

A contribution to the understanding of phylogenetic relationships among species of the genus *Octopus* (Octopodidae: Cephalopoda)

MARÍA SOLEDAD ACOSTA-JOFRÉ^{1,3}, RICARDO SAHADE^{1,3}, JÜRGEN LAUDIEN⁴
and MARINA B. CHIAPPERO^{2,3}

¹ Cátedra de Ecología Marina, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Argentina.

² Cátedra de Genética de Poblaciones y Evolución, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Argentina. E-mail: mchiappero@efn.unc.edu.ar

³ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

⁴ Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany.

SUMMARY: Many species of the genus *Octopus* are important resources for fisheries worldwide. Its approximately 200 species show a strong similarity in structural morphology and a wide diversity in skin coloration and patterning, behaviour and life strategies that have hampered the study of phylogenetic relationships. We used a Bayesian approach to estimate as yet unknown phylogenetic relationships among *O. tehuelchus* from the southwestern Atlantic, new specimens of *O. mimus* (Chile and Peru) and other *Octopus* species, and used Bayes factors to test phylogenetic hypotheses. *O. tehuelchus* was more closely related to the genera *Callistoctopus*, *Grimpella* and *Macroctopus* than to *Octopus*, and therefore its generic placement may need a revision. *O. vulgaris* specimens from Costa Rica (Pacific Ocean) and *O. oculifer* grouped with *O. mimus*. Bayes factors showed positive evidence in favor of this grouping and therefore these individuals could have been misidentified, being in fact *O. mimus*. *O. vulgaris* specimens from the Costa Rican Caribbean were more related to *O. mimus* than to other *O. vulgaris* and could represent a cryptic species. The remaining *O. vulgaris* clustered with *O. tetricus*. Bayes factors found strong evidence against the monophyly of *O. vulgaris* as currently defined, giving statistical support to the monophyly of an *O. vulgaris* s. str. + *O. tetricus* group proposed previously by other authors.

Keywords: *Octopus mimus*, *Octopus tehuelchus*, phylogeny, cytochrome oxidase subunit III, Bayes factors.

RESUMEN: CONTRIBUCIÓN AL CONOCIMIENTO DE LAS RELACIONES FILOGENÉTICAS ENTRE ESPECIES DEL GÉNERO *OCTOPUS* (OCTOPODIDAE: CEPHALOPODA). – Numerosas especies del género *Octopus* constituyen un recurso importante para las pesquerías en todo el mundo. Sus aproximadamente 200 especies presentan a la vez una importante similitud en morfología y una amplia diversidad en el patrón y coloración de la piel, comportamiento y estrategias de vida, lo cual ha dificultado el estudio de las relaciones filogenéticas. En este trabajo estimamos las relaciones filogenéticas entre *O. tehuelchus* del Atlántico sudoccidental (cuyas relaciones con otras especies de la subfamilia son desconocidas), nuevos especímenes de *O. mimus* (Chile y Perú) y otras especies de *Octopus*, utilizando métodos de estimación bayesiana. Además utilizamos tests de factores de Bayes para comprobar hipótesis filogenéticas. *O. tehuelchus* estuvo más cercanamente relacionado a los géneros *Callistoctopus*, *Grimpella* y *Macroctopus* que a *Octopus*, y por lo tanto su ubicación genérica debería ser revisada. Los especímenes de *O. vulgaris* de Costa Rica (Océano Pacífico) y *O. oculifer*, se agruparon junto con *O. mimus*. Los factores de Bayes mostraron evidencia positiva a favor de este agrupamiento, por lo que estos ejemplares podrían representar ejemplares de *O. mimus* mal clasificados. Los especímenes de *O. vulgaris* del Mar Caribe de Costa Rica podrían constituir una especie críptica ya que estuvieron más cercanamente relacionados a *O. mimus* que a los restantes *O. vulgaris*. Estos últimos se agruparon con *O. tetricus*. Los factores de Bayes mostraron una fuerte evidencia a favor de este agrupamiento, dando soporte estadístico a la monofilia de *O. vulgaris* s. str. + *O. tetricus* propuesta previamente por otros autores.

Palabras clave: *Octopus mimus*, *Octopus tehuelchus*, filogenia, citocromo oxidasa subunidad III, factores de Bayes.

INTRODUCTION

The genus *Octopus* (Lamarck 1798) (Cephalopoda: Octopodidae: Octopodinae) includes approximately 200 species, many of which are important resources for fisheries worldwide. The genus is found in shallow waters of all oceans except in the polar regions. It displays a wide diversity in skin coloration, behaviour and life strategies but a strong similarity in structural morphology; as a consequence, phylogenetic relationships, species limits and identification are difficult to establish (Robson 1929, Roper and Hochberg 1988, Hochberg *et al.* 1992, Voight 1994, and reviewed in Guzik *et al.* 2005). In the last few years, molecular techniques have been increasingly applied to increase the accuracy of phylogenetic relationships assessments. For example, Barriga Sosa *et al.* (1995) examined five *Octopus* species from the northern Pacific Ocean using the mitochondrial cytochrome oxidase III (COIII) gene. They confirmed the species-level status of *O. bimaculoides* and *O. bimaculatus*, two two-spotted octopuses that occur sympatrically. Carlini *et al.* (2001), using cytochrome oxidase I, and Guzik *et al.* (2005), using one nuclear (Elongation Factor-1 α) and two mitochondrial (cytochrome oxidase III and cytochrome b) genes, estimated the phylogenetic relationships among several *Octopus* species; these authors found that *Octopus* is not a monophyletic genus, although it includes monophyletic groups. However, Norman and Hochberg (2005), in a revision of the species-level taxonomy of the family Octopodidae, reassigned several of the species considered as belonging to the genus *Octopus* to other genera such as *Amphioctopus*, *Callistoctopus* and *Enteroctopus*, while others were considered as “unplaced” and left for the moment in the genus *Octopus* until a major revision is undertaken.

There are also taxonomic problems regarding the species *O. vulgaris*, whose status as a true cosmopolitan species or as a species complex is uncertain (Guerra *et al.* 2010). Warnke *et al.* (2004) used COIII and 16rRNA genes to clarify the limits and distribution of the species *O. vulgaris*. They revealed that populations from the Mediterranean, the western and eastern Atlantic, Venezuela, Japan and Taiwan form a monophyletic clade, confirming the presence of the species in the north-western Pacific. *O. mimus* was more closely related to *O. bimaculoides* than to *O. vulgaris*. However, as they included few species and specimens in their analyses, monophyly could be an artifact of poor species sampling. Guerra *et al.* (2010), using COI and COIII genes, estimated phylogenetic relationships among a larger sample of species of the genus *Octopus* and included specimens of *O. vulgaris* from more geographic locations. They found that *O. vulgaris* from the southern Indian Ocean clustered with a high bootstrap percentage to those specimens of Warnke (*O. vulgaris* sensu stricto). However, this group clustered with *O. tetricus* from Australia; the remaining specimens of *O. vulgaris* (from the Caribbean Sea and Pacific Ocean)

clustered together with *O. mimus* (from Iquique, Chile, and from the Pacific Ocean near Costa Rica), and *O. oculifer* from the Galapagos Islands, and were placed in the same major cluster as the *O. vulgaris* s. str. plus *O. tetricus*. These authors suggest treating this grouping as an *O. vulgaris* species group. These and other studies (such as the above cited and those of Strugnell *et al.* (2005) and Teske *et al.* (2007)) included only a limited number of specimens from the southern part of South America.

The Gould octopus, *O. mimus*, is an important target of fisheries along the southern Pacific coasts of South America, from central Chile to northern Peru (Rocha and Vega 2003, Cardoso *et al.* 2004). Octopuses from these locations were considered as synonymous with the cosmopolitan species *O. vulgaris*, but were recently recognized again as a separate species by Guerra *et al.* (1999) and by Söller *et al.* (2000), who found more than 12% of nucleotide divergence between the two species. However, these authors only studied three specimens from a single locality (Iquique, northern Chile), all showing the same haplotype. On the Atlantic coast of southern South America, the commercially exploited *O. tehuelchus* or “pulpito” is distributed from southern Brazil to approximately 44°S in Argentina (Iribarne 2009). To date, the phylogenetic relationships of this species with others of the genus *Octopus* remain unknown.

The aim of the present study was to assess the relationships among representatives of *O. mimus* from localities not studied previously (the southeastern Pacific coast of Chile and Peru) and those previously sequenced, in order to estimate their phylogenetic relationships with other *Octopus* species, particularly *O. vulgaris*, and to assess the support for the monophyletic status of *O. vulgaris* through the use of Bayes factors. We also aimed to study the relationships of the poorly known species *O. tehuelchus* from the southwestern Atlantic Ocean (Argentina) with other species of the genus using the COIII gene.

MATERIALS AND METHODS

Specimens

Samples were obtained by artisanal fishing; the species *O. minus* was collected near Coloso (northern Chile; 23°45'36.72''S, 70°27'47.29''W; N=4) and Callao (Peru; 12°04'21.27''S 77°09'41.73''W; N=3). Samples of *O. tehuelchus* were collected in the San Matías Gulf (Argentina; 40°47'11.08''S 64°53'45.52''W; N=3). Tissue samples were obtained from the arm of freshly caught specimens and stored in 90% ethanol at -20°C until DNA extraction.

Sequences of COIII for two specimens of *O. mimus* from Chile and Costa Rica, for 16 *O. vulgaris* from different locations worldwide, and for 42 additional species from the subfamily Octopodinae were obtained from GenBank for comparison (Table 1). Some of

TABLE 1. – Specimens, sampling regions, sample sizes and accession numbers of DNA sequences included in the phylogenetic inferences with COIII. Sequences reassigned to other genera by Norman and Hochberg (2005) that are still identified as *Octopus* in GenBank have their accession numbers underlined; specimens marked as “unplaced” are those of uncertain position but left in the genus until further revision by the same authors. Species used as outgroups are listed at the bottom of the table.

Species	Sampling region	Sample size	Accession number
<i>Abdopus aculeatus</i> (D’Orbigny, 1834)	Orpheus Island, Queensland, Australia	1	<u>AJ628213</u> ^e
<i>Ameloctopus litoralis</i> Norman, 1992	Dudley Point, Northern Territory, Australia	1	AJ628207 ^e
<i>Amphioctopus exannulatus</i> (Norman, 1993)	Lizard Island, Queensland, Australia	1	<u>AJ628223</u> ^e
<i>Amphioctopus kagoshimensis</i> (Ortmann, 1888)	One tree Island, Queensland, Australia	1	<u>AJ628226</u> ^e
	Jogashima Island, Japan	1	AB573193 ⁱ
<i>Amphioctopus marginatus</i> (Taki, 1964)	Northern Sulawesi, Indonesia	1	<u>AJ628232</u> ^e
	Nha Trang, Vietnam	1	AB573196
<i>Amphioctopus mototi</i> (Norman, 1993)	New South Wales, Australia	1	<u>AJ628233</u> ^e
<i>Amphioctopus ocellatus</i> (Gray, 1849)	Tokyo, Japan	1	NC007896 ^f
<i>Amphioctopus ovulum</i> (Sasaki, 1917)	East China Sea, Japan	1	AB573198 ⁱ
<i>Callistoctopus alpheus</i> (Norman, 1993)	One Tree Island, Queensland, Australia	1	<u>AJ628215</u> ^e
<i>Callistoctopus aspilosomatis</i> (Norman, 1993)	One Tree Island, Queensland, Australia	1	<u>AJ628216</u> ^e
	Miyagi Island, Okinawa, Japan	1	AB573205 ⁱ
<i>Callistoctopus bunurong</i> (Stranks, 1990)	St. Leonards Pier, Victoria, Australia	1	<u>AJ628219</u> ^e
<i>Callistoctopus dierythraeus</i> (Norman, 1993)	Magnetic Island, Queensland, Australia	1	<u>AJ628222</u> ^e
<i>Callistoctopus graptus</i> (Norman, 1993)	Townsville, Queensland, Australia	1	<u>AJ628224</u> ^e
<i>Callistoctopus minor</i> Sasaki, 1920 - UNPLACED	East China Sea, Japan	1	AB573201 ⁱ
<i>Callistoctopus luteus</i> (Sasaki, 1929)	Kanagawa, Miura, Japan	1	AB573206 ⁱ
<i>Cistopus indicus</i> (Rapp, 1835)	Taichung Fish Market, Taiwan	1	AJ628208 ^e
<i>Enteroctopus dofleini</i> (Wulker, 1910)	British Columbia, Canada, Pacific Ocean	1	X83103 ^a
<i>Grimpella thaumastocheir</i> Robson, 1928	Pt. Victoria Jetty, South Australia	1	AJ628209 ^e
<i>Hapalochlaena fasciata</i> (Hoyle, 1886)	Moreton Bay, Queensland, Australia	1	AJ628210 ^e
<i>Hapalochlaena maculosa</i> (Hoyle, 1883)	St. Leonards Pier, Victoria, Australia	1	AJ628212 ^e
<i>Hapalochlaena</i> sp. Norman, 2000	Darwin, Australia	1	AJ628211 ^e
<i>Macroctopus maorum</i> Hutton, 1880	Potsea Pier, Victoria, Australia	1	<u>AJ628231</u> ^e
<i>Octopus australis</i> Hoyle, 1885 - UNPLACED	Port Stephens, New South Wales, Australia	1	AJ628217 ^e
<i>Octopus berrima</i> Stranks and Norman, 1993 - UNPLACED	Edithburg Jetty, South Australia	1	AJ628218 ^e
<i>Octopus bimaculatus</i> Verrill, 1883	California, USA, Pacific Ocean	1	X83100 ^a
<i>Octopus bimaculoides</i>	Santa Barbara, CA, USA, Pacific Ocean	1	AJ250482 ^d
	Pickford and McConnaughey, 1949		
<i>Octopus californicus</i> Berry, 1911 - UNPLACED	Ellwood, Santa Barbara Coast, CA, USA, Pacific Ocean	1	AJ250483 ^d
<i>Octopus cyanea</i> Gray, 1849	Queensland, Australia	2	X97955 ^b AJ628220 ^e
<i>Octopus insularis</i> Leite and Haimovici, 2008	Recife, North Brazil, Atlantic Ocean	2	AJ012123 ^c AJ012124 ^c
<i>Octopus kurna</i> Stranks, 1990 - UNPLACED	Port Phillip Bay, Victoria, Australia	1	AJ628227 ^e
<i>Octopus maya</i> Voss and Solis, 1966	Yucatán Peninsula, México	1	GU362546 ^j
<i>Octopus mimus</i> Gould, 1852	Iquique, North Chile, Pacific Ocean	1	AJ012128 ^c
	Coloso, North Chile, Pacific Ocean	2	GU355928 ^h GU355929 ^h
	Callao, Peru, Pacific Ocean	2	GU355927 ^h GU355933 ^h
<i>Octopus ocellate</i> sp.A Norman, 1998	Isla de Cocos, Costa Rica, Pacific Ocean	1	AJ250480 ^d
<i>Octopus oculifer</i> Hoyle, 1904	Victoria, Australia	1	AJ628234 ^e
<i>Octopus pallidus</i> Hoyle, 1885 - UNPLACED	Galapagos Islands, Pacific Ocean	1	AJ628235 ^e
<i>Octopus rubescens</i> Berry, 1953 - UNPLACED	St. Leonards Pier, Victoria, Australia	1	AJ628236 ^e
<i>Octopus salutii</i> Verany, 1839 - UNPLACED	California, USA, Pacific Ocean	1	X83101 ^a
<i>Octopus tehuelchus</i> D’Orbigny, 1834	Banyuls, France, Mediterranean Sea	1	AJ250484 ^d
	San Matías Gulf, Argentina, Southwestern Atlantic Ocean	2	GU355937 ^h GU355938 ^h
<i>Octopus tetricus</i> Gould, 1852	Wallaga Lakes, New South Wales, Australia	1	AJ628237 ^e
	Port Stephens New South Wales, Australia	1	AJ628240 ^e
	Lucky Bay, Western Australia	1	AJ628238 ^e
	Fremantle Warf, Western Australia	1	AJ628239 ^e
<i>Octopus vulgaris</i> Cuvier, 1797	Banyuls, France, Mediterranean Sea	1	AJ012121 ^c
	Lanzarote Is/Senegal, West Africa, Atlantic Ocean	1	AJ250476 ^d
	Knysna Lagoon, South Africa, Indian Ocean	1	AJ250487 ^d
	Tristan da Cunha, Atlantic Ocean	1	AJ250477 ^d
	Itajai, South Brazil, Atlantic Ocean	1	AJ012122 ^c
	Rio de Janerio, Brazil, Atlantic Ocean	1	AJ616312 ^d
	Isla Margarita, Venezuela, Caribbean Sea	1	AJ250478 ^d
	Seto Inland Sea, Japan	1	AJ616311 ^d
	North East Taiwan, Pacific Ocean	1	AJ250479 ^d
	Port Elizabeth, South Africa	1	AJ628241 ^e
	Costa Rica, Pacific Ocean	1	AJ012125 ^c
	Costa Rica, Caribbean Sea	2	AJ012126 ^c AJ012127 ^c

TABLE 1 (cont.). – Specimens, sampling regions, sample sizes and accession numbers of DNA sequences included in the phylogenetic inferences with COIII. Sequences reassigned to other genera by Norman and Hochberg (2005) that are still identified as *Octopus* in GenBank have their accession numbers underlined; specimens marked as “unplaced” are those of uncertain position but left in the genus until further revision by the same authors. Species used as outgroups are listed at the bottom of the table.

Species	Sampling region	Sample size	Accession number
<i>Octopus vulgaris</i> Cuvier, 1797	Southern Indian Ocean	3	FN424384 ^g FN424383 ^g FN424382 ^g
<i>Octopus</i> sp.	Port Elizabeth, South Africa	1	AJ628230 ^e
<i>Octopus</i> sp.	Isla del Coco, Costa Rica, Pacific Ocean	1	AJ250481 ^d
<i>Octopus</i> sp. 5 Norman, 2000	Coconut Wells, Western Australia	1	AJ628225 ^e
<i>Octopus</i> sp. 8 Norman, 2000	Rockingham, Western Australia	1	AJ628228 ^e
<i>Octopus</i> sp. 10 Norman, 2000	Exmouth Gulf, Western Australia	1	AJ628229 ^e
<i>Loligo vulgaris</i> Lamarck, 1789		1	X97961 ^b
<i>Loligo forbesi</i> Steenstrup, 1856		1	X97962 ^b
<i>Loligo reynaudii</i> (D'Orbigny, 1839-1841)		1	X97960 ^b

^a From Barriga Sosa *et al.* 1995; ^b From Bonnaud *et al.* 1997; ^c From Söller *et al.* 2000; ^d From Warnke *et al.* 2004; ^e From Guzik *et al.* 2005; ^f From Akasaki *et al.* 2006; ^g From Guerra *et al.* 2010; ^h This work; ⁱ Unpublished.

these species were placed in the genus *Octopus* at the time of the original publications, but were reassigned to other genera by Norman and Hochberg (2005). These species are in some cases still identified as *Octopus* in GenBank, in which case accession numbers are underlined in Table 1. The species *Loligo forbesi*, *L. vulgaris* and *L. reynaudii* were used as outgroups.

Amplification and sequencing

Total genomic DNA was obtained from tissues by phenol-chloroform extraction and ethanol precipitation (Maniatis *et al.* 1982). DNA was stored in double-distilled water at -20°C until polymerase chain reaction (PCR) amplification. The mitochondrial COIII gene was amplified with the primers 5'-CATTTAGTT-GATCCTAGGCCTTGACC-3' and 5'-CAAACCA-CATCTACAAAATGCCAATATC-3' (Ó'Foighil and Smith 1995). Amplifications were performed in 50- μl reaction volumes containing 15 ng of genomic DNA, 1 unit Taq polymerase (Invitrogen, Brazil), 1 \times reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 2.5 mM MgCl₂, 25 nM of each dNTP (dATP, dCTP, dGTP, dTTP), 3 μM of each primer and double-distilled water to the final volume.

PCR conditions were as follows: 1 cycle of 2 min at 95°C , 30 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 1 min, and extension at 72°C for 1 min; a post-treatment of 7 min at 72°C and a final cooling at 15°C were performed. PCR products were purified and sequenced at the facilities of Macrogen Inc. (Rockville, USA).

Data analysis

Sequences were inspected manually using the program CHROMAS version 2.23 (McCarthy 1998) and aligned using MUSCLE (Edgar 2004). All new sequences were submitted to GenBank; accession numbers are cited in Table 1. Phylogenetic relationships were assessed using the Bayesian approach implemented in MrBayes v3.1.2 (Ronquist and Huelsenbeck

2003). The model of sequence evolution that best fits our data set was chosen among 24 evolutionary models using MrModeltest 2.3 (Nylander 2004), considering both the complete data set and partitioning into the first, second, and third codon positions. The final model selected for the unpartitioned data set and for the first and third codon positions was GTR+I+G (Tavaré 1986). The selected model for the second codon position was GTR+G. The Bayesian analysis was conducted for the unpartitioned and codon position-partitioned data sets by running the Markov chain Monte Carlo with six chains for 3 million generations, on three independent runs. Trees were sampled every 100 generations and the first 25% of them were discarded as burn in. The robustness of clades was estimated by the Bayesian posterior probabilities (BPP). Results of the partitioned and unpartitioned analyses were compared by calculating Bayes factors (Nylander *et al.* 2004). The Bayes factor (B_{10}) is defined as the ratio of the likelihood of the alternative model to the null model. We used the ln of the harmonic mean of the likelihood values sampled from the stationary phase of the MCMC run (obtained from MrBayes) as an estimator of the model likelihoods (Newton and Raftery 1994). The Bayes factor in favour of a model was then calculated as $2\log_e B_{10}$ and the resulting values were interpreted following Kass and Raftery (1995), where values between 2 and 6 indicate positive evidence, between 6 and 10 strong evidence, and greater than 10 very strong evidence against the null model (and complementary, similar negative values, evidence favouring the null model). Additionally, we used Bayes factors to test the unconstrained tree topology versus trees obtained with MrBayes but forcing the monophyly of *O. vulgaris*, *O. mimus*, and *O. vulgaris* plus *O. mimus*.

RESULTS

The aligned sequences were 642 bp long. Four closely related haplotypes were found in *O. mimus*, differing by one or two mutations from each other. These new haplotypes differed by 2 to 4 substitutions from

TABLE 2. – Bayes factor tests for alternative hypotheses of evolution in the genus *Octopus*. The Bayes factor was calculated as twice the natural logarithm of the harmonic mean of the likelihood values sampled from the stationary phase of the MCMC run ($2\log_e B_{10}$).

M0	Models compared	M1	Harmonic mean of ln L		Bayes factor (interpretation*)
			M0	M1	
Unconstrained, non-partitioned tree	Unconstrained tree, partitioned by codon position		-9935.11	-9772.63	324.96 (very strong evidence against M0)
Unconstrained tree, partitioned by codon position	Monophyly of <i>O. vulgaris</i> enforced, partitioned by codon position		-9772.63	-9775.93	-6.6 (strong evidence against M1)
Unconstrained tree, partitioned by codon position	Monophyly of <i>O. mimus</i> enforced, partitioned by codon position		-9772.63	-9775.29	-5.32 (positive evidence against M1)
Unconstrained tree, partitioned by codon position	Monophyly of <i>O. vulgaris</i> and of <i>O. mimus</i> enforced, partitioned by codon position		-9772.63	-9774.92	-4.58 (positive evidence against M1)

* Kass and Raftery (1995)

that of Iquique published by Söller *et al.* (2000). Two haplotypes were found among the three *O. tehuelchus* sequenced, differing in two nucleotidic positions.

The value of the Bayes factor comparing the unconstrained, non-partitioned tree (M_0) versus the unconstrained, codon-partitioned tree (M_1) indicated

very strong evidence against the null model (Table 2). Therefore, we present the topology obtained by considering different evolutionary models for each codon. Subsequent Bayesian analyses (that is, the testing of alternative topologies) were performed considering the codon-partitioned data set.

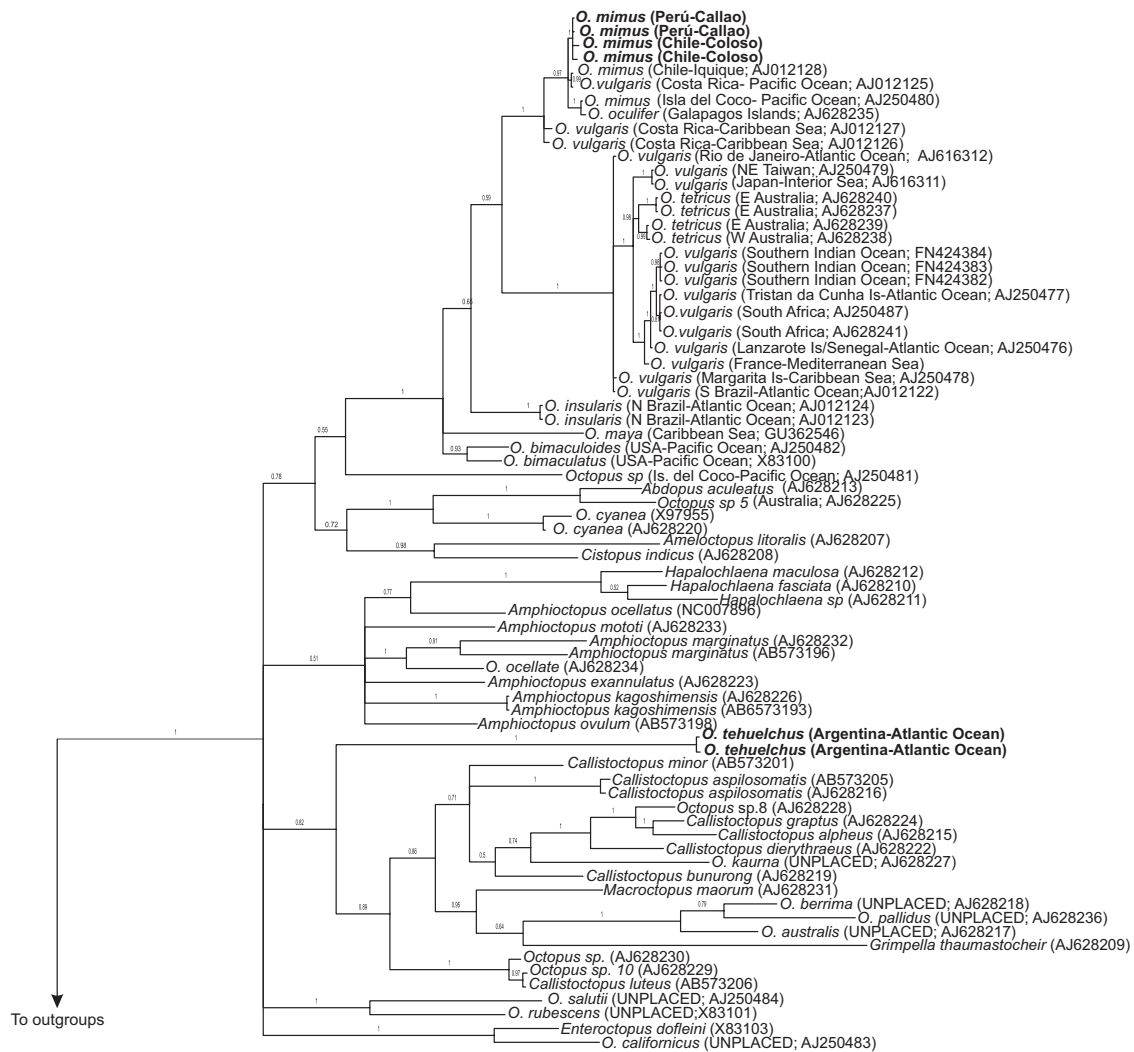


FIG. 1. – Phylogenetic relationships among species of the genus *Octopus* and other genera of the subfamily Octopodinae, estimated using COIII partial sequences and Bayesian inference, considering separate evolutionary models for each codon position. *Loligo* species (*L. vulgaris*, *L. forbesi* and *L. reynaudii*) were used as outgroups (not shown). Numbers on branches are the Bayesian posterior probabilities of the node to the right. Newly sequenced individuals are shown in bold.

O. tehuelchus appears more closely related to the genera *Callistoctopus*, *Grimpella* and *Macroctopus*, as well as to “unplaced” *Octopus* species sensu Norman and Hochberg (2005), than to the clade containing most of the valid species of *Octopus* (Fig. 1). Our *O. mimus* specimens from Chile and Peru form a well supported clade with *O. mimus* from other localities: those from Isla del Coco (Costa Rica) and Iquique (Chile). However, this clade also includes an *O. vulgaris* specimen from the Pacific coast of Costa Rica and *O. oculifer* from the Galapagos Islands with a BPP of 0.97. The sister group of these “Pacific *O. mimus*” are the two *O. vulgaris* from the Caribbean Sea, also with high support. All remaining *O. vulgaris* from various geographic origins cluster in a group that also includes *O. tetricus* specimens from Australia (BPP=1). The specimens named *O. vulgaris* from Recife (northern Brazil) by Söller *et al.* (2000) and Warnke *et al.* (2004) and identified as a new species, *O. insularis*, by Leite *et al.* (2008) were placed as basal to this *O. vulgaris/O. tetricus/O. mimus* clade.

The results of the analyses conducted to evaluate alternative hypotheses of monophyly of *O. vulgaris* and *O. mimus* are summarized in Table 2. Bayes factors show strong evidence against the models in which the monophyly of *O. vulgaris* is enforced, and positive evidence against the monophyly of *O. mimus* and of *O. vulgaris* plus *O. mimus*.

DISCUSSION

In the last few years, studies on the molecular systematics of octopuses have helped to clarify their confusing systematics and phylogenetic relationships and also to identify and describe new species (Norman and Hochberg 2005, Allcock *et al.* 2007, Leite *et al.* 2008, Strugnell *et al.* 2009). In the present study, we focused on the genus *Octopus* using a Bayesian approach to estimate the phylogenetic relationships among published sequences of the COIII gene of *Octopus* species, of *O. tehuelchus* from the southwestern Atlantic Ocean (a species never included in phylogenetic analyses of the genus before), and of individuals of *O. mimus* from newly sampled localities in the Pacific Ocean (Chile and Peru).

The phylogenetic trees showed two major groupings: one included representatives of the genera *Hapalochlaena*, *Grimpella*, *Amphioctopus* and *Callistoctopus* and the “unplaced” *Octopus* species and the other included valid species of *Octopus*.

Species of the genus *Callistoctopus* and *O. karna* formed a well-supported clade, while the “unplaced” *O. pallidus*, *O. berrima* and *O. australis* were more closely related to the genera *Grimpella* and *Macroctopus* than to the valid species of *Octopus*. Specimens of *O. tehuelchus* (a valid species of *Octopus* according to Norman and Hochberg 2005) were included in this major cluster, as the sister group to *Grimpella* and *Callistoctopus* with high support. Therefore, the generic placement of this species may need a revision. The second main cluster

included the majority of the valid species of *Octopus* sensu Norman and Hochberg (2005). Guzik *et al.* (2005) estimated the phylogenetic relationships among species of the genus *Octopus* using one nuclear and two mitochondrial genes, and demonstrated the polyphyly of the genus. However, several species included in the work of Guzik *et al.* were soon afterwards reassigned to other genera by Norman and Hochberg (2005). Nevertheless, the genus still appears to be polyphyletic because species of *Amelooctopus*, *Abdopus* and *Cistopus* are included in our phylogenetic tree within the *Octopus* cluster, although with medium support (BPP=0.78).

O. vulgaris specimens are included in two different, well-supported clades, with *O. insularis* as their sister group. These specimens were considered by Söller *et al.* (2000) and Warnke *et al.* (2004) as part of the *O. vulgaris* species complex, but Leite *et al.* (2008) described them morphologically, established their molecular distinctiveness and phylogenetic position using the 16S rRNA gene, and assigned them the name *O. insularis*. In the papers by Söller *et al.* (2000) these specimens group with other *O. vulgaris* forming a monophyletic clade, but they do not include other species of *Octopus*. In the present work, the position of these specimens in the phylogenetic tree, using COIII and including more species of the subfamily Octopodinae, remains the same as in Leite *et al.* (2008).

The topology of the tree obtained in this study using Bayesian Analysis was very similar to that of Guerra *et al.* (2010), in which specimens of *O. vulgaris* fall within two well-supported groups. One includes the *O. vulgaris* s. str. and the other *O. mimus*, *O. oculifer* from the Galapagos Islands and *O. vulgaris* from Costa Rica (all eastern Pacific localities), with *O. vulgaris* from the Costa Rican Caribbean Sea in a basal position. Bayes factors showed that there is positive support for the topology in Figure 1 against that obtained by enforcing *O. mimus* monophyly, so there is a high probability that the *O. vulgaris* from Costa Rica and the *O. oculifer* specimens are in fact misidentified *O. mimus*. Regarding the Caribbean *O. vulgaris*, Jassoud (2010) sequenced the cytochrome oxidase subunit I gene of 92 *O. vulgaris* specimens from the Caribbean Sea (Curaçao, Dominica, Guadeloupe and Puerto Rico). He found 11% of sequence divergence between these specimens and *O. vulgaris* s. str. and suggest that they represent a distinct genetic lineage within the *O. vulgaris* species complex. In this study, Caribbean *O. vulgaris* appear as more related, and basal to, the *O. mimus* clade than to other *O. vulgaris*, a result very similar to that obtained by Söller *et al.* (2000), with the exception that they did not include *O. oculifer* in their study. Bayes factors showed that there is a strong support for the topology in Figure 1 against that obtained by enforcing *O. vulgaris* monophyly. Taken together, these results argue in favour of the presence of a cryptic species of *Octopus* in the Caribbean Sea different from *O. vulgaris*, *O. insularis* and *O. maya* (Fig. 1), and basal to

O. mimus. As suggested by Söller *et al.* (2000), both “Caribbean *O. vulgaris*” and *O. mimus* could have originated from an ancestral species whose populations were separated by the rise of the Isthmus of Panama about 3 million years ago.

To resolve the incompletely described and wide geographic range of *O. vulgaris*, Warnke *et al.* (2004) estimated phylogenetic relationships among *O. vulgaris* from several localities, including South Africa, Tristan da Cunha (South Atlantic), West Africa, Japan, southern Brazil and the Caribbean, and compared the sequences of the COIII and 16S genes with those of *O. mimus* specimens from Chile and the Pacific coast of Costa Rica. They confirmed a previous result by Söller *et al.* (2000) that *O. mimus* and *O. vulgaris* are separate lineages, and concluded that the monophyly of *O. vulgaris* was supported. However, they included a very limited number of other *Octopus* species. Guerra *et al.* (2010) estimated phylogenetic relationships among *O. vulgaris* specimens from worldwide locations and several other species of *Octopus*. These authors found that *O. vulgaris* from the Mediterranean Sea, eastern Atlantic, Pacific and Indian Oceans (*O. vulgaris* s. str.) clustered together with *O. tetricus* from Western Australia (a species not included in Warnke *et al.* 2004) with high bootstrap support, and proposed considering them as a monophyletic *O. vulgaris* species group. Bayes factors showed that there is strong support for the topology in Figure 1 against that obtained by enforcing *O. vulgaris* monophyly (that is, excluding *O. mimus*, *O. tetricus* and *O. oculifer* from the clade, and including the Costa Rican specimens), giving statistical support to the monophyly of an *O. vulgaris* s. str. + *O. tetricus* group *sensu* Guerra *et al.* (2010).

Our results emphasize the need for a revision of the generic status of *O. tehuetchus*, and more detailed population-level and/or phylogeographic studies in the *O. mimus* and *O. vulgaris* groups, in order to elucidate the number of species present, their limits and geographic ranges, and their phylogenetic relationships. This information will be useful for sustainable management of these important fisheries resources.

ACKNOWLEDGEMENTS

We are very grateful to the teams of the Unidad de Investigaciones de Invertebrados Marinos, Instituto del Mar (IMARPE) of Peru and of the Instituto de Investigaciones Oceánicas (Universidad de Antofagasta) of Chile for their assistance in field work, and to Lorena Storero for providing the *O. tehuetchus* samples. We also thank Juan José Martínez for his advice in data analysis. Financial and logistic support was provided by the Alfred Wegener Institute (Germany), and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina).

This work was carried out in the context of the PhD thesis of M.S. A.-J. at the Universidad Nacional de Córdoba, Argentina.

REFERENCES

- Akasaki T., Nikaido M., Tsuchiya K., Segawa S., Hasegawa M., Okada N. 2006. Extensive mitochondrial gene arrangements in coleoid Cephalopoda and their phylogenetic implications. *Mol. Phylogenet. Evol.* 38: 648-658.
- Allcock A., Strugnell J., Prodöhl P., Piatkowski U., Vecchione M. 2007. A new species of *Pareledone* (Cephalopoda: Octopodidae) from Antarctic Peninsula Waters. *Polar Biol.* 30: 883-893.
- Barriga Sosa I.A., Beckenbach K., Hartwick B., Smith M.J. 1995. The molecular phylogeny of five eastern north Pacific *Octopus* species. *Mol. Phylogenet. Evol.* 4: 163-174.
- Bonnaud L., Boucher-Rodoni R., Monnerot M. 1997. Phylogeny of cephalopods inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 7 (1): 44-54.
- Cardoso F., Villegas P., Estrella C. 2004. Observaciones sobre la biología de *Octopus mimus* (Cephalopoda: Octopoda) en la costa peruana. *Rev. Peru. Biol.* 11: 45-50.
- Carlini D.B., Young R.E., Vecchione M. 2001. A Molecular Phylogeny of the Octopoda (Mollusca: Cephalopoda) Evaluated in Light of Morphological Evidence. *Mol. Phylogenet. Evol.* 21 (3): 388-397.
- Edgar R.C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *Bioinformatics* 5: 113-131. <http://www.drive5.com/muscle>.
- Guerra A., Cortez T., Rocha F. 1999. Redescrpción del pulpo de los changos, *Octopus mimus* Gould, 1835, del litoral chileno-peruano (Mollusca, Cephalopoda). *Iberus* 17(2): 37-57.
- Guerra A., Roura A., González A.F., Pascual S., Chel Y., Pérez-Losada M. 2010. Morphological and genetic evidence that *Octopus vulgaris* (Cuvier, 1797) inhabits Amsterdam and Saint Paul Islands (southern Indian Ocean). *ICES J. Mar. Sci.* 67: 1401-1407.
- Guzik M.T., Norman M.D., Crozier R.H. 2005. Molecular phylogeny of the benthic shallow-water octopuses (Cephalopoda: Octopodinae). *Mol. Phylogenet. Evol.* 37:235-248.
- Hochberg F.G., Nixon M., Toll R.B. 1992. Order Octopoda Leach, 1818. In: Sweeney M.J., Roper C.F.E., Mangold K.M., Clarke M.R., Boletzky S.V. (eds), “*Larval and Juvenile Cephalopods: A Manual for Their Identification*”. Smithsonian Contributions to Zoology 513, Smithsonian Institution Press, Washington, DC, pp. 213-280.
- Iribarne O.O. 2009. Life history and distribution of the small southwestern Atlantic octopus, *Octopus tehuetchus* (d’Orbigny). *J. Zool.* 223: 459-565.
- Jassoud, A.F.J. 2010. Genetic differentiation of eastern and western Atlantic *Octopus vulgaris*. MSc. thesis, University of Puerto Rico, 34 pp.
- Kass R., Raftery A. 1995. Bayes factors and model uncertainty. *JASA* 90: 773-795.
- Leite T.S., Haimovici M., Molina W., Warnke K. 2008. Morphological and genetic description of *Octopus insularis*, a new cryptic species in the *Octopus vulgaris* complex (Cephalopoda: Octopodidae) from the tropical Southwestern Atlantic. *J. Molluscan Stud.* 74: 63-74.
- Maniatis T., Fritsch E.F., Sambrook J. 1982. *Molecular cloning. A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 545 pp.
- McCarthy C. 1998. Chromas ver. 1.45. School of Health Science, Griffith University, Queensland, Australia. <http://www.technlysium.com.au/chromas.html>
- Newton M.A., Raftery A.E. 1994. Approximate Bayesian inference by the weighted likelihood bootstrap (with discussion). *J. R. Statist. Soc. B* 56: 3-48.
- Norman M.D., Hochberg F.G. 2005. The current state of *Octopus* taxonomy. *Phuket Mar. Biol. Cent. Res. Bull.* 66: 127-154.
- Nylander J.A. 2004. MrModeltest. Program distributed by the author. *Evolutionary Biology Centre*, Uppsala University.
- Nylander J.A., Ronquist F., Huelsenbeck J.P., Nieves-Aldrey J.L. 2004. Bayesian phylogenetic analysis of combined data. *Syst. Biol.* 53: 47-67.
- Ó Foighil D.O., Smith M.J. 1995. Evolution of asexuality in the cosmopolitan marine *Lasaea*. *Evolution* 49: 140-150.
- Robson G.C. 1929. *A monograph of the Recent Cephalopoda. Part I, Octopodinae*. Order of the Trustees of the British Museum, London, pp. 236.

- Rocha F., Vega M.A. 2003. Overview of cephalopod fisheries in Chilean waters. *Fish. Res.* 60: 151-159.
- Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- Roper C.F.E., Hochberg F.G. 1988. Behavior and systematics of cephalopods from Lizard Island, Australia, based on color and body patterns. *Malacologia* 29: 153-194.
- Söller R., Warnke K., Saint-Paul U., Blohm D. 2000. Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* (Cephalopoda: Octopodidae). *Mar. Biol.* 136: 29-35.
- Strugnell J., Norman M., Jackson J., Drummond A.J., Cooper A. 2005. Molecular phylogeny of coleoid cephalopods (Mollusca: Cephalopoda) using a multigene approach; the effect of data partitioning on resolving phylogenies in a Bayesian framework. *Mol. Phylogenet. Evol.* 37: 426-441.
- Strugnell J., Voight J.R., Collins P.C., Allcock A.L. 2009. Molecular phylogenetic analysis of a known and a new hydrothermal vent octopod: their relationships with the genus *Benthoctopus* (Cephalopoda: Octopodidae). *Zootaxa* 2096: 442-459.
- Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lec. Math. Life Sci.* 17: 57-86.
- Teske P.R., Oosthuizen A., Papadopoulos I., Baker N.P. 2007. Phylogeographic structure of *Octopus vulgaris* in South Africa revisited: identification of a second lineage near Durban harbour. *Mar. Biol.* 151: 2119-2122.
- Voight J.R. 1994. Morphological variation in shallow-water octopuses (Mollusca, Cephalopoda). *J. Zool.* 232: 491-504.
- Warnke K., Söller R., Blohm D., Saint-Paul U. 2004. A new look at geographic and phylogenetic relationships within the species group surrounding *Octopus vulgaris* (Mollusca, Cephalopoda): indications of very wide distribution from mitochondrial DNA sequences. *J. Zool. Syst. Evol. Res.* 42: 306-312.

Scient. ed.: A. Guerra.

Received February 22, 2011. October 24, 2011.

Published online February 3, 2012.