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PHYLOGEOGRAPHICAL FEATURES OF *OCTOPUS VULGARIS* AND *OCTOPUS INSULARIS* IN THE SOUTHEASTERN ATLANTIC BASED ON THE ANALYSIS OF MITOCHONDRIAL MARKERS

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ABSTRACT The genus *Octopus* occurs in tropical and temperate oceanic waters throughout the world, and currently includes 112 species, although the phylogenetic relationships among the different taxa are still poorly understood. The cosmopolitan *Octopus vulgaris* is one of the most widely analyzed cephalopods in genetic studies, primarily because of its ample range and the problems associated with the morphological identification of specimens, which indicate the possible existence of a species complex with a worldwide distribution. Two large-bodied octopus species—*O. vulgaris* and *Octopus insularis*—are found in the western South Atlantic. The limits of the geographical range of the *O. insularis* are still unclear. The current study is based on a phylogeographic analysis of the 2 species in the South Atlantic, with the objective of confirming their monophyletic status and the limits of their geographical distribution in this region. The analyses were based on the mitochondrial genes *16S rDNA* and *Cytochrome Oxidase subunit I (COI)*. The topologies generated for both genes confirmed the monophyletic status of the 2 species. In the case of *O. vulgaris*, it was possible to confirm the monophyletic status of the specimens from this region relative to those of other areas around the world, although 3 distinct haplogroups were clearly differentiated, corresponding to the Americas, Europe and Africa, and Asia. The differentiation among these 3 groups may be determined by the limitations of the dispersal of paralarvae among continents. Further studies are needed to confirm the possible occurrence of distinct groups in the western South Atlantic, as well as the influence of oceanic currents on the phylogeographical distribution of *O. vulgaris* on the Brazilian coast.

KEY WORDS: phylogeography, *Octopus vulgaris*, *Octopus insularis*, South Atlantic, genetics, mitochondrial DNA

INTRODUCTION

The genus *Octopus* occurs throughout the tropical and temperate regions of the world's oceans (Norman 2003). Approximately 112 species are currently recognized, although the phylogenetic relationships among the different forms are still poorly understood (Norman & Hochberg 2005). The nominal members of this genus present widely varying characteristics, ranging from small-bodied species with large eggs, low fecundity, benthic larvae, and a restricted geographical distribution, such as *Octopus tehuelchus* Orbigny, 1834 (Alves & Haimovici 2011), to large, widely distributed species with high fecundity and pelagic postlarvae, such as *Octopus vulgaris* Cuvier, 1797 (Mangold 1987, Villanueva & Norman 2008). However, recent phylogenetic studies have indicated that *O. tehuelchus*, in fact, is related phylogenetically to *Grimpella* and *Callistoctopus*, not *Octopus* (Acosta-Jofré et al. 2012).

Genetically, *Octopus vulgaris* is one of the most widely studied cephalopod species (Carlini & Graves 1999, Warnke 1999, Warnke et al. 2004, Guzik et al. 2005, Leite et al. 2008), which is a result of a combination of its cosmopolitan distribution and the difficulties of identifying the species based on morphological criteria. Norman (2003) referred to this taxon as a "species complex," and argued that a number of distinct taxa are classified incorrectly as *Octopus vulgaris* in different parts of the world. This has been confirmed in recent years by a number of genetic and morphological studies, principally in the western hemisphere, which resulted in the description of a number of new species, including *Octopus maya* (Voss & Ramirez 1966), *Octopus mimus* (Guerra et al. 1999), and *Octopus insularis* (Leite et al. 2008). The cosmopolitan distribution of *O. vulgaris* has been challenged by some authors (e.g., Mangold 1997, 1998), although its occurrence has been confirmed by the molecular genetic analysis of specimens from coastal waters of the Americas (Warnke et al. 2004, Sales et al. 2007), Africa (Oosthuizen et al. 2004), and Asia (Takumiya et al. 2005).

At least 2 species of large-bodied octopi with small eggs, high fecundity, and pelagic postlarvae occur in the western South Atlantic: *Octopus vulgaris* (Cuvier 1797) and *Octopus insularis* (Leite & Haimovici 2008). The geographical range of *O. insularis*, which was described from specimens collected in

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the vicinity of the oceanic islands off the northeastern coast of Brazil, is now known to include northern South America (Sales et al. 2007).

In the current study, a phylogeographical analysis of these 2 common *Octopus* species from the South Atlantic (*Octopus vulgaris* and *Octopus insularis*) was conducted using molecular mitochondrial markers. This analysis aimed to corroborate the identification of the species and their monophyletic status, as well as confirm their occurrence throughout the study area.

MATERIAL AND METHODS

Samples

Samples of the 2 study species were obtained along the coast of the western Atlantic in Brazil, between the latitudes 03′24′27″ N and 27°08′48.06″ S (Fig. 1). The specimens collected in northern Brazil (Amapá and Pará states) were obtained from the bycatch of fishing for red snapper (*Lutjanus purpureus* Poey 1875) and green lobster (*Panulirus laevicauda* Latreille 1817), as well as from the stomach contents of some red snapper specimens (samples OvuPA 78, OvuPA 173, Ovu 184, OvuAP 225, and AmspPA 86, representing *Amphioctopus* sp.). All other specimens were obtained from commercial fisheries (*Octopus hummelincki* Adam 1936, *Eledone massyae* Voss 1964) or local fish markets (locations provided in Appendix A (Strugnell et al. 2004, Allcock et al. 2006, Teske et al. 2007)). A small fragment of muscle tissue was extracted from 1 of the arms of each animal, and was stored in a freezer in flasks with 100% ethanol until the extraction of the DNA.

Adult specimens were identified based on the specific literature (Roper et al. 1984). Some of these adults, as well as all the material obtained from stomach contents, were fixed in 10% formalin and deposited in the zoological collection of the Oceanographic Museum at Universidade Federal do Rio Grande (FURG). The identification of some of the specimens obtained from stomach contents, which were in an advanced stage of decomposition, and thus lacked the morphological structures necessary for taxonomic analysis, was achieved by comparing the DNA 16S and cytochrome oxidase subunit I (COI) sequences with those available for the study species in GenBank.

Extraction of DNA, Polymerase Chain Reaction, and Sequencing

Total DNA was isolated using the modified phenol/chloroform protocol of Sambrook and Russel (2001). When this approach was unsuccessful, a Wizard Genomics DNA purification kit was used, according to the manufacturer's instructions (Promega Corporation, Madison, WI). In both cases, the tissue was prewashed with 600 µL ultrapure water based on two 2-min centrifugations at 13,000*g* for the removal of excess alcohol.

The primers for the 2 mitochondrial genes (*16S rDNA* and *Cytochrome Oxidase subunit I—COI*) were obtained from the

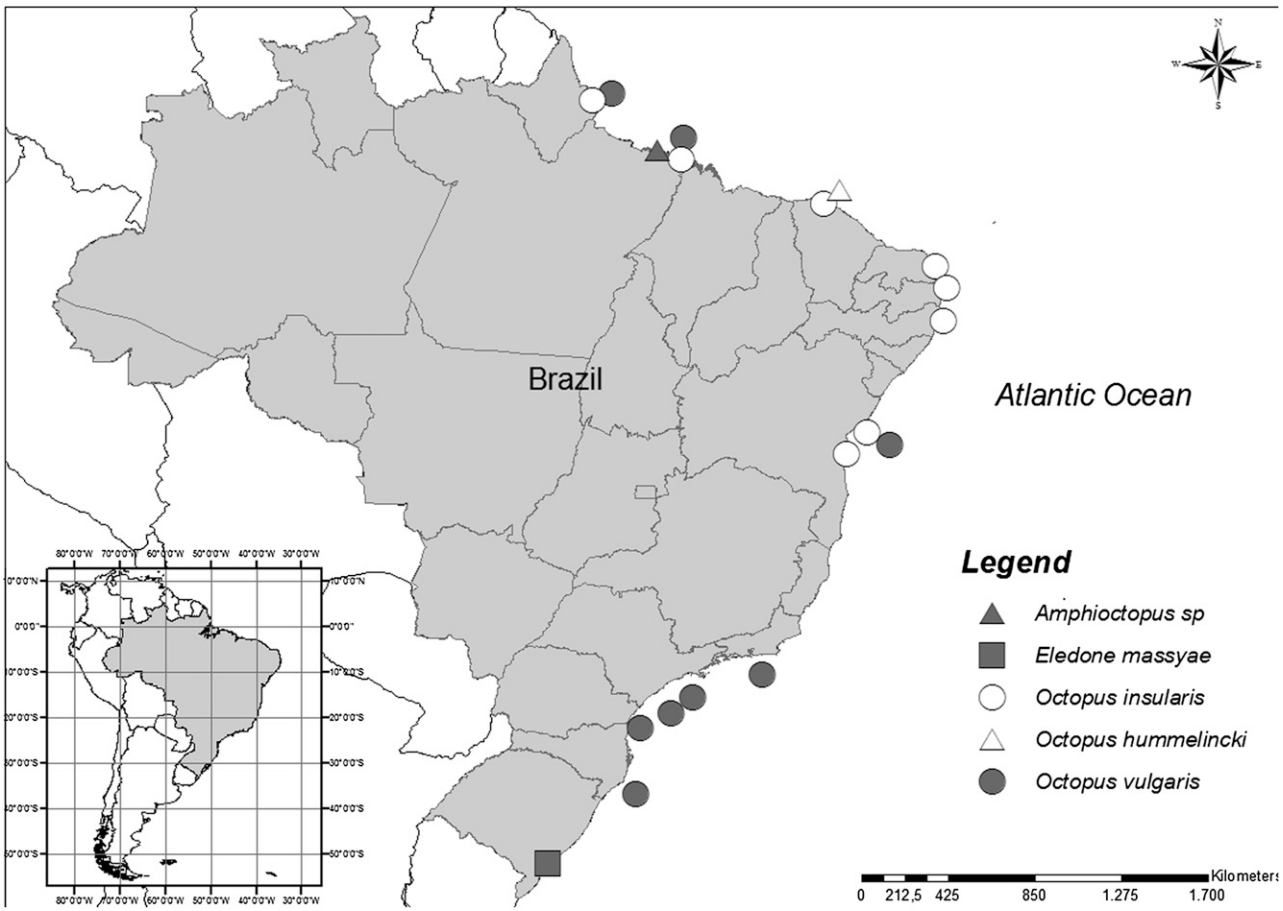

Figure 1. Localities from which specimens analyzed in the current study were collected.

literature (Table 1). Amplification of the *16S* gene was based on the following cycling parameters: 2 min at 94°C for denaturation, followed by 30 cycles of 30 sec at 94°C, 1 min at 51°C for annealing, and 2 min at 72°C for extension, and then 7 min at 72°C for the final extension. For COI, the procedure was 2 min at 94°C for denaturation, followed by 30 cycles of 1 min at 94°C, 1 min at 45.5°C for annealing, 2 min at 72°C for extension, and 7 min at 72°C for the final extension. The polymerase chain reactions for both markers were conducted in a final volume of 25 μL containing 4 μL DNTPs (1.25 mM), 2.5 μL buffer solution (10×), 1 μL $MgCl_2$ solution (50 mM), 80–200 ng total DNA, 0.25 μL each oligonucleotide (200 ng/μL), 0.25 μL AccuPrime *Taq* enzyme polymerase (Invitrogen; 5 U/μL), and sterile bidistilled water to complete the final reaction volume.

Prior to sequencing, the polymerase chain reactions were purified with the ExoSAP-IT enzyme (Amersham Pharmacia Biotech Inc.). Sequencing was conducted using BigDye kit reagents (Applied Biosystems), with the products being read in an ABI 3500 automatic sequencer (Applied Biosystems). Additional sequences from other *Octopus* species (*Octopus vulgaris*, *Octopus insularis, Octopus maya* Voss & Solis 1966, *Octopus mimus* Gould 1852, and *Octopus bimaculoides* Pickford & McConnaughey 1949), as well as *Hapalochlaena maculosa* Hoper & Hochberg 1988, were obtained from GenBank for the comparative analysis of the divergence among sequences and the rooting of the phylogenetic groups (details are provided in Appendix A (Strugnell et al. 2004, Allcock et al. 2006, Teske et al. 2007)).

Phylogenetic and Population Inferences

The DNA sequences were aligned using the ClustalW multiple alignment tool (Thompson et al. 1997) in the BioEdit program v.5.0.6 (Hall 1999). After automatic alignment, each sequence was inspected visually for the correction of possible edition errors. This was especially important in the case of the *16S* gene, which presented a large number of gaps when comparing sequences of the most divergent species.

For the phylogenetic analyses, the optimum evolutionary models were selected using the jModelTest program (Guidon & Gascuel 2003), based on the Akaike information criterion (Akaike 1974) for maximum likelihood (ML) and the Bayesian information criterion for Bayesian inference (BI). The ML analysis was run in PhyML 3.0 (Guidon et al. 2010), with the reliability of the groups being verified using a nonparametric bootstrap analysis with 1,000 replicates (Felsenstein 1985). The Bayesian analysis was run in MrBayes v 3.1.2 (Ronquist & Huelsenbeck 2003). For BIs, the data set was analyzed with a single substitution model (i.e., unpartitioned), and partitioned by gene and codon position (i.e., a separate substitution model was chosen for each of the 3 *COI*s). Partitioned Bayesian analyses were based on the Markov chain Monte Carlo sampling procedure, with 4 simultaneous runs, each consisting of 4 chains (1 cold, 3 heated), and a total run length of 10 million generations, using the parameters of the evolutionary models selected for each partition. The *a posteriori* Bayesian probabilities were selected by the 50% consensus rule, with random starting trees and trees sampled every 5,000 generations after the removal of the trees that appeared to have reached a stationary state, at which the burn-in was verified by the empirical examination of the likelihood values. FigTree v.1.1.2 was used to edit the phylogenetic trees. When the topologies were obtained, the observed clades were considered to be distinct groups for the subsequent calculation of intra- and interspecific divergence values in MEGA 5.04 (Tamura et al. 2011).

For the analysis of *Octopus vulgaris* and *Octopus insularis* populations, the indices of haplotype (*h*) (Nei 1987) and nucleotide diversity (π) (Nei 1987) were estimated in DnaSP, version 5.10 (Librado & Rozas 2009). Arlequin 3.01 (Excoffier et al. 2006) was used to estimate the fixation indices (F_{st}) (Weir & Hill 2002) and to run the hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992), which was based on 1,000 permutations using the Kimura 2P substitution model (Kimura 1980). The *D* (Tajima 1989) and *Fs* (Fu 1997) tests of selective neutrality were run in Arlequin 3.01 (Excoffier et al. 2006). The spatial distribution of haplotypes within the populations was mapped using Haploview (Salzburger et al. 2011).

TABLE 1.

Primers used for the PCR amplification of the 2 genes analyzed in the current study.

Gene	Primers	References
16S	5′-GCCTGCCTGTTTACCAAAAAC-3′	Palumbi et al. (1991)
	5′-CGGTCTGAACTCAGATCACGT-3′	
COI	5′-GGTCAAACAAATCATAAAGA TATTGG-3′	Folmer et al. (1994)
	5′-TAAAATTCAGGGTGACCAAAA AATCA-3′	

RESULTS

A total of 948 bp were sequenced, including 482 for *COI* and 466 for *16S*. The optimum models of substitution selected by jModelTest were TIM3 + G for the *16S* gene (for both ML and BI), whereas for *COI*, different models were selected for ML (GTR + G) and BI (TIM2 + G) for the unpartitioned data set, and TIM 2 + I + G for the codon partitioned data set. Because the topologies produced by the 2 approaches were highly similar, only the ML trees are shown here (Figs. 2 and 3). The monophyletic status of both *Octopus vulgaris* and *Octopus insularis* is clear from the configuration of this tree.

The analysis indicated the presence of a single monophyletic *Octopus vulgaris* clade throughout the study area, with strong statistical support (99% for both ML and BI). Three distinct haplogroups can be discerned in the tree for the 16S gene in both phylogenetic approaches (Fig. 2). Group 1 is formed by specimens from Africa and Europe, whereas group 2 is formed exclusively by specimens from the southeastern Atlantic, including individuals from Venezuela and the coast of Brazil. Group 3 is composed of specimens from Asia (Japan and Taiwan), and is the most basal within the *O. vulgaris* clade. The topologies derived from the analysis of the *COI* gene also confirmed the monophyletic status of this species (statistical support, 99/1), as well as the presence of subgroups, although with a slightly different topology.

Nucleotide divergence between the different *Octopus vulgaris* groups ranged from 1.6–2.1% (Table 2). In turn, *O. vulgaris* diverged from other *Octopus* species by 7.8–9.7% (*Octopus*

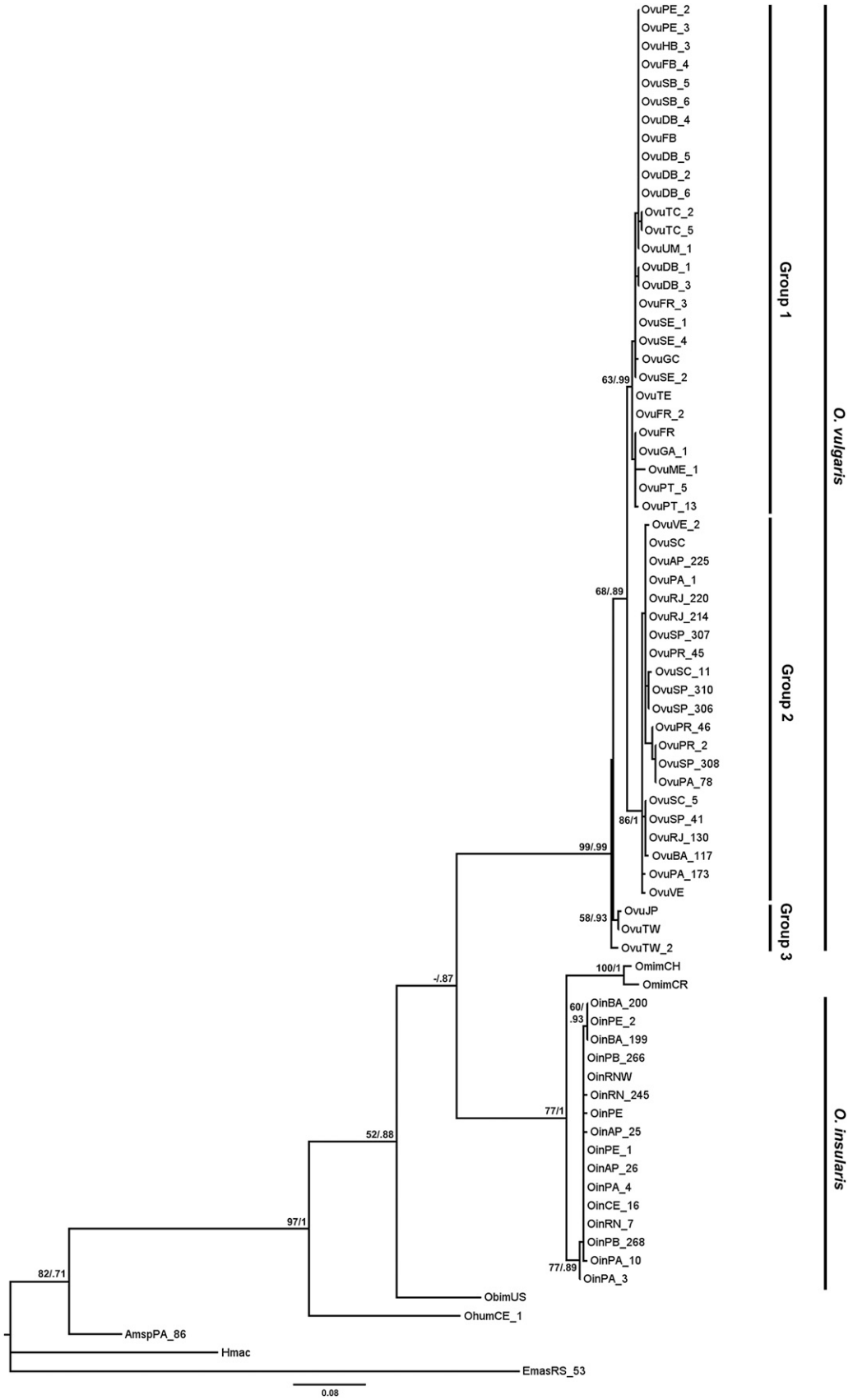

Figure 2. Maximum likelihood phylogenetic tree for the *16S* rDNA mitochondrial gene based on the TIM3 + G evolutionary model selected by the jModeltest program. Only reliability values of more than 50% are shown.

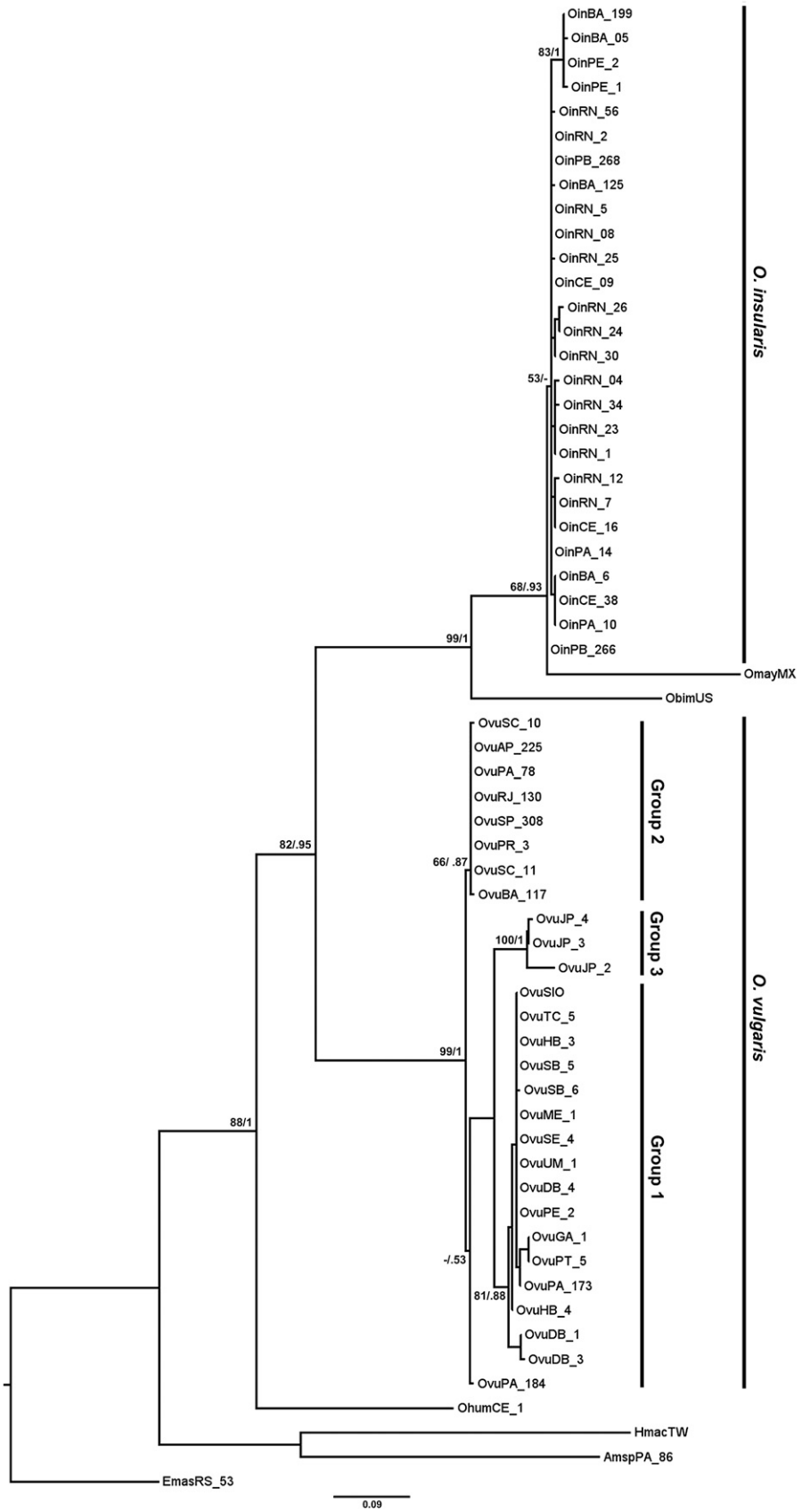

Figure 3. Maximum likelihood phylogenetic tree for the COI mitochondrial gene based on the GTR + G evolutionary model selected by the jMODELTEST program. Only reliability values of more than 50% are shown.

TABLE 2.

Genetic divergence between among species groups identified through the analysis of the *16S* rDNA gene.

Group	1	2	3	4	5	6	7	8	9	10
Group 1		*0.8*	*0.8*	*3.3*	*4.0*	*5.1*	*5.7*	*11.2*	*16.1*	*17.6*
Group 2	**2.0**		*0.7*	*3.1*	*3.7*	*4.9*	*5.6*	*11.2*	*16.1*	*17.2*
Group 3	**2.0**	**1.6**		*2.7*	*3.6*	*4.6*	*6.0*	*11.2*	*16.6*	*17.7*
Octopus bimcauloides	**9.7**	**9.1**	**7.8**		*3.8*	*3.4*	*3.6*	*9.5*	*18.1*	*17.3*
Octopus insularis	**11.2**	**9.7**	**10.1**	**11.2**		*1.8*	*4.5*	*5.8*	*8.7*	*17.8*
Octopus mimus	**12.9**	**12.3**	**11.7**	**10.1**	**5.3**		*6.2*	*9.1*	*12.3*	*18.7*
Octopus hummelincki	**13.2**	**13.5**	**13.5**	**10.4**	**13.2**	**16.5**		*6.1*	*12.8*	*17.2*
Amphioctopus sp.	**14.9**	**15.3**	**14.8**	**15.8**	**13.6**	**16.1**	**13.8**		*5.1*	*15.1*
Hapalochlaena maculosa	**19.0**	**18.6**	**18.5**	**20.9**	**17.2**	**19.6**	**19.4**	**10.4**		*11.1*
Eledone massyae	**26.4**	**25.9**	**26.4**	**22.5**	**24.5**	**26.0**	**24.1**	**19.1**	**18.4**	

The values in bold type (below the diagonal) are the nucleotide divergence values (percent); values in italics (above the diagonal) are SDs.

TABLE 3.

Genetic divergence among the species groups identified through the analysis of the *COI* gene.

Group	1	2	3	4	5	6	7	8	9	10
Group 1		*0.7*	*0.7*	*2.6*	*2.2*	*2.6*	*2.3*	*3.2*	*3.2*	*2.8*
Group 2	**2.6**		*0.6*	*2.8*	*2.3*	*2.7*	*2.4*	*3.2*	*3.2*	*2.8*
Group 3	**3.2**	**2.6**		*2.7*	*2.3*	*2.7*	*2.5*	*3.1*	*3.3*	*2.9*
Octopus bimcauloides	**16.9**	**18.8**	**18.3**		*1.9*	*2.0*	*2.7*	*3.4*	*3.4*	*3.3*
Octopus insularis	**13.7**	**14.8**	**15.0**	**11.2**		*1.4*	*2.5*	*3.3*	*3.4*	*3.2*
Octopus mimus	**17.8**	**18.8**	**18.9**	**12.8**	**8.6**		*2.6*	*3.6*	*3.7*	*3.2*
Octopus hummelincki	**15.1**	**15.2**	**16.6**	**17.8**	**16.3**	**18.3**		*3.2*	*3.0*	*2.8*
Amphioctopus sp.	**20.2**	**20.1**	**20.3**	**23.3**	**22.0**	**24.4**	**21.5**		*2.8*	*3.2*
Hapalochlaena maculosa	**21.1**	**21.7**	**22.8**	**23.3**	**23.0**	**25.1**	**19.6**	**18.7**		*3.0*
Eledone massyae	**19.9**	**19.2**	**20.6**	**22.0**	**22.0**	**22.6**	**19.3**	**22.2**	**20.8**	

The values in bold type (below the diagonal) are the nucleotide divergence values (percent); values in italics (above the diagonal) are SDs.

bimaculoides), 9.5–11.2% (*Octopus insularis*), 11.7–12.9% (*Octopus mimus*), and 13.2–13.6% (*Octopus hummelincki*). The lowest genetic divergence between 2 species was 5.2% for *O. insularis* and *O. mimus*. Genetic divergence among genera ranged from 10–26%.

The species *Octopus insularis* was also clearly monophyletic (77/1) based on the *16S* sequences, but closely related phylogenetically to *Octopus mimus* from the Pacific Ocean, as indicated by the low genetic divergence recorded between the species (Fig. 2, Table 2). The *COI* sequences also confirm the monophyletic status of this species. Because *COI* sequences were not available for *Octopus mimus*, *Octopus maya* was the closest species to *O. insularis* in this phylogenetic analysis, followed by *Octopus bimaculoides*, *Octopus vulgaris*, and *Octopus hummelincki* (Fig. 3, Table 3).

Based on the identification of the 3 subgroups in *Octopus vulgaris*, 3 geographical divisions were established (Appendices B and C): group 1, specimens from the western hemisphere; group 2, specimens from Europe and the eastern Atlantic; and group 3, specimens from Asia. The databases for the *16S* and *COI* genes include some unique samples, of which the number varies according to the number of taxa included in the analysis (63 in *16S* and only 46 in *COI*). In the case of the 16S gene, the *O. vulgaris* subgroups presented high values for both genetic and haplotype diversity, ranging from 0.81–1.00 (Table 4). Group 1 presented the largest number of polymorphic sites, followed by groups 2 and 3. However, the highest haplotype diversity was recorded in the Asian group (group 3), the lowest in the African group (group 2), and none of the haplotypes were shared by the different populations.

Nucleotide diversity varied from 0.005 (for groups 1 and 2)–0.007 (for group 3). The haplotype networks generated from the sequences upheld the 3 subgroups, corresponding to their geographical distribution (Fig. 4).

This was confirmed by the high values obtained for the AMOVA and F_{st} analyses, which indicate more divergence between than within populations (Table 5). In addition, all the between-population values for Φst were significant ($P < 0.05$), with the greatest differentiation found between the populations of groups 1 and 3 (Table 6). The Φst values obtained for 16S also presented some differences in comparison with those for COI. Although all the values for COI were highly significant ($P < 0.05$), the highest divergence was obtained for groups 1 and 2, and the lowest between groups 2 and 3. This gene also returned highly significant AMOVA and F_{st} values for the *Octopus vulgaris* groups (Table 7). The distribution of polymorphic sites was also distinct in comparison with 16S. Group 3 presented 20 polymorphic sites, even though only 4 specimens were sequences, whereas the African group, despite being represented by 17 specimens, had the lowest number of polymorphic sites (Table 7). The *COI* gene also showed highly significant AMOVA and F_{st} values for the *O. vulgaris* groups (Table 8). The Φst values obtained for the *16S* also presented some differences in comparison with those for *COI*. While all the values for *COI* were highly significant ($P < 0.05$), the highest divergence was obtained for groups 1 and 2, and the lowest between groups 2 and 3 (Table 9). The Φst values for *COI* also presented certain differences in comparison with *16S*. All the

TABLE 4.

Diversity indices derived from the sequences of the *16S* rDNA gene analyzed for the different *Octopus vulgaris* populations analyzed in the current study.

Group	*n*	PS	*H*	*Pi*	Tajima's *D*	Fu's *Fs*
Group 1	27	10	0.85 (0.042)	0.005 (0.000)	–0.468	–1.686
Group 2	33	10	0.81 (0.047)	0.005 (0.006)	–1.359	–2.021
Group 3	3	5	1.00 (0.272)	0.007 (0.002)	0.000	0.587
Total	63	24	0.90 (0.021)	0.014 (0.000)	–0.451	–2.887

N, number of individuals; PS, polymorphic sites; H_s haplotype diversity; *Pi*, nucleotide diversity; Tajima's *D*, value of Tajima's statistics; Fu's *Fs*, Value of Fu's statics; PS, polymorphic sites. Standard deviation values are in parenthesis.

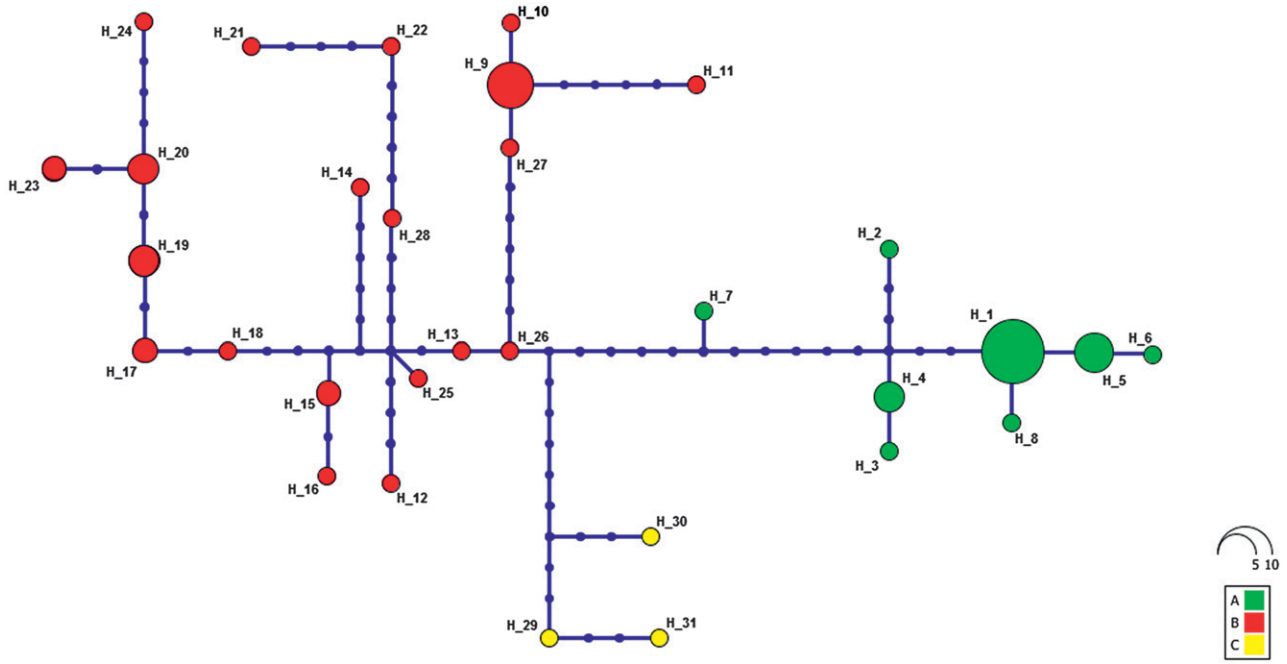

Figure 4. Haplotype genealogy for the mitochondrial 16S rDNA gene based on the maximum likelihood tree derived from the TIM3 + G evolutionary model. Specimens from the Americas (A; green), Europe and Africa (B; red), and from Asia (C; yellow).

values were highly significant ($P < 0.05$), with the highest value being recorded between groups 1 and 2 (Table 9). In contrast with the *16S* gene, however, a number of haplotypes were shared between groups 2 and 3. It is also interesting to note that 1 specimen from group 1 (OvuPA 173–H_3) was closely related to group 2 (Fig. 5), as observed in the phylogenetic tree generated for this gene (Fig. 3).

DISCUSSION

Octopus vulgaris

The phylogenetic analyses presented here confirmed the monophyletic status of *Octopus vulgaris*, with 3 well-defined continental groups. It is important to note that even though these groups are well defined and structured, the level of divergence among them is lower than that found typically between closely related species. The monophyletic status of the samples from the western hemisphere is especially important here, given that the largest number of specimens were obtained from this region. The existence of well-supported clades within the species indicates that each geographical region may support its own distinct *O. vulgaris* lineage. The occurrence of this species in the southeastern Indian Ocean was also confirmed recently, based on molecular markers and morphometric analyses, although some parameters were distinct from those presented by European specimens, such as a narrower head, smaller funnel, and larger number of suckers on the hectocotylus (Guerra et al. 2010).

Differentiation at the population level in cephalopods and, on a more ample temporal scale—speciation—may be derived from genetic, anatomic, physiological, or behavioral incompatibilities, reflecting the dispersal capacity of the planktonic larvae and/or the migratory potential of the adults (O'Dor 1988). The dispersal capacity of the juveniles depends on their size at the time of hatching and during the planktonic phase. The larger the juveniles, the shorter the planktonic phase, and the faster the transition to the adult lifestyle, when dispersal capacity is reduced (Boletzky 1987, Vecchione 1987).

Oceanic currents may limit the dispersal potential of the *Octopus vulgaris* paralarvae, restricting their migration among different regions. Previous studies of this species found little evidence of geographical differentiation or genetic distance among populations, nor of possible morphological differentiation

TABLE 5.

Results of the analysis of molecular variance and the fixation index (F_{st}) for the *16S* rDNA gene in populations of *Octopus vulgaris*.

Octopus vulgaris	16S	
Source of the variation	% of Variation	F_{st}
Between populations	62.44	0.62*
Within populations	37.56	

* Significant $P < 0.05$.

TABLE 6.

Estimates of genetic differentiation among *Octopus vulgaris* populations based on the Φst values for the mitochondrial *16S* rDNA gene.

	Group 1	Group 2
Group 2	0.640*	—
Group 3	0.811*	0.511*

* $P < 0.05$.

TABLE 7.

Diversity indices derived from the sequences of the *COI* gene analyzed for the different *Octopus vulgaris* populations analyzed in the current study.

Group	*n*	PS	*H*	*Pi*	Tajima's *D*	Fu's *Fs*
Group 1	16	14	0.45 (0.151)	0.004 (0.002)	–1.976 (0.921)	0.408 (1.640)
Group 2	26	9	0.58 (0.093)	0.004 (0.001)	–1.999 (0.004)	0.158 (1.047)
Group 3	4	20	1.00 (0.177)	0.021 (0.006)	–0.697 (0.921)	0.353 (0.626)
Ovu total	46	35	0.79 (0.042)	0.016 (0.001)	–0.133	1.296

N, number of individuals; PS, polymorphic sites; *H*, haplotype diversity; *Pi*, nucleotide diversity; Tajima's *D*, value of Tajima's statistics; Fu's *Fs*, value of Fu's statics; PS, polymorphic sites. Standard deviation values are in parenthesis.

consistent with the existence of distinct populations of *O. vulgaris* in different geographical regions. However, Vidal et al. (2010) recently found marked differences in the distribution of chromatophores in *O. vulgaris* paralarvae from the northeastern (Galicia, Spain) and southwestern Atlantic (southern Brazil), which reinforce the findings of the current study. These authors suggested the possible existence of distinct geographical populations of the species, or a cryptic species similar to *O. vulgaris*, reinforcing the need for the analysis of genetic divergence levels. In the current study, specimens from the same areas—Spain and Santa Catarina, in Brazil—do not diverge genetically to a degree consistent with species-level differentiation. However, 1 specimen from Pará, in northern Brazil (OvuPA 184, Fig. 3) was quite distinct phylogenetically from the other samples from the southwestern Atlantic, which indicates the possible presence of a cryptic species in the South American *O. vulgaris* species complex.

Murphy et al. (2002) analyzed microsatellites in *Octopus vulgaris* populations from the northwestern coast of Africa and found highly significant genetic structuring among specimens from Mauritania and the western Sahara. In a second microsatellite study, Cabranes et al. (2007) compared populations from the eastern Atlantic and the Mediterranean, and found a general trend for increasing genetic differentiation with increasing geographical distance, although the tendency was not upheld at distances of less than 200 km. Moreira et al. (2011) identified 4 subpopulations of *O. vulgaris* off southern Brazil, once again, with a tendency for greater genetic differentiation between geographically more distant populations.

The haplotype network for the COI gene also identified a close relationship between 1 individual from group 1 (OvuPA 173) and the members of the European and Asian groups. Initial evidence of intercontinental genetic similarity among a number of *Octopus* species was recorded by Warnke et al. (2004), who analyzed many of the specimens of *Octopus vulgaris* included in the current study (from all 3 groups), and also confirmed the monophyletic status of the species, with well-supported differentiation among continents (bootstrap values of 70–100), which is consistent with the results of the current study.

Octopus insularis

In a phylogenetic comparison between *Octopus vulgaris* from Europe and *Octopus mimus* from Central and South America, Soller et al. (2000) found that some specimens from the northern South Atlantic were genetically distinct from both species. These specimens were then formally described as a new species, *Octopus insularis* (Leite et al. 2008). The geographical range of this species was originally thought to be restricted to the oceanic islands off northeastern Brazil, although Sales et al. (2007) had collected specimens from the northern extreme of the South Atlantic. The results of the current study indicate that the species is distributed throughout the northern coast of Brazil, ranging as far south as Bahia, on the east coast.

This study also confirms the monophyly of the species as well as its affinities with some sympatric *Octopus* species. The range of this species is influenced by a number of different oceanic (the South Equatorial Current and the Equatorial Countercurrent) and continental (northern Brazilian and Brazilian) currents, which may favor the dispersal of the pelagic paralarvae toward both the open sea and coastal areas (Scheltema 1986, Lumpkin & Garzoli 2005). Based on the *16S* gene, *Octopus mimus* was the *Octopus* species most closely related to *Octopus insularis* (with the lowest divergence for any 2 representatives of the genus), although in the *COI* topology (which did not include *O. mimus*), *Octopus maya* is the sister species of *O. insularis*. The low levels of genetic divergence observed here indicate that either *O. maya* or *O. mimus* may have shared the most recent common ancestor with *O. insularis*.

The genetic structuring found in both *Octopus vulgaris* and *Octopus insularis*, together with the pattern reported for other

TABLE 8.

Results of the analysis of molecular variance and the fixation index (F_{st}) for the *COI* gene in populations of *Octopus vulgaris*.

O. vulgaris	*COI*	
Source of the variation	% of the Variation	F_{st}
Between populations	79.00	0.79*
Within populations	21.00	

* Significant $P < 0.05$.

TABLE 9.

Estimates of genetic differentiation among *Octopus vulgaris* populations based on the Φst values for the mitochondrial *COI* gene.

	Group 1	Group 2
Group 2	0.838*	—
Group 3	0.739*	0.697*

* $P < 0.05$.

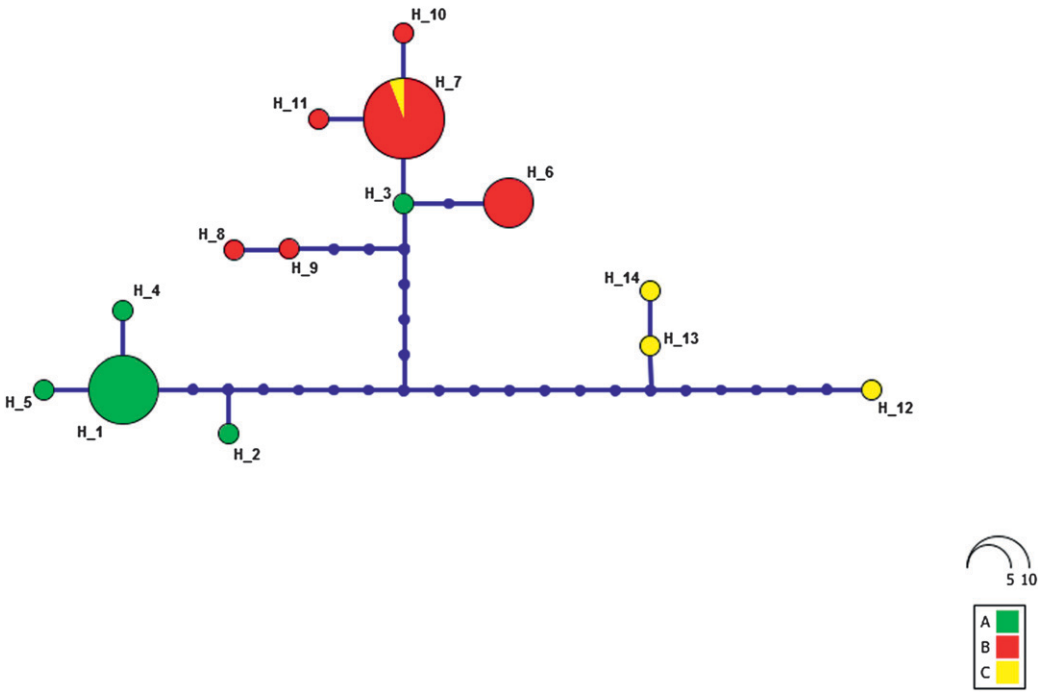

Figure 5. Haplotype genealogy for the mitochondrial COI gene, based on the maximum likelihood tree derived from the GTR + G evolutionary model. Specimens from the Americas (A; green), Europe and Africa (B; red), and from Asia (C; yellow).

Octopus species (Murphy et al. 2002, Cabranes et al. 2007, Doubleday et al. 2009, Moreira et al. 2011), indicate that the association between genetic and geographical distances is a common feature of this genus. Specific factors such as direct internal fertilization (Kayes 1974, Mather 1988), a solitary lifestyle, and the reduced dispersal capacity of the adults (Hanlon & Messenger 1996) may combine to favor the genetic structuring of the populations of these animals.

The current study amplifies the geographical distribution of *Octopus insularis* along the Atlantic coast of South America, and confirms the monophyletic status of *Octopus vulgaris* throughout its worldwide range. The findings also generate an important question: Are the genetic differences among the *O. vulgaris* lineages consistent with species-level differentiation? The levels of nucleotide divergence found here (>1% for *16S* and ~3% for *COI*) can certainly be considered evidence of supporting a taxonomic revision of this species, although this is a complex question that requires a more detailed analysis of a much wider samples of populations representing the different geographical lineages.

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APPENDIX A

List of specimens analyzed in the current study, showing their geographical origin, source, markers analyzed, and code numbers.

Species	16S	COI	Origin	Reference	Code
Octopus insularis	1	—	Cabo Norte-AP	Current study	OinAP 25
O. insularis	1	—	Cabo Norte-AP	Current study	OinAP 26
O. insularis	1	—	Bragança-PA	Current study	OinPA3
O. insularis	1	1	Bragança-PA	Current study	OinPA10
O. insularis	2	1	Bragança-PA	Current study	OinPA14
O. insularis	—	1	Fortaleza-CE	Current study	OinCE09
O. insularis	1	1	Fortaleza-CE	Current study	OinCE16
O. insularis	—	—	Fortaleza-CE	Current study	OinCE36
O. insularis	—	1	Fortaleza-CE	Current study	OinCE38
O. insularis	—	1	Natal-RN	Current study	OinRN1
O. insularis	—	1	Natal-RN	Current study	OinRN2
O. insularis	—	1	Rio Grande do Norte	Current study	OinRN04
O. insularis	—	1	Natal-RN	Current study	OinRN5
O. insularis	1	1	Natal-RN	Current study	OinRN7
O. insularis	—	1	Rio Grande do Norte	Current study	OinRN08
O. insularis	—	1	Natal-RN	Current study	OinRN12
O. insularis	—	1	Natal-RN	Current study	OinRN23
O. insularis	—	1	Natal-RN	Current study	OinRN24
O. insularis	—	1	Natal-RN	Current study	OinRN25
O. insularis	—	1	Natal-RN	Current study	OinRN26
O. insularis	—	1	Natal-RN	Current study	OinRN30
O. insularis	—	1	Natal-RN	Current study	OinRN34
O. insularis	—	1	Natal-RN	Current study	OinRN56
O. insularis	1	—	Natal-RN	Current study	OinRN245
O. insularis	1	1	Baia da Traição-PB	Current study	OinPB266
O. insularis	1	1	Baia da Traição-PB	Current study	OinPB268
O. insularis	1	1	Recife-PE	Current study	OinPE1
O. insularis	1	1	Recife-PE	Current study	OinPE2
O. insularis	—	1	Barra Grande -BA	Current study	OinBA05
O. insularis	—	2	Barra Grande -BA	Current study	OinBA06
O. insularis	—	1	Barra Grande-BA	Current study	OinBA125
O. insularis	1	1	Salvador-BA	Current study	OinBA199
O. insularis	1	—	Salvador-BA	Current study	OinBA200
Octopus sp.	EF093793†	—	Natal-RN	Leite et al. (2008)	OinRNW
Octopus vulgaris	AJ390315†	—	Recife-PE	Warnke et al. (2004)	OinPE
O. vulgaris	1	1	Cabo Norte-AP	Current study	OvuAP225
O. vulgaris	1	—	Bragança-PA	Current study	OvuPA1
O. vulgaris	1	1	Bragança-PA	Current study	OvuPA173
O. vulgaris	1	1	Bragança-PA	Current study	OvuPA78
O. vulgaris	3	3	Bragança-PA	Current study	OvuPA79
O. vulgaris	—	1	Bragança-PA	Current study	OvuPA184
O. vulgaris	1	1	Salvador-BA	Current study	OvuBA117
O. vulgaris	1	1	Rio de Janeiro-RJ	Current study	OvuRJ130
O. vulgaris	3	3	Rio de Janeiro-RJ	Current study	OvuRJ131
O. vulgaris	1	—	Rio de Janeiro-RJ	Current study	OvuRJ214
O. vulgaris	3	—	Rio de Janeiro-RJ	Current study	OvuRJ219
O. vulgaris	1	—	Rio de Janeiro-RJ	Current study	OvuRJ220
O. vulgaris	—	3	Rio de Janeiro-RJ	Current study	OvuRJ280
O. vulgaris	—	3	Juréia-SP	Current study	OvuSP24
O. vulgaris	3	—	Juréia-SP	Current study	OvuSP40
O. vulgaris	1	—	Juréia-SP	Current study	OvuSP41
O. vulgaris	1	3	Guarujá-SP	Current study	OvuSP306
O. vulgaris	1	—	Guarujá-SP	Current study	OvuSP307
O. vulgaris	1	1	Guarujá-SP	Current study	OvuSP308
O. vulgaris	1	—	Guarujá-SP	Current study	OvuSP310
O. vulgaris	1	—	Guaratuba-PR	Current study	OvuPR2
O. vulgaris	—	1	Guaratuba-PR	Current study	OvuPR3

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APPENDIX A

continued

Species	16S	COI	Origin	Reference	Code
O. vulgaris	—	3	Paranaguá-PR	Current study	OvuPR43
O. vulgaris	1	—	Paranaguá-PR	Current study	OvuPR45
O. vulgaris	1	—	Paranaguá-PR	Current study	OvuPR46
O. vulgaris	1	—	Cabo de Santa Marta-SC	Current study	OvuSC5
O. vulgaris	3	—	Cabo de Santa Marta-SC	Current study	OvuSC6
O. vulgaris	—	3	Cabo de Santa Marta-SC	Current study	OvuSC10
O. vulgaris	1	1	Cabo de Santa Marta -SC	Current study	OvuSC11
O. vulgaris	1	1	Portugal	Current study	OvuPT5
O. vulgaris	—	—	Portugal	Current study	OvuPT11
O. vulgaris	—	—	Portugal	Current study	OvuPT12
O. vulgaris	—	3	Portugal	Current study	OvuPT13
O. vulgaris	3	—	Portugal	Current study	OvuPT37
O. vulgaris	3	—	Portugal	Current study	OvuPT40
O. vulgaris	AJ390317†	—	Taiwan	Warnke et al. (2004)	OvuTW
O. vulgaris	AJ252771†	—	Taiwan	Hundelot (unpubl.)	OvuTW2
O. vulgaris	AJ616307†	—	Japan	Warnke et al. (2004)	OvuJP
O. vulgaris	AJ616308†	—	Rio de Janeiro-RJ	Warnke et al. (2004)	OvuRJ
O. vulgaris	AJ390314†	—	Itajaí-SC	Warnke et al. (2004)	OvuSC
O. vulgaris	AJ390316†	—	Venezuela	Warnke et al. (2004)	OvuVE
*O. vulgaris**	AJ252770†		Venezuela	Hudelot (unpubl.)	OvuVE2
O. vulgaris	DQ683247†	DQ683221†	Galícia, Spain	Teske et al. (2007)	OvuGA1
O. vulgaris	DQ683248‡	DQ683222‡	Galícia-Spain	Teske et al. (2007)	OvuGA2
O. vulgaris	DQ683249‡	DQ683223‡	Galícia,Spain	Teske et al. (2007)	OvuGA4
O. vulgaris	AJ390310†	—	France	Warnke et al. (2004)	OvuFR
O. vulgaris	DQ683234†	DQ683214†	Durban, South Africa	Teske et al. (2007)	OvuDB1
O. vulgaris	DQ683235†	DQ683215‡	Durban, South Africa	Teske et al. (2007)	OvuDB2
O. vulgaris	DQ683236†	DQ683216†	Durban, South Africa	Teske et al. (2007)	OvuDB3
O. vulgaris	DQ683237†	DQ683217†	Durban, South Africa	Teske et al. (2007)	OvuDB4
O. vulgaris	DQ683238†	DQ683218‡	Durban, South Africa	Teske et al. (2007)	OvuDB5
O. vulgaris	DQ683239†	DQ683219‡	Durban, South Africa	Teske et al. (2007)	OvuDB6
O. vulgaris	DQ683250†	DQ683227†	Mediterranean	Teske et al. (2007)	OvuME1
O. vulgaris	DQ683228†	DQ683212†	Porto Elizabeth, South Africa	Teske et al. (2007)	OvuPE2
O. vulgaris	DQ683229†	DQ683213‡	Porto Elizabeth, South Africa	Teske et al. (2007)	OvuPE3
O. vulgaris	DQ683232†	DQ683210†	Struisbaai, South Africa	Teske et al. (2007)	OvuSB5
O. vulgaris	DQ683233†	DQ683211†	Struisbaai, South Africa	Teske et al. (2007)	OvuSB6
O. vulgaris	DQ683230†	DQ683208†	Hout Bay, South Africa	Teske et al. (2007)	OvuHB3
O. vulgaris	DQ683231†	DQ683209†	Hout Bay, South Africa	Teske et al. (2007)	OvuHB4
O. vulgaris	DQ683240†	DQ683220†	Umhlanga, South Africa	Teske et al. (2007)	OvuUM1
O. vulgaris	AJ390312†	—	False Bay, South Africa	Warnke et al. (2004)	OvuFB
O. vulgaris	DQ683244†	DQ683224‡	Senegal	Teske et al. (2007)	OvuSE1
O. vulgaris	DQ683245†	DQ683225‡	Senegal	Teske et al. (2007)	OvuSE2

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APPENDIX A

continued

Species	16S	COI	Origin	Reference	Code
O. vulgaris	DQ683246†	DQ683226†	Senegal	Teske et al. (2007)	OvuSE4
O. vulgaris	DQ683241†	DQ683205‡	Tristan da Cunha	Teske et al. (2007)	OvuTC2
O. vulgaris	DQ683242‡	DQ683206‡	Tristan da Cunha	Teske et al. (2007)	OvuTC3
O. vulgaris	DQ683243†	DQ683207†	Tristan da Cunha	Teske et al. (2007)	OvuTC5
O. vulgaris	AJ616309†	—	Greece	Warnke et al. (2004)	OvuGC
O. vulgaris	—	FN424381†	Saint Paul Islands, Southern Indian Ocean	Guerra et al. (2010)	OvuSIO
O. vulgaris	—	AB191269†	Japan	Takumiya et al. (2005)	OvuJP2
O. vulgaris	—	AB430548†	Japan	Kaneko and Kubodera (unpubl.)	OvuJP3
O. vulgaris	—	AB052253†	Japan	Minataka et al. (unpubl.)	OvuJP4
O. vulgaris	AJ252777†	—	France	Hudelot (unpubl.)	OvuFR2
O. vulgaris	AJ252778†	—	France	Hudelot (unpubl.)	OvuFR3
O. vulgaris	AJ252773†	—	Tenerife	Hudelot (unpubl.)	OvuTE
O. vulgaris	—	—	France	Allcock et al. (2006)	OvuFR4
Octopus bimaculoides	AJ390321§	AF377967§	Santa Barbara, USA	Warnke et al. (2004), Carlini et al. (2001)	ObimUS
Octopus californicus	AJ390222§	AF377968§	Santa Barbara, USA	Warnke et al. (2004), Carlini et al. (2001)	OcalUS
Octopus mimus	AJ390918§/ AJ390919§	—	Iquique, Chile; Isla de Cocos, Costa Rica	Warnke et al. (2004)	OmimCR/ OmimCH
Octopus maya	—	GU362545§	Mexico	Juarez et al. (unpubl.)	OmayMX
Octopus hummelinck	2	2	Ceará	Current study	OhumCE1
Amphioctopus sp.	2	2	Pará	Current study	AmspPA86
Eledone massyae	2	2	Cassino, Rio Grande do Sul	Current study	EmasRS53
Hapalochlaena maculosa	AY545107§	AB430531§	Unknown, Taiwan	Strugnell et al. (2004), Kaneko and Kubodera (unpubl.)	Hmac/Hmac/Hmac

* Specimens of unknown geographical origin (GenBank records).
† Specimens included in the phylogeographical analysis only.
‡ Specimens included in the population analysis only.
§ Specimens included in the phylogenetic analysis only.

APPENDIX B

Specimens in which the different 16S rDNA haplotypes identified in the current study were recorded.

Haplotype	*n*	Code	Origin
Hap_1	13	OvuAP_225, OvuPA_1, OvuPA_78, OvuPA_79, OvuRJ_220 OvuRJ_131,OvuSP_308, OvuSP_310, OvuPR_2 OvuPR_45 OvuPR_46 OvuSC_6, OvuSC	America
Hap_2	1	OvuPA_173	America
Hap_3	1	OvuBA_117	America
Hap_4	3	OvuRJ_130 OvuSP_41 OvuSC_5	America
Hap_5	5	OvuRJ_219 OvuRJ_214 OvuSP_40 OvuSP_306 OvuSP_41	America
Hap_6	1	OvuSC_11	America
Hap_7	1	OvuVE	America
Hap_8	1	OvuVE_2	America
Hap_9	7	OvuPT_37, OvuPT_40, OvuPT_5, OvuGA_4, OvuGA_2 OvuGA_1 OvuFR	Europe, Africa
Hap_10	1	OvuPT_13	Europe, Africa
Hap_11	1	OvuME	Europe, Africa
Hap_12	1	OvuSE_4	Europe, Africa
Hap_13	1	OvuSE_2	Europe, Africa
Hap_14	1	OvuSE_1	Europe, Africa
Hap_15	2	OvuTC_5, OvuTC_3	Europe, Africa
Hap_16	1	OvuTC_2	Europe, Africa
Hap_17	2	OvuUM_1, OvuDB_2	Europe, Africa
Hap_18	1	OvuDB_6	Europe, Africa
Hap_19	3	OvuDB_5 OvuSB_5 OvuFB	Europe, Africa
Hap_20	3	OvuDB_4 OvuHB_3 OvuPE_2	Europe, Africa
Hap_21	1	OvuDB_3	Europe, Africa
Hap_22	1	OvuDB_1	Europe, Africa
Hap_23	2	OvuSB_6, OvuPE_3	Europe, Africa
Hap_24	1	OvuHB_4	Europe, Africa
Hap_25	1	OvuGC	Europe, Africa
Hap_26	1	OvuFR_2	Europe, Africa
Hap_27	1	OvuFR_3	Europe, Africa
Hap_28	1	OvuTE	Europe, Africa
Hap_29	1	OvuTW	Asia
Hap_30	1	OvuTW_2	Asia
Hap_31	1	OvuJP	Asia

APPENDIX C

Specimens in which the different COI haplotypes identified in the current study were recorded.

Haplotype	*n*	Code	Origin
Hap_1	12	OvuAP_225, OvuPA_78, OvuPA_79, OvuRJ_130, OvuRJ_131, OvuRJ_280, OvuSP_308, OvuSP_306, OvuSP_24, OvuPR_3, OvuPR_43, OvuSC_11	America
Hap_2	1	OvuPA_184	America
Hap_3	1	OvuPA_173	America
Hap_4	1	OvuBA_117	America
Hap_5	1	OvuSC_10	America
Hap_6	6	OvuPT_13, OvuPT_5, OvuPT_12, OvuGA_4, OvuGA_2, OvuGA_1	Europe, Africa
Hap_7	17	OvuME_1, OvuSE_4, OvuSE_2, OvuSE_1, OvuUM_1, OvuDB_6, OvuDB_5, OvuDB_4, OvuDB_2, OvuPE_3, OvuPE_2, OvuSB_5, OvuHB_3, OvuTC_5, OvuTC_3, OvuTC_2, OvuSIO	Europe, Africa, Asia
Hap_8	1	OvuDB_3	Europe, Africa
Hap_9	1	OvuDB_1	Europe, Africa
Hap_10	1	OvuSB_6	Europe, Africa
Hap_11	1	OvuHB_4	Europe, Africa
Hap_12	1	OvuJP_2	Asia
Hap_13	1	OvuJP_3	Asia
Hap_14	1	OvuJP_4	Asia