Morphological and genetic evidence that *Octopus vulgaris* Cuvier, 1797 inhabits Amsterdam and Saint Paul Islands (southern Indian Ocean)

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Guerra, Á., Roura, Á., González, Á. F., Pascual, S., Cherel, Y., and Pérez-Losada, M. 2010. Morphological and genetic evidence that *Octopus vulgaris* Cuvier, 1797 inhabits Amsterdam and Saint Paul Islands (southern Indian Ocean). – ICES Journal of Marine Science.

The coastal octopus at Saint Paul and Amsterdam Islands is *Octopus vulgaris* Cuvier 1797. Meristic and morphological characters, along with phylogenetic analysis of COI and COIII DNA sequences, were used to identify 11 animals collected in 2000 or 2001. The range of the species is therefore expanded to include the oceanic islands of the central southern Indian Ocean. The trees also depicted the genus *Octopus* as polyphyletic and *O. vulgaris* sense Cuvier or *sensu stricto* as monophyletic.

Keywords: biogeography, DNA sequences, *Octopus vulgaris*, southern Indian Ocean.

accepted 18 March 2010.

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Introduction

The benthic octopus fauna of the southern Indian Ocean, including the oceanic islands, is among the poorest known worldwide (Voight, 1998). Toll (1998) recognized just 12 species-level taxa as valid among the 25 nominal species and subspecies from the Indian Ocean.

To date, it is not clear whether *Octopus vulgaris* is a true cosmopolitan species or simply a complex of species that has been treated as a single species in the literature. Taxonomic analyses are complicated by the fact that although *O. vulgaris* is the type species of the genus, type specimens were not designated by Cuvier nor was a type locality indicated in the original description, and the material identified by Cuvier and other early cephalopod workers such as Lamarck is not extant (Mangold and Hochberg, 1991); a neotype has not yet been designed and deposited, and the geographic distribution of the *O. vulgaris* group is not fully known. Currently, *O. vulgaris* sense Cuvier, 1797 (hereafter *sensu stricto*; *s. str.*) is considered to inhabit the Mediterranean Sea, the eastern Atlantic coast from southern England to southwestern Africa, the Azores, the Canary Islands, the Cape Verde Islands, the St Helena Islands, and many localities from the western Atlantic (Mangold, 1998; Norman, 2000). Two phylogenetic analyses of mitochondrial DNA COIII (Warnke *et al.*, 2004) and COI, 12S rRNA and 16S rRNA genes (Takumiya *et al.*, 2005) showed that the species is also present in waters of Taiwan and Japan. Warnke *et al.* (2004) also demonstrated that the monophyly of *O. vulgaris s. str.* was supported by high bootstrap values (79–100%). Using COI and 16S rRNA data, Teske *et al.* (2007) found that the species was present on both sides (Atlantic and Indian Oceans) of South Africa, but two specimens from Durban (Indian Ocean) were genetically so different that they could represent an undescribed species. To address this problem, those authors suggested that further sampling was needed in regions from which no genetic data were yet available, such as East Africa, India, and Southeast Asia. With this aim in mind, we study the octopus present at Amsterdam Islands (AI) and Saint Paul Islands (SPI; Southern Indian Ocean) using morphological, meristic, and genetic data.

Material and methods

In all, 11 specimens were collected in sublittoral waters (5–40 m) around AI (37°50′S 77°31′E) and SPI (38°43′S 77°32′E) in the southern Indian Ocean. Animals were collected by trapping and also as bycatch from the baited trap fishery targeting the St Paul's rock lobster *Jasus paulensis*.

Morphological study

Animals were frozen at −20°C and transported to the laboratory, where they were defrosted at room temperature (18°C), then preserved in 70% ethanol. Measurements and counts were carried out on preserved animals, following Roper and Voss (1983), Mangold (1998), and Huffard and Hochberg (2005), except for sucker counts. The last included all suckers, instead of just the proximal half of the arm. The characters recorded and the relevant abbreviations are listed in Table 1.

Unless otherwise stated, all measurements are in millimetres and weights in grammes. Small structures such as the ligula, calamus, and spermatophores were measured with an ocular

Table 1. Abbreviations (Abb) and definitions of the measurements and indices used (for more detail, see Roper and Voss, 1983; Mangold, 1998; Huffard and Hochberg, 2005).

Abb	Character	Definition	Abb	Character	Definition
AF	Arm formula	–	LnD	Lens diameter	–
AL	Arm length (L, left side; R, right side; 1, dorsal; 2, latero-dorsal; 3, latero-ventral; 4, ventral)	–	LnDI	Lens diameter index	Diameter of the eye lens as a percentage of ML
ALIa	Arm length index 1	Arm length/ML × 100	MAI	Mantle arm length index	ML length/longest arm length × 100
ALIb	Arm length index 2	Length of the longest arm as a percentage of the TL/TL	MAI	Mantle arm length index	ML length/longest arm length × 100
ASC	Arm sucker count	Number of suckers of each designated arm	ML	Dorsal ML	–
CaL	Calamus length	–	MW	Mantle width	–
CaLI	Calamus length index	Calamus length/LL × 100	MWI	Mantle width index	Mantle width/ML × 100
FFuL	Free funnel length	–	OAI	Opposite arm length index	HcA length/normal third arm length × 100
FFuLI	Free funnel length index	Length of the free region of the funnel/Ml × 100	PA	Pallial aperture	–
FuLI	Funnel length index	Funnel length/ML × 100	PAI	Pallial aperture index	Pallial aperture/ML × 100
GiLC	Number of gill lamellae per demibranch	–	SD	Sucker diameter	Diameter of the most enlarged sucker
HcL	HcA length	–	SDI	Enlarged sucker diameter index	Enlarged sucker diameter/ML × 100
HcAI	HcA index	Length of the HcA/ML × 100	TL	TL	–
HW	Head width		TBW	Total body weight	–
HWI	Head width index	Head width/ML × 100	TBW	Total body weight	–
LL	Ligula length	–	WD	Web depth (A to E sectors sections)	–
LLI	Ligula length index	Ligula length/HcL × 100	WDI	Web depth index	Web deepest depth/longest arm length × 100

micrometer in a binocular microscope. The animals collected were classified into four maturity stages (MS): 1, immature; 2, nearly mature; 3, mature; and 4, spawning, according to Guerra (1975). The chromatic and skin texture components were described following Hanlon (1988) and Mather and Mather (1994).

Molecular study

Tissue samples were taken from the arms of specimen 3 from SPI and specimens 10 and 11 from AI (Tables 2 and 3), all preserved in 70% ethanol. Total DNA was extracted using a QIAGEN DNeasy® tissue kit following the manufacturer's instructions. Two regions of the mitochondrial COIII (547 bp) and COI (682 bp) genes were amplified using the primers Ooc3F and Ooc3R (Guzik *et al.*, 2005) and HCO-LCO (Folmer *et al.*, 1994), respectively.

The PCRs were set up in a 25-μl reaction volume containing 2.5 μl of 10 × *Taq* buffer, 0.5 μl of 10 mM dNTPs, 0.75 μl each of two 10 mM primers, 0.125 μl of *Taq* (ROCHE), and 1 μl of DNA (200 ng). Amplifications were carried out using a Perkin–Elmer 9600 thermal cycler with the following cycling conditions: an initial denaturation at 96°C for 3 min, followed by 40 cycles of 95°C for 30 s, 50°C for 45 s, and 72°C for 1 min, followed by an extension at 72°C for 5 min. PCR products were resolved by 1.5% agarose gel electrophoresis, visualized by ethidium bromide fluorescence, purified using a MANU 030 PCR clean plate kit. Automated sequences were generated in both directions from different runs on an Applied Biosystems (ABI) 377XL automated sequencer using the ABI BigDye Ready Reaction Kit, following the standard cycle sequencing protocol, but using 1/16th of the suggested reaction size.

Phylogenetic inference

To assess the systematic position of the octopuses from SPI (SPI3) and AI (AI10 and AI11; Table 1), our three COI sequences were analysed in combination with 69 COI sequences from GenBank including 21 *Octopus* species and another 18 Octopoda Incirrata taxa (Figure 1). Further, our three COIII sequences were combined with 58 COIII sequences from GenBank including 33 *Octopus* species and another four incirrate genera (Figure 2). Nucleotide sequences were aligned using MAFFT v5.7 (Katoh *et al.*, 2005) under the global pairwise alignment algorithm and using default settings. Best-fit models of evolution were selected using the Akaike Information Criterion (Akaike, 1973) as implemented in Modeltest 3.7 (Posada and Crandall, 1998). The GTR + Γ + I model (COI: base frequencies = 0.288, 0.166, 0.160, 0.386, substitution rates = 3.78, 4.05, 6.49, 1.12, 41.94, shape parameter = 0.938, invariable sites = 0.465; COIII: base frequencies = 0.296, 0.186, 0.110, 0.408, substitution rates = 3.24, 6.53, 8.39. 0.79, 105.52, shape parameter = 0.707, invariable sites = 0.459) was chosen for both genes. Maximum likelihood genetic searches were performed in GARLI v0.951 (Zwickl, 2006) under the default settings (see GARLI manual for a description). Trees were rooted using midpoint rooting. Confidence in the resulting relationships was assessed using the non-parametric bootstrap procedure (Felsenstein, 1985) with 2000 bootstrap replicates. Genetic divergence among *Octopus* taxa was estimated using corrected (GTR + Γ + I) genetic distances. All DNA sequences were deposited in GenBank under the Accession Numbers FN424379–424384.

Table 2. Measurements and counts (for parameter abbreviations, see Table 1) on the octopuses from Saint Paul (SPI) and Amsterdam (AI) Islands.

Parameter	SPI 1	SPI 2	SPI 3	SPI 4	AI 5	AI 6	AI 7	AI 8	AI 9	AI 10	AI 11
Sex	M	M	F	M	M	M	F	M	F	M	M
MS	4	4	2	4	3–4	4	3	4	2	4	4
ML	175	200	140	167	146	210	190	177	175	210	135
WT	2 687	4 293	1 569	2 802	1 754	3 527	2 326	2 142	2 783	2 946	1 290
TL	930	1 180	790	970	822	1 331	1 120	920	950	1 087	822
MW	110	160	90	125	97	123	115	107	101	127	85
HW	52	72	43	35	48	50	49	53	53	62	39
LnD	10	15	6	13	6	9	14	10	9	11	11
PA	70	84	55	79	65	98	80	89	84	90	58
FuL	65	76	58	53	53	87	65	61	61	58	59
FFuL	52	49	42	21	36	51	44	43	43	51	30
AL1/AR1	530/670 b	835/617 r	465/495 r	527 b/600	565 r/535	860/921	765/261 r	710/700 r	532 r/667	807 r/720 r	434 r/510 r
AL2/AR2	650/720 b	955/1 075	642/626 r	813/721 r	746/690	1 034/1 130	947/861 r	818 r/783	805/488 b	860 b/885 r	553 r/574 r
AL3/R3(Hc)	619 b/630	890/735	754/725b	843 b/571	706/528	1 052/796	917/887	751 r/545	786/825	695 b/753	653 b/550
AL4/AR4	660/630	847/860	550/492	440 b/613	615/687	917/904 r	832 b/810	752/714	521/682	696 r/670r	571 r/561r
AF	–	2.3.4.1	3.2.4.1	–	2.3.4.1	3.2.4.1	2.3.4.1	–	2.3.1.4	–	–
ASC1L/R	256/–	278/–	216/–	–/300	/236	315/296	316/–	304/–	–/308	–/–	–/–
ASC2R/L	218/–	350/342	300/–	336/–	290/290	332/320	342/–	–/322	326/–	–/–	–/–
ASC3R/HASC	–/192	297/182	334/–	–/162	316/182	330/176	320/–	–/186	342/–	–/178	–/188
ASC4R	302/308	352/76	–/399	–/260	356/314	336/–	–/374	362/362	276/368	–/–	–/–
SD	39	43	19	30	30	36	23	34	28	34	32
LL	5.4	6.5	–	4.7	4.2	7.6	–	5.8	–	6.9	4.6
CaL	2.5	1.9	–	2.1	2.2	2.1	–	1.6	–	2.3	2.1
WD A	97	102	89	66	80	102	71	100	88	91	52
WD B	140	130	98	145	120	159	139	153	92	180	111
WD C	135	151	120	154 r	121	195	192	181	146	210	120
WD D	130	150	125	156	104	153	163	101	154	129	119
WD E	105	118 r	60	79	74	97	125	80 r	102	126	103
WF	B.C.D.E.A	C.D.B.E.A	D.C.B.A.E	D.C.B.E.A	C.B.D.A.E	C.B.D.A.E	C.D.B.E.A	C.B.D.A.E	D.C.E.B.A	C.B.D.E.A	C.D.B.E.A
GiLC	9	10	9	10	v	9–10	9–10	v	v	9	9

Table 3. Indices (for parameter abbreviations, see Table 1) from the octopuses caught at Saint Paul (SPI) and Amsterdam (AI) Islands.

Parameter	SPI 1	SPI 2	SPI 3	SPI 4	AI 5	AI 6	AI 7	AI 8	AI 9	AI 10	AI 11
ALIaL2	371.43	477.50	458.57	486.83	510.96	492.38	498.42	–	460.00	–	–
ALIaL3	–	445.00	538.57	–	483.56	500.95	482.63	–	449.14	–	–
ALIaL4	377.14	423.50	392.86	–	421.23	436.67	–	424.86	297.71	–	–
ALIaR1	–	–	–	359.28	366.44	438.57	–	–	381.14	–	–
ALIaR2	–	537.50	–	–	472.60	538.10	–	442.37	–	–	–
HcAI/ALIaR3	360.00	367.50	–	341.92	361.64	379.05	–	307.91	–	358.57	407.41
ALIaR4	360.00	430.00	351.43	367.07	470.55	–	426.32	403.39	389.71	–	–
ALIb	77.42	80.93	77.73	86.90	–	79.03	84.55	–	–	–	–
CaLI	67.57	34.55	–	56.76	52.38	38.18	–	37.21	–	58.97	58.33
FuLi	37.14	38.00	41.43	31.74	36.30	41.43	34.21	34.46	34.86	27.62	43.70
FFuLI	29.71	24.50	30.00	12.57	24.66	24.29	23.16	24.29	24.57	24.29	22.22
HcAI	360.00	367.50	–	341.92	361.64	379.05	466.84	307.91	471.43	358.57	407.41
HWI	29.71	36.00	30.71	20.96	32.88	23.81	25.79	29.94	30.29	29.52	28.89
LLI	0.85	0.88	–	0.82	0.80	0.95	–	1.06	–	0.92	0.84
LnDI	5.71	7.50	4.29	7.78	4.11	4.29	7.37	5.65	5.14	5.24	8.15
MAI	–	18.60	18.57	19.81	19.57	18.58	20.06	–	21.21	–	–
MWI	62.86	80.00	64.29	74.85	66.44	58.57	60.53	60.45	57.71	60.48	62.96
OAI	–	82.58	–	74.78	75.66	–	–	–	–	–	–
PAI	40.00	42.00	39.29	47.31	44.52	46.67	42.11	50.28	48.00	42.86	42.96
SDI	22.29	21.50	13.57	17.96	20.55	17.14	12.11	19.21	16.00	16.19	23.70
WDI	–	14.05	16.58	–	16.22	17.26	20.27	–	18.67	–	–

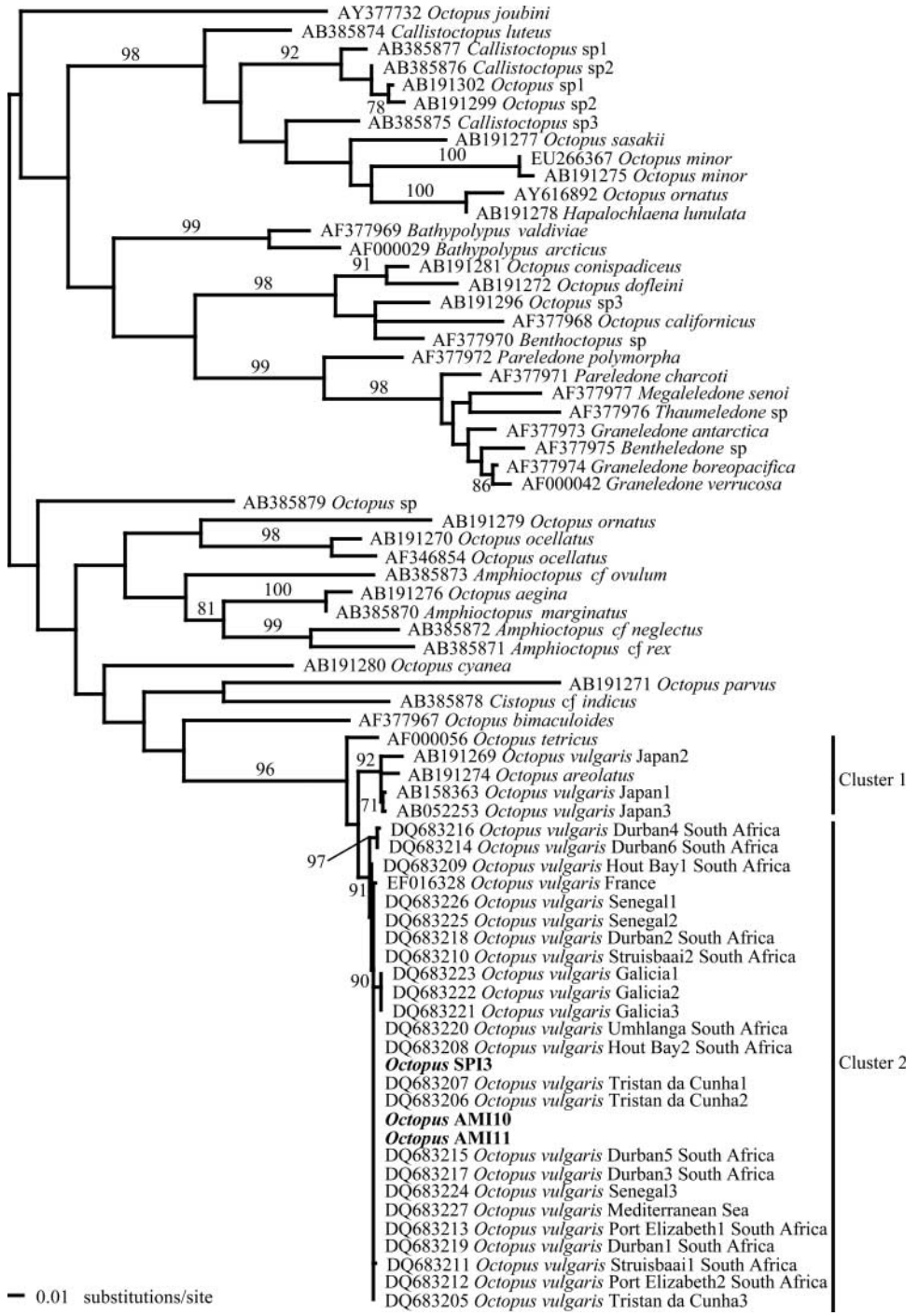

Figure 1. COI maximum likelihood tree. Branch lengths are shown proportional to the amount of change along the branches. Bootstrap values ≥70% are shown for each node. Specimens from Saint Paul (SPI3) and Amsterdam (AI10 and AI11) Islands are shown emboldened. **Q3**

Results

Description

Tables 2 and 3 list the measurements, counts, and indices of the 11 animals examined (all medium to large size adults; up to 210 mm mantle length, ML, of both sexes, and up to 4300 g total weight, and at least 1300 mm total length, TL). Other characters not included in the tables are as follows: there are two thick cartilaginous stylets (0.2 mm diameter, 18.8 mm long, in a male of 78 mm ML); the terminal organ or penis is moderately long and with a small and rounded diverticulum; there are no ocelli; the skin is firm and smooth in preserved specimens; the colour pattern and skin sculpture in preserved animals do not differ from *O. vulgaris s. str.* specimens preserved in the same manner; there are six supraocular papillae, two in the anterior region of the eyes, two large (horns) in the middle, and two of medium size in the posterior region of the eyes.

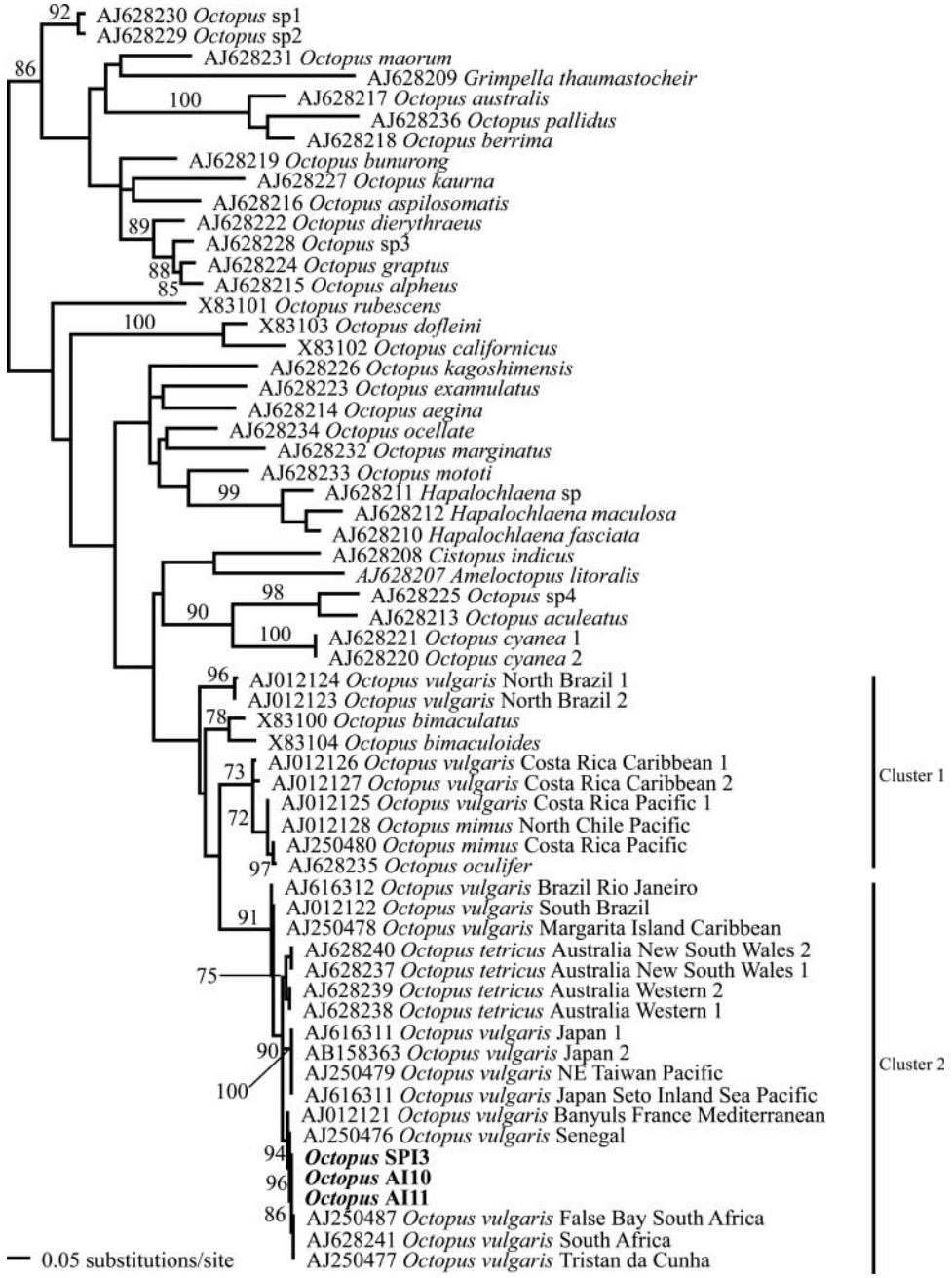

Figure 2. COIII maximum likelihood tree. Branch lengths are shown proportional to the amount of change along the branches. Bootstrap values ≥70% are shown for each node. Specimens from Saint Paul (SPI3) and Amsterdam (AI10 and AI11) Islands are shown emboldened.

Molecular analysis

Both the COI and the COIII maximum likelihood trees (Figures 1 and 2, respectively) indicate that the three octopods sampled from SPI (SPI3) and AI (AI10, AI11) are genetically similar to *O. vulgaris s. str*. The maximum likelihood trees show SPI and AI specimens in a short branch-length clade containing other, confidently identified, specimens of *O. vulgaris s. str*. This assemblage is supported by bootstrap values ≥70% in both trees. Our COIII tree also shows that *O. vulgaris* from South Africa and Tristan da Cunha are the closest relatives to the three SPI and AI animals and that this relationship is supported by 85% bootstrap values. Our trees show that the genus *Octopus* is polyphyletic and that *O. vulgaris s. str*. is monophyletic.

COI mean genetic distances between the SPI/AI octopods and *O. vulgaris s. str*. (cluster 1), the *Octopus* in cluster 2, and the rest of the *Octopus* outside these two clusters (Figure 1) were 0–0.012, 0.037–0.051, and 0.318–0.638, respectively. COIII mean genetic distances between the SPI/AI octopods and *O. vulgaris s. str*. (cluster 1), the *Octopus* in cluster 2, and the rest of the *Octopus* outside these two clusters (Figure 2) were 0.002–0.057, 0.188–0.363, and 0.354–1.835, respectively. These estimates again demonstrate that the octopods from SPI and AI are genetically

similar or identical to other *O. vulgaris s. str.*, and different from other *Octopus* species.

Discussion

The measurements, counts, indices, and other characters, such as the presence of papillae or the lack of ocelli (Tables 2 and 3), generally match *O. vulgaris s. str.* from the Mediterranean Sea (Mangold, 1998). The few characters that do not match, e.g. the narrower head, the smaller funnel, more hectocotylized arm (HcA) suckers, ligula slightly smaller, and the calamus slightly larger, could be attributed to preservation, or perhaps to local adaptation. The main differences between the animals analysed and preserved *Octopus cyanea* Gray, 1849, is the absence of ocelli (see redescription by Norman, 1991). Moreover, the results of Guzik *et al.* (2005) provide strong support for the absence of a close phylogenetic relationship between the *O. vulgaris* group and *O. cyanea*, although the latter species shares a number of morphological features with the *O. vulgaris* group, including large body size, small male reproductive structures, and enlarged suckers.

The phylogenetic and genetic divergence estimates indicate that the octopuses from AI and SPI belong to *O. vulgaris s. str.* and confirm that COI and COIII are useful for inferring evolutionary relationships and distinguishing among closely related octopuses (Söller *et al.*, 2000; Warnke *et al.*, 2004; Guzik *et al.*, 2005). Our maximum likelihood trees show that the three study specimens (SPI3, AI10, and AI11) clustered with *O. vulgaris* from the Mediterranean Sea, France, Galicia (NW Iberian Peninsula), Senegal, Tristan da Cunha, and South Africa. All these regions are within the typical geographic range of *O. vulgaris s. str.* (Mangold, 1998). All specimens then clustered with *O. vulgaris* from Japan and Taiwan, south Brazil, Rio de Janeiro, and Venezuela, which are also areas where *O. vulgaris s. str.* has been recorded (Warnke *et al.*, 2004). Our trees also show that the three study specimens are phylogenetically different from *O. cyanea*, as previously indicated by Guzik *et al.* (2005).

The known distribution area of *O. vulgaris s. str.* was the Mediterranean Sea, the eastern Atlantic (from southern England to southwestern Africa), the Azores, the Canary Islands, Cape Verde, St Helena, the Tristan da Cunha Islands, the southeast coast of South Africa in the Indian Ocean, and the northwestern Pacific, namely the waters of Taiwan and Japan (Mangold, 1998; Warnke *et al.*, 2004). Our results extend the distribution of the species to the oceanic islands of the central southern Indian Ocean.

In the COI maximum likelihood tree (Figure 1), *Octopus areolatus* de Haan, 1839, which is a synonym (Norman and Hochberg, 2005) of *Amphioctopus fansiao* (d'Orbigny, 1839), fell within the *O. vulgaris* clade. This could be due to: (i) misidentification of *O. areolatus* in GenBank, (ii) misidentification of *O. vulgaris* from Japan, (iii) that *O. vulgaris* from Japan is a new species, or (iv) a case of incomplete lineage sorting, i.e. when the topology of the gene trees may differ from that of the species tree (Mossel and Roch, 2010).

In the COIII maximum likelihood tree (Figure 2), *Octopus tetricus* Gould 1852 from Australian waters and *Octopus oculifer* Hoyle 1904 (AJ628235) clustered with *O. vulgaris s. str.* Both are recognized species (Norman and Hochberg, 2005) and, based on the descriptions of Robson (1929) and Stranks (1998), both are morphologically very different from our study specimens. However, interestingly, both *O. tetricus* and *O. oculifer* appeared within the *O. vulgaris* clade (98% bootstrap support) in the consensus tree of Guzik *et al.* (2005). All this molecular evidence reinforces the argument suggested by those authors that *O. tetricus* and *O. oculifer* are members of the *O. vulgaris* group and therefore should be treated as true representatives of the genus *Octopus*.

Other taxa found within the *O. vulgaris* clade (Figures 1 and 2) came from Costa Rica, both Pacific and Caribbean sides (AJ012125–AJ12127), northern Brazil (AJ012123 and AJ012124), Rio de Janeiro (AJ616312), southern Brazil (AJ012122), and Isla Margarita, Venezuela (AJ250478). Therefore, from our results, the animals from Rio de Janeiro, southern Brazil, and Isla Margarita are *O. vulgaris s. str.*, agreeing with the findings of Warnke *et al.* (2004). However, the current distribution of *Octopus* spp. in the western Atlantic is unclear. Clarifying the geographic range of *O. vulgaris s. str.* in these waters, as well as its phylogenetic relationship with other octopuses such as *Octopus insularis* (Leite and Hamovici 2008) or *Octopus maya* (Voss and Solis 1966), requires further study.

Our results and those of Warnke *et al.* (2004) show that *O. vulgaris s. str.* is monophyletic. The analyses performed by Guzik *et al.* (2005) go further, however, suggesting that the *O. vulgaris* species group, including *O. oculifer* from Galapagos, *O.* cf. *tetricus* from Western Australia, *O. tetricus* from New South Wales, and *O.vulgaris s. str.* from Port Elizabeth in South Africa, which were the species used by those authors, is also monophyletic. However, because that species group may contain other species such as *O. insularis* (Leite *et al.*, 2008), further study is needed to test whether the *O. vulgaris* species group will hold its monophyletic status when all species are analysed together.

Finally, our phylogenetic trees also show that the genus *Octopus* is polyphyletic. This agrees with the results of Guzik *et al.* (2005), who demonstrated that the genus contains a number of distinct and divergent clades and that the systematics of the subfamily Octopodinae require major revision.

Acknowledgements

We thank Elizabeth Shea for her useful comments on a first draft of this manuscript, Nicolas Gasco and the crew of "l'Austral" for collecting the samples, and María Teresa Fernández and Manuel Garci (IIM, Vigo) for technical support. The study was partially supported by a FCT grant (PTDC/BIA-BEC/098553/2008) to MP-L. ÁR was supported by a fellowship (Introduction to Research and *JAE* pre-doctoral) from the CSIC.

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doi:10.1093/icesjms/fsq040