

MORPHOLOGICAL AND GENETIC DESCRIPTION OF *OCTOPUS INSULARIS*, A NEW CRYPTIC SPECIES IN THE *OCTOPUS VULGARIS* COMPLEX (CEPHALOPODA: OCTOPODIDAE) FROM THE TROPICAL SOUTHWESTERN ATLANTIC

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ABSTRACT

A medium-sized *Octopus* species is described based on material collected in shallow equatorial waters around the oceanic islands of Fernando de Noronha Archipelago, Rocas Atoll, St Peter and St Paul Archipelago and the mainland of northeastern Brazil. The new species, *Octopus insularis*, is described morphologically, and also characterized by the large mitochondrial subunit ribosomal RNA gene (mt 16S rDNA). The new species has relatively short and stout arms, rugose reddish brown skin in preserved specimens, 8 to 11 gill lamellae on the outer demibranchs, small ligula, characteristic symmetrical radula, spermatophore and beak, small eggs and high fecundity (213,000 oocytes under 1.5 mm diameter). The habitats and skin patterns of living animals are briefly described. The new species differs both morphologically and genetically from *Octopus vulgaris* in the Mediterranean, Venezuela and southern Brazil.

INTRODUCTION

The benthic octopus fauna of the western Atlantic, including the oceanic islands, is among the least known throughout the world (Voight, 1998). Haimovici, Perez & Santos (1994) recorded the following five species of the genus *Octopus* living in reef systems and associated shallow-water habitats in the tropical southwestern Atlantic: *Octopus vulgaris* Cuvier, 1797, *Callistoctopus macropus* (Risso, 1826), *Octopus filosus* Howell, 1868 (now known as *Octopus hummeli* Adam, 1936), *Paroctopus joubini* Robson, 1929 and *Amphioctopus burryi* (Voss, 1950).

More recently, Leite (2002) and Leite & Haimovici (2006) found a mid-sized octopus, not previously distinguished from *Octopus vulgaris*, that was common in the shallow waters around the Rocas Atoll, Fernando de Noronha Archipelago, St Peter and St Paul Archipelago and also on beach rocks and reef systems along the northeastern coast of Brazil. This octopus, more robust than *Octopus vulgaris*, did not fit descriptions of the species in recent reviews of the western and eastern Atlantic (Mangold, 1998; Voss & Toll, 1998; Norman & Hochberg, 2005).

Warnke (1999) studied DNA sequences of the genus *Octopus* from the Southern Hemisphere using the mitochondrial genes cytochrome oxidase subunit III (COIII) and 16S rRNA and found the genotype of a specimen collected in Recife (northeastern Brazil) to differ from that one of *Octopus vulgaris* from the Mediterranean and southern Brazil.

In this paper, we describe this new species as *Octopus insularis*, distinguishing it both morphologically and genetically from *Octopus vulgaris* from the Mediterranean and southern Brazil. The similarities and differences among other *Octopus* species in the tropical western Atlantic are discussed.

MATERIAL AND METHODS

Collection sites

A total of 61 specimens were collected in the shallow coastal waters of Rio Grande do Norte state (RN) ($5^{\circ}47'42''S$; $35^{\circ}12'34''W$), Pernambuco state (PE) ($8^{\circ}03'14''S$; $34^{\circ}52'52''W$) and from three oceanic island groups in the northeast of Brazil: Fernando de Noronha Archipelago (FN) ($03^{\circ}51'S$; $32^{\circ}25'W$), Rocas Atoll (RA) ($3^{\circ}05'S$, $33^{\circ}40'W$) and St Peter and St Paul Archipelago (SPSP) ($0^{\circ}55'00''$; $29^{\circ}20'76''W$); the last is a remote group of 15 small rocky islands lying just north of the Equator on the mid-Atlantic ridge, 1,100 km from the Brazilian mainland (Fig. 1). The island groups lie within the Equatorial Surface Water with a mean temperature of $27.7^{\circ}C$ and mean salinity of 35.9‰ in the upper 50 m water column (Travassos *et al.*, 1999).

The specimens were collected at depths of up to 20 m by hand or with a drag hook, during snorkeling or SCUBA diving on the steep bottom slopes of the St Peter and St Paul Archipelago. Octopuses were also caught in traps set at depths from 25 to 45 m.

Morphological study

The specimens were fixed in 10% formalin and preserved in 70% ethanol. Some of them were cooled or frozen for at least 24 h before fixation to avoid contraction. Measurements, counts and indices followed Roper & Voss (1983) and Huffard & Hochberg (2005), except for sucker counts; these included all suckers, rather than only those in the basal half.

The following abbreviations are used for measurements and indices:

AFL: left arm formula

AFR: right arm formula

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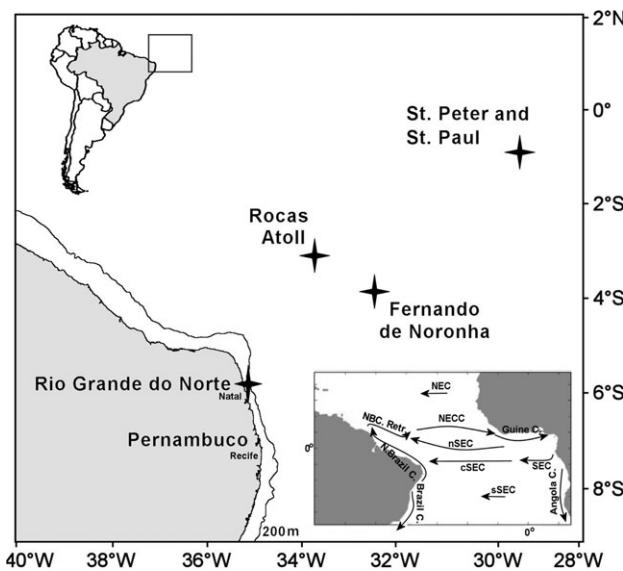


Figure 1. Collection sites of *Octopus insularis* examined (stars): Rio Grande do Norte (RN), Pernambuco (PE), Rocas Atoll (AR), Fernando de Noronha Archipelago and St Peter and St Paul Archipelago. Small inset shows the main currents (NEC, North Equatorial Current; NECC, North Equatorial Countercurrent; SEC, South Equatorial Current; nSEC, South Equatorial Current, north direction; cSEC, South Equatorial Current, central direction; Brazil Current; Angola Current; Guinea Current; N. Brazil C, North Brazil Current; NBC Retr., North Brazil Current).

- ASC: arm sucker count (number of suckers of each designated arm)
- AWI: arm width index (arm width at the widest point on stoutest arm/ML × 100)
- CaLI: calamus length index (calamus length/LL × 100)
- FLI: funnel length index (funnel length/ML × 100)
- GILC: gill lamellae count per demibranch
- HWI: head width index (head width/ML × 100)
- LLI: ligula length index (ligula length/HL × 100)
- ML: dorsal mantle length
- MWI: mantle width index (mantle width/ML × 100)
- OAI: opposite arm length index (hectocotylized arm/normal third arm length × 100)
- PAI: pallial aperture index (pallial aperture/ML × 100); ALI: arm length index (arm length/TL × 100);
- MAI: mantle arm index (ML/longest arm length × 100)
- PLI: terminal organ (penis) length index (terminal organ length/ML × 100)
- SDeI: enlarged sucker diameter index (enlarged sucker diameter/ML × 100)
- SDnI: largest normal sucker diameter index (largest normal sucker diameter/ML × 100)
- SpLI: spermatophore length index (spermatophore length/ML × 100)
- SpRLI: sperm reservoir length index (sperm reservoir length/spermatophore length × 100)
- SpWI: spermatophore width index (spermatophore width/spermatophore length)
- TL: total length
- WDI: web depth index (web more depth/longest arm length × 100)
- WF: web formula
- WT: total preserved weight

Unless stated otherwise, all measurements are in millimeters and weights in grams. Small structures such as ligula, calamus,

spermatophores and eggs were measured with an ocular micrometer in a binocular microscope. The nonparametric *U*-test was used to compare indices, and unless stated otherwise, a 5% rejection level ($P < 0.05$) was accepted as statistically significant. Body patterns were photographed during dives. The chromatic and skin texture components were described following Hanlon (1988) and Mather & Mather (1994).

The collected specimens were classified in four maturity stages: I (immature), II (nearly mature), III (mature) and IV (spawning). All specimens used at the description (Table 1A, B) were in stages III or IV.

Acronyms used for institutions:

LABIPE: Laboratory of Fishery Biology, Oceanography Department, Universidade Federal do Rio Grande do Norte (UFRN), Brazil

MORG: Oceanographic Museum Prof. Eliézer C. Rios, Fundação Universidade Federal do Rio Grande (FURG), Brazil

MNRJ: National Museum of Rio de Janeiro, Universidade Federal do Rio de Janeiro (UFRJ), Brazil

MUSP: Museum of Universidade Federal de São Paulo (USP), Brazil

SBMNH: Santa Barbara Museum of Natural History, Santa Barbara, California, USA

USNM: National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

Molecular study

Digestive gland tissue was collected from eight *Octopus insularis* n. sp. from the coast of Rio Grande do Norte and the three oceanic islands, Rocas Atoll, Fernando de Noronha Archipelago and St Peter and St Paul Archipelago. The genomic DNA was isolated from ethanol-methanol-preserved digestive gland tissues by standard proteinase K, phenol-chloroform extraction (Sambrook, Fritsch & Maniatis, 1989). The mitochondrial 16S rRNA sequence was amplified by polymerase chain reaction (PCR), utilizing the amplification Kit PuReTaq Ready-To-Go PCR beads (Amersham Biosciences), in 25 µl reactions containing 0.25 µl of each primer (L1987: 5'-GCCTCGCCTG TTTACCAAAAC-3'; H2609: 5'-CGGTCTGAACTCAGAT-CACGT-3') (Palumbi *et al.*, 1991), 1.0 µl of DNA template (100 µg/µl) and 23.5 µl of distilled water. PCR conditions were as follows: 94°C (2 min), 30 cycles of 94°C (30 s), 49°C (30 s), 72°C (55 s) and a final extension of 72°C (2 min).

Samples were sequenced using the Big Dye terminator cycle sequencing reaction kit (Applied Biosystems Inc.) on an ABI Prism377 automated DNA sequencer, following the manufacturer's instructions. All templates were sequenced in both directions. Sequences of 16S rRNA were aligned using CLUSTAL W (Thompson *et al.*, 1997) and improved by eye using BioEdit v.5.0.6 (Hall, 1999).

All sequences of *O. insularis* samples were deposited in the EMBL database (Nucleotide Sequence Database) (accession numbers: EF093793, Rio Grande do Norte (RN), NE Brazil; EF093791, Fernando de Noronha Archipelago; EF093790, Rocas Atoll, and EF093792, St Peter and St Paul Archipelago).

The *O. insularis* n. sp. sequence was compared with sequences of *Octopus vulgaris* from three other geographic regions: France (Genbank no.: AJ390310), Isla Margarita, Venezuela (Genbank accession no.: AJ390316), Rio de Janeiro, SE Brazil (Genbank accession no.: AJ390314). A specimen from Recife (PE), Brazil, recorded as *O. vulgaris* in Genbank, with the accession number AJ390315, was shown to be genetically similar to the new species. One additional sequence of *Octopus mimus* (Genbank accession no.: AJ390318) was selected to test the monophyly of '*Octopus vulgaris*' from the Brazilian coast (see Supplementary data).

Table 1A. Measurements and indices of the male holotype and paratypes of *Octopus insularis* new species (see Methods for abbreviations and details of fixation and preservation of the specimens).

| | Holotype | Paratype | Paratype | Paratype | Paratype |
|------------------------|----------------------|----------------------|--------------------------|--------------------------|----------------------|
| Museum collection | MORG | MUSP | MNRJ | USNM | SBMNH |
| Reg. number | 49524 | 44648 | 10368 | 1100783 | 369710 |
| Conservation procedure | Fresh-formol-alcohol | Fresh-formol-alcohol | Fresh-ice-formol-alcohol | Fresh-ice-formol-alcohol | Fresh-formol-alcohol |
| Collection site | ASPSP | FN | FN | FN | FN |
| Sex | M | M | M | M | M |
| Maturity | III | III | III | III | III |
| WTc | 588 | 820 | 942.4 | 538.6 | 797 |
| TL | 510 | 392 | 494 | 430 | 430 |
| ML | 104 | 102 | 114 | 94 | 100 |
| HWI | 48.0 | 48 | 37 | 35 | 47 |
| MWI | 81.2 | 71 | 59 | 84 | 68 |
| FLI | 41.8 | 35 | 33 | 42 | 40 |
| PAI | 54.1 | 44 | 44 | 49 | 44 |
| MAI | 29.2 | — | 33 | 31 | 36 |
| ALI | 69.8 | * | 70 | 72 | 65 |
| OAI | 64.4 | * | * | — | 91 |
| ASC (third right) | 96 | * | 142 | — | 122 |
| ASC (Min.–Max.) | 104–223* | * | 222–237* | — | 100* |
| AWI | 13.8 | — | 13 | 15 | 16 |
| AFR | 4123 | * | * | 4231 | * |
| AFL | 4321 | * | * | — | * |
| SDnI | — | 11 | 9 | 10 | 11 |
| SDel | — | 15 | * | 13 | 16 |
| WDI | 26.1 | — | — | 28 | — |
| WF | BCDEA | * | * | DCBEA | DCBE |
| LLI | 1.5 | — | 1.4 | 1.7 | — |
| CaLI | 43 | 41 | 56 | 44 | — |
| SpLI | — | 39 | 32 | 27 | 43 |
| GILC | 9 | 10 | 8 | 10 | 10 |

*Missing or regenerating arms.

The choice of these taxa was based on the hypothesis of phylogenetic relationships proposed by Warnke *et al.* (2002, 2004).

Octopus mimus was used as an outgroup in all analyses. Maximum parsimony (MP; Fitch, 1977), maximum likelihood (ML; Felsenstein, 1981) and neighbour-joining (NJ; Saitou & Nei, 1987) analyses were conducted with PAUP* v.4.0b10 (Swofford, 2002). Heuristic searches were carried out by stepwise taxon addition (100 replicates), combined with tree-bisection-reconnection (TBR) as the branch-swapping algorithm. Bootstrap and Jackknife analyses were performed to assess the resulting topology and were based on 10,000 replicates of the heuristic search previously described.

MODELTEST 3.0 (Posada & Crandall, 1998) was used to determine the optimal model of nucleotide evolution for the dataset. Thus, an ML analysis was performed based on the TnR+G model of gene evolution.

SYSTEMATIC DESCRIPTION

Family Octopodidae d'Orbigny, 1840

Subfamily Octopodinae d'Orbigny, 1840

Genus *Octopus* Cuvier, 1797

Synonym: 'vulgaris species-group' *sensu* Robson, 1929

Amended diagnosis: Adults: Benthic, small to large octopodids; ML to 300 mm; weight to 10 kg. Mantle muscular, globose to

rounded ovoid; skin muscular and tight, without longitudinal wrinkles or folds; skin with distinct patch and groove system which appears as dark trellis or reticulate pattern; ocelli (false eye spots) present in some species, often with complex blue iridescent ring; dorsal mantle white spots present; frontal white spot complex present; fixed pattern of four long primary papillae in diamond pattern in mid-region of dorsal mantle; one long primary papilla at posterior end of dorsal mantle; one long and two shorter primary papillae over each eye; mantle aperture wide. Funnel organ W-shaped, large, limbs subequal. Interbrachial aquiferous (water) pore system absent. Stylets present, long, chitinous (non-mineralized). Cranial cartilage present; head nearly as wide as mantle. Arms stout, medium length, 3–5 times length of mantle; lateral arms longest. Suckers biserial, small to large; modifications of male arm tips (suckerless ridges, elongate filaments, etc) absent; 1–3 enlarged suckers present on arms 2 and 3 in adults of both sexes (especially conspicuous in males). Right arm III hectocotylized, slightly shorter than opposite arm; copulatory organ with distinct ligula and calamus; ligula minute to small, lengths <3% of arm length; ligula conical, copulatory groove shallow, smooth; calamus of medium length, >50% of ligula length. Crop with anteriorly projecting diverticulum; intestine long with distinct U-shaped loop. Terminal organ apex (penis) short, slender, with small rounded diverticulum. Spermatophores small, unarmed; typically shorter than length of mantle. Female reproductive tract without accessory seminal receptacle (i.e. distal oviduct adjacent to oviducal gland may be slightly swollen but

Table 1B. Measurements and indices of the female paratypes of *Octopus insularis* new species (see Methods for abbreviations and details of fixation and preservation of the specimens).

| | Paratype | Paratype | Paratype | Paratype | Paratype |
|------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Museum collection | MORG | MUSP | MNRJ | USNM | SBMNH |
| Reg. number | 49525 | 44649 | 10369 | 1100782 | 369711 |
| Conservation procedure | Fresh-formol–alcohol | Fresh-formol–alcohol | Fresh-formol–alcohol | Fresh-formol–alcohol | Fresh-formol–alcohol |
| Collection site | ASPSP | ASPSP | FN | FN | FN |
| Sex | F | F | F | F | F |
| Maturity | III | II | III | IV | III |
| WTc | 1329.8 | 856.2 | 634 | 650 | 613 |
| TL | 470 | 465 | 390 | 440 | 407 |
| ML | 120 | 102 | 95 | 104 | 95 |
| HWI | 42 | 47 | 47 | 46 | 47 |
| MWI | 63 | 70 | 74 | 73 | 79 |
| FLI | 35 | 39 | 33 | 33 | 38 |
| PAI | 42 | — | 48 | 44 | 42 |
| MAI | 38 | 29 | 34 | 34 | 33 |
| ALI | 68 | 75 | 72 | 69 | 71 |
| ASC (Min.–Max.) | | 215* | 208–241* | 104–212* | |
| AWI | * | 18 | 15 | 10 | 12 |
| AFR | * | 3421 | 3412 | * | 3 = 421 |
| AFL | * | 3421 | 3412 | * | 3 = 421 |
| SDnI | 11 | 10 | — | 8 | 10 |
| WDI | 29 | 26 | — | — | — |
| WF | CDBAE | CDBEA | BCDAE | CDBEA | DCBEA |
| GiLC | 9 | 9 | 10 | 11 | 10 |

*Missing or regenerating arms.

does not envelop gland), oviducal glands distinctly bipartite, spermatheca present, visible in dark proximal region, radiating braiding chambers visible in distal half. *Spawned eggs:* Size variable, chorion capsules range from small (2–4 mm long) to large (10–18 mm). Attached to substrate in festoons. *Hatchlings:* Planktonic or benthonic. Kölliker organs (bristles) present during development. In species with planktonic stages mantle bluntly ovoid (not rounded or elongate and pointed); arm lengths subequal throughout paralarval development, with fine tips; arms typically with 3–4 suckers at time of hatching; ventral mantle densely covered with chromatophores; dorsal mantle clear, devoid of epithelial chromatophores. *Behaviour:* Species in this genus are crepuscular; territorial; occupy hard-substrate dens; feed primarily on crustaceans and shelled molluscs that are typically drilled.

Octopus insularis new species Leite & Haimovici

Type material: Holotype: male (mature) 104 mm ML; behind Belmont Island, St Peter and St Paul Archipelago, Brazil ($0^{\circ}55'00''$; $29^{\circ}20'76''W$); on bedrock, 10 m depth; coll. T.S. Leite, by hand during SCUBA dive, April 2004; MORG 49524. Paratypes: one female (mature) 120 mm ML; St Peter and St Paul Archipelago ($0^{\circ}55'00''$; $29^{\circ}20'76''W$); on bedrock, 10 m depth; coll. F. Callipo, by hand during SCUBA dive; September 2001; MORG 49525. one male (mature) 102 mm ML; between Rata and Middle Islands, Fernando de Noronha Archipelago ($32^{\circ}24'36''W$, $03^{\circ}49'00''S$); under rocks, 10 m depth; coll. T.S. Leite, by hand during SCUBA dive; August 2001; MUSP 44648. one female (nearly mature) 102 mm ML; St Peter and St Paul Archipelago ($0^{\circ}55'00''$; $29^{\circ}20'76''W$); on bedrock, 15 m depth; coll. F. Callipo, by hand during SCUBA dive; August 2001; MUSP 44649. one male (mature) 114 mm ML, Ressurreta behind Rata Island, Fernando de Noronha

Archipelago ($32^{\circ}24'30''W$, $03^{\circ}49'00''S$); under rocks, 8 m depth; coll. T. Leite, by hand during SCUBA dive; July 2000; MNRJ 10368. one female (mature) 95-mm ML, Buraco do Inferno behind Rata Island, Fernando de Noronha Archipelago ($32^{\circ}23'00''W$, $03^{\circ}48'36''S$); 10 m depth; coll. T. Leite, by hand during SCUBA dive; August 2001; MNRJ 10369. one male (mature) ML 94 mm; Buraco da Rachel behind Fernando de Noronha Island, Fernando de Noronha Archipelago ($32^{\circ}24'00''W$, $03^{\circ}50'12''S$); on bedrock and sand, 2 m depth; coll. T. Leite, by hand during snorkeling; USNM 1100783; one female (spawning) ML 104 mm; Ressurreta behind Rata Island, Fernando de Noronha Archipelago ($32^{\circ}24'30''W$, $03^{\circ}49'00''$); under rocks, 8 m depth; coll. T. Leite, by hand during SCUBA dive; August 2001; USNM 1100782; one male (mature) 100 mm ML; Buraco do Inferno behind Rata Island, Fernando de Noronha Archipelago ($32^{\circ}23'00''W$, $03^{\circ}48'36''$); 10 m depth; coll. T. Leite, by hand during SCUBA dive; August 2001; SBMNH 369710. one female (mature) 95 mm ML; Ressurreta behind Rata Island, Fernando de Noronha Archipelago, ($32^{\circ}24'30''W$, $03^{\circ}49'00''$); under rocks, 8 m depth; coll. T. Leite, by hand during SCUBA dive; August 2001; SBMNH 369711 (Table 1A, B).

Etymology: The name *insularis* refers to the presence of the species in the waters around all the oceanic islands off northeastern Brazil.

Other examined material: one male (mature) 105 mm ML; St Peter and St Paul Archipelago ($0^{\circ}55'00''$; $29^{\circ}20'76''W$); under rocks, 10 m depth; coll. T. Leite; April 2004 (LABIPE). one male (mature) 102 mm ML; coast of Rio Grande do Norte state ($5^{\circ}47'42''S$; $35^{\circ}12'34''W$); coll. fisherman; July 2005; LABIPE. one female (nearly mature) 80 mm ML; Sueste Bay, Fernando de Noronha Archipelago; over bedrock, 1 m depth; coll. T. Leite; February 2001; LABIPE. one female (nearly

Table 2. Measurements and indices of other examined material of *Octopus insularis* new species (see Methods for abbreviations and details of fixation and preservation of the specimens).

| Museum collection | LABIPE | LABIPE | LABIPE | LABIPE |
|-------------------|--------|--------|--------|--------|
| Collection site | RN | ASPSP | FN | RN |
| Sex | M | M | F | F |
| Maturity | III | III | II | II |
| WTc | 844 | 983 | 363.8 | 910 |
| TL | 475 | 485 | 360 | 530 |
| ML | 102 | 105 | 80 | 93 |
| HWI | 43 | 37.2 | 42 | 48 |
| MWI | 89 | 64.7 | 72 | 95 |
| FLI | 35 | 39.9 | 41 | 49 |
| PAI | 48 | 53.3 | 48 | 54 |
| MAI | 30 | 26.3 | 29 | 24 |
| ALI | 72 | 82.5 | 76 | 76 |
| OAI | — | 94.3 | — | — |
| ASC (3R) | * | — | — | — |
| AWI | 133 | — | 13 | 14 |
| AFR | — | 2341 | 3421 | — |
| AFL | — | 2134 | 3421 | 4213 |
| SDnI | 12.7 | — | 12 | 12 |
| SDeI | 15.8 | — | — | — |
| WDI | 24 | 22.5 | 22 | 23 |
| WF | CDBEA | CBADE | CDBEA | CDBAE |
| LLI | 1.3 | — | — | — |
| CaLI | 50 | — | — | — |
| SpLI | — | — | — | — |
| GILC | 11 | — | 11 | 11 |

*Missing or regenerating arms.

mature) 93 mm ML; coast of Rio Grande do Norte state (5°47'42" S; 35°12'34" W); coll. fisherman; July 2005; LABIPE (Table 2).

Diagnosis: Medium to large-sized adults (ML less than 120 mm; to 1,330 g total weight); mantle wide (MWI 60–95) (min–max); head wide (HWI 37–48); arms thick (AWI 9–18), relatively short (ALI 65–76); web moderately deep (WDI 22–29); third right arm of males hectocotylized, moderately long (length 91–94% of opposite arm); ligula small (LLI 1.3–1.7); calamus relatively long (CLI 41–56); gills with 8–11 lamellae per hemibranch, most frequently 10. Small papillae on ventral surfaces of mantle, head and web, larger ones on dorsal mantle and head; one large primary papilla over each eye, two or three additional smaller ones. Skin of the dorsal surface specimens extremely rugose in preserved. Colours of recently caught animals yellowish brown to reddish brown. Ocelli absent.

Description: The following description is based on holotype (male) and seven mature paratypes (3 females and four males) (Table 1A, B), plus two additional mature males (Table 2). Medium to large-sized adults up to 120 mm and 1,330 g total weight. Broad saccular mantle with muscular wall; two thick cartilaginous stylets (0.2 mm diameter, 18.8 mm long, in a male of 78 mm ML). Pallial aperture of moderate width (PAI 42–54). Head wide (HWI 37–48). Eyes moderately prominent (Fig. 2A). Funnel tubular (FLI 32–49). Well defined W-shaped funnel organ; lateral limbs longer; all four limbs of equal width (Fig. 2B). Arms thick (AWI 9–18), relatively short (ALI 65–76; MAI 24–38); AFL: III > IV > II > I; AFR:

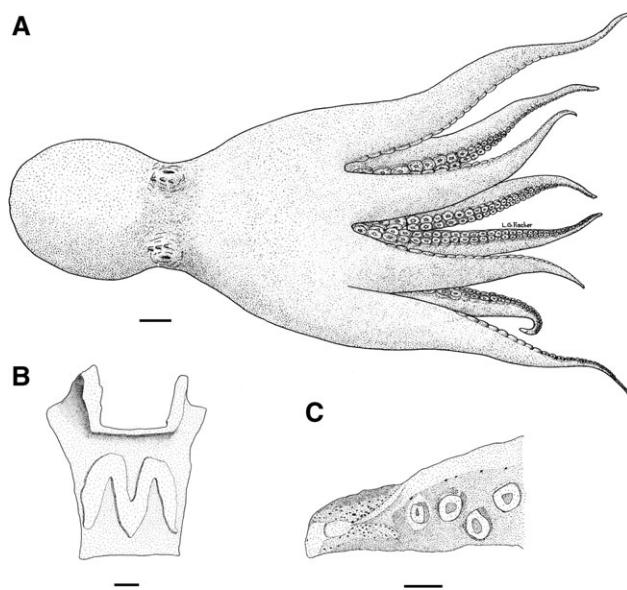


Figure 2. *Octopus insularis* new species. **A.** Dorsal view of a 120 mm ML male. **B.** W-shaped funnel organ, from 78 mm ML male. **C.** Hectocotylus of a 78 mm ML male. Scale bars: **A** = 2 cm; **B** = 0.5 cm; **C** = 1 mm.

II > III > IV > I.). Third right arm of males hectocotylized, shorter than opposite one (OAI 91–94), bearing 96–142 suckers. Well defined spermatophoric groove along hectocotylized arm ends at base of relatively long calamus (CLI 41–56). Small ligula (LLI 0.6–1.4) (Fig. 2C). Total number of suckers on the normal arms 220 to 238. Normal sucker diameter moderate (SDnI 9–13 ♂; 8–14 ♀). Mature males with 2–4 enlarged suckers at the 8th or 9th row on arms II and III (SDeI 10–15). Web moderately deep (WDI 20–25–30); shallower between dorsal arms; deeper between ventral arms (most common web formulae C > D > B > E > A and D > C > B > E > A). External hemibranchs have stout gills, with 8–11 lamellae.

Typical *Octopus* digestive tract (Fig. 3A). Large buccal mass; pair of flattened, medium-sized, anterior salivary glands joined by salivary ducts to posterior portion of buccal mass; posterior salivary glands triangular. Narrow oesophagus followed by crop diverticulum; stomach wide. Spiral caecum connected by two ducts to large digestive gland; ink sac embedded in digestive gland surface. Intestine long, curved, ending in muscular rectum with anal flaps. Strong beaks; prominent rostrum and thick wings (Fig. 3B, C). Rachidian tooth on a half radula; two lateral teeth, one marginal tooth, one marginal plate, one lateral cusp on each side of rachidian tooth with a symmetric seriation every two or three teeth (A 2–3); cusp on outer margin of first lateral tooth; second lateral tooth triangular, almost symmetrical; marginal tooth thin, curved; marginal plate small, flat (Fig. 3D).

Reproductive system (Fig. 4). Ovary large, round in mature females; two proximal oviducts, short, thin; two small oviducal glands, spherical, dark; longer distal oviducts; over 213,000 oocytes (largest diameter less than 1.5 mm) in ovary of a single mature female. Testis large, broad in mature males; vas deferens narrow, long, of numerous turns and wrapped in membranous sac. Vas deferens opening in long spermatophore gland, curved accessory gland, both opening in an atrium linked to Needham's sac; terminal organ small, tubular (PLI 15–16); diverticulum spherical (Fig. 4B). Spermatophores medium-sized (SpLI 32–43), narrow, without swelling (SpWI: 1.2–1.6); medium-sized sperm masses (SpRLI 33–35) (Fig. 4C). Maximum of 66 spermatophores in storage sac of a mature male.

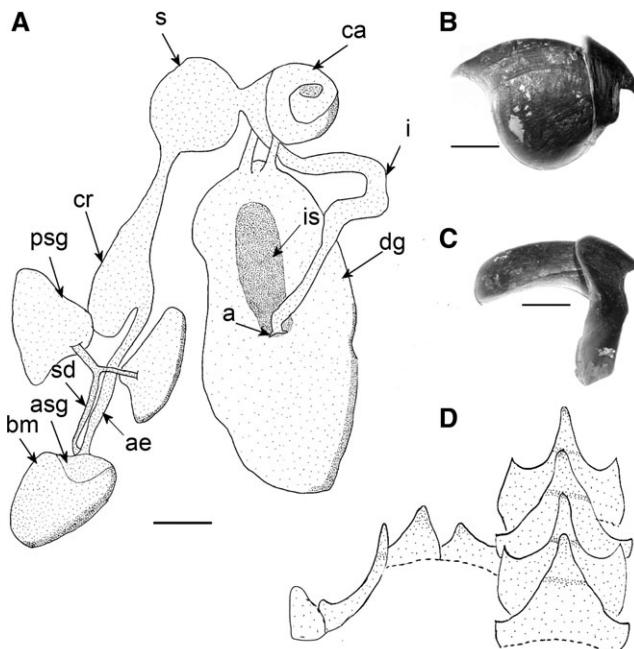


Figure 3. *Octopus insularis* new species. **A.** Digestive tract from 78 mm ML male. **B.** Upper beak. **C.** Lower beak of a 95 mm ML. **D.** Radula from female of 120 mm ML. Abbreviations: a, anus; ae, anterior oesophagus; asg, anterior salivary gland; bm, buccal mass; ca, caecum; cr, crop; dg, digestive gland; i, intestine; is, ink sac; psg, posterior salivary gland; s, stomach; sd, salivary duct. Scale bars: **A** = 1 cm; **B** and **C** = 0.5 cm.

Skin rugose on dorsal surface in specimens fixed without previous freezing (Fig. 5A). Ventral surface less rugose than dorsal. In specimens frozen for some time before fixation, the skin is smoother on dorsal surface (Fig. 5B). Colour varying from yellowish brown to dark purplish on dorsal surface and from cream to brownish on ventral. Ventral surfaces of mantle, head and web covered with well-spaced papillae, dorsal mantle and head with larger papillae. One large cirrus and additional smaller ones over each eye.

Body colour of living animals in the environment varying from yellow to reddish-brown, usually cream-brown (Fig. 5C). Skin with patch and groove trellis arrangement. Patches light brown; grooves dark brown; chromatophores in grooves larger and fewer in number than those that cover patches. Five chromatophore colours were identified (Black, Brown, Red, Yellow and White), plus leucophores. Brown and White were widespread throughout the body, while all other colours showed some concentration on different parts of body. Red was common to ventral parts of arms and edge of suckers. Yellow was more common in areas around eyes. A blue-green colour (from leucophores) was common in the ventral mantle during swimming.

The most common body patterns observed were Mottle, Blotch, Dorsal Light-Ventral Blue-Green and Uniform Dark (Leite & Mather, in press). Seven of the chromatic components observed were restricted to specific areas of the body: (1) the white V at the proximal part of the first arms; (2) bluish-green around the eyes; (3) alternate bars on distal parts of dorsal arms; (4) bar across eye, usually dark but sometimes red or white; (5) alternate light/dark around eye; (6) dark blotch above eye; and (7) purple around suckers (Leite & Mather, in press). Some chromatic components were common to *Octopus vulgaris* (Mather & Mather, 1994), such as frontal white spots, mantle white spots, arm bars, eye bar, black hood, and few were distinct components, such as the blue-green colour and alternate light and dark bars around the eye.

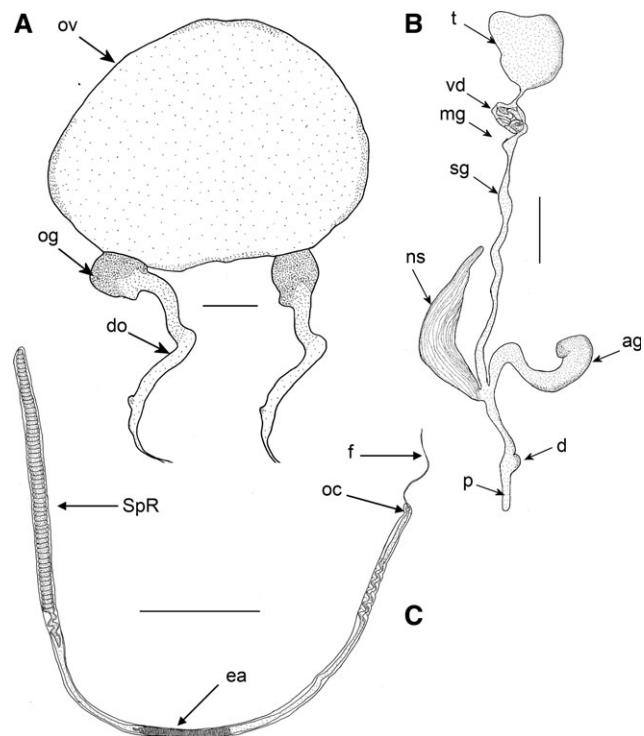


Figure 4. *Octopus insularis* new species. **A.** Frontal view of the reproductive system of a 120 mm ML female. **B.** Reproductive system of a 78 mm ML male. **C.** Spermatophore of a 114 mm ML male. Abbreviations: ag, accessory gland; d, diverticulum; do, distal oviduct; ea, ejaculatory apparatus; f, flagellum; mg, mucilaginous gland; ns, Needham's sac; oc, oral ca; og, oviductal gland; ov, ovary; p, terminal organ; sg, spermatophore gland; SpR, sperm reservoir; t, testis; vd, vas deferens. Scale bars: **A** = 1 cm; **B** = 1 cm; **C** = 5 mm.

Small papillae were spread throughout the body, while the large papillae occurred usually on the dorsal mantle or on the proximal-dorsal area of the first arms (Fig. 5B). Leite & Mather (in press) described the body patterns associated with different behaviours of living animals.

Sexual dimorphism: The comparison of morphometric indices between males and females showed sexual dimorphism, with significant differences only in the arm width and the normal sucker diameter (*U*-test, $P < 0.05$). Males showed thicker arms and larger normal suckers than females. These two sex-linked differences may be correlated: the arms must be thicker to carry bigger suckers. Males reach sexual maturity at a smaller size (78 mm ML) than females (95 mm ML).

Habitat: In Fernando de Noronha Archipelago small specimens, mostly immature and almost mature females (58–90 mm ML), were collected in shallow water and in rock pools; larger specimens, mainly maturing and mature males (78–116 mm ML) were found during deeper dives, up to a depth of 20 m on reefs and out of tidal range. In St Peter and St Paul Archipelago catches included almost mature and mature males and females (78–116 mm ML) collected mainly in the traps at depths of over 20 m. In Rocas Atoll mature and juvenile octopuses were found in shallow waters within the encircling coral reef. Along the mainland shelf maturing and mature specimens (102–116 mm ML) were caught by local fishermen at depths of 5–15 m on reefs 30 km off the coast, while juveniles (54–97 mm ML) were caught in shallow waters (less than 2 m) on rocks and on reef patches on sedimentary rocks (Leite &

OCTOPUS INSULARIS N.S.P.



Figure 5. *Octopus insularis* new species. **A.** Preserved specimen (120 mm ML). **B.** Living specimen (ML), in its natural habitat at Fernando de Noronha Archipelago. Scale bar: **B** = 10 cm.

Haimovici, 2006). The species is found on reefs, bedrock, rubble, gravel and sand beds and rocky bottoms, regardless of the presence of algae, but never on sandy and muddy bottoms (Leite, 2007).

Distribution: *Octopus insularis* is the species most frequently found in shallow waters in coastal areas off Rio Grande do Norte (RN) ($5^{\circ}47'42''S$; $35^{\circ}12'34''W$) and Pernambuco (PE) ($8^{\circ}03'14''S$; $34^{\circ}52'52''W$) states and in the three oceanic island groups off northeastern Brazil: Fernando de Noronha Archipelago ($03^{\circ}51'S$; $32^{\circ}25'W$), Rocas Atoll ($3^{\circ}05'S$; $33^{\circ}40'W$) and St Peter and St Paul Archipelago ($0^{\circ}55'00''S$; $29^{\circ}20'76''W$) (Fig. 1).

MOLECULAR ANALYSIS

Fragments 438 bp in length were obtained from the mitochondrial 16S rRNA gene of eight specimens of *Octopus insularis* n. sp. Due to asymmetry and bad fluorescent signals in the sequencing reactions, some nucleotides ($n = 16$) were omitted from the analysis. Estimated base frequencies were $A = 0.3472$, $C = 0.0859$, $G = 0.1809$ and $T = 0.3857$. The transition/transversion ratio (Ts:Tv) was 1.796. The relationship between sequence distance and Ts:Tv ratio indicated that the dataset was not saturated. No sequence diversity was found among the samples from the Brazilian oceanic islands of Rocas Atoll, Fernando de Noronha Archipelago and St Peter and St Paul Archipelago. The average distance of 16S rRNA sequences among *Octopus insularis* and the other three samples of *O. vulgaris* from the Atlantic and the Mediterranean was 7.22%. For 16S rRNA sequences compared among all sampled *Octopus insularis* haplotypes and *O. mimus*, the overall divergence was 4.6% (Table 3).

A well-supported topology was found based on MP analysis. A monophyletic cluster (bootstrap $P = 100\%$) included specimens of *O. insularis* from the oceanic islands off northeastern Brazil, from Rio Grande do Norte and included the specimen previously recorded as *O. vulgaris* from Pernambuco (Fig. 6).

DISCUSSION

Comparison with related taxa

Until recently, *Octopus vulgaris* Cuvier, 1797 was considered to be the only medium-large species found along the Brazilian coast, besides *Callistoctopus macropus* (Haimovici et al., 1994; Voight, 1998). However, although in general resembling *Octopus vulgaris*, adult *Octopus insularis* n. sp. have some distinct characteristics (Table 4): smaller size at maturity (120 mm max ML, vs 250 mm max ML for *O. vulgaris*; see Mangold, 1983), shorter relative length of the arms (65–76 vs 76.3–88.7 ALI), deeper web (24–29 vs 16.5–18.5 WDI), fewer suckers on the

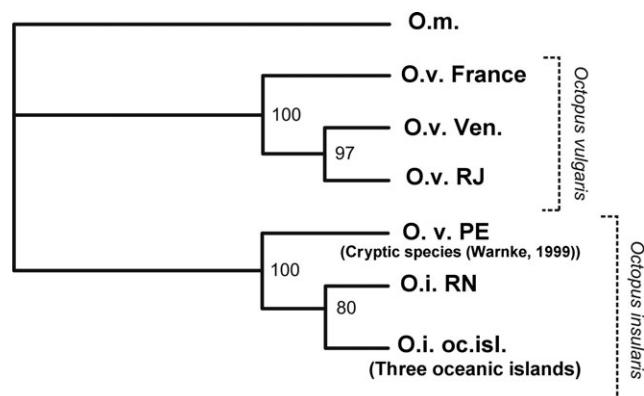


Figure 6. Phylogenetic tree of the *Octopus* 16S rRNA gene sequence. Maximum parsimony analysis. Parsimony searches were done with 10,000 bootstrap re-sampling replications. Percentages are bootstrap probabilities. Tree length = 73; consistency index = 0.986; retention index = 0.989; 46 parsimony-informative characters. Abbreviations: O.m., *Octopus mimus*; O.v. Ven, *Octopus vulgaris* from Isla Margarita, Venezuela; O.v. France, *Octopus vulgaris* from the Mediterranean, France; O.v. RJ, *Octopus vulgaris* from Rio de Janeiro; O.v. PE, *Octopus vulgaris* from Recife, Pernambuco; O.i. RN, *Octopus insularis* from Rio Grande do Norte; O.i. oc. isl., *Octopus insularis* from Brazilian oceanic islands (Rocas Atoll, Fernando de Noronha Archipelago and St Peter and St Paul).

hectocolyzed arm (95–142 vs 144–168), larger calamus (41–56 vs 45–52 CaLI), distinct radula (A 2–3 vs A2–A5; Mangold, 1998), stronger beaks with prominent rostrum and thick wings, and smaller spermatophores.

Octopus insularis becomes stouter with growth; it has negative allometric growth of arm length (slope 0.773), and positive allometric growth of arm width (slope = 1.190) with ML, for a size range of 32–120 mm ML (Leite, 2007). This trend does not occur with *Octopus vulgaris* for which Voight (1991) obtained regression slopes of 1.119 and 0.995 between arm length and arm width with ML for a size range of 13–185 mm of ML. This difference makes it easier to discriminate larger specimens of the two species.

The studies of Warnke (1999) and Söller et al. (2000), analysing the genetic variability of the genus *Octopus* in the Mediterranean Sea, western Atlantic and East Pacific using COIII and 16S rRNA sequences, suggested the existence of a cryptic species of the *O. vulgaris* complex in northeastern Brazil, based on a sample of two specimens from Recife (Pernambuco state). The specimens from Recife were genetically different from the *O. vulgaris* from Venezuela, Southern Brazil and the Mediterranean, and similar to *O. insularis* n. sp., *Octopus insularis* appears to be the most common medium-sized octopus caught

Table 3. Pairwise comparisons of the averages distances of 16S gene among the *Octopus* samples.

| | O.m. | O.v. Ven | O.v. PE | O.v. RJ | O.v. France | O.i. RN | O.i. Oc. Isl |
|---------------|---------|----------|---------|---------|-------------|---------|--------------|
| O.m. | — | | | | | | |
| O.v. Ven | 0.0821 | — | | | | | |
| O.i. PE. | 0.04598 | 0.07356 | — | | | | |
| O.v. RJ | 0.08238 | 0.00000 | 0.07373 | — | | | |
| O.v. France | 0.08447 | 0.01766 | 0.07551 | 0.01778 | — | | |
| O.i. RN | 0.04598 | 0.07126 | 0.00229 | 0.07143 | 0.07094 | — | |
| O.i. Oc. Isl. | 0.04608 | 0.07143 | 0.00229 | 0.07159 | 0.07110 | 0.00000 | — |

Abbreviations: O.m., *Octopus mimus*; O.v. Ven, *Octopus vulgaris* from Isla Margarita, Venezuela; O.v. PE, *Octopus vulgaris* from Recife, PE; O.v. RJ, *Octopus vulgaris* from Rio de Janeiro; O.v. France, *Octopus vulgaris* from the Mediterranean coast of France; O.i. RN, *Octopus insularis* from Rio Grande do Norte, Brazil; O.i. oc. isl., *Octopus insularis* from Brazilian oceanic islands (Rocas Atoll, Fernando de Noronha Archipelago and Saint Peter and Saint Paul).

Table 4. A morphological comparison between *Octopus insularis* new species and *Octopus cf. vulgaris* from southern Brazil and Mediterranean.

| Index | <i>Octopus vulgaris</i> Mediterranean | | <i>Octopus cf. vulgaris</i> southern Brazil | | <i>Octopus insularis</i> northeastern Brazil | |
|-----------------------------------|---------------------------------------|------|--|------|--|------|
| | Mangold (1998) ($N > 200$) | | Leite & Haimovici (unpublished) ($N = 16$) | | This paper ($N = 18$) | |
| | Min. | Max. | Min. | Max. | Min. | Max. |
| Dorsal mantle length (ML) | 20 | 250 | 102 | 172 | 90 | 144 |
| Head width index (HWI) | 32.5 | 53.5 | 26 | 37 | 20 | 52 |
| Arm longest length index (ALLI) | 76.3 | 88.7 | 65 | 83 | 65 | 76 |
| MAI: mantle arm index (MAI) | 18 | 25.2 | 17 | 35 | 24 | 38 |
| Arm formula | 3241/3 = 241 | 2341 | 3412/3421 | | | |
| Sucker normal index (SDnI) | 12.5 | 13.5 | 6 | 11 | 8 | 14 |
| Normal arm sucker count (ASC) | – | – | 102 | 222 | 102 | 237 |
| ASC (third right) | 140 | 180 | 112 | 221 | 96 | 142 |
| Deeper web index (WDI) | 16.5 | 18.5 | 17 | 25 | 24 | 39 |
| Web formula | CDBEA | – | CDBEA/DCBEA | – | CDBEA/DCBEA | – |
| Opposite arm index (OAI) | 75.2 | 82 | 73 | 87 | 81 | 94 |
| Ligula length index (LLI) | 1.2 | 2.1 | 1 | 1 | 0.6 | 1.4 |
| Calamus length index (CaLI) | 47 | 52 | 29 | 39 | 41 | 56 |
| Spermatophore length index (SpLI) | 31 | 81 | 24 | 24 | 32 | 43 |
| Radula | A2–A5 | B | A 2–3 | | | |
| Preserved skin | No information | | Smooth with primary papillae | | Rugose with primary and secondary papillae | |

along the mainland shelf and the oceanic islands of northeastern Brazil.

Octopus mimus Gould, 1852 was identified as *Octopus vulgaris* for a long time and was only recently redescribed (Guerra, Cortez & Rocha, 1999). Its distribution is from northern Peru to San Vicente in Chile. Warnke (1999) considered this species genetically more similar to the *Octopus* sp. from Recife than to *O. vulgaris* from Venezuela, Southern Brazil and the Mediterranean. However, *O. mimus* can be separated from *O. insularis* based on the following features: larger enlarged suckers (SDeI 16.1–16.9 vs 11–16), smaller calamus (CAI 33 vs 41–76), fewer lamellae per demibranchia (GiLC 7–8 vs 8–11) and presence of light ocellus (absent in *O. insularis*).

Having established that the new species is distinct from *O. vulgaris* and *O. mimus*, it must also be compared with other *Octopus* from the southwestern Atlantic, described by Robson (1929) in his monograph on the cephalopods from the British Museum collection, and by Palacio (1977) in his review of the Brazilian cephalopod fauna. More recently, Voss & Toll (1998) and Norman & Hochberg (2005) reviewed the nomenclature and taxonomy of octopus in the western Atlantic.

There is a strong likelihood that specimens from Fernando de Noronha examined by Robson (1929: 63–65) and identified as *O. rugosus* Bosc, 1792, belong to the species described here. Following Robson, this species has a wide head, relatively short arms, rough skin and symmetric rachidian teeth, and a distribution in the Atlantic, Indian and Pacific Oceans. No holotype or type locality was designated by Robson, and he stated that the wide geographical range made it likely that several species were combined under this name. For this reason, *Octopus rugosus* is not considered a valid species (Voss & Toll, 1998) and its status is unresolved (Norman & Hochberg, 2005).

Voss & Toll (1998) raised *Octopus rugosus* var. *sanctaehelena* Robson, 1929 to species level, based on Robson's description of a single 'distorted and dissected' female of unrecorded size and maturity from the island of St Helena (16°S; 5°45'W). Robson (1929) characterized it as having a very narrow head (and mantle for *O. rugosus*) and deep web (33%), and skin covered with multifid papillae, pentastellate on the sides of the body,

those on the dorsum often forming longitudinal ridges and those on the arms and web becoming smaller, rougher and more densely packed. He counted 11 gill filaments. Ian Gleadall (personal communication) examined this specimen in the Natural History Museum, London (BMNH 1868.3.12.1; collector J.C. Mellis; locality St. Helena). According to Gleadall, the specimen is flaccid and in poor condition, internal organs are missing and only three of the arm tips are intact. The colour is yellowish-grey with darker dorsal surfaces, the ventral surface of web sector A is very dark, and on the dorsal skin there is a distinct patch-and-groove system; the dorsal mantle length is approximately 85 mm and the head width is 26 mm; complete arms are L3 of 219 mm, R1 of 163 mm and R2 of 200 mm. The web membrane depths are A: 35 mm, B: 51/50, C: 62/56, D: 70/64 and E: 52 mm. The indices derived from these characters fit both *O. vulgaris* and *O. insularis* and so we cannot rule out that this specimen could belong to either of these two species. Voss & Toll (1998: 468) justified the elevation of *O. sanctaehelena* to full species status based on what they considered the only definitive character in Robson's account: the gill lamellae count (11), which they considered 'not attributable to *O. vulgaris* or other described species from those islands'. However, the total of eleven gill lamellae is within the range of *O. vulgaris* both from the Mediterranean (Mangold, 1998) and from Brazil (Leite, 2002). The status of *O. sanctaehelena* requires further study.

Octopus verrucosus Hoyle, 1885 was described based on a male from an island in the Tristan da Cunha group (37°6'44"S, 12°16'56"W) (Robson, 1929: 74–75). According to Robson, the distorted specimen which he examined was medium-sized, with a combination of characters of *O. vulgaris* and *O. rugosus* and some individual particularities, such as shallow web (18%), minute ligula (LLI 0.8), acutely pointed calamus half the length of the ligula, with a median groove and three transverse ridges. The skin of the dorsal surface of the mantle and head, as well as the aboral surface of web, was covered with dense irregular secondary papillae up to several mm in diameter in the nuchal region. There were papillae in two lines along the dorsal side of the mantle, smaller papillae on the ventral side and single supra-ocular primary papillae. The colour in preservation was dull purplish grey, darker dorsally, the ventral side

considerably lighter (Robson, 1929; Voss & Toll, 1998). *Octopus insularis* can be distinguished from this species by its deeper web, wider head, and longer ligula and calamus length.

Octopus occidentalis Hoyle, 1886 was originally described based on a small female (ML 55 mm) from Ascension Island (7.95°S, 14.37°W). Palacio (1977) redescribed this species from the holotype and a second female from Trindade Island (20°30'52"S; 29°19'50"W) as a small species with a broad head (HWI 75), prominent eyes and two primary papillae over each eye. The arms are long (ALI 76) and stout (AWI 31), web shallow (*vs* WDI 18); funnel organ W-shaped with the inner pads noticeably more slender than the outer ones, and radula shows A-3 seriation. The skin surface is rough with clusters of minute secondary papillae over the head, ocular region, web and to a lesser extent the dorsal surface of the arms. Males of this species are unknown. *Octopus occidentalis* was previously considered to be a synonym of *O. rugosus* by Robson (1929: 63–74) and of *O. vulgaris* by Pickford (1945) (Palacio, 1977; Voss & Toll, 1998). Although this species has some characteristics in common with *O. insularis* n. sp., such as a wide head and moderately long and stout arms, it differs in web depth, shape of the funnel organ and radula seriation, and no minute secondary papillae were observed on its skin.

Other species of the subfamily Octopodinae previously described from the southwestern Atlantic also differ from the new species. *Octopus hummelincki* Adam, 1936 has a smaller adult size and most specimens have ocelli on the web under the eyes, it also has different spermatophores, ligula, radula and skin colour and textures (Burgess, 1966; Voss & Toll, 1998; Leite & Haimovici, 2006). Recently, the specific name *O. hummelincki* was maintained for this common small ocellate octopus from the Caribbean and western Atlantic and the older name *O. filosus* suppressed (Gleadall, 2004; ICZN, 2006). *Octopus maya* Voss & Solís Ramírez, 1966 can reach larger adult size (to 4 kg), the dermal sculpture in preservation is granular, with paired ocelli between the eyes and at the base of arms II and III (Hanlon, 1988; Voss & Toll, 1998). *Octopus briareus* Robson, 1929 has a smaller but well developed ligula (LLI 3–4), with lateral fringing membranes and central ridge and about 12–16 transverse laminae, smaller calamus (CLI 28–32), distinct web formula (A = B = C=D>E), fewer gill lamellae (6–8), larger mature eggs (10–14 mm long by 4–5 mm wide) and the colour in life is iridescent blueish-green (Robson, 1929; Voss & Toll, 1998). *Octopus tehuelches* D'Orbigny, 1835 has a smaller adult size, longer arms with fewer suckers (about 100), females bear large eggs up to 18 mm in diameter, and the mantle, even in preserved animals, is smooth (Palacios, 1977; Voss & Toll, 1998). *Callistoc-topus macropus* (Risso, 1826) has a larger adult size, a narrower mantle, longer and thinner arms, shallower web and smooth skin with a distinct red and white colouration on body and arms (Mangold, 1998; Leite & Haimovici, 2006). In fact, because *C. macropus* was described from the Mediterranean, the specimens found in the western Atlantic off Brazil could be a distinct species, and critical revision is required. *Macrotritopus deflippi* Verany, 1851 has a smaller adult size, a narrower mantle, longer and thinner arms, shallower web and smooth skin with pallid colour (Mangold, 1998; Leite & Haimovici, 2006).

Octopus geryonea Gray, 1849 has been considered a form of *O. rugosus* (Robson, 1929: 73), a *nomen dubium* (Voss & Toll, 1998) or a synonym of *O. americanus* (Norman & Hochberg, 2005). Robson (1929) referred to the type of *O. geryonea* as a 'worn specimen from Bahia, probably an old animal'. The holotype is a large female and was described based on few characters. It can be distinguished from *O. insularis* n. sp. by its smooth mantle (rugose in *O. insularis*), the granular head, base of arms and aboral surface, and the single supraocular cirrus (Voss & Toll, 1998). *Octopus americanus* Baker in Denys de Montfort,

1802 has been variously considered a *nomen dubium* (Voss & Toll, 1998), an unresolved species or a potential name for western Atlantic *O. 'vulgaris'* (Norman & Hochberg, 2005). There is no holotype. Although this species has an inadequate description, some of the characteristics, such as small size and finely papillose skin (Voss & Toll, 1998), do not fit the new species *O. insularis*.

Molecular data

There have been a variety of interspecific *Octopus* studies utilizing different mitochondrial genes. For COIII '*Octopus rubescens*' Berry, 1953 showed a sequence 16.3–17.4% divergent from *Enteroctopus dofleini* (Wülker, 1910), '*Octopus californicus*' (Berry, 1912), *Octopus bimaculoides* Verrill, 1883 and *Octopus bimaculatus* Pickford & McConaughey, 1949. However, the two latter species were only 5.5% divergent from each other (Sosa *et al.*, 1995). Different mitochondrial genes display distinct rates of divergence. Our results suggest that *O. vulgaris* and *O. insularis* are distinct species, showing divergence of 7.2% between 16S rRNA haplotypes of *O. vulgaris* from Mediterranean, Caribbean and South Atlantic compared with those of *O. insularis* (including misidentified '*Octopus vulgaris*' from Recife; Warnke, 1999). The average nucleotide divergence between these octopuses is almost two-fold greater than that between *O. insularis* and *O. mimus* from the Pacific (4.6%). The phylogenetic analysis (Fig. 6) does not support clustering of *O. insularis* and *O. vulgaris* as a monophyletic group. The genetic distance estimates for 16S rRNA among the *Octopus* samples suggest a closer phylogenetic relationship between *O. insularis* and *O. mimus* than between *O. insularis* and *O. vulgaris*. Warnke (1999), Söller *et al.* (2000) and Warnke *et al.* (2004) also found more genetic similarity between *O. mimus* and '*O. vulgaris*' from Recife, northern Brazil (i.e. *O. insularis*) than between *O. mimus* and *O. vulgaris* from Venezuela, southern Brazil and the Mediterranean Sea.

In addition, our data indicate that the new species is distributed without detectable differentiation among the oceanic islands (Rocas Atoll, Fernando de Noronha Archipelago and St Peter and St Paul Archipelago). A close relationship was found between the lineage from the oceanic islands and haplotypes from the northeast coast of Brazil (RN and PE). These samples present the smallest level of sequence divergence in the dataset (Table 3). This result should be verified with further, preferably nuclear, genes.

Geographic distribution

The most common octopus species recorded throughout all the Brazilian coast has previously been identified as *Octopus vulgaris* (Haimovici *et al.*, 1994). This is a cosmopolitan species in temperate and tropical waters with unknown distributional limits, as reported by Roper, Sweeney & Nauen (1984). The wide distribution of this species is questionable, and a neotype is being designated from the Mediterranean (F.G. Hochberg, personal communication). Norman (2003) assumed that *O. vulgaris* was a complex of a number of closely similar octopus species in tropical to temperate waters around the world. The confirmation of this new species in the shallow waters around the Brazilian oceanic islands and along the northeastern coast confirms previous evidence of the existence of an additional species in the *O. vulgaris* complex.

The small size of the oocytes and high fecundity observed for *O. insularis* are characteristic of an octopus species with planktonic paralarvae (Hochberg, Nixon & Toll, 1992), which should give this species a high potential for dispersion. This is consistent with the wide distribution range. The equatorial region of the southwestern Atlantic is under the influence of

several currents (Fig. 1): South Equatorial Current and Equatorial Counter Current in the oceanic region, and North Brazil Current and Brazil Current along the continent. These currents may favour pelagic paralarval dispersal in various directions in both the oceanic and coastal regions (Scheltema, 1986; Lumpkin & Garzoli, 2005). Therefore, the distribution of *O. insularis* could potentially include the shallow waters of the continental shelves, banks, seamounts and islands in the western Atlantic Ocean. Recently, samples of *Octopus* from the continental shelf off Para and Amapá states ($0^{\circ}42'3.38''N$) in northern Brazil have been found to show the same 16S rRNA gene sequence as *O. insularis* in most specimens, and in a few the sequence of *O. vulgaris* (Sales *et al.*, 2007). One specimen from Bahia state in central Brazil ($12^{\circ}50'7.69''S$), examined by one of the authors (T. Leite), has been identified as *O. insularis*.

Although *O. vulgaris* was not present in our samples, a break in its distribution with no occurrence in the northeastern Brazil is unlikely, as it also has planktonic paralarvae and is present in southern and northern Brazil and in Venezuela (Warnke, 1999; Warnke *et al.*, 2004; Sales *et al.*, 2007). Its scarcity in the shallow waters of both mainland and oceanic islands in northeastern Brazil indicates the possibility of an exclusion mechanism or an adaptive advantage that favours *O. insularis*.

Unlike some reef fishes that show biogeographical (Rocha, 2003) and genetic (Rocha *et al.*, 2005) links between Caribbean and Brazilian oceanic islands, the octopus fauna of the Brazilian oceanic islands shows more similarity with the Brazilian mainland (Leite & Haimovici, 2006). Despite the difference between habitats of the Brazilian oceanic islands and the mainland, the presence of this new species in both suggests that insularity is not the principal factor determining distribution, since there is hard substrate in both regions and they are connected by the South Equatorial current.

Interestingly, *O. insularis* was the only octopus recorded in the minute St Peter and St Paul Archipelago (Leite & Haimovici, 2006). The low diversity of the Archipelago may be explained by its isolation, the small area of shallow water less than 50 m in depth (less than 0.5 km^2) and poor diversity of habitats (Edwards & Lubbock, 1983). It also suggests a higher adaptability of *O. insularis* to this environment when compared to other highly fecund tropical octopuses with small eggs. The stouter and shorter arms and deeper web may play a role in this adaptability. Studies of feeding ecology, which are being carried out, may clarify this point.

Because of the prevailing currents, St Peter and St Paul Archipelago has been considered to be an impoverished outpost of the Brazilian Province. The reef fish fauna more closely resembles that of the Brazilian Province than that of the other mid-Atlantic Ridge islands of Ascension and St Helena (Feitosa *et al.*, 2003). Thus, there is a chance of dispersal of the *O. insularis* from the American continent towards the tropical oceanic islands. On the other hand, the South Atlantic Gyre may connect spawning points of the Mid-Atlantic Islands and even the West African shelf to the tropical eastern shelf of South American.

Sympatric speciation of highly fecund species with pelagic hatchlings, such as both *O. vulgaris* and *O. insularis*, is unlikely. A cue to the geographic origin of *O. insularis* may be found in its genetic similarity to *O. mimus*. The rise of the Central American Isthmus forming a marine barrier occurred approximately 3 million years ago (Coates *et al.*, 1992). A common Atlantic–Pacific ancestral population of these two species of octopus may have begun diverging at that time and attained 4.6% divergence. This divergence is similar to that for 16S rRNA sequences of geminate species of fish of the genus *Centropomus* (Tringali *et al.*, 1999). The recent finding of *O. insularis* and *O. vulgaris* on both the southern and northern margins of the Amazon plume (Sales *et al.*, 2007) strengthens this suggestion. More studies on the distribution, ecology and paralarval

retention and distribution will be necessary to contribute to understanding of the biogeography and phylogenetic relationships of this new species.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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