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Conus pennaceus: a phylogenetic analysis of the Mozambican molluscan complex

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The genus *Conus* has over 500 species and is the most species-rich taxon of marine invertebrates. Based on mitochondrial DNA, this study focuses on the phylogenetics of *Conus*, particularly the *pennaceus* complex collected along the Mozambican coast. Phylogenetic trees based on both the 16S and the 12S ribosomal genes grossly revealed the same groupings: one that clustered the individuals from the north (Pemba, Nacala and Island of Mozambique), another that grouped the individuals from the central/southern coast (Pomene/Massinga and Inhambane) and a third that assembled the specimens from the central/southern islands (Bazaruto and Inhaca). The 16SrRNA-based trees further distinguished the northern group into a Nacala group and a Pemba+Island of Mozambique group. In all trees, *C. p. bazarutensis* and *C. lohri* collected on the central/southern islands grouped separately from the *C. p. bazarutensis* and *C. lohri* collected on the central/southern coast, suggesting a genetic similarity between these species. Likewise, *C. praelatus* revealed greater proximity to *C. pennaceus* from Pemba. Although different topologies were produced by each gene (low bootstrap support in some nodes), we support the hypothesis of a southward ancestral colonisation pattern, indicated by the 16SrRNA trees. The common ancestor would have shifted from planktonic to non-planktonic larval development and this weak vagility would have promoted the divergence between north and south specimens. Our results suggest that the separation of these groups might have been a relatively recent event, and part of the current morphological variability could be the outcome of phenotypic plasticity and/or ecological adaptation.

Keywords: complex, *Conus pennaceus*, mitochondrial genes, morphological diversity, Mozambique

Introduction

The genus *Conus* Linnaeus 1758 is the most species-rich taxon of marine invertebrates currently known. It includes more than 500 species that range in habitat from tropical to subtropical areas (Kohn 1990). The importance of this genus resides both in its species richness and high level of endemism, which are evident in its wide morphological diversity, and in the production of toxins (conotoxins) that are used by the animals to paralyse their prey (Röckel et al. 1995). *Conus* species are dioic and have internal fertilisation. The larvae are generally veliger and planktotrophic, although in some species, such as *Conus pennaceus*, larvae are reported to be benthic and lecitotrophic (Perron and Corpuz 1982).

The wide geographic distribution of *Conus*, mainly in the Indo-Pacific region where 60% of all the genus biodiversity is retained (Röckel et al. 1995), explains why it occurs in many countries (GBIF 2008). In Mozambique (South-East Africa), the genus ranges from 10°20′ S to 26°50′ S, along a coastline of nearly 2 500 km where there are several major bays. Several *Conus* species occur along this coast, including the *Conus pennaceus* Born 1778 species complex (Röckel et al. 1995), whose large morphological variability is exclusive to Mozambique and Hawaii (Nishi and Kohn 1999). Röckel et al. (1995) described two main geographical areas of distribution: (1) the North of Mozambique, from the Tanzania border to Pomene, where specimens of *C. pennaceus* cohabit with its synonymous species *Conus elisae* Kiener 1845 and *Conus praelatus* Hwass in Bruguière 1792; and (2) the South of Mozambique, where the subspecies *Conus pennaceus bazarutensis* Fernandes & Monteiro 1988 is found from the Bazaruto Archipelago to Massinga, and the species *Conus lohri* Kilburn 1972 extends from south of Massinga to KwaZulu-Natal in South Africa. Röckel et al. (1995) consider these two latter species as two forms of the same subspecies, but other malacologists find them too different to be acknowledged as synonymous. However, although not officially documented, Mozambican malacologists further distinguish this complex, alleging that the specimens from the localities of Pemba and Nacala are

morphologically distinct and should thus be regarded as two different forms.

Conus pennaceus shells generally exhibit a white ground colour, commonly suffused with greyish blue to greyish violet, but other tones such as yellowish to pink cream, orangey to light brown and pinkish to red are possible. The shells characteristically have a network of lines (from thick to thin) woven into patterns of triangles, the dimensions of which vary according to subspecies and forms. In the North of Mozambique, the shells cover practically the entire range of the species' morphology. However, the line patterns are thin and delimit clear patches of shell, allowing a better view of the background colour (Figure 1). The same morphology is found in the synonymous sympatric species *C. praelatus* and *C. elisae*. On Bazaruto Island (in the South of Mozambique), specimens of *C. p. bazarutensis* have shells with a greyish blue to greyish violet ground colour, adorned with a reduced pattern of brown to blackish line network. Similar morphology is found on the specimens from Massinga (in the South of Mozambique), although the ground colour is pinkish cream and the brown reticulate pattern is more reduced. From Inhambane (south of Massinga) to KwaZulu-Natal (South Africa), the specimens were described as *C. lohri*, although its morphology is not different from *C. p. bazarutensis* from Bazaruto Island and Massinga. The sole criterion used to distinguish both species is the translucency of the periostracum, a thin membrane that protects the shell (Röckel et al. 1995).

Conus textile Linnaeus 1758 shells generally have a white ground colour, sometimes with mixtures of blue, violet, orange, pink or beige. In the Western Indian Ocean, the line network on the shells of this species varies from the characteristic pattern to a very thin mesh that might not include the spiral. This species has been shown to be phylogenetically close to *Conus dalli* Stearns 1873 and *Conus episcopatus* da Motta 1982 (Duda and Kohn 2005, Bandyopadhyay et al. 2008); the latter species being described as similar to *C. pennaceus* (Röckel et al. 1995) (Figure 1).

Notwithstanding the large number of *Conus* species, many of them are negatively impacted by human activities, such as overexploitation, rapid coastal development, pollution and global warming, which all contribute to habitat degradation — particularly the coral reefs communities. Further, the uncontrolled cone collection has contributed to global depletion, in spite of its importance to local tourism (Obura et al. 2002, Chivian et al. 2003, Whittingham et al. 2003).

According to the 1996 IUCN study, four of the only five assessed *Conus* species (*Conus africanus* Kiener 1845, *Conus cepasi* Trovao 1975, *Conus nobrei* Trovao 1945 and *Conus zebroides* Kiener, 1845) are classified as Vulnerable, whereas the fifth species (*Conus kohni* McLean and Nybakken 1979) has the status of Data Deficient (IUCN 2007).

For many years the *pennaceus* complex has puzzled both professional and amateur malacologists, making the taxonomic status of several formerly described species dubious. Indeed, *C. pennaceus* alone has 13 synonymous species and seven subspecies (Röckel et al. 1995). More and new information should be provided in order to better understand this species' complex. The aim of this study was to provide genetic information on the *pennaceus* complex and to compare it with the current morphological classification in order to better understand the distinction between the species.

Material and methods

A total of 22 *Conus* specimens was collected along the coasts of Mozambique and southern Angola. The 20 Mozambican specimens were collected at the localities of Pemba (two specimens of *C. pennaceus*, form of Pemba), Nacala (seven specimens of *Conus pennaceus*, form of Nacala), Island of Mozambique (one specimen of *C. praelatus*), Bazaruto Island (two specimens of *C. p. bazarutensis*), Pomene/Massinga (three specimens of *C. p. bazarutensis*), Inhambane (two specimens of *C. lohri*) and Inhaca Island (two specimens of *C. lohri* and one specimen of *C. textile*) (Figures 1, 2; Table 1). All these selected localities are included in, or are relatively close to, bays. The two Angolan specimens, *C. dealbatus* A. Adams 1854 and *C. franciscoi* Röckel & Rolán 2000, were collected north of Namibe. All captured specimens were preserved in absolute ethanol.

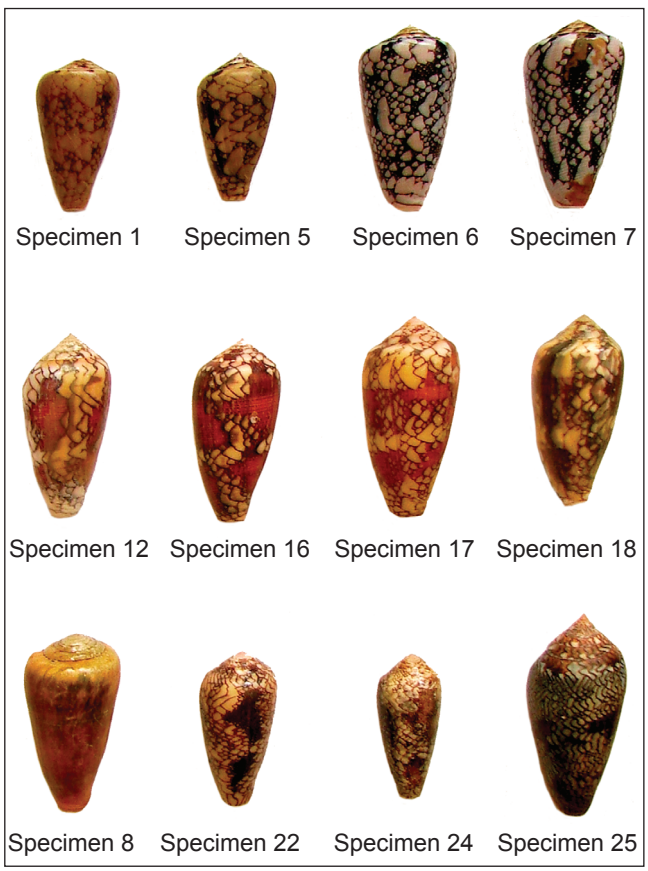

Figure 1: Examples of Mozambican specimens of *Conus*, of which some were successfully amplified on this study: specimens 1–7 — *C. p. bazarutensis* (specimen 5 = CPB5, specimen 6 = CPB6); specimen 8 — *C. lohri* (CL1); specimens 12–18 — *C. pennaceus*, Nacala form (specimen 13 = CPP2, specimen 14 = CPP3, specimen 15 = CPP4, specimen 16 = CPP5, specimen 17 = CPP6, specimen 18 = CPP7); specimen 22 — *C. pennaceus*, Pemba form (CPpe1); specimen 24 — *C. praelatus* (CPra); specimen 25 — *C. textile* (CT)

DNA extraction, PCR amplification and sequencing

Several protocols were tested to extract DNA from the foot tissue, but difficulties arose because of the high levels of mucopolysaccharides present in molluscan tissues (Winnepenninckx et al. 1993). DNA was obtained with the Omega Bio-Tek Mollusc DNA extraction kit. However, not all samples were successfully extracted using this method and thus were excluded from further analysis. The initial grinding step with liquid nitrogen was eliminated and in the final step, the DNA pellet was eluted in 30 µl of TE buffer. Fragments of both mitochondrial DNA genes 16S rRNA and 12S rRNA were amplified by polymerase chain reaction (PCR) with the following universal primers:

- 16Sar [CGCCTGTTTATCAAAAACAT] and 16Sbr [CCGGTCTGAACTCAGATCACGT] (Palumbi 1996);
- 12 SH [TGACTGCAGAGGGTGACGGGCGGTGTGT] and 12 SL [CAAACTGGATTAGATACCCCACTAT] (Paulo et al. 2008).

PCR amplifications were performed in a final volume of 12.5 µl, containing 1× Taq buffer (Fermentas), 2.5 µM $MgCl_2$ (Fermentas), 1 µg $µl^{-1}$ BSA, 0.1 µM dNTP's, 1 µM of each primer, 0.25U Taq DNA Polymerase (Promega), and 2–5 µl of DNA template. The amplification of the 16S rRNA fragments had an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 30 s at 94 °C, 1 min at 55–64 °C (annealing temperature) and 1 min at 72 °C. The same conditions were conducted for the fragments of 12S rRNA, except for the annealing temperature which varied between 47 and 51 °C.

All amplified fragments were purified (SureClean) and sent to Macrogen Inc. for sequencing. Fragments of 500–600 base pairs (bp) and 400–500 bp were obtained for the 16S and 12S rRNA genes respectively.

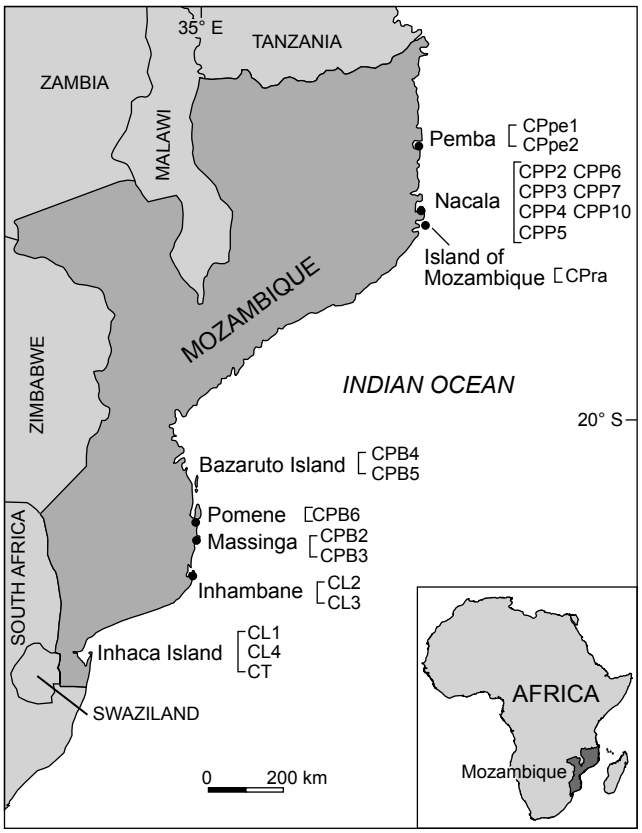

Figure 2: Map of Mozambique with the sampled locations and the names of the analysed specimens

Table 1: Details from the specimens collected: species code and name, origin (locality and country of capture), gene fragments amplified and GenBank code

Species code	Species name	Locality	Country	Gene fragments amplified	GenBank accession number (16S/12S)
CPpe1	*Conus pennaceus* (Pemba form)	Pemba	Mozambique	16S/12S	GQ424502/GQ424506
CPpe2	*Conus pennaceus* (Pemba form)	Pemba	Mozambique	16S/12S	GQ424503/GQ424507
CPra	*Conus praelatus*	Island of Mozambique	Mozambique	16S/12S	GQ424504/GQ424519
CPP2	*Conus pennaceus* (Nacala form)	Nacala (Relanzapo)	Mozambique	16S/12S	GQ424489/GQ424509
CPP3	*Conus pennaceus* (Nacala form)	Nacala (Relanzapo)	Mozambique	16S/12S	GQ424490/GQ424510
CPP4	*Conus pennaceus* (Nacala form)	Nacala (Relanzapo)	Mozambique	16S/12S	GQ424491/GQ424511
CPP5	*Conus pennaceus* (Nacala form)	Nacala (Relanzapo)	Mozambique	16S/12S	GQ424492/GQ424512
CPP6	*Conus pennaceus* (Nacala form)	Nacala (Melala)	Mozambique	16S/12S	GQ424493/GQ424513
CPP7	*Conus pennaceus* (Nacala form)	Nacala (south of Relanzapo)	Mozambique	12S	GQ424514
CPP10	*Conus pennaceus* (Nacala form)	North of Nacala	Mozambique	16S	GQ424494
CPB2	*Conus pennaceus bazarutensis*	Pomene/Massinga	Mozambique	16S/12S	GQ424499/GQ424515
CPB3	*Conus pennaceus bazarutensis*	Pomene/Massinga	Mozambique	12S	GQ424516
CPB4	*Conus pennaceus bazarutensis*	Bazaruto Island	Mozambique	16S	GQ424500
CPB5	*Conus pennaceus bazarutensis*	North of Bazaruto Island	Mozambique	16S/12S	GQ424501/GQ424517
CPB6	*Conus pennaceus bazarutensis*	Pomene	Mozambique	12S	GQ424518
CL1	*Conus lohri*	South of Inhaca Island (Ponta Abril)	Mozambique	16S/12S	GQ424495/GQ424508
CL2	*Conus lohri*	Inhambane	Mozambique	16S	GQ424496
CL3	*Conus lohri*	Inhambane	Mozambique	16S	GQ424497
CL4	*Conus lohri*	South of Inhaca Island	Mozambique	16S	GQ424498
CT	*Conus textile*	Inhaca Island	Mozambique	16S/12S	GQ424505/GQ424520
Cdeal	*Conus* sp. cf. *dealbatus*	North of Namibe	Angola	12S	GQ424521
Cfrans	*Conus* sp. cf. *franciscoi*	North of Namibe	Angola	12S	GQ424522

Complications were encountered when trying to obtain DNA fragments for every sample with both sets of primers, most likely as a result of the PCR inhibitors. This was the case for the two Angolan specimens, which only amplified for the 12S rRNA gene. New sets of primers were designed using the amplified fragments and other sequences from GenBank, but this was unsuccessful. As a result, the set of amplified sequences for each gene was uneven.

Another gene, the cytochrome oxidase I (COI), was also tested because of its rapid mutation rate and resulting high variability between species (Hajibabaei et al. 2005). However, neither the universal primers (Shearer and Coffroth 2008)

(LCO1490 [ATTCAACCAATCATAAAGATATTGG];

HCO2198 [TAAACTTCTGGATGTCCAAAAAATCA])

nor the designed primers were able to amplify gene fragments.

Phylogenetic analysis

For the outgroup, we used two species from the Turridae family, which are phylogenetically close to Conidae: *Fusiturris similis* (GenBank accession number EU827197 for the complete mitochondrial genome) and *Lophiotoma cerithiformis* (accession numbers EU682307 [16SrRNA] and EU682298 [12SrRNA]). Other *Conus* species were included in the analysis to provide better scope of the genus, but we were unable to produce the same dataset for both genes on account of the unavailability of sequences on the GenBank: for the 16SrRNA trees, *C. dalli* (EU078935) and *C. episcopatus* (EU078937) from the Philippines; for the 12SrRNA trees, *C. antoniomonteroi* (AY726440) from Cape Verde and *C. ventricosus* (AY726489) from Portugal. Also included in this latter dataset were *C. dealbatus* and *C. franciscoi*, both sequenced in this study. The only sequence of *C. pennaceus* (AF174190) available online (16S) was tested on our dataset, but it did not return any new relevant information because of its relatively small size (374 bp), and is not shown here.

The DNA sequences were verified manually with Sequencher 4.05 (GeneCodes) and aligned with Clustal_X 2.0 (Thompson et al. 1997). The most appropriate evolutionary model that best explains the genetic divergence was determined using Modeltest 3.7 (Posada and Crandall 1998). The phylogenetic trees were obtained with PAUP* 4.0b10 (Swofford et al. 1996) for the methods of maximum parsimony (MP), maximum likelihood (ML) and neighbour joining (NJ) (maximum likelihood distance selected). For the parsimony analyses, gaps were treated as a fifth character state and alternatively were deleted, although no significant differences were found between both analyses. In all analyses, the data were resampled 1 000 times using the bootstrap technique to evaluate the robustness of the nodes of the phylogenetic trees.

The Bayesian inference (BI) analysis was performed with MrBayes 3.1.2. (Ronquist and Huelsenbeck 2003) using the method of Metropolis Coupled Markov Chain Monte Carlo (MCMCMC), estimated for 1.5×10^6 generations, sampled every 100 generations from the stationary phase, and with a burn-in of the first 1 500 trees. For each dataset, the model selection was carried out with MrModeltest v2.2 and implemented, according to Nylander (2004).

The entire set of analyses was carried out for each fragment individually and for the concatenation of both fragments using 'Concatenator' (Pina-Martins and Paulo 2008). Additional chi-square (χ^2) tests were performed to verify if the DNA base proportion were significantly different among the sequences. The incongruence length distance (ILD) test was conducted to assess the level of differences among the gene fragments and therefore assure the possibility of concatenation (Farris et al. 1995, Cunningham 1997).

The consistency index (CI; Kluge and Farris 1969), retention index (RI; Farris 1989) and the homoplasy index (HI; Archie 1989) were also calculated to describe the amount of homoplasy of the tree. Additionally, the software Network 4.5 (Bandelt et al. 1999) was used for the analysis of the populations' haplotypes.

Results

Phylogenetic analysis

A total of 34 sequences was obtained (17 for each gene; Table 1), which were subsequently blasted to confirm the gene identification. The amplification resulted in fragments with 530 bp for the 16S rRNA and 425 bp for the 12S rRNA. Sequences were aligned with different values of gap-opening and gap-extension penalties. However, for the final analysis, the default values of 15 and 6.66 (gap-opening and gap-extension penalties respectively) were preferred on account of the higher average score value. The alignment was then checked manually.

The base composition did not differ among the sequences of the 12S rRNA ($\chi^2 = 4.07$, df = 60, $p = 1.00$). Likewise, the 16S rRNA sequences did not show significant differences ($\chi^2 = 5.49$, df = 60, $p = 1.00$), as did the concatenated dataset ($\chi^2 = 3.10$, df = 39, $p = 1.00$).

Datasets for 16S and 12S genes were analysed separately and concatenated together, despite significant differences between both datasets ($p = 0.01$) revealed by the ILD test.

The 16S rRNA parsimony analyses generated 254 trees of score 198 with 135 variable sites, of which 76 were parsimoniously informative (outgroup excluded). The homoplasy of the tree was low (CI = 0.8240; RI = 0.8472; HI = 0.1760). The 12S rRNA parsimony analyses generated two trees of score 216 with 142 variable sites, of which 81 were parsimoniously informative (outgroup excluded). The homoplasy of the tree was also low (CI = 0.8426; RI = 0.8509; HI = 0.2464). The concatenated dataset resulted in six trees for the parsimony analyses with tree score of 350. The total number of variable sites was 288, of which 124 were parsimoniously informative (outgroup excluded). Similarly, the concatenated tree had low homoplasy scores (CI = 0.9371; RI = 0.8675; HI = 0.1317).

According to the Akaike information criterion (AIC; Swofford et al. 1996), the best evolutionary model for the 16S rRNA and the concatenated fragments was the TVM+I+G with gamma parameters of 0.3560 and 0.6679 respectively and transition/transversion rates of 3.43 and 4.47 respectively. For the 12S rRNA, the best evolutionary model was the TVM+G with a gamma parameter of 0.2698 and a transition/transversion rate of 4.22.

The groupings found in the trees resulting from each method were generally similar for each gene, although the topological relationships were not always congruent, as shown by the low bootstrap values of some nodes (Figures 3, 4).

A single optimal ML 16S rRNA phylogenetic tree (−ln L 1 559.17) was obtained under the selected evolutionary model (Figure 3). Within the *pennaceus* complex, the bootstrap values of some nodes were not consistently high across methods of analysis and some were unresolved. However, it was possible to detect some clustering: one group included *C. lohri* specimens from Inhambane (CL2 and CL3) and one *C. p. bazarutensis* specimen from Pomene/ Massinga (CPB2) (central/southern coastal group); another, in the central/southern islands group, included the *C. p. bazarutensis* specimens from Bazaruto Island (CPB4 and CPB5) and *C. lohri* from Inhaca Island (CL1 and CL4), these two forming a monophyletic group; another group included all specimens from Nacala (CPP); and the remaining specimens from Pemba (CPpe1 and CPpe2) and the Island of Mozambique (CPra) appeared as a polytomy at the base of the *C. pennaceus* complex. Nevertheless, on the MP, NJ and BI analyses, this last group clustered with the specimens from Nacala (bootstrap or posterior probabilities of 60%, 89% and 58% respectively). The 16S trees were not congruent in the evaluation of basal/derived groups within the *C. pennaceus* species complex group. The highest distance within the *pennaceus* complex for this gene occurred between the specimens CPB2 and CPpe1 (1.8%, corrected by the maximum likelihood distance).

A single optimal ML 12S rRNA phylogenetic tree (−ln L 1 411.66) was obtained under the selected evolutionary model (Figure 4) and revealed clustering within this complex: one group (northern) contained the specimens from the Island of Mozambique (CPra), Pemba (CPpe1 and CPpe2) and Nacala (CPP), except for CPP6 which was found in another group along with the specimens of *C. p. bazarutensis* from Pomene and Massinga (CPB2, CPB3 and CPB6). On the ML and Bayesian trees, *C. lohri* (CL1) and *C. p. bazarutensis* (CPB5) from Inhaca Island and Bazaruto Island respectively were part of a polytomy at the base of the *C. pennaceus* complex group. However, for the MP analysis, CL1 and CPB5 did not occupy a basal position but were rather grouped with the specimens from the north. The highest distance found for this gene within this complex was 1.7% (corrected by the maximum likelihood distance) between both specimens CL1 and CPra, and the specimens CPB2, CPB3, CPB6 and CPP6.

The concatenated dataset did not produce trees with significant differences (Figure 5). Among the *pennaceus* complex, there were two main groups, one clustering the individuals from the north — although a closer proximity was found between the Pemba form and *C. praelatus* (BI value of 96) — and the other grouping the specimens from the south. In the latter, the individuals from the islands (CL1 and CPB5) were distinguished from the specimen from the coast (CPB2), with high probability support (>83). On this analysis, the CT specimen was placed outside the complex, but its relation to specimens within the *pennaceus* group was uncertain.

Both 16S and 12S trees showed the monophyly of the *C. pennaceus* species complex in relation to *C. textile* (Figures 3 and 4) with high bootstrap and posterior probabilities values (>84%), except with the 16S ML analysis in which there was a polytomy of this complex with *C. textile*, *C. dalli* and *C. episcopatus*. On the 16S BI, the complex was monophyletic, but the posterior probability value was low (56%). On the 16S trees, *C. textile* from Inhaca Island (CT) grouped with *C. dalli* from the East Pacific with high bootstrap and Bayesian credibility values (Figure 3). Although also from the East Pacific, *C. episcopatus* either grouped with the CT+*C. dalli* group or with the *C. pennaceus* complex group. On the 12S trees, CT grouped with the *pennaceus* complex with high bootstrap/probability (Figure 4). The Angolan specimens (*C. dealbatus* and *C. franciscoi*) appeared to be genetically more similar to the complex than the Cape Verde and

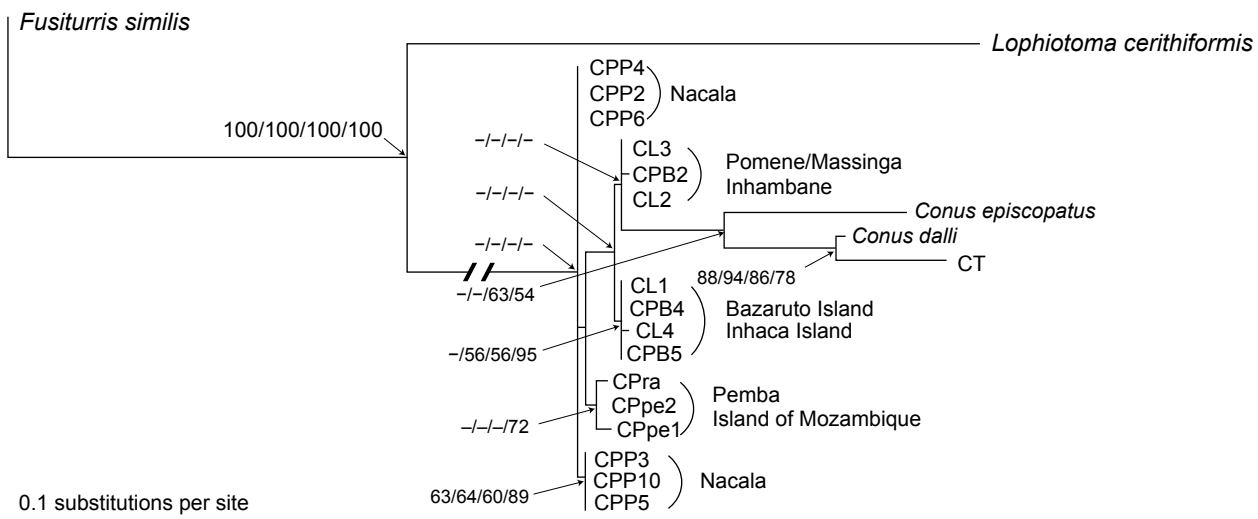

Figure 3: Optimal maximum likelihood 16S rRNA phylogenetic tree. The arrows indicate the values of bootstrap for the analyses of maximum parsimony, neighbour joining and maximum likelihood respectively. The fourth value corresponds to the Bayesian credibility value

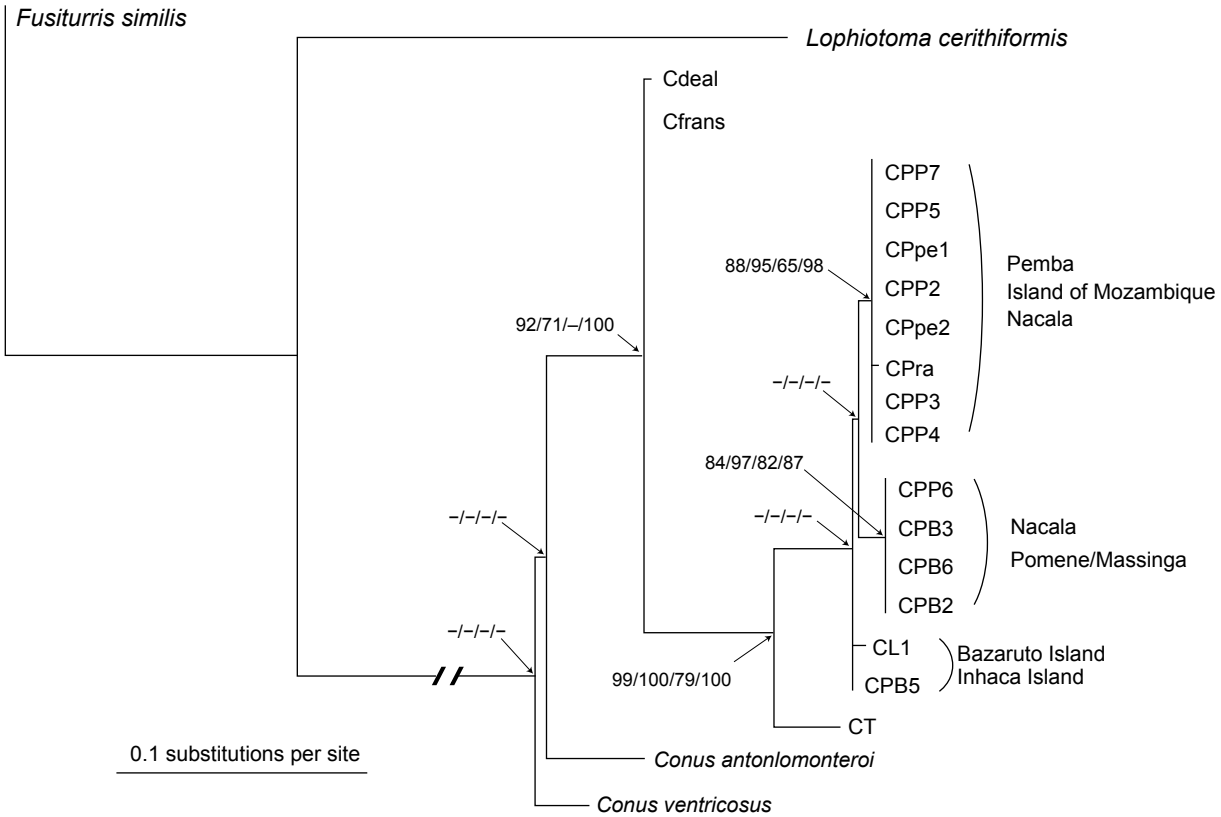

Figure 4: Optimal maximum likelihood 12S rRNA phylogenetic tree. The arrows indicate the values of bootstrap for the analyses of maximum parsimony, neighbour joining and maximum likelihood respectively. The fourth value corresponds to the Bayesian credibility value

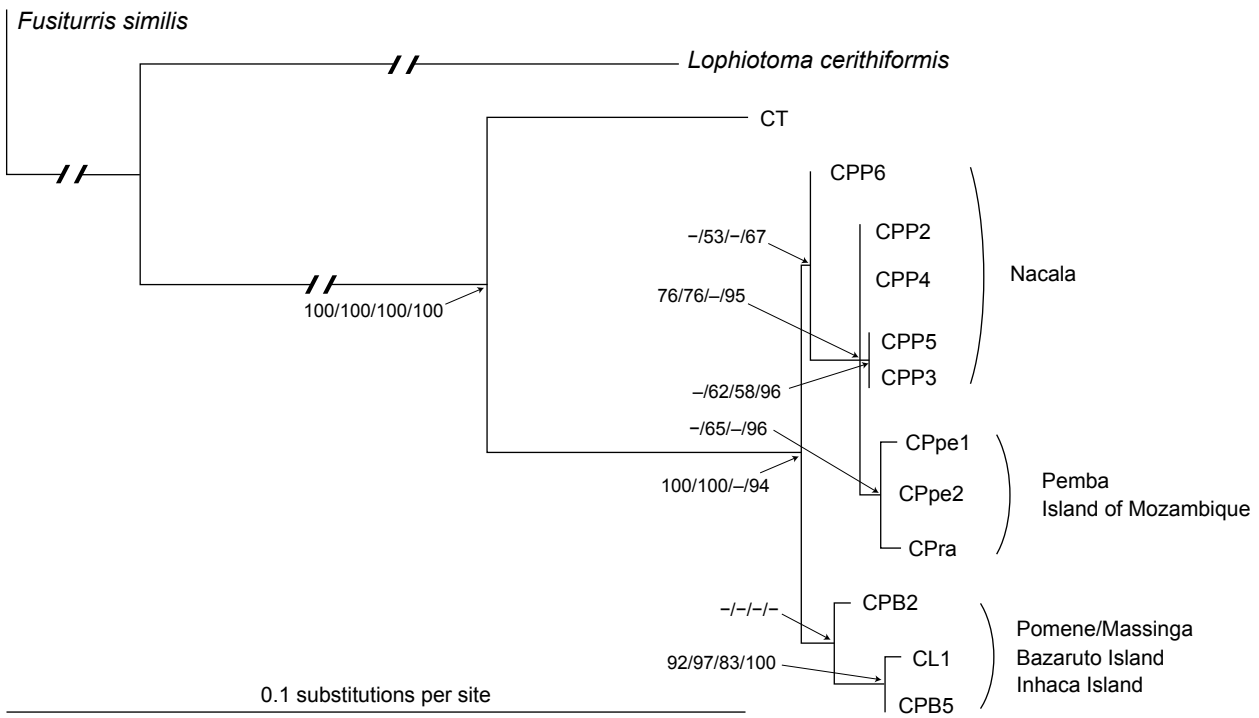

Figure 5: Optimal maximum likelihood of the concatenated dataset. The arrows indicate the values of bootstrap for the analyses of maximum parsimony, neighbour joining and maximum likelihood respectively. The fourth value corresponds to the Bayesian credibility value

Portuguese haplotypes (*C. antoniomonteroi* and *C. ventricosus* respectively).

To understand the haplotype diversity within the *C. pennaceus* complex, median-joining networks were determined. In general, the networks obtained from each gene fragment recovered the same groups as the phylogenetic trees (Figures 6 and 7), supporting the notion that the genetic distinction between the groups is considerable. Similar to the 16S rRNA phylogenetic trees, the 16S rRNA network showed two distinct groups: a northern group, which clustered the specimens from Nacala and those from Pemba and the Island of Mozambique; and a central/southern group, which included the haplotypes from Inhambane and Pomene/Massinga and the islands of Bazaruto and Inhaca. The separation into two subgroups of this latter group was more difficult to identify in the network than in the trees. The groups obtained from the 12S rRNA network were identical to those revealed by the 12S rRNA phylogenetic trees: one group with specimens from Nacala, Pomene and Massinga; another with specimens from Pemba, Nacala and the Island of Mozambique; and a group that included the specimens from Bazaruto Island and Inhaca Island.

Discussion

The phylogenetic trees and the haplotype networks obtained for both 16S and 12S rRNA genes revealed a close genetic relationship between *C. lohri* and *C. p. bazarutensis*, as well as between *C. praelatus* and *C. pennaceus* (forms of Pemba and Nacala). There was a clear genetic differentiation in both genes between northern and southern Mozambican populations of the *C. pennaceus* species complex, with the exception of one haplotype from Nacala (CPP6) in the 12S dataset, which was the same haplotype found in the south (Pomene and Massinga). Within the *C. lohri* and *C. p. bazarutensis* group, the haplotypes from Inhaca Island (*C. lohri*) and Bazaruto Island (*C. p. bazarutensis*) were differentiated from the remaining haplotypes found in Inhambane (*C. lohri*) and in Pomene and Massinga (*C. p. bazarutensis*).

Nevertheless, the results from the 12S rRNA gene fragments revealed fewer groups because of the merging of both northern groups into one group (Island of Mozambique, Nacala and Pemba).This weaker resolution might be the result of the slower mutation rate of the 12S rRNA gene (Palumbi 1996), thus allowing the prevalence of ancestral characteristics.

The specimen *C. textile* was highly differentiated from the *C. pennaceus* species complex (between 6.35% and 7.35% for the 16S and 4.75% and 5.70% for the 12S) and groups with the Eastern Pacific reference samples (16S dataset). When including the Atlantic *Conus* species from Angola, Cape Verde and Algarve (12S dataset), *C. textile* and *C. pennaceus* complex formed a monophyletic group in relation to these. Angolan species appeared to be less differentiated from the Mozambican group than from the Cape Verde and Algarve species.

The small sample size and the limited number of localities sampled do not allow inferences to be made on the origin and direction of the colonisation in this group of species. However, some hypotheses can be considered. In one scenario, the *Conus* ancestor that originated the *C. textile*, *C. dalli* and *C. episcopatus* group would have also branched into the *pennaceus* species complex group. The divergence of this group would have started with *C. lohri* and *C. p. bazarutensis* in the central/southern islands of Mozambique (Bazaruto

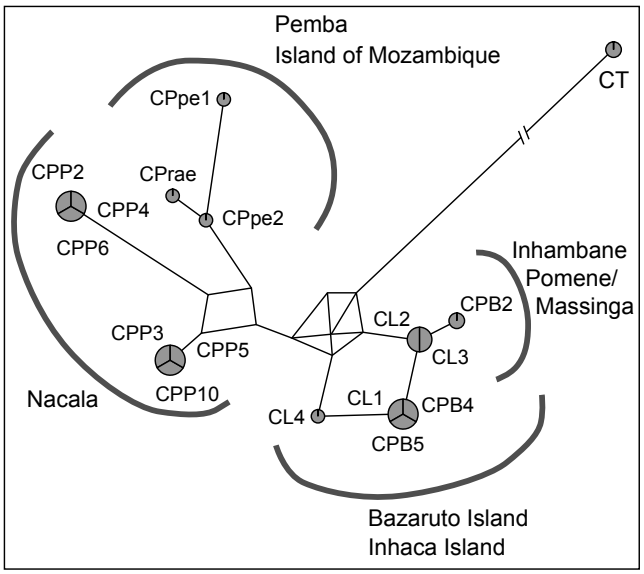

Figure 6: Median-joining network of the 16S rRNA haplotypes. The size of each circle is related to the number of specimens that share the same haplotype. In general, this analysis recovered the same groups as the 16S rRNA phylogenetic tree, although there seems to be a weak distinction between the groups from Inhambane and Pomene/ Massinga (Central Mozambique) and Bazaruto and Inhaca islands

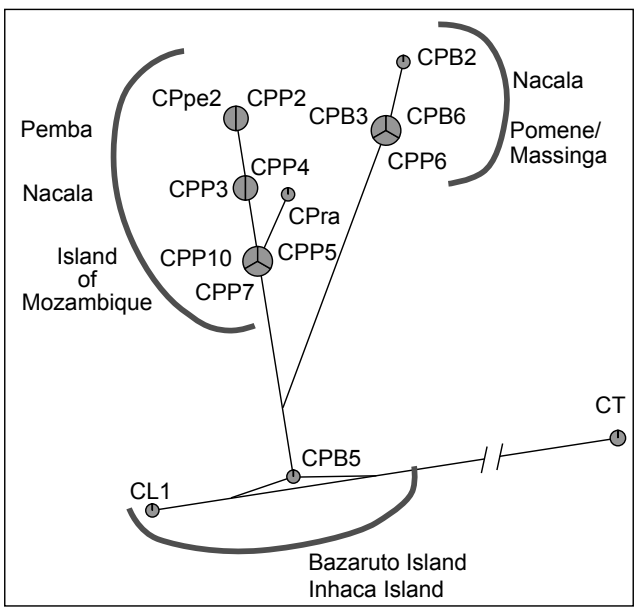

Figure 7: Median-joining network of the 12S rRNA haplotypes. The size of each circle is related to the number of specimens that share the same haplotype. Similarly to the 12S rRNA phylogenetic tree, this analysis recovered three groups

and Inhaca) and the same ancestor would have moved coastward and northward. On the central/southern coast, it would have further differentiated in genetically distinct forms of *C. lohri* and *C. p. bazarutensis,* and to the north it would have originated the Pemba and Nacala forms. However, our results also show the opposite scenario, which suggests that the common ancestor would have started its divergence southwards, and the last species to be differentiated would have been *C. lohri* and *C. p. bazarutensis*. This latter scenario seems the most plausible, if the oceanic currents that characterise the Mozambique Channel are considered. This current was originally thought to be a continuous flow that moved southwards, but more recent studies have demonstrated that the entire region is influenced by eddies resulting from the East Madagascar Current and the South Equatorial Current (Sete et al. 2002, Lutjeharms 2006, Hoguane 2007). The number of eddies in the channel is unknown, but it is believed to be around seven per year. Saetre (1982) (cited by Hoguane 2007) divided these eddies into three anticyclonic and four cyclonic cells along the Mozambican coast; the latter as a result anticyclonic eddies (Figure 8). The first anticyclonic cell occurs in the north, from Rovuma to Nacala, followed by the second anticyclonic cell from Angoche to Vilanculos. The third cell extends from Inhambane to the region bordering South Africa. At certain times of the year, the three cells appear to be interspersed by cyclonic cells (Hoguane 2007). In the Delagoa Bight, the water mass shifts back northwards (Figure 8; cyclonic cell d), therefore barely contributing to the Agulhas Current that moves southwards (Sete et al. 2002, Lutjeharms 2006, Hoguane 2007). These current dynamics may have caused the distinction between populations from the coast and those from the islands. Non-planktonic forms have often arisen from planktonic forms — but the opposite is rare — and such species do not form a monophyletic group (Duda and Palumbi 1999). Therefore, shifts in development mode would be very common within *Conus* (Duda and Palumbi 1999), which could help explain the dispersal of the ancestor of this complex. The weak vagility of the benthic larval stages of the *C. pennaceus* species (Cunha et al. 2005) would have promoted the distinction between the North and South of Mozambique considering the distance and the lack of a constant oceanic current between those regions.

If coupled to this transport, the hypothesis of local adaptation could explain the morphological diversity observed in the complex. The phenotypic variability of *C. pennaceus*, evident through the variety of colour patterns and shape, could indicate polymorphisms, as with the species *Littorina saxatilis*. In Galiza, Spain, two ecotypes are frequently found on rocky surfaces: one ridged and banded (RB) that colonises the upper-level shores (larger shells) and one smooth and unbanded (SU) that inhabits the lower-level shores (smaller shells) and thus is exposed to currents. Notwithstanding the morphological distinction, mitochondrial and nuclear data do not reveal significant differences between both ecotypes that colonise the same bay. However, the same ecotype collected from different isolated bays has been demonstrated to be genetically different from each other (Quesada et al. 2007), suggesting the possible influence of the environment on the emergence of polymorphisms. This may be the case in the

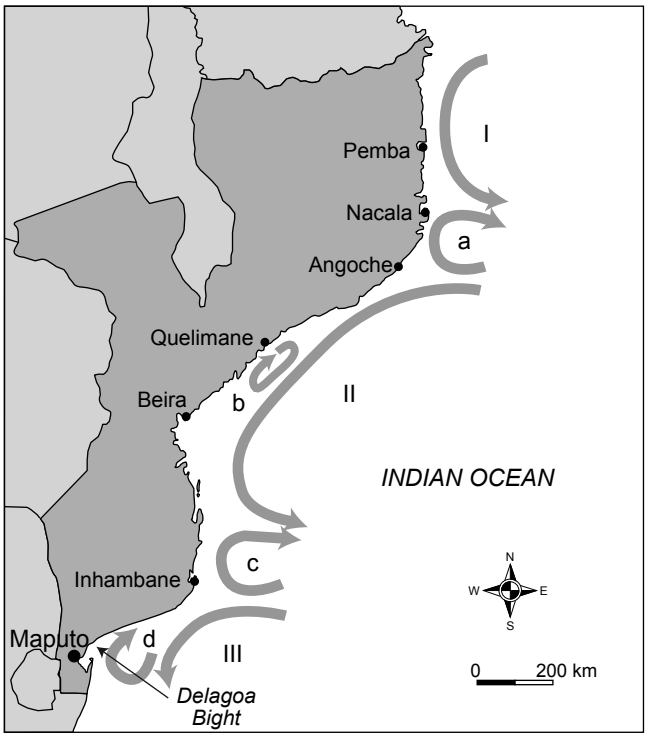

Figure 8: Map depicting the anticyclonic (I–III) and cyclonic (a–d) eddies along the Mozambique Channel (adapted from Saetre 1982 in Hoguane 2007)

pennaceus complex. If so, the ancestral group would have colonised the coast of Mozambique and developed different morphological types that resulted from local adaptation. Gene flow would have occurred continuously between some populations, but without interfering with the genes responsible for the ecological adaptation (Hendry 2009).

Another scenario would be the existence of a sole species that had remarkable morphological variety resulting from local accommodation. The entire complex would be equivalent to one species with high phenotypic plasticity, being able to manifest different phenotypes while maintaining the genetic information. An alternative hypothesis could be allied to either scenario. Regardless of the intensity of the gene flow, the *C. pennaceus* divergence into different subspecies and forms might be a recent event. The early stages of speciation, which are currently occurring, would be evident between the northern and central/southern populations. Within each region, there might be further divergence, explaining the distinction made by local malacologists between the Pemba and Nacala forms in the north.

When considering species conservation, it is not only important to be mindful of the preservation of the genetic background, which depicts the evolution of the taxa, but also the adaptive potential of the species, which is evident through the phenotypic variability (Moritz 2002).

The dynamics of the *C. pennaceus* is not yet fully understood. The rapid degradation of the environment by human activity compromise the sustainability of the species, therefore further studies should be conducted.

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