Genetic analysis of the *Octopus vulgaris* population on the coast of South Africa

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This study on *Octopus vulgaris* focused on the COIII gene region of mitochondrial DNA. Sequences from 21 samples from the Eastern Cape, and 14 samples from the Western Cape, were compared to determine whether different populations exist along the South African coast. A 380-bp segment of the COIII region of mtDNA was amplified using the polymerase chain reaction with specific designed primers. Phylogenetic inference was made using maximum parsimony (MP), maximum likelihood (ML), and distance-based methods. All sequences conformed to a single haplotype. Lack of variation within and between east and west coast samples precluded further population genetic analysis. The sequence obtained in this study was also compared with other sequences lodged in the Genbank database. Phylogenetically, the South African *O. vulgaris* is closely related to *O. vulgaris* from Senegal (0.67% divergence) and the Mediterranean (1.51% divergence). Within the Mediterranean group, *O. vulgaris* from South Africa displayed less sequence divergence from Senegalese and Mediterranean individuals than *O. vulgaris* from Venezuela (3.85%) and Taiwan (3.87%). These data do not, therefore, refute the hypothesis of a single *O. vulgaris* genetic population around the coast.

Introduction

Octopus vulgaris has been identified as a marine resource for a possible new commercial fishery in South Africa.[1,2] The use of genetic methods in fishery population studies is an important facet of both fisheries stock assessment and management.[3] Failure to detect stock structures within a population can lead to biological changes, influence productivity rates and cause loss of genetic diversity within a species.[4–6] This in turn can lead to ineffective fisheries management as the underlying functioning of the population is not understood and the response of the stock to management measures cannot be predicted.[3] The aims of this study were to investigate the genetic variation within South African *O. vulgaris* in the mitochondrial DNA (mtDNA) COIII region, to determine population structure and, also, to derive the phylogenetic relatedness of South African and Mediterranean *O. vulgaris.*

Recently, there have been significant advances made in the field of genetic stock identification.[3] Genetic markers have increasingly been used in the identification of finfish stocks.[7–12] By comparison, the use of genetic methods in cephalopod population studies has been modest[13–16] and genetic studies on octopus specifically have focused mainly on phylogeny.[17–20] As one of the most intensively studied cephalopod species,[21] *O. vulgaris* is still undergoing taxonomic revision. Long considered to be globally distributed,[22] it has now been restricted to the Mediterranean and Eastern Atlantic through morphological description.[21,23] However, species previously classified as *O. vulgaris* have recently been separated from the *O. vulgaris* complex and described as a separate species through genetic studies[19,20] to support the morphological taxonomic revision.

The molecular analyses of octopus phylogeny have focused on allozymes, and mtDNA (gene coding regions: COI, COII, COIII, 16s rRNA, etc.). Studies on octopus, and *O. vulgaris* in particular, have focused on the COIII gene region of mtDNA,[17–19,24] with DNA sequences available from individuals from several regions including South Africa. Given that the taxonomic classification of the South African species is uncertain,[25] it is essential to determine the phylogenetic status as well as population structure of the species, because of the proposed fishery for *O. vulgaris.*[1,2,38]

Materials and methods

Sample collection

Octopus vulgaris was collected intertidally and by scuba diving from various sites around the South African coast (Fig. 1). Approximately 3–5 cm of arm tip was removed from each specimen, after which the octopus was released. The samples were preserved in 95% ethanol. No morphological differences were observed between specimens, therefore no voucher specimens were collected.

DNA extraction, PCR and sequencing

The total genomic DNA was extracted using a QIAGEN DNeasy® tissue kit; after addition of the ATL buffer and proteinase K, the tissue was incubated overnight at 55°C before the protocol was completed. The elute was tested on agarose gel to determine DNA yield.

Initial primers were designed (R. Kirby, Rhodes University) to amplify 700 base pairs (bp) of the COIII region; however, no products were amplified. A second set of primers was then designed (M. Jivaji, Rhodes University) to match conserved regions within the COIII sequences available in Genbank for *O. vulgaris.* A 380-bp segment of the COIII region of mitochondrial DNA was amplified using the polymerase chain reaction (PCR) with these specific designed primers: mtDNA forward 5´-ACCATAATTCAATGTGACGTGATATT-5´ and mtDNA reverse 5´-AAATAGAAAATGATGCTTCTATATATTCTAA-3´ supplied by Inqaba Biotech (Pretoria). The PCR reactions were set up in 25 μl reaction volume, containing: 2.5 μl 10 mM NH_4 buffer, 2.5 μl 50 mM $MgCl_2$, 2.5 μl BSA, 2.0 μl 100 mM dNTPs, 1 μl primer (3.2 pmol), 0.4 μl *Taq* (BIOTAQ™ DNA Polymerase) and 1–5 μl DNA. Thermal cycling was hot-started (96°C for 5 min, held at 85°C while *Taq* was added), followed by five cycles of 93°C (50 s), 45°C (50 s) and 72°C (1 min), followed by 28 cycles of 93°C (50 s), 50°C (30 s) and 72°C (1 min). The last step of 72°C was prolonged for 5 min.[19] The amplified product was cut out of agarose gel and purified using a Promega Wizard® SV GEL and PCR clean-up

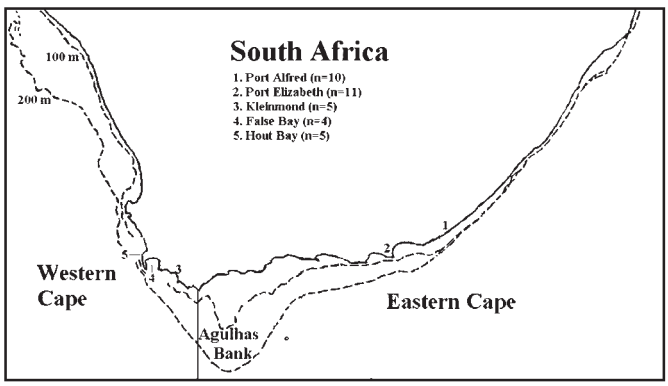

Fig. 1. Collection sites of *Octopus vulgaris* around the South African coast. The number of samples collected per site is indicated in brackets.

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system. DNA was sequenced in one direction (forward) using BigDye® Terminator v. 3.1 Cycle Sequencing kit and run on an ABI 3100 genetic analyser (PE AppliedBiosystems).

Data analysis

Sequences from 21 samples from the Eastern Cape and 14 samples from the Western Cape were compared to determine whether different populations exist along the South African coast. Sequences were aligned using CLUSTAL W in BioEdit,[26] with further modifications by eye. Analyses were carried out with both 595-bp region (published sequences only) and the 380-bp region (published plus sequence from this study).

Phylogenetic inference was made using maximum parsimony (MP), maximum likelihood (ML) and distance-based methods in PAUP* 4.0b10[27] and MEGA version 2.1.[28] Distance analysis was performed using Neighbor Joining (NJ), assuming a Kimura 2-parameter and HKY85 model[29] of sequence evolution. Analysis for MP was a heuristic search algorithm, with tree-bisection, branch swapping and random sequence addition. Ten additional replicates were analysed for distance and parsimony searches. Analysis for ML was similar with heuristic searches, tree-bisection, branch swapping and stepwise additions. Confidence was assessed by 1000 bootstrap resamplings for distance and parsimony with 100 iterations for the maximum likelihood approach. *Loligo vulgaris reynaudii* (EMBL/Genbank accession number: X97960) was used as an out-group to root all trees.

Results

Sequence comparisons

No sequence variation was observed between individuals from the same region or between individuals from the Eastern (n = 21) and Western Cape (n = 14) regions. All sequences conformed to a single haplotype. Lack of variation within and between east and west coast samples precluded further population genetic analysis.

The sequence obtained in this study was an exact match of a previous *O. vulgaris* COIII sequence from False Bay, South Africa, and from Tristan da Cunha lodged by Warnke[30] in Genbank (accession numbers SA: AJ250487; TdC: AJ250477). The South African COIII sequence from the present study was compared with a range of *O. vulgaris* and other octopus species COIII sequences lodged in the EMBL/Genbank database (Table 1). The 380-bp COIII region of *O. vulgaris* sequenced in this study is illustrated in Fig. 2.

Phylogenetic trees

All three tree construction methods revealed a similar topology for both the 380-bp and 595-bp regions (Figs 3 and 4, 380 bp region shown only). Results from the MP and NJ tree topologies are

```
O. v. 5 (Mediterranean)          1   TCGAGAAAGTACATTTCAAGGTTTCCACACATCCAAAGTA 40
O. v. 1 (S.A. this study)        1   ........................................ 40
O. v. 2 (South Africa)           1   ........................................ 40
O. v. 3 (Tristan da Cunha)       1   ........................................ 40
O. v. 4 (Senegal)                1   ........................................ 40
O. v. 6 (Taiwan)                 1   .....................C..........T...... 40
O. v. 7 (Venezuela)              1   C.....................T..T.......... 40
O. v. 8 (North Brazil)           1   C........A.............T..T.....T.....C 40
O. v. 9 (North Brazil)           1   C........A.............T..T.....T.....C 40
O. v. 10 (Costa Rica, Caribbean  1   C........A.............T..T.....T..G..T 40
O. v. 11 (Costa Rica, Caribbean  1   C........A.............T..T.....T..G..T 40
O. v. 12 (Costa Rica, Pacific)   1   C........A.............T..T.....T..G..T 40
O. m. 1 (Costa Rica, Pacific)    1   C........A.............T........T..G..T 40
O. m. 2 (North Chile)            1   C........A.............T..T.....T..G..T 40
O. o. (California, USA)          1   .........A.............T..T.....T...... 40
O. a. (California, USA)          1   .........A...........C..T..T............ 40
O.d.                             1   .........A.....C.....A..T.....TA.T.....T 40
Loligo (X97960)                  1   C........A...........C.A...T...CTA.....C 40

O. v. 5 (Mediterranean)          41  TATAATGGCCTTCGATGAGGTATAATACTTTTTATTATTT 80
O. v. 1 (S.A. this study)        41  .....................................C. 80
O. v. 2 (South Africa)           41  .....................................C. 80
O. v. 3 (Tristan da Cunha)       41  .....................................C. 80
O. v. 4 (Senegal)                41  ........................................ 80
O. v. 6 (Taiwan)                 41  ............................C.......... 80
O. v. 7 (Venezuela)              41  ........T.....................C.......... 80
O. v. 8 (North Brazil)           41  .....C..AT.A..................C.......... 80
O. v. 9 (North Brazil)           41  .....C..AT.A..................C.......... 80
O. v. 10 (Costa Rica, Caribbean  41  ........GT.A.......................... 80
O. v. 11 (Costa Rica, Caribbean  41  ........GT.A.......................... 80
O. v. 12 (Costa Rica, Pacific)   41  ........GT.A.......................... 80
O. m. 1 (Costa Rica, Pacific)    41  ........GT.A.......................... 80
O. m. 2 (North Chile)            41  ........GT.A.......................... 80
O. o. (California, USA)          41  ........AT.A.....................C..... 80
O. a. (California, USA)          41  .....C..AT.A.......................... 80
O.d.                             41  ........GT.A.................A......G.A. 80
Loligo (X97960)                  41  .CGTTA..TA.A...AT...G...G.TT.A.....C.CC. 80

O. v. 5 (Mediterranean)          81  CAGAAGTATGTTTCTTTTTTGCTTTCTTTTGAGCCTATTT 120
O. v. 1 (S.A. this study)        81  ................................T..... 120
O. v. 2 (South Africa)           81  ................................T..... 120
O. v. 3 (Tristan da Cunha)       81  ................................T..... 120
O. v. 4 (Senegal)                81  ................................T..... 120
O. v. 6 (Taiwan)                 81  ........................................ 120
O. v. 7 (Venezuela)              81  ........................................ 120
O. v. 8 (North Brazil)           81  .......T.....T..........T........T..... 120
O. v. 9 (North Brazil)           81  .......T.....T..........T........T..... 120
O. v. 10 (Costa Rica, Caribbean  81  ............T.....................T..... 120
O. v. 11 (Costa Rica, Caribbean  81  ............T.....................T..... 120
O. v. 12 (Costa Rica, Pacific)   81  ............T.....G.....T........T..... 120
O. m. 1 (Costa Rica, Pacific)    81  ............T..........T........T..... 120
O. m. 2 (North Chile)            81  ............T.....G.....T........T..... 120
O. o. (California, USA)          81  ............T..C........T........A..... 120
O. a. (California, USA)          81  ............T..........T........G..... 120
O.d.                             81  .....A.T.....T..C.....C..T........T..C.. 120
Loligo (X97960)                  81  .C..........T...G.......T........T..... 120

O. v. 5 (Mediterranean)          121 CCATAGTAGTTTAGCTCCTAATATAGATATTGGATCATGT 160
O. v. 1 (S.A. this study)        121 T..C................................... 160
O. v. 2 (South Africa)           121 T..C................................... 160
O. v. 7 (Venezuela)              241 ...T.............T..................... 280
O. v. 8 (North Brazil)           241 ...T..T..........T..C...T..........A.C 280
O. v. 9 (North Brazil)           241 ...T..T..........T..C...T..........A.C 280
O. v. 10 (Costa Rica, Caribbean  241 .........A........T.....................C 280
O. v. 11 (Costa Rica, Caribbean  241 .........A........T.....................C 280
O. v. 12 (Costa Rica, Pacific)   241 ......T..A........T......T..........A.C 280
O. m. 1 (Costa Rica, Pacific)    241 ......T..A........T......T..........A.C 280
O. m. 2 (North Chile)            241 ......T..A........T......T..........A.C 280
O. o. (California, USA)          241 ......T........T..T..................A.. 280
O. a. (California, USA)          241 ......T..........T..................A.C 280
O.d.                             241 ...T..A........T.....C..........GA...A.A 280
Loligo (X97960)                  238 .A.A..A..............CG.A.T...C.CGG.A.. 277

O. v. 5 (Mediterranean)          281 TTAAAATCCGCAACTCACTCTATAATTATTACTATTTCCT 320
O. v. 1 (S.A. this study)        281 ........................................ 320
O. v. 2 (South Africa)           281 ........................................ 320
O. v. 3 (Tristan da Cunha)       281 ........................................ 320
O. v. 4 (Senegal)                281 ........................................ 320
O. v. 6 (Taiwan)                 281 ........................................ 320
O. v. 7 (Venezuela)              281 .....................C.................. 320
O. v. 8 (North Brazil)           281 C................T.....................T. 320
O. v. 9 (North Brazil)           281 C................T.....................T. 320
O. v. 10 (Costa Rica,Caribbean   281 C................T.........G.......C.... 320
O. v. 11 (Costa Rica,Caribbean   281 C................T.........G.A.....C.... 320
O. v. 12 (Costa Rica, Pacific)   281 C................T.........G.M.....C..T. 320
O. m. 1 (Costa Rica, Pacific)    281 C................T..........C.....C..T. 320
O. m. 2 (North Chile)            281 C................T.........G.A.....C..T. 320
O. o.(California, USA)           281 C....................C.....C.....C..C.... 320
O. a.(California, USA)           281 C..............................C..C..C.... 320
O.d.                             281 .....TC.A..T.T..........C...C..C..CA.A. 320
Loligo (X97960)                  278 CAC.T.GAAT.T..C..A..C...GCAT.G..G...ATT. 317
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Continued on p. 605

Fig. 2. The 380-bp COIII region of *Octopus vulgaris* sequenced in this study, aligned and compared to octopus COIII sequences from other regions.

presented. Four distinct clusters were visible. The South African and Tristan da Cunha haplotypes fell within the Mediterranean cluster also containing results for Senegal, Taiwan and Venezuela. A second cluster consisted of the *O. vulgaris/Octopus mimus* complex from Costa Rica and North Chile, whereas the last two groups were formed by *O. vulgaris* from North Brazil and *Octopus bimaculatus/bimaculoides* from California. All these clusters were supported by high bootstrap values (92–100%).

Average sequence divergence was calculated within and between groups. Within the Mediterranean group, *O. vulgaris* (595 bp) from South Africa displayed less sequence divergence from Senegal (0.67%) and Mediterranean (1.51%) individuals than *O. vulgaris* from Venezuela (3.85%) and Taiwan (3.87%). Sequence divergences (595 bp) within the groups were as follows: Mediterranean 2.73%, Costa Rica group 2.31%, Brazil group 0.19%, and the California group 16.47%.

Distance matrixes using a Kimura-2 parameter (Table 2) and Jukes-Cantor, HKY 85 (data not shown) models produced similar values. These distance data also support the close relation (0.01–0.0 = no divergence) between the South African, Tristan da Cunha, Senegal and Mediterranean sequences.

Fig. 2 (*continued*)

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O. v. 5 (Mediterranean)          321 TAGGATTTTATTTTACAATCCTTCAAATATTAGAATATAT 360
O. v. 1 (S.A. this study)        321 ...................T..................... 360
O. v. 2 (South Africa)           321 ...................T..................... 360
O. v. 3 (Tristan da Cunha)       321 ...................T..................... 360
O. v. 4 (Senegal)                321 ........................................... 360
O. v. 6 (Taiwan)                 321 ....G.................C...........G..... 360
O. v. 7 (Venezuela)              321 ...................T..C..................... 360
O. v. 8 (North Brazil)           321 ..........C..............G.GA.......... 360
O. v. 9 (North Brazil)           321 ..........C..C...........G.GA.......... 360
O. v. 10 (Costa Rica,Caribbean   321 ............C........C..................... 360
O. v. 11 (Costa Rica,Caribbean   321 ............C........................... 360
O. v. 12 (Costa Rica, Pacific)   321 ............C........................... 360
O. m. 1 (Costa Rica, Pacific)    321 ............C........................... 360
O. m. 2 (North Chile)            321 ............C........................... 360
O. o. (California, USA)          321 ..........C........T..A..................... 360
O. a. (California, USA)          321 ..........C........T..C..................... 360
O.d.                             321 ..................T.TT.A......A.......... 360
Loligo (X97960)                  318 ....TACA..C..C...T.T......GCTGA.......T. 357

O. v. 5 (Mediterranean)          361 AGAAGCATCATTTTCTATTT 380
O. v. 1 (S.A. this study)        361 .................... 380
O. v. 2 (South Africa)           361 .................... 380
O. v. 3 (Tristan da Cunha)       361 .................... 380
O. v. 4 (Senegal)                361 .................... 380
O. v. 6 (Taiwan)                 361 .................... 380
O. v. 7 (Venezuela)              361 .................... 380
O. v. 8 (North Brazil)           361 .................... 380
O. v. 9 (North Brazil)           361 .................... 380

O. v. 10 (Costa Rica,Caribbean   361 ..................C. 380
O. v. 11 (Costa Rica,Caribbean   361 ..................C. 380
O. v. 12 (Costa Rica, Pacific)   361 ..................C. 380
O. m. 1 (Costa Rica, Pacific)    361 ..................C. 380
O. m. 2 (North Chile)            361 ..................C. 380
O. o.(California, USA)           361 .................... 380
O. a.(California, USA)           361 ...G................ 380
O.d.                             361 T................C. 380
Loligo (X97960)                  358 ...G..TC.G.....C..C. 377
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Discussion

No distinction could be made between *O. vulgaris* collected on the east (Port Alfred to Port Elizabeth) and west (Hout Bay to Kleinmond) coasts of South Africa on the basis of genetic variation in the COIII region. These data do not, therefore, refute the hypothesis of a single *O. vulgaris* genetic population around the coast. However, a similar study, conducted on abalone from around South Africa, did find a significant difference between the east (Wave Crest to Struisbaai: haplotype diversity = 0.31) and west coasts (Dassen Island to Quin Point: haplotype diversity = 0.78).[31] However, when sites in close proximity to Cape Agulhas were compared, the east (Struisbaai: haplotype diversity = 0.69) versus west (Cape Point: haplotype diversity = 0.86) distinction could not be made, indicating mixing around this cape.

Population genetic studies on several other species have not shown genetic variation between west and east coasts. These

Table 1. Abbreviations, origin and accession numbers of DNA sequences for the various species compared.

Species	Abbreviation	Origin	Accession number
Octopus vulgaris	*O. v.1*	South Africa, this study	—
	O. v. 2	South Africa	AJ250487
	O. v. 3	Tristan da Cunha	AJ250477
	O. v. 4	Senegal	AJ250476
	O. v. 5	Mediterranean	AJ012121
	O. v. 6	Taiwan	AJ250479
	O. v. 7	Venezuela	AJ250478
	O. v. 8	North Brazil	AJ012123
	O. v. 9	North Brazil	AJ012124
	O. v. 10	Costa Rica, Caribbean	AJ012126
	O. v. 11	Costa Rica, Caribbean	AJ012127
	O. v. 12	Costa Rica, Pacific	AJ012125
Octopus mimus	*O. m.1*	Costa Rica, Pacific	AJ250480
Octopus mimus	*O. m.2*	North Chile	AJ012128
Octopus bimaculoides	*O. o.*	U.S.A., California	AJ250482
Octopus bimaculatus	*O. a.*	U.S.A., California	X83100
Octopus dolfeini	*O. d.*		X83103
Loligo vulgaris reynaudii	*L. r.*		X97960

Table 2. Pairwise distances between haplotypes based on the Kimura-2 parameter model. Haplotype abbreviations as in Table 1.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Loligo																	
2	O.v. 5	0.38	–															
3	O.v. 1	0.36	0.01	–														
4	O.v. 7	0.36	0.04	0.04	–													
5	O.v. 11	0.35	0.10	0.10	0.09	–												
6	O.v. 10	0.35	0.12	0.12	0.10	0.01	–											
7	O.v. 12	0.34	0.14	0.14	0.12	0.03	0.03	–										
8	O.v. 9	0.32	0.13	0.13	0.11	0.08	0.09	0.08	–									
9	O.v. 8	0.33	0.12	0.13	0.11	0.09	0.09	0.08	0.00	–								
10	O.m. 2	0.34	0.14	0.14	0.12	0.03	0.03	0.00	0.08	0.08	–							
11	O. d.	0.36	0.23	0.22	0.22	0.20	0.20	0.20	0.20	0.20	0.20	–						
12	O.v. 4	0.37	0.01	0.01	0.04	0.10	0.11	0.13	0.12	0.12	0.13	0.22	–					
13	O.v. 6	0.37	0.03	0.04	0.04	0.11	0.11	0.14	0.14	0.13	0.14	0.23	0.03	–				
14	O.m. 1	0.34	0.13	0.13	0.12	0.03	0.03	0.01	0.08	0.08	0.01	0.19	0.12	0.13	–			
15	O.o.	0.35	0.12	0.12	0.10	0.09	0.09	0.09	0.09	0.09	0.09	0.18	0.12	0.12	0.09	–		
16	O.a.	0.34	0.12	0.12	0.11	0.09	0.09	0.09	0.09	0.08	0.09	0.19	0.12	0.13	0.09	0.05	–	
17	O.v. 3	0.36	0.01	0.00	0.04	0.10	0.12	0.14	0.13	0.13	0.14	0.22	0.01	0.04	0.13	0.12	0.12	–

studies focused on demersal teleosts[32,33] and benthic invertebrates[34] with planktonic eggs and larvae, indicating free movement of larvae between the west and east coasts. *O. vulgaris* produces planktonic larvae, and oceanographic studies along the South African coast have suggested that the dispersal of ichthyoplankton may occur on a large scale.[35]

Molecular genetic analysis of cephalopods has, however, been hampered by low levels of variability.[16] The COIII region examined here might be too conserved, i.e. there is not enough genetic variation present to show differences between populations and other criteria such as microsatellite DNA markers should be explored to investigate population structures. Microsatellite markers are now available for octopus and have been used in a population study on octopus in northwest Africa.[36,37] Genetic variability detected by the microsatellites indicated that more than two genetically distinct populations exist off the northwest coast of Africa.[37] Microsatellites were also successfully employed to distinguish population structuring in squid (*Loligo forbesi*), where other techniques involving mtDNA and allozymes failed to detect differences.[16] The South African *O. vulgaris,* based on data from this study, should be treated as a single population for fishery management purposes until more in-depth genetic research can be conducted.

Phylogenetically, the South African *O. vulgaris* is closely related to *O. vulgaris* from Senegal (0.67% divergence) and the Mediterranean (1.51% divergence). These results are similar to those found by Söller *et al.*[19] and Warnke *et al.*[20] The latter[20] further postulated that populations from France, Senegal, South Africa, Tristan da Cunha and Venezuela are from the same taxon. However, the high sequence divergence (this study, 2.86–3.87%) between the Mediterranean (including South Africa, Senegal) and Taiwan and Venezuela might support conclusions drawn from morphological data by Mangold and Hochberg,[23] and Mangold,[21] that indicated a Mediterranean and eastern Atlantic distribution only. Nevertheless, further taxonomic classification of octopus should take morphology, reproductive and molecular data into consideration to ensure an all-round approach to systematic analyses.

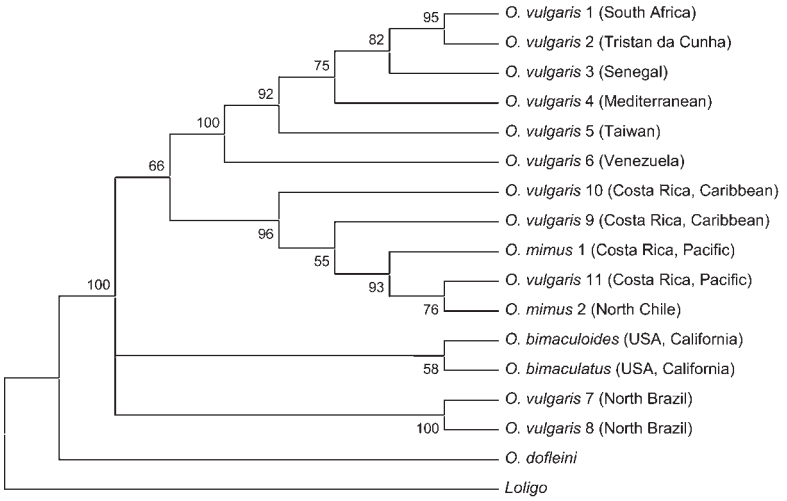

Fig. 3. Maximum parsimony tree (in MEGA, Branch & bound) constructed from aligned *Octopus vulgaris* and a range of other octopus species mtDNA COIII sequences. [Bootstrap values (%) after 1000 replications.]

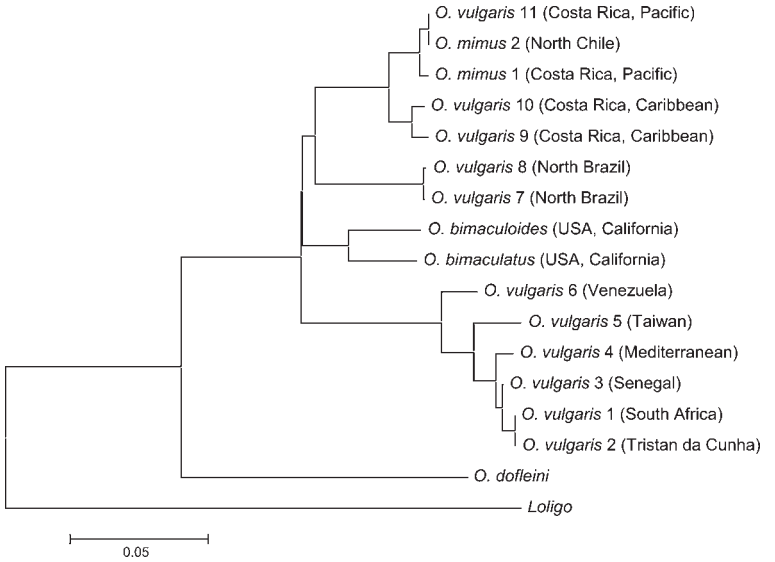

Fig. 4. Neighbor-Joining (assuming Kimura-2 parameter model) tree constructed from aligned *Octopus vulgaris* and a range of other octopus species mtDNA COIII sequences in MEGA.[28] [Bootstrap values (%) after 1000 replications.] Scale bar indicates branch length.

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1. Smith C.D. (1999). *Population biology and ecology of octopus in the southwestern Cape: A study towards the establishment of a small-scale octopus fishery.* MSc thesis, University of Cape Town.
2. Oosthuizen A. (2004). *A development and management framework for a new* Octopus vulgaris *fishery in South Africa.* Ph.D. thesis, Rhodes University, Grahamstown.
3. Begg G.A., Friedland K.D. and Pearce J.B. (1999). Stock identification and its role in assessment and fisheries management: an overview. *Fish. Res.* **43**, 1–8.
4. Altukhov Y.P. (1981). The stock concept from the viewpoint of population genetics. *Can. J. Fish. Aquat. Sci.* **38**, 1523–1538.
5. Smith P.J., Francis R.I.C.C. and McVeagh M. (1991). Loss of genetic diversity due to fishing pressure. *Fish. Res.* **10**, 309–316.
6. Stephenson R.L. (1999). Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. *Fish. Res.* **43**, 247–249.
7. Bartley D.M., Bentley B., Olin P.G. and Gal G.A.E. (1992). Population genetics structure of the coho salmon (*Oncorhynchus kisutch*) in California. *Cal. Fish. Game* **78**, 88–104.
8. Beacham T.D. Withler R.E. and Gould A.P. (1995). Biochemical genetic stock identification of chum salmon (*Oncorhynchus keta*) in southern British Columbia. *Can. J. Fish. Aquat. Sci.* **42**, 437–448.
9. Imsiridou A., Apostolisdis A.P., Durand J-D., Briolay J., Bouvet Y. and Triantaphyllidis C. (1998). Genetic differentiation and phylogenetic relationships among Greek chub *Leuciscus cephalus* L. (Pisces, Cyprinidae) populations as revealed by RFLP analysis of mitochondrial DNA. *Biochem. Sys. Ecol.* **26**, 415–429.
10. Shaklee J.B., Beacham T.D., Seeb L. and White B.A. (1999). Managing fisheries using genetic data: case studies from four species of Pacific salmon. *Fish. Res.* **43**, 45–78.
11. Katsarou E. and Nævdal G. (2001). Population genetics of the roughhead grenadier, *Macrourus berglax* L., in the North Atlantic Ocean. *Fish. Res.* **51**, 207–215.
12. Smith P.J., Robertson S.G., Horn P.L., Bull B., Anderson O.F., Stanton B.R. and Oke C.S. (2002). Multiple techniques for determining stock relationships between orange roughy, *Hoplostethus atlanticus,* fisheries in the eastern Tasman Sea. *Fish. Res.* **58**, 119–140.
13. Carvallo G.R., Thompson A. and Stoner A.L. (1992). Genetic diversity and population differentiation of the shortfin squid *Illex argentinus* in the south-west Atlantic. *J. Exp. Mar. Biol. Ecol.* **158**, 105–121.
14. Brierly A.S., Rodhouse P.G., Thorpe J.P. and Clarke M.R. (1993). Genetic evidence of population heterogeneity and cryptic speciation in the ommastrephid squid *Martialia hyadesi* from the Patagonian shelf and Antarctic Polar Frontal Zone. *Mar. Biol.* **116**, 593–602.
15. Norman J., Murphy J.M., Pierce G. and Boyle P.R. (1994). Preliminary molecular genetic analysis of stock structure in the squid *Loligo forbesi* (Steenstrup). *ICES Shelfish Committee CM 1994/K.* **23**, 1–8.

16. Shaw P.W., Pierce G.J. and Boyle P.R. (1999). Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi,* demonstrated with microsatellite DNA markers. *Mol. Ecol.* **8**, 407–417.
17. De Los Angeles Barriga Sosa I., Beckenbach K., Hartwick E.B. and Smith M.J. (1995). The molecular phylogeny of five eastern North Pacific octopus species. *Molec. Phylogenet. Evol.* **4**, 163–174.
18. Bonnaud, L., Boucher-Rodoni R. and Monnerot M. (1997). Phylogeny of cephalopods inferred from mitochondrial DNA sequences. *Molec. Phylogenet. Evol.* **7**, 44–54.
19. Söller R., Warnke K., Saint-Paul U. and 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.
20. Warnke K., Söller R., Blohm D. and Saint-Paul U. (2000). Rapid differentiation between *Octopus vulgaris* Cuvier (1797) and *Octopus mimus* Gould (1852), using randomly amplified DNA. *J. Zool. Syst. Evol. Res.* **38**, 119–122.
21. Mangold K. (1997). *Octopus vulgaris*: Review of the biology. In Proc workshop on: *The fishery and market potential of Octopus in California,* University of Southern California, eds M.A. Lang, and F.G. Hochberg, pp. 85–90. Smithsonian Institution, Washington, D.C.
22. Mangold K. 1983. *Octopus vulgaris.* In *Cephalopod Life Cycles,* vol. 1, ed. P.R. Boyle, pp. 335–364. Academic Press, London.
23. Mangold K. and Hochberg F.G. (1991). Defining the genus *Octopus*: redescription of *Octopus vulgaris. Bull. Mar. Sci.* **49**, 1–665.
24. Warnke K.(1999). *Diversität des Artenkomplexes Octopus cf. vulgaris Cuvier, 1797 in beziehung zu seiner Verbreitung an der Ost- und Westküstes Lateinamerikas.* Ph.D. thesis, Universität Bremen, Shaker Verlag, Aachen.
25. Roeleveld M.A.C. (1998). The status and importance of cephalopod systematics in southern Africa. *S. Afr. J. mar. Sci.* **20**, 1–16.
26. Hall T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* **41**, 95–98.
27. Swofford D.L (1998). PAUP: Phylogenetic analysis using parsimony, Ver. 4.0d64. Smithsonian Institution, Washington, D.C.
28. Kumar S., Tamura K. and Nei M. 2001. MEGA: Molecular Evolutionary Genetics Analysis, Ver. 1.01. Pennsylvania State University, University Park.
29. Hasegawa M., Kishino H. and Yano T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **21**, 32–38.
30. Warnke K.(1999). AJ250487, AJ250477. Direct submission to Genbank database, 20-OCT-1999, Biotechnology & Mol. Genetics (FB2-UFT), University of Bremen/Center for Tropical Marine Ecology, Leobener Str., Bremen, 28359, Germany.
31. Evans B.S., Sweijd N.A., Bowie R.C.K., Cook P.A. and Elliott N.G. (2004). Population structure of the perlemoen *Haliotis midae* in South Africa: evidence of range expansion and founder events. *Mar. Ecol. Prog. Ser.* **270**, 163–172.
32. Grant W.S., Leslie R.W and Becker I. (1987). Genetic stock structure of southern African hakes, *Merluccius capensis* and *M. paradoxus. Mar. Ecol. Prog. Ser.* **41**, 9–20.
33. Leslie R.W. and Grant W.S. (1990). Lack of Congruence between genetic and morphological stock structure of the southern African anglerfish *Lophuis vomerinus. S. Afr. J. mar. Sci.* **9**, 379–398.
34. Lombart A.T. and Grant W.S. (1986). Biochemical population genetics of the black mussel *Choromytilus meridionalis. S Afr. J. Zool.* **21**, 131–135.
35. Roberts M.J. and Van Den Berg M. (in press). ADCP measured currents along the Tsitsikamma coast (South Africa) and potential transport of squid paralarvae and ichthyoplankton. *African Journal of Marine Science.*
36. Greatorex E.C., Jones C.S., Murphy J., Key L.N., Emery A.M. and Boyle P.R. (2000). Microsatellite markers for investigating population structure in *Octopus vulgaris* (Mollusca: Cephalopoda). *Mol. Ecol.* **9**(5), 641–142.
37. Murphy J.M., Balguerías L.N. and Boyle P.R. (2002). Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the northwest African coast. *Bull. Mar. Sci.* **72**(1), 545–553.
38. Oosthuizen A. (2004). Economic feasibility of an experimental octopus fishery in South Africa. *S. Afr. J. Sci.* **100**, 595–602.