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

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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 otros especímenes 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 y como estos especímenes podrían representar especímenes mal clasificados. Los especímenes de *O. vulgaris* del Mar Caribe de Costa Rica podrían constituir una especie criptica 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-1a) 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. minus* 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. minus* (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. minus*, 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. minus* 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. minus* 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
| Species                                      | Accession number | Sampling region                              | Sample size | Sample size |
|----------------------------------------------|------------------|----------------------------------------------|-------------|-------------|
| Abdopus aculeatus (D’Orbigny, 1834)          |                  | Orphus Island, Queensland, Australia         | 1           |             |
| Ameloctopus litoralis Norman, 1992            |                  | Dudley Point, Northern Territory, Australia   | 1           |             |
| Amphioctopus examinatus (Norman, 1993)        |                  | Lizard Island, Queensland, Australia         | 1           |             |
| Amphioctopus kagoshimensis (Ortmann, 1888)   |                  | One tree Island, Queensland, Australia       | 1           |             |
| Amphioctopus marginatus (Taki, 1964)         |                  | Nha Trang, Vietnam                           | 1           |             |
| Amphioctopus motoi (Norman, 1993)            |                  | Dapoli, India                                | 1           |             |
| Amphioctopus ocellatus (Gray, 1849)          |                  | Tokyo, Japan                                 | 1           |             |
| Amphioctopus ovulatus (Sasaki, 1917)         |                  | East China Sea, Japan                        | 1           |             |
| Callistoctopus alpheus (Norman, 1993)        |                  | One Tree Island, Queensland, Australia       | 1           |             |
| Callistoctopus asynchronous (Norman, 1993)   |                  | One Tree Island, Queensland, Australia       | 1           |             |
| Callistoctopus bunurong (Stranks, 1990)      |                  | St. Leonards Pier, Victoria, Australia       | 1           |             |
| Callistoctopus dierythraeus (Norman, 1993)   |                  | Magnetic Island, Queensland, Australia       | 1           |             |
| Callistoctopus gratipes (Norman, 1993)       |                  | Townsville, Queensland, Australia            | 1           |             |
| Callistoctopus minor Sasaki, 1920 - UNPLACED |                  | East China Sea, Japan                        | 1           |             |
| Callistoctopus luteus (Sasaki, 1929)         |                  | Kanagawa, Miura, Japan                       | 1           |             |
| Cistopus indicus (Rapp, 1835)                |                  | Taichung Fish Market, Taiwan                 | 1           |             |
| Enteroctopus dolfini (Walker, 1910)          |                  | British Columbia, Canada, Pacific Ocean      | 1           |             |
| Grimpella thasmostocheir Robson, 1928        |                  | Pt. Victoria Jetty, South Australia          | 1           |             |
| Hapalochlaena fasciata (Hoyle, 1886)         |                  | Moreton Bay, Queensland, Australia           | 1           |             |
| Hapalochlaena maculosa (Hoyle, 1883)         |                  | St. Leonards Pier, Victoria, Australia       | 1           |             |
| Hapalochlaena sp. Norman, 2000               |                  | Darwin, Australia                             | 1           |             |
| Macroctopus maorum Hartman, 1980             |                  | Portsea Pier, Victoria, Australia             | 1           |             |
| Octopus australis Hoyle, 1885 - UNPLACED     |                  | Port Stephens, New South Wales, Australia     | 1           |             |
| Octopus bernina Stranks and Norman, 1993 -   |                  | Edithburg Jetty, South Australia             | 1           |             |
| Octopus bimaculatus Ver ill, 1883            |                  | California, USA, Pacific Ocean               | 1           |             |
| Octopus bimaculoides Pickford and McConnaughey, 1949 |        | Santa Barbara, CA, USA, Pacific Ocean        | 1           |             |
| Octopus californicus Berry, 1911 - UNPLACED  |                  | Ellwood, Santa Barbara Coast, CA, USA, Pacific Ocean | 1           |             |
| Octopus cyanoe Gray, 1849                    |                  | Queensland, Australia                        | 2           |             |
| Octopus insularis Leite and Haimovici, 2008  |                  | Recife, Brazil, Atlantic Ocean               | 2           |             |
| Octopus kouma Stranks, 1990 - UNPLACED       |                  | Port Phillip Bay, Victoria, Australia         | 1           |             |
| Octopus maya Voss and Solis, 1966            |                  | Yucatan Peninsula, Mexico                    | 1           |             |
| Octopus mimus Gould, 1852                    |                  | Iquique, North Chile, Pacific Ocean          | 1           |             |
| Octopus marmoratus Berry, 1953 - UNPLACED    |                  | Coloso, North Chile, Pacific Ocean           | 2           |             |
| Octopus pallidus Hoyle, 1885 - UNPLACED      |                  | San Matias Gulf, Argentina, Southwestern     | 2           |             |
| Octopus salutii Verany, 1839 - UNPLACED      |                  | British Columbia, Canada, Pacific Ocean      | 1           |             |
| Octopus tehuechus D’Orbigny, 1834            |                  | Lanzarote Is/Senegal, West Africa, Atlantic Ocean | 1           |             |
| Octopus tetricus Gould, 1852                 |                  | Wallaga Lakes, New South Wales, Australia     | 1           |             |
| Octopus vulgaris Cuvier, 1797                |                  | Port Stephens, New South Wales, Australia     | 1           |             |
|                                          |                  | Lucky Bay, Western Australia                | 1           |             |
|                                          |                  | Fremantle Warf, Western Australia            | 1           |             |
|                                          |                  | Banyuls, France, Mediterranean Sea           | 1           |             |
|                                          |                  | Lanzarote Is/Senegal, West Africa, Atlantic Ocean | 1           |             |
|                                          |                  | Knysna Lagoon, South Africa, Indian Ocean    | 1           |             |
|                                          |                  | Tristan da Cunha, Atlantic Ocean             | 1           |             |
|                                          |                  | Itajai, South Brazil, Atlantic Ocean         | 1           |             |
|                                          |                  | Rio de Janeiro, Brazil, Atlantic Ocean       | 1           |             |
|                                          |                  | Isla Margarita, Venezuela, Caribbean Sea     | 1           |             |
|                                          |                  | Sieto Island Sea, Japan                      | 1           |             |
|                                          |                  | Port Elizabeth, South Africa                 | 1           |             |
|                                          |                  | Costa Rica, Pacific Ocean                    | 2           |             |
|                                          |                  | Costa Rica, Caribbean Sea                    | 2           |             |

Table 1. – Specimens, sampling regions, sample sizes and accession numbers of DNA sequences included in the phylogenetic inferences with COII. 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.
From Barriga Sosa Data analysis Inc. (Rockville, USA). purified and sequenced at the facilities of Macrogen cooling at 15°C were performed. PCR products were 1 min; a post-treatment of 7 min at 72°C and a final at 95°C, 30 cycles of denaturation at 94°C for 30 s, to the final volume. dTTP), 3

mented in MrBayes v3.1.2 (Ronquist and Huelsenbeck were assessed using the Bayesian approach imple-

bers are cited in Table 1. Phylogenetic relationships sequences were submitted to GenBank; accession num-

gram CHROMAS version 2.23 (McCarthy 1998) and

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 under- lined 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 COII gene was amplified with the primers 5´-CATTTAGTT-GATCCTAGGCCTTGACC-3´ and 5´

ing Bayes factors (Nylander 2004), comparing the 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 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 (B10) 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 2loge B10 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

| Species | Sampling region | Sample size | Accession number |
|---------|-----------------|-------------|-----------------|
| Octopus vulgaris Cuvier, 1797 | Southern Indian Ocean | 3 | FN424384* |
| Octopus sp. | Port Elizabeth, South Africa | 1 | A628230* |
| Octopus sp. | Isla del Coco, Costa Rica, Pacific Ocean | 1 | A6250481* |
| Octopus sp. 5 Norman, 2000 | Coconut Wells, Western Australia | 1 | A628225* |
| Octopus sp. 8 Norman, 2000 | Rockingham, Western Australia | 1 | A628228* |
| Octopus sp. 10 Norman, 2000 | Exmouth Gulf, Western Australia | 1 | A628229* |
| Loligo vulgaris Lamarck, 1789 | | 1 | X97962* |
| Loligo forbesi Steenstrup, 1856 | | 1 | X97962* |
| Loligo reynaudii (D’Orbigny, 1839-1841) | | 1 | X97960* |

* From Barriga Sosa et al. 1995; † From Bonnaud et al. 1997; ‡ From Stöller et al. 2000; § From Wernke et al. 2004; ¶ From Guzik et al. 2005; ‖ From Akasai et al. 2006; ′ From Guerra et al. 2010; This work; ‰ Unpublished.

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| Loligo forbesi Steenstrup, 1856 | | 1 | X97962* |
| Loligo reynaudii (D’Orbigny, 1839-1841) | | 1 | X97960* |

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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 (2log b). Mα.

| Models compared | Harmonic mean of ln L | Bayes factor (interpretation) |
|-----------------|-----------------------|-------------------------------|
| M0 | M1 | M0 | M1 |
| Unconstrained, non-partitioned tree | Unconstrained tree, partitioned by codon position | -9935.11 | -9772.63 | (very strong evidence against M0) | 324.96 |
| Unconstrained tree, partitioned by codon position | Monophyly of O. vulgaris, enforced, partitioned by codon position | -9772.63 | -9775.93 | (very strong evidence against M0) | 6.6 |
| Unconstrained tree, partitioned by codon position | Monophyly of O. vulgaris, enforced, partitioned by codon position | -9772.63 | -9775.93 | (very strong evidence against M0) | 6.6 |
| Unconstrained tree, partitioned by codon position | Monophyly of O. vulgaris and of O. minus, enforced, partitioned by codon position | -9772.63 | -9774.92 | (very strong evidence against M0) | 4.58 |

* Kass and Raftery (1995)

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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.

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 (M0) versus the unconstrained, codon-partitioned tree (M1) indicated
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 Hapalochaena, Grimpella, Amphioctopus and Callistoctopus and the “unplaced” Octopus species and the other included valid species of Octopus.

Species of the genus Callistoctopus and O. kaurna 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 Ameloctopus, 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. minus. As suggested by Söller et al. (2000), both “Caribbean O. vulgaris” and O. minus 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 COII and 16S genes with those of O. minus specimens from Chile and the Pacific coast of Costa Rica. They confirmed a previous result by Söller et al. (2000) that O. minus 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. minus, O. tetricus and O. ocuifer 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. tehuelchus, and more detailed population-level and/or phylogeographic studies in the O. minus 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.

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