

**GENETIC DIFFERENTIATION OF EASTERN AND WESTERN ATLANTIC *OCTOPUS VULGARIS***

By

ALEXANDRE F.J. JASSOUD

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**Approved by:**

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Dr. Nikolaos Schizas, PhD  
Chairman, Graduate Committee

---

Date

---

Dr. Paul Yoshioka, PhD  
Member, Graduate Committee

---

Date

---

Dr. Richard S. Appeldoorn, PhD  
Member, Graduate Committee

---

Date

---

Dr. Juan Carlos Martínez Cruzado, PhD  
Representative of Graduate Studies

---

Date

---

Dr. Nilda E. Aponte, PhD  
Director

---

Date

## ABSTRACT

*Octopus vulgaris* is an important species in cephalopod fisheries and also in understanding population regulation in marine invertebrates. Despite its importance, little is known about its distribution and genetic connectivity among populations. The distribution of *O. vulgaris* is cosmopolitan, but this assumption has been recently challenged. In order to address this issue and learn more about the population structure of this species, the patterns of genetic variation of geographically distant populations were compared: the Caribbean Sea (Puerto Rico, Guadeloupe, Curacao, and Dominica), the European Atlantic Ocean (Spain), the Mediterranean Sea (Spain, France, and Greece), Atlantic Africa (Senegal and South Africa), and Japan. A portion of the Cytochrome Oxidase Subunit I (COI) gene was used to determine the genetic differences among the populations. Bayesian COI genealogies resulted in three monophyletic groups: Caribbean, Eurafrican and Japanese. The Japanese lineage is more closely related to the Eurafrican than the Caribbean lineage. A parsimony network analysis of 17 COI haplotypes showed that the three groups do not share common haplotypes. Within the Caribbean lineage, the most common haplotype is shared by all the populations except for Curaçao. The most common haplotype in the Eurafrican group is shared by all populations. The haplotype parsimony network indicated that the Eurafrican lineage and the Japanese group are relatively close (14 mutations), while the Caribbean lineage is isolated from the two other groups. The Caribbean octopus exhibits an average uncorrected divergence of 11.5% compared to the Eurafrican and Japan octopus, whereas the latter groups are only 3.1% different. The amount of divergence observed between the Caribbean *Octopus vulgaris* and other geographically distant populations suggests the presence of a Caribbean cryptic species and questions the claim that *O. vulgaris* is a cosmopolitan species.

Keywords: Population structure, *Octopus vulgaris*, Caribbean, Eastern Atlantic

## RESUMEN

*Octopus vulgaris* es una especie importante en la pesca de cefalópodos y en la regulación de la población de los invertebrados marinos. A pesar de su importancia, poco se conoce acerca de su distribución y flujo genético. Es aceptado comúnmente que su área de dispersión es del Oeste Atlántico hasta Japón, pero investigadores en el campo han cuestionado esta repartición. Para responder a esta interrogante y conocer más sobre el flujo genético de esta especie, una investigación basada en genética fue utilizada entre diferentes poblaciones del mundo: el Mar Caribe (Puerto Rico, Guadalupe, Curaçao y Dominica), el Océano Atlántico (España), el Mar Mediterráneo (Francia, España y Grecia), África (Senegal y África del Sur) y Japón. Una porción del gene Citocromo Oxidasa Subunidad I (COI) fue utilizada para determinar las diferencias en población en las diferentes regiones. Un análisis bayesiano de los especímenes resultó en un árbol parafilético con tres grupos: el grupo caribeño, el grupo euroafricano y el grupo japonés. El grupo japonés está más cercano al grupo euroafricano que del grupo caribeño. Un análisis de haplotipos demostró que ninguno de los grupos comparten haplotipos en común. Dentro del grupo caribeño, el haplotipo más común es compartido entre todos los grupos con excepción de Curaçao. El haplotipo más común en el grupo euroafricano está presente en todas las regiones. La red de haplotipos parsimonio indicó que el linaje euroafricano y el grupo japonés son relativamente cercanos (14 mutaciones), mientras que el linaje caribeño está aislado de los otros dos grupos. El pulpo caribeño exhibe un promedio de la divergencia no corregida de 11.5% comparado con el pulpo japonés y el euroafricano, donde estos últimos dos grupos son diferentes por solamente un 3.1%. La magnitud de la divergencia observada entre el pulpo caribeño y otras poblaciones geográficamente distantes sugiere la presencia de especies crípticas y pone en cuestión el enunciado de que *O. vulgaris* es una especie cosmopolita.

Palabras clave: Estructura de la población, *Octopus vulgaris*, el Caribe, el Atlántico del Este

## RESUME

*Octopus vulgaris* tient une place importante dans la pêche des céphalopodes, et aussi dans la régulation des populations d'invertébrés marins. Malgré son importance, peu de chose sont connues sur son aire de répartition et les connections entre les populations. Il est communément admis que son aire de dispersion vas de l'Atlantique Ouest jusqu'au Japon, cette répartition a été remise en cause récemment par la communauté scientifique. Afin de répondre à cette remise en compte et connaitre un peu plus sur le flux génétique de cette espèce, une approche génétique a été utilisée entre différentes populations dans les Caraïbes (Porto Rico, Guadeloupe, Curaçao et la Dominique), l'océan Atlantique est (Espagne), la mer Méditerranée (Espagne, France et Grèce), l'Afrique (Sénégal et Afrique du Sud) et le Japon. Une portion du gène Cytochrome Oxydase Sous-unité 1 a été utilisée pour déterminer les différences entre les différents emplacements. Une analyse Bayésienne des individus montre un arbre paraphylétique constitué de trois groupes distincts : le groupe des Caraïbes, le groupe Eurafricain et le groupe Japonais. Le groupe Japonais est plus proche du groupe Eurafricain que ne l'est le groupe Caribéen. Une analyse des haplotypes a été réalisée et montre que aucun des trois groupes ne partagent d'haplotypes communs. À l'intérieur du groupe Caribéen l'haplotype le plus commun est partagé par toutes les populations à l'exception de Curaçao. L'haplotype le plus commun dans le groupe Eurafricain est partagé par toutes les populations. La réalisation d'un réseau parsimonique des haplotypes révèle que le groupe Eurafricain et japonais sont relativement proches (14 mutations les séparent) tandis que le groupes Caribéen est totalement isolé des deux autres groupes. Le groupe caribéen montre une divergence non corrigée moyenne de 11.5% comparé au poulpe eurafricain et japonais, alors que ces derniers ne sont éloignés que de 3.1%. La grande différence observée entre la pieuvre commune des caraïbes et des autres localités géographiques suggèrent la présence d'une espèce cryptique et remet en question le fait que *O. vulgaris* soit une espèce cosmopolite.

Mots-clés: Structure de la population, *Octopus vulgaris*, les Caraïbes, l'Atlantique Est

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## 1. INTRODUCTION

A stone carving from the Bronze Age found in Knossos (Crete, Greece) indicates that humans were consuming octopus at least since 1700 B.C. (Pendlebury and Evans, 2003). Nowadays, *Octopus vulgaris* is one of the most important species in the cephalopod fishery. World catches attributed to *O. vulgaris* were low before the industrial fishery of the 1970's with annual catch values lower than 10K tons per year. The 1970's marked the beginning of the industrialization and data collection in regard to the octopus fishery. The 1970's through the 1980's showed a peak in octopus harvest with more than 100K tons per year. *Octopus* spp. is in highest demand in Italy (importing 50.9K tons), Japan (44.7K tons), and Spain (42.6K tons), however octopus is also a popular food item in Caribbean and South American countries. In 2008, the *O. vulgaris* fishery represented approximately US\$292 millions of the total amount of the octopus fishery valued on US\$1.3 billion. During the last five years the wholesale prices of octopus in Spain have fluctuated from about €6 to €16/kg (FAO, 2010). *Octopus vulgaris* populations are overfished and new regulations have been applied in an attempt to recover the fishery (FAO GlobeFish 2010). During the last 10 years, efforts for developing aquaculture techniques have been initiated (Rodríguez et al., 2006; Vaz-Pires et al., 2004) to provide octopus to European, Asian and South American markets.

The common octopus, *Octopus vulgaris*, is thought to have a cosmopolitan distribution and can be found between 50°N to 50°S from the east coast of America to Japan, although it is not present in the islands of South-East Asia and Oceania (Figure 1). There are several studies on the life history of larvae based on behaviour and morphology from hatchment to settlement (Nixon and Mangold, 1998; Villanueva, 1995; Villanueva et al., 1995). The planktonic post-hatching stage is termed paralarvae (Young and Harman, 1988) and differs in morphology and ecology from older

juveniles (Jereb and Roper, 2005). The paralarval stage lasts about 40-55 days and ends once a critical weight is reached (Iglesias et al., 2004). At this point, the arms start to develop and the animal switches to a sedentary benthic lifestyle (Villanueva, 1995). Adult *O. vulgaris* are opportunistic and active predators and play an important role in regulating many populations of molluscs, arthropods, and teleosts (Hanlon and Messenger, 1996). Upon feeding, it discards hard structures from its food outside its lair. These discarded piles are named middens (Hanlon and Messenger, 1996) and are used by scientists to study the invertebrates that inhabit the area and provide a more accurate inventory of species diversity for molluscs, arthropods, and fishes (Anderson et al., 2008).

Figure 1. Distribution of *Octopus vulgaris* (FAO, 2008). Modified from <http://world-map.nl>



Despite the commercial importance of *O. vulgaris*, its distribution and genetic population structure are relatively unknown (Hanlon and Messenger, 1998). The octopus family is assumed to originate from the late Cretaceous in Lebanon, where the fossils resemble modern octopus (Fuchs et al., 2009). Drilled mollusk shells attributed to *O. vulgaris* dated from the Pleistocene

were found in the Mediterranean Sea (Bromley, 1993). During this period the sea level fluctuated, reaching at the highest point more than 85 meters above the current sea level (Emig and Geistdoerfer, 2004). At such high sea level *O. vulgaris* could potentially have reached the Red Sea and further spread to the Indian Ocean. Before the opening of the Suez Canal, the Red Sea was isolated for approximately 500 thousand years from the Mediterranean Sea. Furthermore, it remains unknown how *O. vulgaris* reached the Caribbean waters. Some gastropod genera such as *Cymatium*, *Charonia*, *Tonna*, *Phalium*, and *Thais* have an ampho-Atlantic distribution and it has been hypothesized that veligers of these species can successfully transverse the Atlantic Ocean (Scheltema, 1971; Levinton, 2001). Similarly, the long duration of *O. vulgaris* paralarvae (40-55 days) and the presence of rapid water mass as hurricanes could potentially explain the presence of the species on both sides of the Atlantic.

The first study on *O. vulgaris* populations was done by Rees (1950) in Great Britain, who suggested that octopus populations in Great Britain are maintained only by immigration. The paralarvae probably moved into the area because of the British Channel Current which determines the abundance of the early stages. Several population studies of *O. vulgaris* have been done for fishery stock assessment (HataNa/Ka, 1979; Rees and Lumby, 1954) and to determine its distribution (Guerra, 1981; Rees, 1950). Studies on the genetic population structure of *O. vulgaris* began with Söller et al. (2000), who described the phylogenetic relatedness of Caribbean and European *O. vulgaris* with five other closely related octopi species. When they examined the connectivity patterns between Caribbean and European locations, they concluded that the *O. vulgaris* from Caribbean are phylogenetically closer to *O. bimaculoides* than to *O. vulgaris* from Europe, except for the south Brazilian *O. vulgaris* which is closer to the European octopus than to the North Brazilian one. Söller et al. (2000) concluded that *O.*

*vulgaris* is a paraphyletic species. Following this study, Oosthuizen et al. (2004) compared African and European populations to those in Taiwan and the Caribbean Sea. The study showed little divergence between Europe and Africa. In South Africa, they detected two different lineages, (East and West), and concluded for those locations that the specimens came from the same taxon. The comparison between Taiwanese vs. Venezuelan specimens showed a divergence of 2.86-3.87% in Cytochrome Oxidase Subunit III (COIII), suggesting that the distribution of *O. vulgaris* is limited to Europe and West African coast. Specimens from Costa Rica and Brazil were phylogenetically closer to *O. mimus* and *O. bimaculoides*, respectively, than to *O. vulgaris* (Oosthuizen et al., 2004). Teske et al. (2007) showed the presence of genetic subdivision in South Africa by the detection of a local population in Durban (Cytochrome Oxidase Subunit I; COI). They formulated two hypotheses to explain this pattern: a) the Durban population could be the only one that survived to the settlement of a new lineage in the area and b) the Durban population was based on octopi with a new haplotype brought to the area within ballast waters. Casu et al. (2002) demonstrated with microsatellite markers that the Mediterranean octopus population does not form a single panmictic unit and they are not isolated-by-distance genetically. Based on mitochondrial data, Warnke et al. (2004) suggested that the distribution of *O. vulgaris* extends beyond the Mediterranean and the eastern Atlantic, including the western Atlantic and the northwestern Pacific. The Caribbean specimens of *O. vulgaris* in all previous studies are limited in number and have shown puzzling phylogeographic patterns, with some specimens from Venezuela resembling those of Europe and western Africa and other specimens from Costa Rica more closely related to the congenetic species *O. mimus* (Warnke et al., 2004). the use of alternative genes, besides those of COI, COIII and 16S, may shed more light on the investigation of the phylogeography of *O. vulgaris* populations.

## **OBJECTIVES**

The objective of this study is to estimate the connectivity of *O. vulgaris* populations between Caribbean vs. European and African populations.

H<sub>0</sub>: There is no genetic exchange between eastern Atlantic and western Atlantic populations of *O. vulgaris*.

H<sub>A</sub>: There is genetic exchange between eastern Atlantic and western Atlantic populations of *O. vulgaris*.

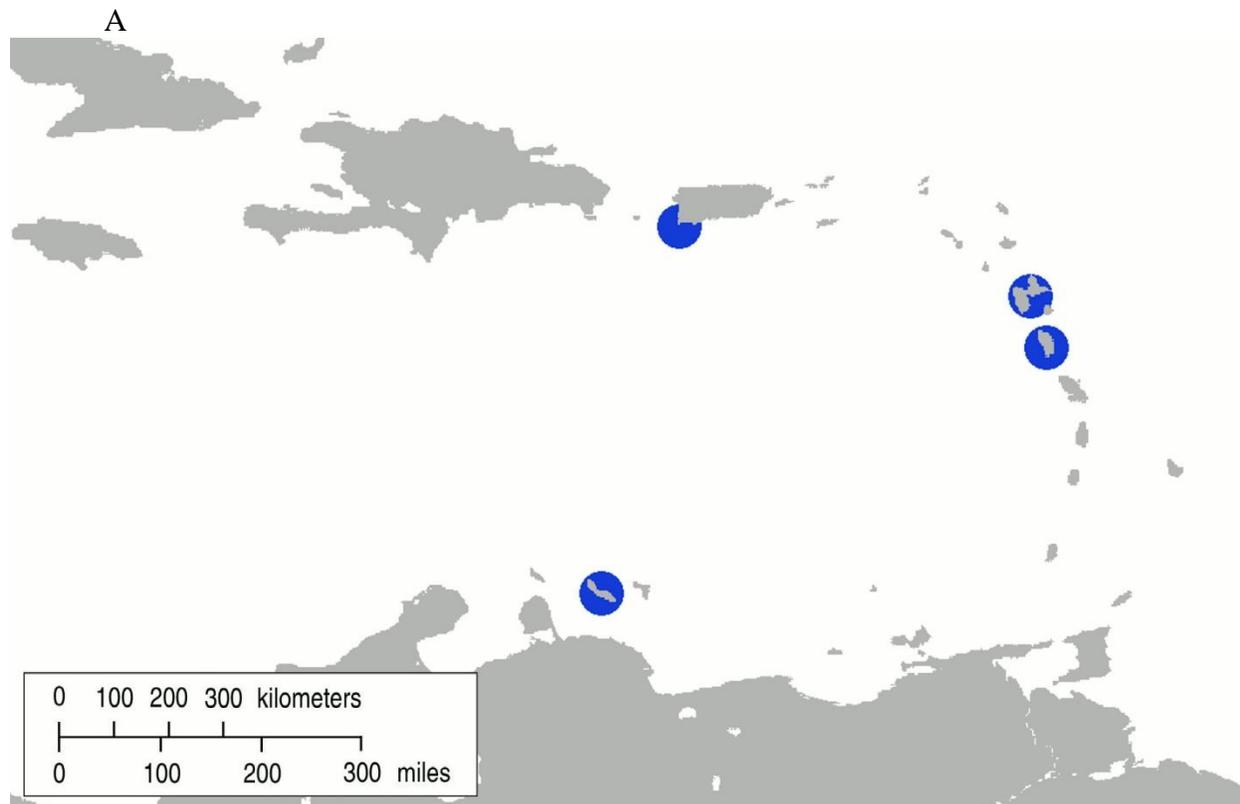


Figure 2. Map of collecting locations. A) Caribbean locations; B) European locations. Modified from <http://world-map.nl>.

## 2. MATERIALS AND METHODS

### Specimens

*Octopus vulgaris* was collected by snorkelling and scuba diving from Puerto Rico, Dominica, Guadeloupe, Curaçao, France and Greece (Table 4 and Figure 2). Samples from Atlantic Spain were purchased from fishermen. Approximately 3–5 cm of arm tip was removed from each specimen, after which the octopus was released, when possible, except for the Atlantic Spain samples, where gills were collected instead. Samples were preserved in 100% ethanol. Voucher tissue of the specimens has been deposited in the Museum of Marine Invertebrates, Isla Magueyes, University of Puerto Rico Mayagüez. Additional COI sequences of *O. vulgaris* from Mediterranean, Senegal, South Africa, Tristan da Cunha, St. Paul and Amsterdam Islands (South Indian Ocean) and Japan were downloaded from GenBank (Table 5 and Figure 2).

### DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted using a QIAGEN DNeasy Tissue Kit following the manufacturer's instructions. The primers LCO1490 and HCO2198 (Folmer et al., 1994) were designed to amplify a large portion of the Cytochrome Oxidase Subunit I (COI) region. All PCR reactions were setup in 25 µl reaction volumes, containing: 1–6 µl of DNA, 12.5 µl of 2X PCR Taq MasterMix (Gentaur) and 1 µl of each primer (10 nM). The profile of the PCR amplification of COI were as follows: 95°C for 3 min followed by 10 cycles at 95°C (15 s), 45°C (45 s) and 72°C (1 min), followed by another 20 cycles of 95°C (15 s), 48°C (45 s) and 72°C (1 min). The amplified product was purified with the Exosap procedure or with the QiaQuik PCR Clean Kit (Qiagen). All successful PCR products were processed for sequencing using the Big Dye 3.1

Terminator Cycle Sequencing Kit and the ethanol precipitate products were loaded into an ABI 3130xl 16-capillary Genetic Analyzer. DNA products were sequenced from both directions. All DNA sequences have been submitted to GenBank (Accession Numbers XXXXXX-XXXXXX).

#### Data analysis

DNA trace files were imported in CodonCode Aligner for base calling, quality assessment, contig assembly, visualization and manual editing. Aligned sequences were imported to DnaSP v5 (Librado and Rozas, 2009) and Arlequin v3.5 (Excoffier and Lischer, 2010) for general statistical analysis. DNA neutrality tests such as Tajima's  $D$  (Tajima, 1989) and Fu's  $F_s$  (Fu, 1997) were computed to test for deviation from the neutral model of molecular evolution (Kimura, 1968). The most appropriate model of DNA substitution for each marker was estimated with the Bayesian Criterion in jModelTest 3.06 (Posada, 2008) and PhyML (Guindon and Gascuel, 2003) and was applied to the Maximum Likelihood (ML) and Bayesian inference (BI), Analysis of Molecular Variance (AMOVA) tests and pairwise  $F_{ST}$  comparisons. For population structure analysis,  $\Phi_{ST}$  were calculated based on the average number of pairwise nucleotide differences within and between island populations in Arlequin and network analysis of haplotypes in TCS 1.21 (Clement et al., 2000). The significance of  $\Phi$ -statistics was assessed by 10,000 permutations of groups and haplotypes. COI sequences were imported into PAUP\* 4.0b10 (Swofford, 2002) to construct ML genealogies and to MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003) to construct the MCMC-based BI genealogies. Bayesian analysis was run for 3,000,000 generations, four independent chains, sampling every 1000 generations and discarding 15% of the sampled trees. Clade support for ML was evaluated with 100 bootstrap replicates (Felsenstein, 1985) using the fast step-wise search and for BI with posterior

probabilities (pP).

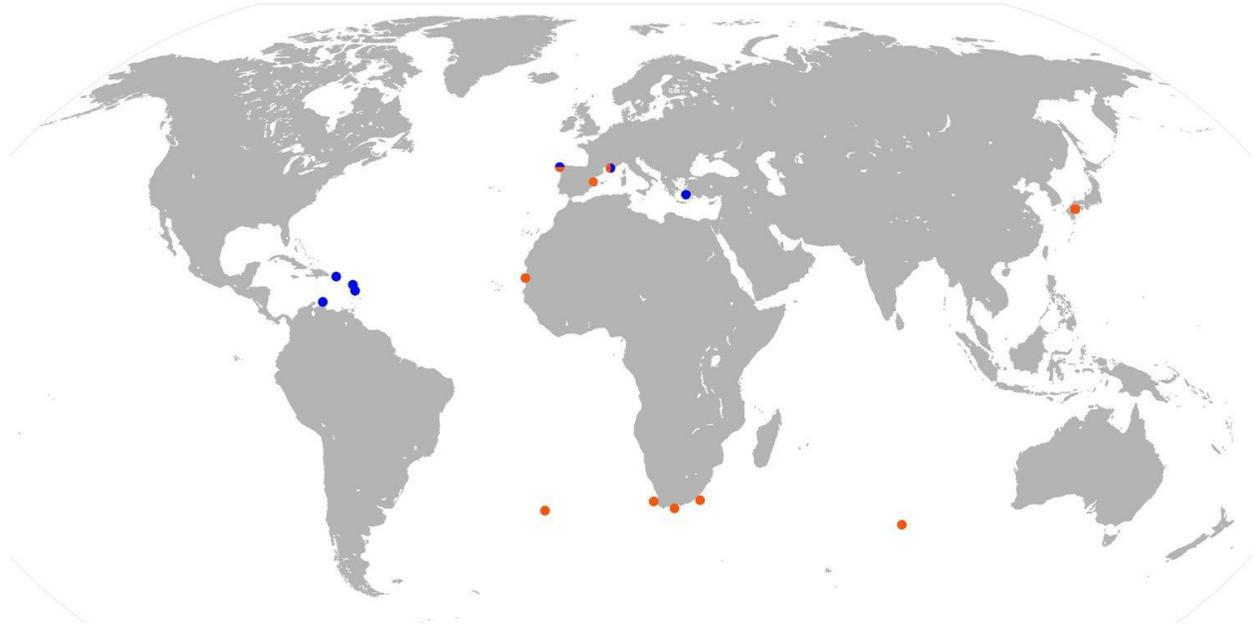


Figure 3. World map of the sequences analysed. The blue dots represent the collecting locations. The orange dots represent GenBank locations. Modified from <http://world-map.nl>.

## RESULTS

After end-trimming, a 467 bp portion of the mitochondrial gene COI from *O. vulgaris* was analysed from 92 specimens from Mediterranean, western Atlantic, greater Caribbean area, Indian Ocean and Pacific Ocean (Figure 3). The final data set consisted of new sequences and sequences downloaded from GenBank (Table 5). Seventeen haplotypes were identified, four of which originated in the Caribbean (Figure 4). Thirteen of the haplotypes were singletons. The highest values of haplotype diversity were observed in the Japanese and E. Atlantic specimens (Table 1). Haplotype 1, the most commonly found haplotype ( $n = 34$ ) was found in all locations except the Caribbean and Japan (Figure 4). The second most common haplotype was found only in Atlantic Spain. The most common Caribbean haplotype ( $n = 19$ ) was found in Puerto Rico, Guadeloupe and Dominica. The nucleotide diversity indices  $\theta_\pi$  and  $\theta_w$  were highest in the Caribbean, E. Atlantic and Japan (Table 1). In the Caribbean, the haplotype diversity is principally due to the curaçao specimen who as a high mutation rate at the beginning of the first 200 bases but only on the third codon and they are silent mutations. The neutrality test Tajima's D was significantly negative in the Caribbean and Atlantic Spain indicating a possible recent

population expansion. The best model of DNA substitution as suggested by jModelTest was the Transitional Substitution Model plus Invariants (TIM2+I) for the COI sequences. The specific attributes of the model are as follows: Lset base = (0.2743 0.1973 0.1485 0.3799), nst = 6, rmat = (227.3245 128.7255 227.3245 1.0000 1210.5827 1.0000), rates = equal, and pinvar = 0.6230. Uncorrected average pairwise differences among populations indicated that the Caribbean is 11.6-11.7% divergent compared to Mediterranean, Atlantic and Japanese samples of *O. vulgaris* (Table 2). The sequence divergence between Japan and Mediterranean and E. Atlantic was 3.1%, indicating a close genetic relationship between these two regions. All *O. vulgaris* populations were at least 14.5% divergent from the closely related *O. briareus* (Table 2).

Table 1. Genetic diversity and summary statistics of *Octopus vulgaris* based on COI sequences. N = number of samples, H = number of haplotypes, S = segregation sites, Hd = haplotype diversity  $\pm$  Standard Deviation (SD),  $\theta_\pi$  = nucleotide diversity  $\pm$  SD,  $\theta_w$  = Watterson's Theta  $\pm$  SD, and Tajima's D. Significant values are represented by \*\*\*, \*\* and \* for  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively.

Localities	N	H	S	Hd	$\theta_\pi$	$\theta_w$	Tajima's D	Fu's Fs
<b>Caribbean</b>	22	4	40	0.260 $\pm$ 0.120	0.00584 $\pm$ 0.00484	0.01762 $\pm$ 0.00653	-2.56676***	3.02983
<b>E. Atlantic + Mediterranean</b>	61	9	12	0.608 $\pm$ 0.040	0.00418 $\pm$ 0.00044	0.00549 $\pm$ 0.00210	-0.68299	-0.93754
<b>E. Atlantic</b>	45	8	11	0.623 $\pm$ 0.050	0.00443 $\pm$ 0.00059	0.00539 $\pm$ 0.00217	-0.52962	-0.47007
<b>Spain, Atl. Mediterranean</b>	26	4	5	0.222 $\pm$ 0.106	0.00082 $\pm$ 0.00048	0.00281 $\pm$ 0.00148	-2.00199*	-1.92850*
<b>Japan</b>	16	2	4	0.125 $\pm$ 0.106	0.00107 $\pm$ 0.00091	0.00258 $\pm$ 0.00151	0.00151*	1.24657
	6	4	9	0.800 $\pm$ 0.172	0.00642 $\pm$ 0.00312	0.00844 $\pm$ 0.00467	-1.42284	0.23500

Table 2. Average pairwise divergence (%) among populations of *O. vulgaris* COI sequences based on *p*-distance. The closely related species *Octopus briareus* has been included for comparison. Med+E. Atlantic = Mediterranean and Atlantic samples.

	Med+E. Atlantic	Caribbean	Japan
Med+ E. Atlantic	-		
Caribbean	11.6	-	
Japan	3.1	11.7	-
<i>O. briareus</i>	14.9	15.6	14.7

Pairwise  $\Phi_{ST}$ s comparisons strongly supported the distinctness of the Caribbean samples (Table 3). Additionally, the specimens from Japan were differentiated from all others except those of Senegal and Tristan da Cunha. The non-significant differentiation probably occurred because of the small sampling size in the two Atlantic locations. The Atlantic Spain samples were significantly different than those from the Mediterranean and those of South Africa (Table 3). The Bayesian genealogy of the COI gene indicates the differentiation between the Caribbean against all other locations (Figure 5). The Japanese octopi formed a monophyletic clade which is more closely related to the octopi from those in Mediterranean, E. Atlantic, Tristan da Cunha and the three specimens from St. Paul and Amsterdam Islands in the South Indian Ocean (Figure 6 A and C). All specimens from the Mediterranean Sea (Spain, France and Greece) together with those from Senegal, Tristan da Cunha and some from South Africa formed a non-differentiating clade (Figure 6 B). Within that clade, all octopi from Atlantic Spain formed a tightly clustered clade. Two specimens from South Africa form a sister clade of the Mediterranean/E. Atlantic clade (Figure 6 B).

Table 3. Populations pair-wise  $\Phi_{ST}$ s based on *Octopus vulgaris* COI sequences.  $\Phi_{ST}$  values were corrected by the Tamura-Nei model of substitution, the most complex substitution model in Arlequin 3.5, and were based on 10,100 permutations. Tristan = Tristan da Cunha. Asterisks indicate significant pair-wise differences (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

	Caribbean	Japan	S. Indian Ocean	Mediterranean	Atl. Spain	S. Africa	Tristan
Caribbean	--						
Japan	0.95134***	--					
S. Indian Ocean	0.95583***	0.85516*	--				
Mediterranean	0.96803***	0.92009***	-0.19403	--			
Atl. Spain	0.97355***	0.94469***	0.88406**	0.84912***	--		
S. Africa	0.95768***	0.85045***	-0.11472	0.06882	0.75281***	--	
Tristan	0.95583***	0.85516	0	-0.19403	0.88406***	-0.11472	--
Senegal	0.95583***	0.85516	0	-0.19403	0.88406***	-0.11472	0

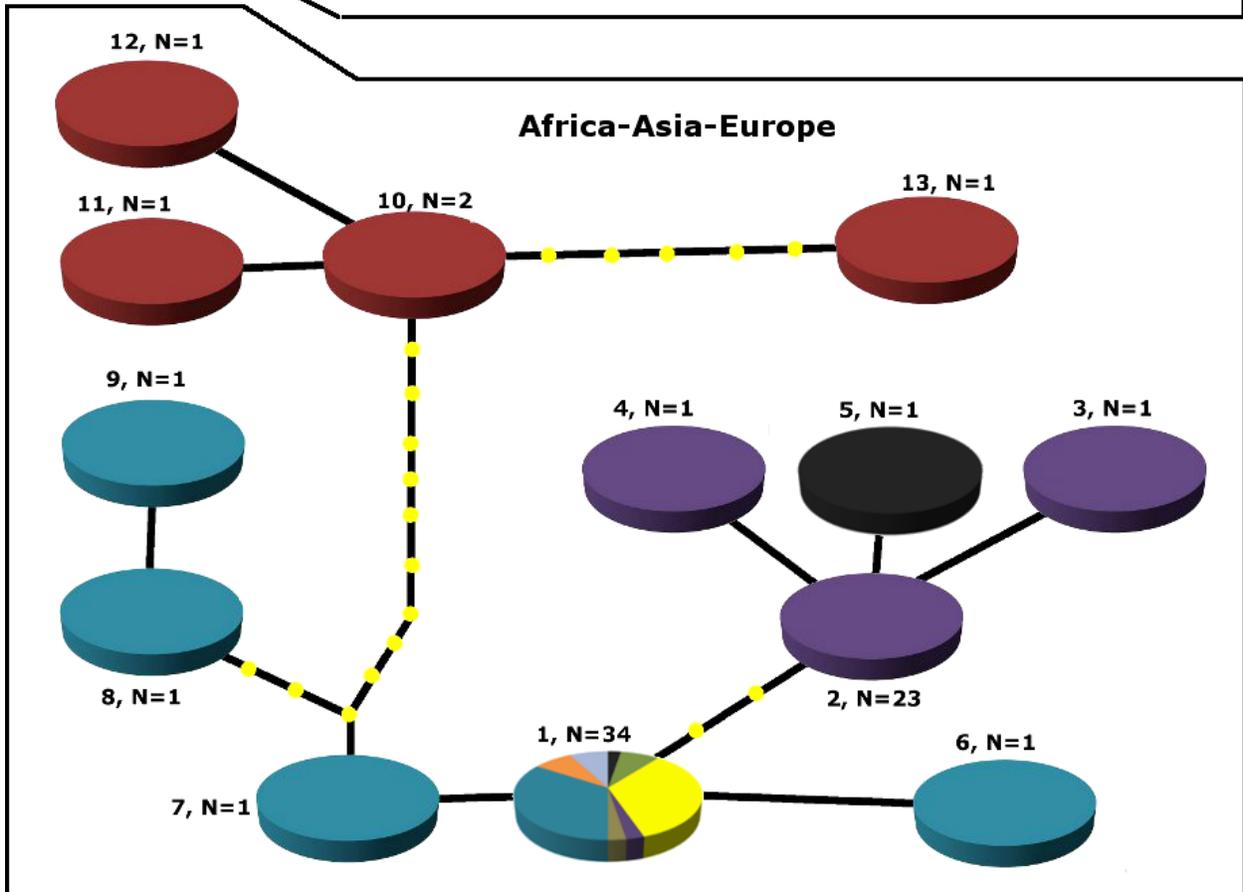
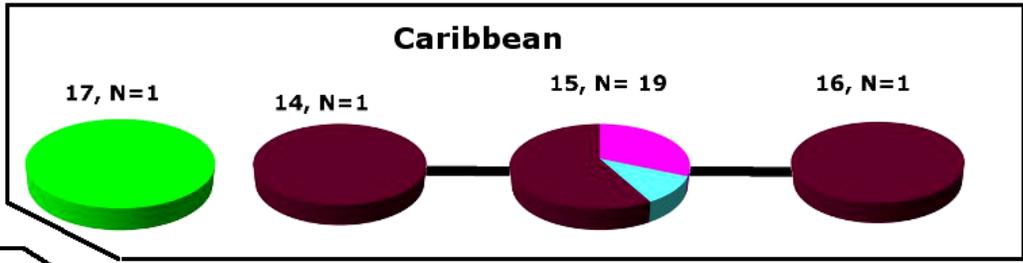
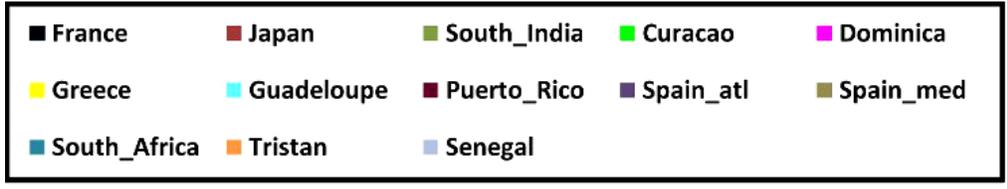


Figure 4. Parsimony network tree of 17 haplotypes of *O. vulgaris* based on COI. Each circle represents a unique haplotype. Each haplotype has a numerical identifier and the number of samples sharing a particular haplotype is indicated. The minimum number of mutational steps is represented by the small yellow dots.

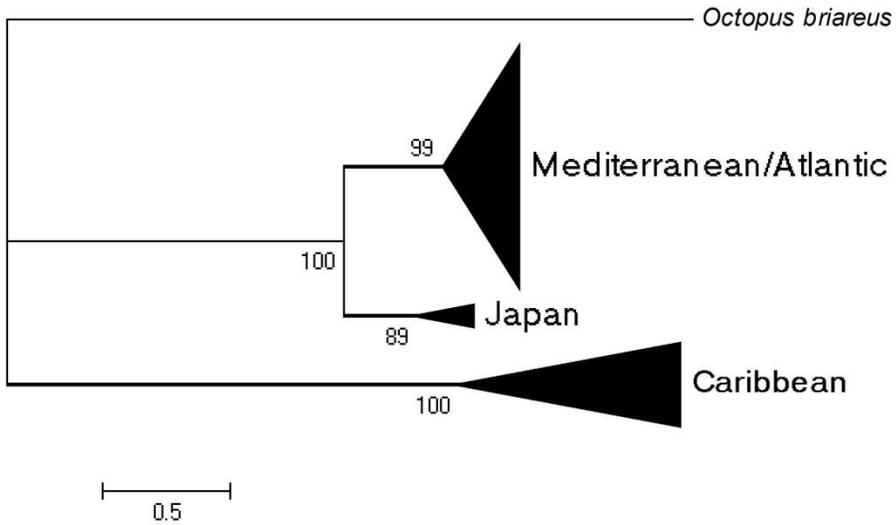
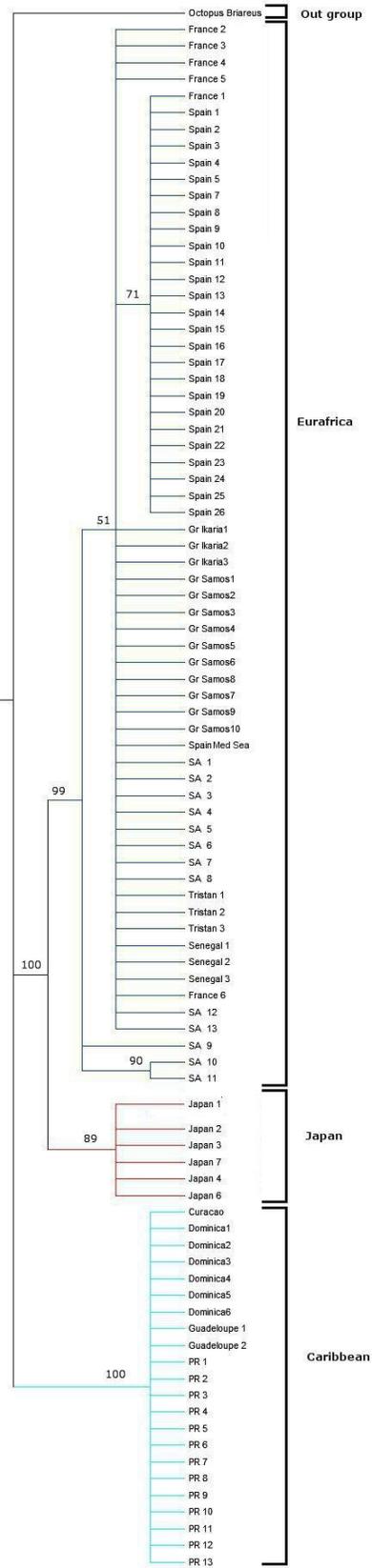


Figure 5. Bayesian genealogy of *Octopus vulgaris* based on COI. The TIM2+I nucleotide substitution model was applied to the Bayesian analysis. Posterior probabilities are shown above branches. The congeneric *Octopus briareus* was used as the outgroup. The width of the triangle is due to the number of sequences; the length of the triangle is due to the divergence between the sequences within the group.



A

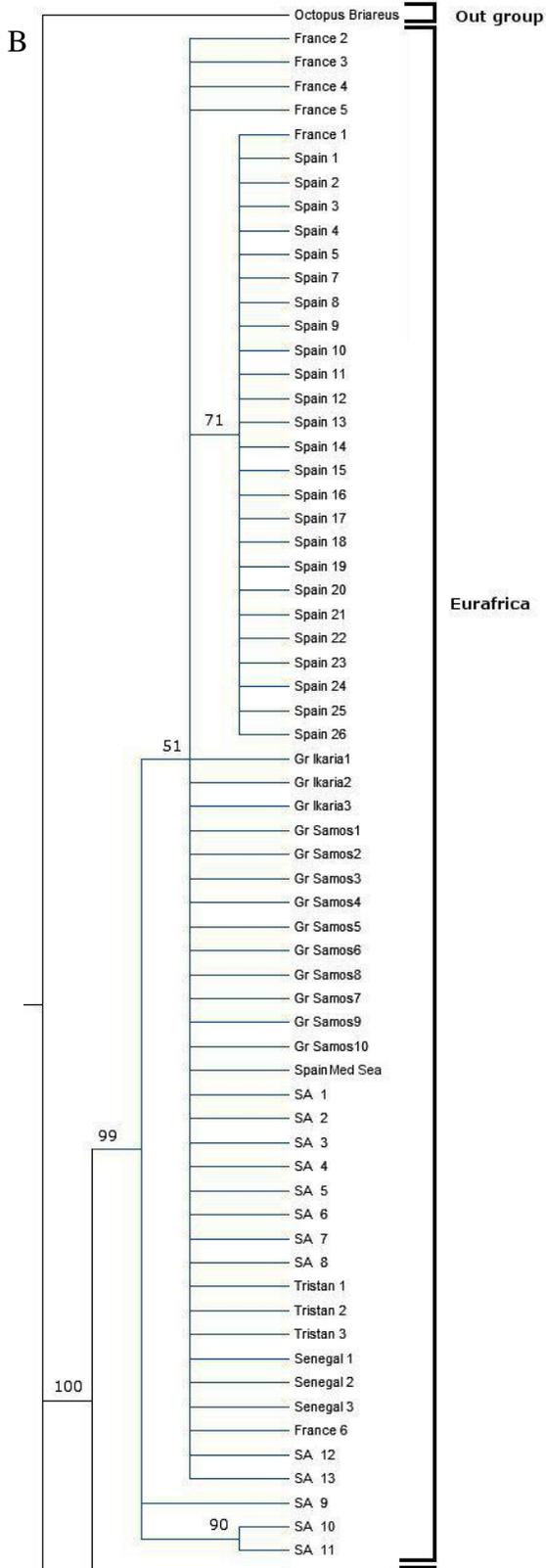
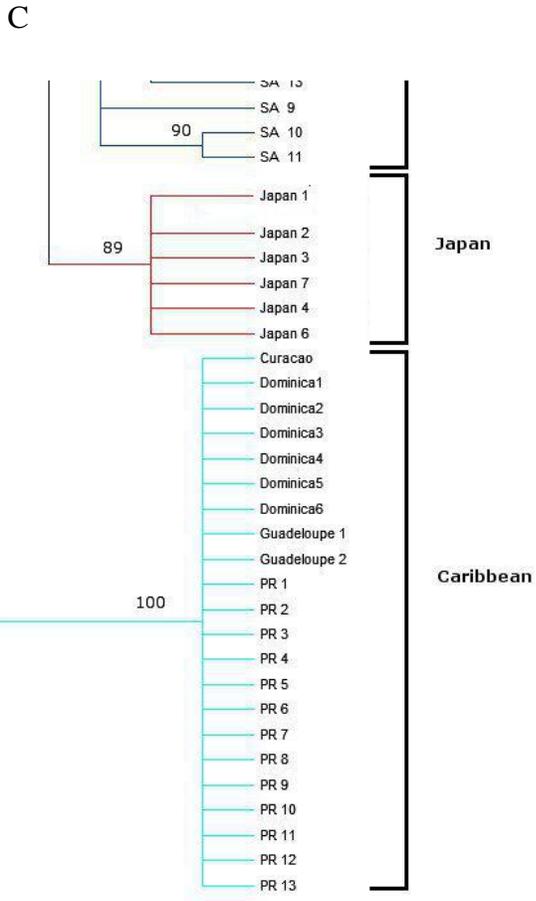


Figure 6. Maximum Likelihood genealogy of *Octopus vulgaris* based on COI. The TIM2+I nucleotide substitution model was applied to the phylogenetic analysis. The congeneric *Octopus briareus* was used as the outgroup. A) Whole tree; B) Detail of the Atlantic and Mediterranean clade; C) detail of the Japanese and Caribbean clade.



## DISCUSSION

This study assessed the genetic differentiation of Caribbean populations against what has been proposed as the traditional distribution of the common octopus *O. vulgaris* with the aim to further our understanding of the population genetics and evolutionary history of this commercially important species. The most important finding was the significant population structure we observed between western and eastern Atlantic (including Mediterranean) populations, which were previously thought as panmictic. The parsimony network and the phylogenetic analyses of the mitochondrial COI argued against an amphi-Atlantic distribution of the common octopus. The Caribbean lineage identified by the genetic analyses represents a lineage of *Octopus vulgaris* that is more genetically distant to all other *O. vulgaris* populations but still less divergent between any *O. vulgaris* and the congeneric species *O. briareus*. The second important pattern that emerges from the results is the close genetic relationship of *O. vulgaris* in Mediterranean, Eastern Atlantic locations (Spain, Senegal and South Africa), including the isolated Atlantic islands of Tristan da Cunha. While pairwise  $\Phi_{STs}$  differentiated the Atlantic Spain samples, both the phylogenetic and the parsimony network analysis indicated that they are imbedded in the Mediterranean/east Atlantic clade. The differences between the Atlantic Iberian octopus and Mediterranean octopus could be partially explained by the flow of coastal currents. The surface current near the Strait of Gibraltar may prevent paralarvae to disperse out of the Mediterranean Sea because water primarily enters from the Atlantic Ocean to the Mediterranean Sea. There is very little Mediterranean Outflow in the opposite direction as deeper, colder high salinity water enters the Atlantic Ocean from the Mediterranean Sea (Reid, 1994). The Atlantic inflow of water does not explain though the fact that the Mediterranean

specimens are closely related to those of the Atlantic Africa, so presumably there is higher gene flow from/to the south than from/to the north of Gibraltar towards the Mediterranean. Camariñas, the location of the Atlantic Iberian samples is at the extreme North-West of Spain, where the Portugal Current flows southward (Perez et al., 2001), perhaps limiting the paralarvae from south to move northward on the Iberian peninsula.

Our analysis confirmed the presence of two genetically different lineages of *O. vulgaris* in southern Africa, previously identified by Teske et al (2007). Surprisingly samples from the South Indian islands of St. Paul and Amsterdam yielded identical sequences with those from the Mediterranean and Atlantic coast of Africa. A possible way to explain these data is through the periodic water exchange between the Indian and Atlantic oceans through the ‘Agulhas leakage’, which is the Agulhas current transporting planktonic (e.g. larvae) species into the Cape basin and beyond (Peeters et al., 2004). The lack of population differentiation among these locations reflects the ability for dispersal of octopi is sufficiently high to prevent population divergence. The Japanese specimens were genetically more similar to those from the Mediterranean/east Atlantic clade than to the Caribbean ones.

The degree of differentiation observed between the *O. vulgaris* populations separated by the Atlantic Ocean (up to 14.5%, p-distance) is exceptionally high in comparison with previous studies. The intra-specific difference among various populations of *O. vulgaris* for the mtCOIII is 3.9% and it is 1.6% for the mt16S (Warnke et al., 2004). The mtCOIII and mt16S alignment displayed similar patterns of nucleotide differences than the mtCOI. The genetic distances observed between some populations of *O. vulgaris* exceed those between other closely related species and several other cephalopod species. For comparison, the 16S divergence between *O. vulgaris* and *O. insularis* was estimated to be 7.2% (Leite et al., 2008). The high levels of

sequence divergence in *O. vulgaris* suggest a long-term isolation of populations, allowing for independent evolutionary trajectory of different mitochondrial lineages.

Most genetic studies of “cosmopolitan” marine organisms reveal a trend toward high levels of gene flow across oceanic regions, therefore high levels of connectivity among populations (Grant and Bowen, 1998; Waples, 1998). If the cosmopolitan species attain continuous distributions, they exhibit less population differentiation than species with discontinuous distributions (Graves, 1998). The population genetics portrait that begins to emerge from this and previous studies is of a widespread species that inhabits the Mediterranean, the temperate Atlantic Europe, the Atlantic Africa, some mid-Atlantic and south Indian Ocean islands and Japan. Undoubtedly, more samples from the Indian and the Pacific Ocean will modify the suggested distribution of *O. vulgaris*. *Octopus vulgaris* is also distributed in the Caribbean but the observed COI divergence challenges the notion that this is a cohesive species. These results indicate that the extensive gene flow and widespread genetic homogeneity predicted from the possession of a pelagic paralarval dispersal stage in *O. vulgaris* may not necessarily be realised in natural populations, and that gene flow and paralarval dispersal may be regionally restricted. In addition, our results agree with the emerging hypothesis that the widely distributed *Octopus vulgaris* is a complex of morphologically indistinguishable species.

## **Selection**

Since *O. vulgaris* inhabits coastal areas with different climates and environments, the patterns of genetic variation in *O. vulgaris* may be the result of natural selection. Both selective sweeps (fixation of an advantageous mutation that also fixes any linked neutral polymorphism) and background selection (selection against deleterious mutations that also removes any linked

neutral polymorphism) can greatly reduce levels of genetic variation. Selective sweeps that are associated with local adaptation will remove variation only over the local geographic area, increase population differentiation, and promote the presence of more widespread polymorphisms. It is possible that when *O. vulgaris* reached the Caribbean adaptation to the new environmental conditions shaped the genetic makeup of the mitochondrial genome. The positive value of Fu's  $F_s$  in the Caribbean samples is suggesting a deficiency of alleles, as would be expected from a recent population bottleneck, however the result is not significant. The neutrality tests Tajima's  $D$  and Fu's  $F_s$  are consistently negative in the Atlantic Spain samples, showing an excess number of alleles, as would be expected from a recent population expansion or from genetic hitchhiking. A recent selective sweep exerts an effect on patterns of variation at linked neutral loci very similar to that of a recent population expansion.

### **Fisheries Genetics of *O. vulgaris***

A major objective of fisheries genetics is the identification of genetically distinct populations. The amount and geographic distribution of genetic variability is determined not only by contemporary levels of gene flow, but also by demographic processes, population history, and selection. Separating the effects of demography and selection can be difficult, especially in marine organisms where high fecundity, high variance in reproductive success and environmental instability are frequent. The Caribbean *O. vulgaris* represents a distinct genetic lineage within the *O. vulgaris* species complex. Even though the current study was not designed to address the species status of the Caribbean specimens, the large amounts of divergence challenges our notion of a globally distributed species. From the management point of view, at least in the eastern Caribbean, there appears to be one panmictic population, however, this

suggestion should be tested with additional molecular markers. Conservation issues in the Caribbean are complex, since the Caribbean consists of several nations, each with its own unique fishing demands and fishery regulations. The underlying theme is that octopus is a very popular food choice. It is only third in importance compared to other commercially important invertebrate species (e.g. *Panurilus argus* and *Strombus gigas*) in the Caribbean, Central and South America. Most likely the most southern distribution of the Caribbean lineage is North Brazil and the most northern limits of its distribution are the Carolinas. Our data agrees with the assertion that the common octopus *O. vulgaris* is a complex of several closely related, rather indistinguishable species in tropical to temperate waters (Norman, 2003).

Table 4. Species name and location of samples collected in the present study.

<b>Species</b>	<b>Country</b>	<b>Location</b>	<b>Latitude</b>	<b>Longitude</b>
<i>Octopus briareus</i>	Curaçao	Near Marine aquarium	12°04'53"	68°53'26" W
<i>Octopus briareus</i>	Curaçao	Near Marine aquarium	12°04'53"	68°53'26" W
<i>Octopus vulgaris</i>	Curaçao	Near Marine aquarium	12°04'53"	68°53'26" W
<i>Octopus vulgaris</i>	Dominica	Champagne Beach	15°14'41"	61°22'22" W
<i>Octopus vulgaris</i>	Dominica	Champagne Beach	15°14'41"	61°22'22" W
<i>Octopus vulgaris</i>	Dominica	Champagne Beach	15°14'41"	61°22'22" W
<i>Octopus vulgaris</i>	Dominica	Champagne Beach	15°14'41"	61°22'22" W
<i>Octopus vulgaris</i>	Dominica	Champagne Beach	15°14'41"	61°22'22" W
<i>Octopus vulgaris</i>	Dominica	Champagne Beach	15°14'41"	61°22'22" W
<i>Octopus vulgaris</i>	Guadeloupe	Le Gosier	16°12'07"	61°29'16" W
<i>Octopus vulgaris</i>	Guadeloupe	Le Gosier	16°12'07"	61°29'16" W
<i>Octopus vulgaris</i>	Puerto Rico	La Parguera	17°56'32"	67°04'34" W
<i>Octopus vulgaris</i>	Puerto Rico	La Parguera	17°56'40"	67°02'22" W
<i>Octopus vulgaris</i>	Puerto Rico	La Parguera	17°56'40"	67°02'20" W
<i>Octopus vulgaris</i>	Puerto Rico	La Parguera	17°56'40"	67°02'25" W
<i>Octopus vulgaris</i>	Puerto Rico	La Parguera	17°56'40"	67°02'27" W
<i>Octopus vulgaris</i>	Puerto Rico	La Parguera	17°56'40"	67°02'27" W
<i>Octopus vulgaris</i>	Puerto Rico	Margarita	17°55'30"	67°06'20" W
<i>Octopus vulgaris</i>	Puerto Rico	Margarita	17°55'19"	67°07'20" W
<i>Octopus vulgaris</i>	Puerto Rico	Margarita	17°55'13"	67°06'20" W
<i>Octopus vulgaris</i>	Puerto Rico	Margarita	17°55'20"	67°06'40" W



Table 5. Location of collections, specimen ID and Genbank accession numbers of COI sequences used in this study. Med = Mediterranean Sea, Atl = Atlantic Ocean, Ind = Indian Ocean, Pac = Pacific Ocean.

<b>Location, specimen ID</b>	<b>GenBank No.</b>	<b>Source</b>
Banyuls, France 2 (Med)	EF016328	Allcock et al. 2006
Spain 6 (Med)	DQ683227	Teske et al. 2007
Spain 24 (Atl)	DQ683221	Teske et al. 2007
Spain 25 (Atl)	DQ683222	Teske et al. 2007
Spain 26 (Atl)	DQ683223	Teske et al. 2007
Senegal 1 (Atl)	DQ683224	Teske et al. 2007
Senegal 2 (Atl)	DQ683225	Teske et al. 2007
Senegal 3 (Atl)	DQ683226	Teske et al. 2007
South Africa 1	DQ683208	Teske et al. 2007
South Africa 2	DQ683215	Teske et al. 2007
South Africa 3	DQ683213	Teske et al. 2007
South Africa 4	DQ683217	Teske et al. 2007
South Africa 5	DQ683210	Teske et al. 2007
South Africa 6	DQ683211	Teske et al. 2007
South Africa 7	DQ683219	Teske et al. 2007
South Africa 8	DQ683220	Teske et al. 2007
South Africa 9	DQ683209	Teske et al. 2007
South Africa 10	DQ683214	Teske et al. 2007
South Africa 11	DQ683216	Teske et al. 2007
South Africa 12	DQ683218	Teske et al. 2007
South Africa 13	DQ683212	Teske et al. 2007
Tristan da Cunha 1 (Atl)	DQ683205	Teske et al. 2007
Tristan da Cunha 2 (Atl)	DQ683206	Teske et al. 2007
Tristan da Cunha 3 (Atl)	DQ683207	Teske et al. 2007
St. Paul and Amsterdam Is. 3 (Ind)	FN424379	Guerra et al. (In Review)
St. Paul and Amsterdam Is. 4 (Ind)	FN424380	Guerra et al. (In Review)
St. Paul and Amsterdam Is. 5 (Ind)	FN424381	Guerra et al. (In Review)
Japan 1 (Pac)	AB158363	Yokobori et al. 2004
Akashi, Futami, Japan 2 (Pac)	AB430546	Kaneko & Kubodera, unpublished
Kanagawa, Misaki, Japan, 3 (Pac)	AB430547	Kaneko & Kubodera, unpublished
Japan 4 (Pac)	AB052253	Minakata et al. unpublished
Japan 6 (Pac)	AB191269	Takumiya et al. 2005
East China Sea, Japan 7 (Pac)	AB430548	Kaneko & Kubodera, unpublished

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