Antarctica	GQ843980	73°42′49.41″ S	167°12′09.54″ W	Allcock et al. 2011
Adelieledone polymorpha	Antarctica	GU073522	66°45′09.12″ S	145°18′33.06″ E	Undheim et al. 2010
Argonauta nodosa	Victoria, Australia	AF000028	35°41′26.93″ S	134°55′59.60″ E	Carlini & Graves 1999
Octopus tetricus	New South Wales, Australia	AF000056	33°00′46.05″ S	152°52′16.40″ E	Carlini & Graves 1999
Octopus insularis	Fernando de Noronha, Brazil	—	03°50′15.46″ S	032°25′25.45″ W	This study

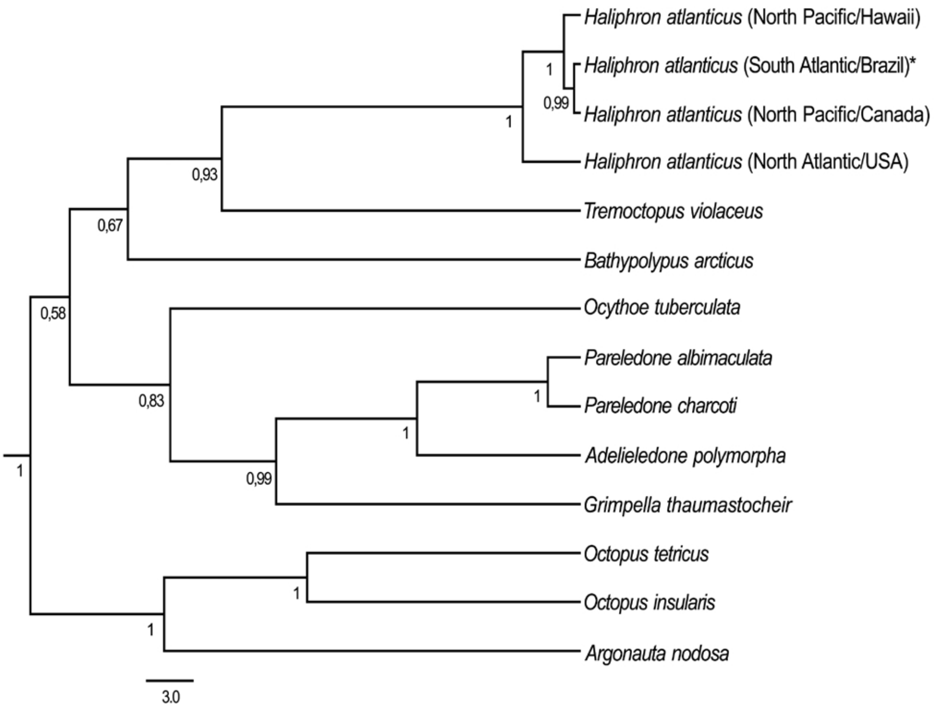

Figure 3. Molecular phylogeny constructed using Bayesian inference for a partial fragment of the Cytochrome Oxidase I gene. The Bayesian posterior probabilities are shown below nodes. The specimen collected in this study is highlighted with an asterisk.

Table 2. Evolutionary divergence between sequences (COI) conducted using the Kimura 2-parameter model. The genetic distances among *Haliphron atlanticus* specimens used in this study are shown in bold.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	*H. atlanticus* (South Atlantic, Brazil)													
2	*H. atlanticus* (North Pacific, Canada)	**0.003**												
3	*H. atlanticus* (North Pacific, Hawaii)	**0.005**	**0.008**											
4	*H. atlanticus* (North Atlantic, USA)	**0.029**	**0.031**	**0.027**										
5	*Tremoctopus violaceus*	0.199	0.199	0.202	0.213									
6	*Bathypolypus arcticus*	0.212	0.212	0.215	0.203	0.214								
7	*Ocythoe tuberculata*	0.213	0.210	0.210	0.201	0.218	0.205							
8	*Grimpella thaumastocheir*	0.194	0.194	0.189	0.197	0.257	0.186	0.179						
9	*Adelieledone polymorpha*	0.196	0.193	0.193	0.185	0.197	0.168	0.186	0.163					
10	*Pareledone albimaculata*	0.191	0.191	0.188	0.177	0.218	0.165	0.182	0.146	0.083				
11	*Pareledone charcoti*	0.196	0.196	0.194	0.183	0.233	0.168	0.182	0.144	0.088	0.015			
12	*Octopus tetricus*	0.235	0.235	0.238	0.224	0.226	0.199	0.182	0.212	0.188	0.211	0.214		
13	*Argonauta nodosa*	0.246	0.249	0.249	0.239	0.264	0.214	0.219	0.223	0.219	0.224	0.224	0.205	
14	*Octopus insularis*	0.223	0.223	0.226	0.223	0.204	0.214	0.223	0.237	0.185	0.195	0.205	0.140	0.223

violaceus Delle Chiaje, 1830, a widespread pelagic species found in the Atlantic Ocean (PP = 0.93). Carlini et al. (2001) and Strugnell et al. (2005) also found a close relationship between these species, using morphological evidence and multi-gene approach, respectively.

Both phylogenetic reconstruction and pairwise genetic divergence show that the specimen recorded in South Atlantic is closely related to individuals from North Pacific, Hawaii (K2P = 0.005), and Canada (K2P = 0.003) (Table 2). However, there is a greater divergence, between 2.9%–3.1%, among these specimens and the giant octopus of North Atlantic, which is about twice the distance among congeneric species, 1.5% between *Pareledone charcoti* Joubin, 1905 and *P. albimaculata* Allcock, 2005.

The specimen from Fernando de Noronha archipelago is the first reliable record of the genus *Haliphron* in Southwest Atlantic. To date, *H. atlanticus* was only recorded in Brazilian waters through beaks identified from stomach contents of long-distance foraging marine predators, as sharks and tunas (Santos & Haimovici 2002; Vaske-Júnior 2005). Apparently, the fragment was not regurgitated by large predators, because it was large, fresh, and well pigmented. This fragment also did not drift from a distant area by oceanic currents, because it would have quickly been eaten by pelagic migrants, such as sharks, tunas and whales, or seabirds such as albatrosses. We believe that part of the giant octopus was predated around the Fernando de Noronha archipelago and the remaining fragment drifted in the water column until arriving in shallow waters.

Phylogenetic reconstruction confirmed the taxonomic identity of the specimen fragment within the monotypic *Haliphron* clade. Furthermore, the analysis of pairwise genetic divergence showed intraspecific variation lower than 1% (except the North Atlantic specimen), which is consistent with barcoding proposals in many coleoid cephalopods (Dai et al. 2012). However, the K2P distances among *H. atlanticus* from North Atlantic and the others specimens were between 2.7% to 3.1%, similar to interspecific variation between octopod congeners (Kaneko et al. 2011). In a molecular identification study of 54 octopods species, Undheim et al. (2010) found that variation between congeners was in most cases higher than 2%, but ranged from 1.0% to 7.7%. Allcock et al. (2011) also verified small interspecific variation in COI sequences of the *Pareledone* genus (mean 2.2% K2P). These evidence suggest that *Haliphron* is not monospecific with, at least, two species, both represented in the Atlantic Ocean. O'Shea (2004) had also suggested an additional species of *Haliphron* based on morphological differences among specimens from New Zealand and Hawaii. Further morphological and molecular systematics studies using more specimens are needed to confirm this hypothesis.

The giant *H. atlanticus* is usually, but not exclusively, associated with islands or continental slopes (O'Shea 2004). According to some studies, *H. atlanticus* might pass periods of its life cycle alternating between the open waters and sea bottom (Thore 1949; Alvariño & Hunter 1981; Willassen 1986; O'Shea 2004). Furthermore, some captures with nets suggest that *H. atlanticus* may undertake diel vertical migration (Finn 2014). Thus, the oceanic islands, an oasis on the open sea, can provide a productive environment for feeding and/or spawning activity for this species.

The new record of *H. atlanticus* in Fernando de Noronha archipelago will contribute for global systematic studies about this rarely encountered giant octopus. In addition, it should be pointed out the importance of oceanic islands to the deep-sea fauna, reinforcing the need to protect these unique environments for the maintenance of marine biodiversity.

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