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Patterns of Cladogenesis in the Venomous Marine Gastropod Genus *Conus* from the Cape Verde Islands

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Abstract.—Isolated oceanic archipelagos are excellent model systems to study speciation, biogeography, and evolutionary factors underlying the generation of biological diversity. Despite the wealth of studies documenting insular speciation, few of them focused on marine organisms. Here, we reconstruct phylogenetic relationships among species of the marine venomous gastropod genus *Conus* from the Cape Verde archipelago. This small island chain located in the Central Atlantic hosts 10% of the worldwide species diversity of *Conus*. Analyses were based on mtDNA sequences, and a novel nuclear marker, a megalin-like protein, member of the low-density lipoprotein receptor gene family. The inferred phylogeny recovered two well-defined clades within *Conus*. One includes Cape Verde endemic species with larger shells, known as the "venulatus" complex together with *C. pulcher* from the Canary Islands. The other is composed of Cape Verde endemic and West Africa and Canary Island "small" shelled species. In both clades, nonendemic *Conus* were resolved as sister groups of the Cape Verde endemics, respectively. Our results indicate that the ancestors of "small" and "large" shelled lineages independently colonized Cape Verde. The resulting biogeographical pattern shows the grouping of most Cape Verde endemics in monophyletic island assemblages. Statistical tests supported a recent radiation event within the "small shell" clade. Using a molecular clock, we estimated that the colonization of the islands by the "small" shelled species occurred relatively close to the origin of the islands whereas the arrival of "large" shelled *Conus* is more recent. Our results suggest that the main factor responsible for species diversity in the archipelago may be allopatric speciation promoted by the reduced dispersal capacity of nonplanktonic lecithotrophic larvae. [Allopatry; biogeography; *Conus*; molecular clock; speciation.]

Ever since Darwin visited the Galápagos (September 1835) and observed their great species diversity, oceanic archipelagos have been considered natural laboratories for the study of evolution and the generation of biological diversity (Wallace, 1880; Mayr, 1942; Carlquist, 1965; Emerson, 2002). Isolation is the key to an understanding of island evolutionary biology (Hubbell, 1968). The entire biota of an oceanic island may derive from few initial colonization events followed by rapid radiations that lead to high levels of endemism and ecomorphological specializations. Well-documented examples of extraordinary insular speciation include *Drosophila* flies (Baker and DeSalle, 1997), *Tetragnatha* spiders (Gillespie, 2004) and honeycreepers (James, 2004) in Hawaii, Darwin's finches (Grant, 1999) in Galápagos, *Anolis* lizards (Losos et al., 1998) in the Greater Antilles, and *Nesotes* beetles (Rees et al., 2001) in the Canary Islands. Despite the wealth of studies documenting insular speciation, few of them are focused in marine organisms (Kay and Palumbi, 1987; Johnson et al., 2001; Robertson, 2001; Vallejo, 2001), and textbook examples are largely wanting.

Cape Verde is an oceanic archipelago located in the Central Atlantic separated from the nearest mainland (Senegal, West Africa) by about 450 Km. This archipelago comprises 10 islands plus eight islets (Fig. 1) that are organized into two chains that are believed to become progressively younger towards the West due to the Eastward movement of the North Atlantic oceanic lithospheric plate (Plesner and Wilson, 1998). According to available K–Ar and ^{40}Ar–^{39}Ar geochronological data, the westernmost islands of Brava and Santo Antão are the youngest (5.9 ± 0.1 and 7.56 ± 0.56 million years, respectively), whereas the easternmost islands of Sal (25.6 ± 1 million years), Maio (21.1 ± 6.3 million years), and Santiago (10.3 ± 0.6 million years) represent the oldest islands of the chain (Griffiths et al., 1975; Grunau et al., 1975; Mitchell-Thomé, 1976; Stillman et al., 1982; Mitchell et al., 1983; Carracedo, 1999; Torres et al., 2002). Age estimates of the remnant five islands, however, are still needed in order to confirm the trend in age progression of both chains from east to west (Plesner et al., 2002).

Cape Verde together with the Canary Islands, Madeira, and the Azores belong to the Macaronesian biogeographic region, being the southernmost of the archipelagos. Darwin also visited Cape Verde during his voyage around the world, and he considered these volcanic islands to be "utterly sterile" in terms of terrestrial habitats but most interesting in terms of marine organism diversity (Darwin, 1845). In contrast to this early observation, the few evolutionary studies centered on Cape Verde archipelago have focused on terrestrial (e.g., Brown and Pestano, 1998; Carranza et al., 2000; Brehm et al., 2001; Hille et al., 2003) rather than marine (e.g., van der Strate et al., 2002) fauna. Furthermore, remarkable putative marine radiations occurring in Cape Verde such as those observed in gastropods belonging to the subfamily Rissoininae (29 endemic species; Rolán and Luque, 2000) and the benthic venomous snail genus *Conus* (49 endemic species; Rolán, 1980, 1990) have received little attention.

Cape Verde *Conus* represent about 10% of the worldwide species diversity in the genus (Kohn and Perron, 1994). Of the 52 described species of *Conus* in Cape Verde, only three (*C. ermineus*, *C. genuanus*, and *C. tabidus*) are nonendemics (Monteiro et al., 2004) (Table 1). The great diversity of *Conus* in Cape Verde, and not in other Macaronesian islands (only two extant species are found in the rest of Macaronesia, specifically in the Canary Islands), could be related to the reduced number of natural competitors, such as members of the family Turridae or Terebridae (both families belonging to the Superfamily

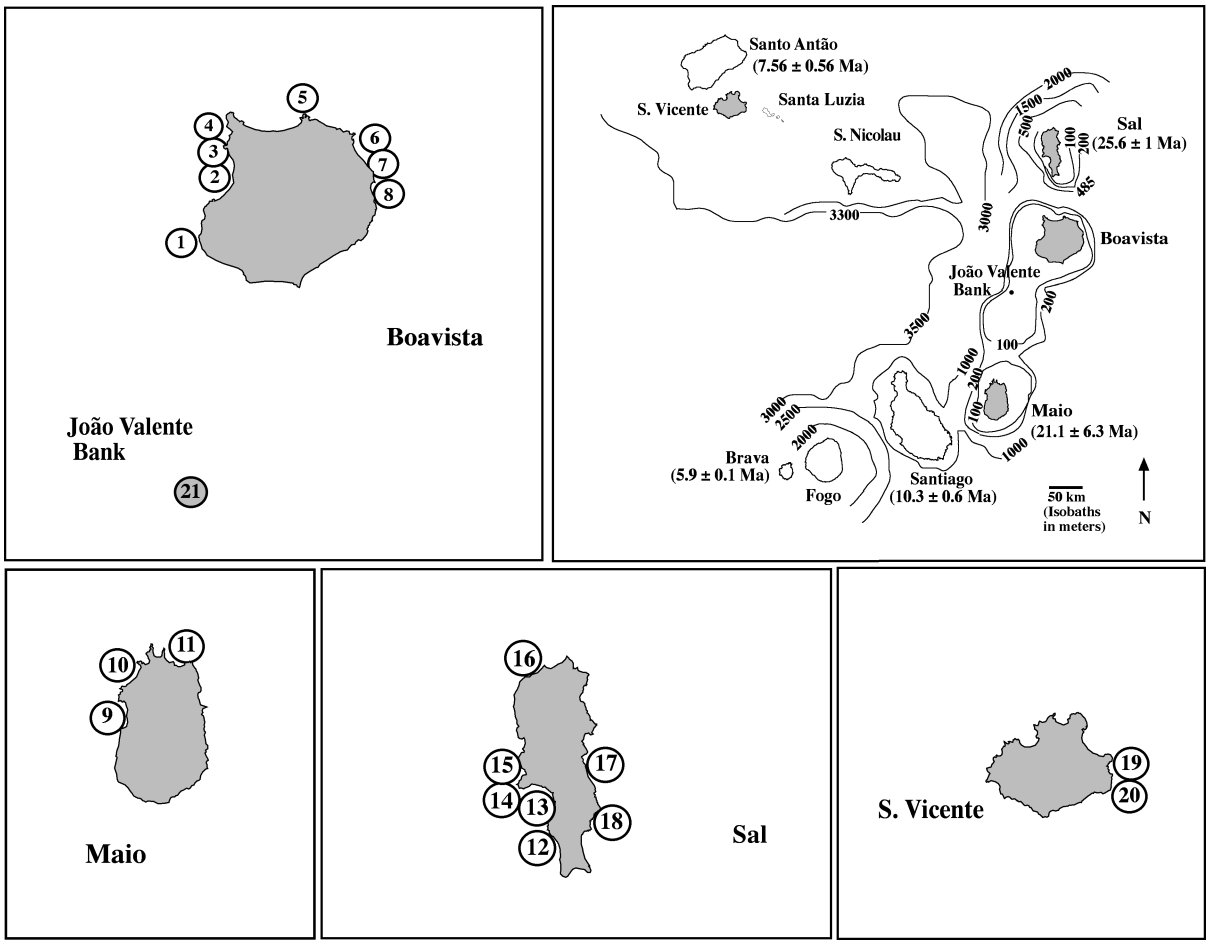

FIGURE 1. Map and bathymetry of the Cape Verde archipelago. Numbers indicate sample localities as listed in Table 1.

Conoidea), in the archipelago (Rolán, 1992). Two main morphological groups based on shell size are found in the archipelago (Röckel et al., 1980; Rolán, 1992). One group includes species with shells of an average individual size between 10 and 29 mm, which henceforth we will refer as "small" shelled species. The other group includes species with shells of an average individual size between 35 and 75 mm, which henceforth we will refer as "large" shelled species (also known as "*venulatus*" complex). In the "small shell" group, all species except two (*C. irregularis* and *C. josephinae*) are confined to a single island or even a single bay within an island (Table 1) (Monteiro et al., 2004). Certain species belonging to the "large shell" group can be found on several islands, and usually in more than one bay on a given island (Table 1). All Cape Verde endemic species are preferentially vermivorous (prey on polychaete annelids) (Röckel et al., 1980; Rolán, 1992).

The evolutionary factors underlying the generation of the remarkable species diversity of *Conus* in Cape Verde remain poorly understood. The presence (planktotrophy) or absence (lecithotrophy) of a pelagic larval stage may play a significant role in dispersal, species range, and rate of speciation in marine gastropods (Jablonski, 1986), and could be a major factor promoting diversification of *Conus* in Cape Verde. All endemic species of *Conus* in Cape Verde exhibit a nonplanktonic lecithotrophic developmental mode (Rolán, 1992; Kohn and Perron, 1994) that likely reduces the dispersal capability of the larvae, as well as gene flow among populations.

Further understanding of the origin and dynamics of speciation of the highly diverse genus *Conus* in Cape Verde can only be addressed within a robust phylogenetic framework (Emerson, 2002). Thus far, the only phylogenetic study focusing on Cape Verde *Conus* was based on 39 morphological characters including radula, larval shell, egg capsule and shell morphology (Rolán, 1992). The results of this study indicated that *Conus* species could be grouped based on shell size, but failed to recover any further phylogenetic or biogeographic structure. In general, phylogenetic studies of *Conus* based on morphology have been seriously hindered by convergence (Röckel et al., 1995). In this regard, phylogenetic studies based on sequence data may be helpful in resolving evolutionary patterns in *Conus*. However, thus far, molecular studies have been focused on the evolution of peptide neurotoxins ("conotoxins") (Duda and Palumbi, 2000; Conticello et al., 2001; Espiritu et al., 2001; Oliveira, 2002;

TABLE 1. List of species used in this study, species distribution, individual average of shell size, GenBank accession numbers, and respective vouchers.

Conus species	Bay	Island/country	Map location	Species distribution	Individual average shell size (mm)	GenBank accession number			Voucher
						12S + VAL + 16S	Cyt *b*	Nuclear	
C. antoniomonteiroi	Parda	Sal	17	Sal (East)	17	AY726440	AY998563	AY726493	MNCN/ADN: 6987
C. ateralbus	Calheta Funda	Sal	12	Sal (SW)	47	AY726441	AY998564	AY726494	MNCN/ADN: 6988
C. boavistensis	Ervatão cidade	Boavista	3	Boavista	14	AY726442	AY998565	AY726495	MNCN/ADN: 6989
C. borgesi	Porto Ferreira	Boavista	8	Boavista (NE)	25	AY726443	AY998566	AY726496	MNCN/ADN: 6990
C. crotchii	Morro Areia	Boavista	1	Boavista (SE)	23	AY726445	AY998567	AY726498	MNCN/ADN: 6991
C. cuneolus	Murdeira	Sal	13	Sal	23	AY726446	AY998568	AY726499	MNCN/ADN: 6992
C. damottai damottai	Gatas	Boavista	6	Boavista (1 bay)	18	AY726447	AY998569	AY726500	MNCN/ADN: 6993
C. damottai galeao	Navio Quebrado	Maio	11	Maio (1 bay)	21	AY726448	AY998570	AY726501	MNCN/ADN: 6994
C. decoratus	Saragarça	São Vicente	20	São Vicente (1 bay); Santa Luzia	24	AY726449	AY998571	AY726502	MNCN/ADN: 6995
C. delanoyi	Gatas	Boavista	6	Boavista (Gatas; Derrubado)	25	AY726450	AY998572	AY726503	MNCN/ADN: 6996
C. derrubado	Derrubado	Boavista	5	Boavista (1 bay)	23	AY726451	AY998573	AY726504	MNCN/ADN: 6997
C. diminutus	Teodora	Boavista	4	Boavista (NW)	12	AY726452	AY998574	AY726505	MNCN/ADN: 6998
C. evorai	Ilheu Galeão-Gatas	Boavista	6	Boavista (1bay)	17	AY726454	AY998576	AY726506	MNCN/ADN: 6999
C. fantasmalis	Porto Cais	Maio	10	Maio (1 bay)	25	AY726455	AY998577	AY726507	MNCN/ADN: 7000
C. felitae	Rabo Junco	Sal	14	Sal (Rabo Junco; J. Petinha)	13	AY726456	AY998578	AY726508	MNCN/ADN: 7001
C. fontonae	Joaquim Petinha	Sal	15	Sal (W)	19	AY726457	AY998579	AY726509	MNCN/ADN: 7002
C. fuscoflavus	Derrubado	Boavista	5	Boavista (NW)	24	AY726458	AY998580	AY726510	MNCN/ADN: 7003
C. genuanus	João Valente	Submarine Bank	22	Sal; Boavista; Santiago; S. Vicente; J. Valente West Africa (Senegal to Angola); S. Tomé e Príncipe	43	AY726459	AY998581	AY726511	MNCN/ADN: 7004
C. grahami grahami	Calhau	S. Vicente	19	São Vicente (1 bay)	21	AY726460	AY998582	AY726512	MNCN/ADN: 7005
C. guanche	Tenerife	Canarias	—	Canary Islands	27	AY726461	AY998583	AY726513	MNCN/ADN: 7006
C. iberogermanicus	Derrubado	Boavista	5	Boavista (West)	25	AY726462	AY998584	AY726514	MNCN/ADN: 7007
C. infinitus	Pau Seco	Maio	9	Maio (North)	20	AY726463	AY998585	AY726515	MNCN/ADN: 7008
C. irregularis	Gatas	Boavista	6	Boavista (NW); Maio (NW)	29	AY726464	AY998586	AY726516	MNCN/ADN: 7009
C. josephinae	Teodora	Boavista	4	Boavista (NW); Maio (NW)	23	AY726465	AY998587	—	MNCN/ADN: 7010
C. longilineus	Rabo Junco	Sal	14	Sal (East)	15	AY726466	AY998588	AY726517	MNCN/ADN: 7011
C. lugubris	Saragarça	S. Vicente	20	São Vicente (1 bay)	21	AY726467	AY998589	AY726518	MNCN/ADN: 7012
C. luquei	Praia Canto	Boavista	7	Boavista (1 bay)	25	AY726468	AY998590	AY726519	MNCN/ADN: 7013
C. maioensis	Porto Cais	Maio	10	Maio (Porto Cais; Navio Quebrado)	27	AY726469	AY998591	AY726520	MNCN/ADN: 7014
C. mercator	Dakar - Senegal	Senegal	—	Senegal	29	AY726470	AY998592	AY726521	MNCN/ADN: 7015
C. messiasi	Derrubado	Boavista	5	Boavista (1 bay)	24	AY726471	AY998593	AY726522	MNCN/ADN: 7016
C. miruchae	Palhona	Sal	16	Sal (Calhau; Saragarça); Santa Luzia (West)	10	AY726472	AY998594	AY726523	MNCN/ADN: 7017
C. mordeirae	Mordeira	Sal	13	Sal (1 bay)	24	AY726473	AY998595	AY726524	MNCN/ADN: 7018
C. navarroi calhetae	Navio Quebrado	Maio	11	Maio (NW)	23	AY726474	AY998596	AY726525	MNCN/ADN: 7019
C. navarroi navarroi	Calhau	S. Vicente	19	S. Vicente (Calhau; Saragarça); Santa Luzia (SE)	15	AY726475	AY998597	AY726526	MNCN/ADN: 7020
C. pseudocuneolus	Serranegra	Sal	18	Sal (East)	27	AY726476	AY998598	AY726527	MNCN/ADN: 7021
C. pulcher siamensis	Tenerife	Canarias	—	Canary Islands; West Africa	43	AY726477	AY998599	AY726528	MNCN/ADN: 7022
C. raulsilvai	Porto Cais	Maio	10	Maio (Pau Seco; Navio Quebrado)	25	AY726478	AY998600	AY726529	MNCN/ADN: 7023
C. regonae	Joaquim Petinha	Sal	15	Sal	21	AY726479	AY998601	AY726530	MNCN/ADN: 7024
C. salreiensis	Teodora	Boavista	4	Boavista (NW)	21	AY726481	AY998602	—	MNCN/ADN: 7025
C. serranegrae	Serranegra	Sal	18	Sal (1 bay)	14	AY726482	AY998603	AY726531	MNCN/ADN: 7026
C. teodorae	Teodora	Boavista	4	Boavista (1 bay)	22	AY726484	AY998605	AY726532	MNCN/ADN: 7027
C. trochulus	Ervatão cidade	Boavista	3	Boavista (West)	50	AY726485	AY998606	AY726533	MNCN/ADN: 7028
C. trochulus "nivifer"	Ervatão cidade	Boavista	3	Boavista (NW); Maio (North); Santiago (SE)	45	AY726486	AY998607	AY726534	MNCN/ADN: 7029

C. ventricosus	Algarve	Portugal	—	South Portugal; Mediterranean	28	AY726489	AY998608	AY726536	MNCN/ADN: 7030
C. guanche	Western Sahara	Morocco	—	West Africa; South Portugal; Mediterranean	25	AY726490	AY998609	AY726537	MNCN/ADN: 7031
C. venulatus	Derrubado	Boavista	5	Sal (East); Boavista; Maio (NW); Santiago (East)	75	AY726487	AY998610	AY726535	MNCN/ADN: 7032
C. venulatus "nivifer"	Praia Canto	Boavista	7	Boavista (NW); Maio (N); Sal (NE)	47	AY726488	AY998611	—	MNCN/ADN: 7033
C. xicoi	Luanda, 15 Km N	Angola	—	Luanda—Angola	24	AY726492	AY998612	AY726539	MNCN/ADN: 7034
C. sp. 1	João Valente	Submarine bank	21	—	35	AY726444	AY998613	AY726497	MNCN/ADN: 7035
C. sp. 2	João Valente	Submarine bank	21	—	37	AY726491	AY998614	AY726538	MNCN/ADN: 7036
Outgroups									
C. ermineus	Sal Rei	Boavista	2	Boavista; Sal; Maio; S. Vicente; Caribbean; North Brasil; West Africa (Senegal to Angola);	57	AY726453	AY998575	–	MNCN/ADN: 7037
C. tabidus	João Valente	Bank	21	From Senegal down to Angola	32	AY726483	AY998604	—	MNCN/ADN: 7038
C. sp. 3	João Valente	Submarine bank	21	—	30	AY726480	AY998615	—	MNCN/ADN: 7039
Species not sampled									
C. curralensis	—	—	—	Santa Luzia (NW)	19	—	—	—	—
C. bellulus	—	—	—	Santa Luzia (West); S. Vicente (Saragarça)	18	—	—	—	—
C. saragasae	—	—	—	Santa Luzia (West); S. Vicente (Saragarça; Calhau)	17	—	—	—	—
C. grahami luziensis	—	—	—	Santa Luzia (South)	25	—	—	—	—
C. verdensis verdensis	—	—	—	Santiago (NW)	18	—	—	—	—
C. roeckeli	—	—	—	Boavista (NE)	26	—	—	—	—
C. anthonyi	—	—	—	Sal	10	—	—	—	—
C. verdensis furnae	—	—	—	Brava (NE)	18	—	—	—	—

Species in grey belong to the group of "large" shell.

Duda and Kohn, 2005), shifts in developmental modes, and feeding ecology (Duda and Palumbi, 1999, 2004; Monje et al., 1999; Duda et al., 2001) in species from the Indo-Pacific and the Caribbean Sea.

In the present study, we examined phylogenetic relationships among most of the endemic species of *Conus* from Cape Verde, and with respect to species from West Africa, Canary Islands, and Algarve (south of Portugal). Phylogenetic reconstruction was based primarily on mitochondrial sequence data including cytochrome *b*, 12S rRNA, tRNA-Val, and 16S rRNA genes. We also inferred phylogenetic relationships among *Conus* species based on a novel nuclear marker, a megalin-like protein, a member of the low-density lipoprotein receptor gene family.

The reconstructed molecular phylogeny was used to test whether (1) the species diversity of *Conus* in Cape Verde results from one or multiple successful colonizations to the archipelago; (2) divergence of "small" and "large" shells predated speciation processes in the archipelago; and (3) nonplanktonic lecithotrophy could have enhanced allopatric speciation of *Conus* in Cape Verde by restricting larval dispersal. In addition, we dated major cladogenetic events of *Conus* in Cape Verde in order (1) to establish the tempo and sequence of island colonization that led to the current diversity of these marine gastropods in the archipelago, and (2) to analyze whether the pattern obtained is consistent with the geological age of the islands.

Material and Methods

DNA Sources and Extraction

A total of 41 out of the 49 valid Cape Verde endemic species of *Conus* (Rolán, 1990 and references therein) as well as three undescribed species (*Conus* sp 1, *Conus* sp 2, and *Conus* sp 3) were collected from São Vicente, Sal, Boavista, Maio, and João Valente submarine bank (25 miles SW of Boavista) during 2002 to 2003 (Fig. 1, Table 1). The taxonomic status of the few reported specimens from Fogo, São Nicolau, and Santo Antão (Rolán, 1992) is still controversial. Hence, they were not included in this study. In addition, all three nonendemic species (*C. ermineus*, *C. genuanus*, and *C. tabidus)* that occur in Cape Verde, and several species from Senegal, Western Sahara, Angola, Canary Islands, and Algarve were analyzed in this study (Table 1). Taxonomy followed Rolán (1980, 1990, 1992). Whenever possible, and to avoid misleading taxonomic classifications, specimens were collected from type localities. Sampling sites are shown in Figure 1. All specimens were preserved in 98% ethanol. Total genomic DNA was extracted from muscle tissue with a DNA Easy extraction Kit (Qiagen).

PCR Amplification and Sequencing

A mitochondrial fragment of 1713 bp (including the 3′ end of the 12S rRNA, the complete tRNA-Val, and the 5′ portion of the 16S rRNA genes) was obtained by PCR amplification of three overlapping fragments with the primers shown in Table 2. In addition, a partial 335-bp fragment of the mitochondrial cytochrome *b* gene, and partial 452-bp portion of the nuclear megalin-like lipoprotein gene were PCR amplified with the primers given in Table 2. Mitochondrial sequence data were obtained from 53 *Conus* species, whereas the nuclear fragment was sequenced in 46 taxa because PCR amplification was unsuccessful in four ingroup species, and all outgroup taxa (Table 1). All PCR amplifications were conducted in 25-μL reactions containing 7.5 mM Tris-HCl (pH 9.0), 2 mM $MgCl_2$, 0.4 mM of each dNTP, 0.4 μM of each primer, template DNA (10 to 100 ng), and Taq DNA polymerase (1 unit, Biotools), using the following program: 1 cycle of 5 min at 94°C, 35–40 cycles of 30 s at 94°C, 30–60 s at 45–50°C, and 60 s at 72°C, and finally, 1 cycle of 5 min at 72°C.

TABLE 2. Polymerase chain reaction primers used for amplification of the mitochondrial 12S rRNA, tRNA-Val, 16S rRNA, cytochrome *b*, and the nuclear fragment of a megalin-like protein in the genus *Conus*.

Primer	Reference
12S rRNA; tRNA-VAL; 16S rRNA	
L1067	Kocher et al., 1989
H1478	Kocher et al., 1989
16Sar	Palumbi, 1996
16Sbr	Palumbi, 1996
CONUS 12S-F (GGT GAA GAT GGG TTA CAA TTA	This study
CONUS 16S-R (CTA CCT TTG CAC GGT CAG AGT	This study
CYT *b*	
151F	Merrit et al., 1998
270R	Merrit et al., 1998
272R	Merrit et al., 1998
Nuclear lipoprotein	
CONUS nuc-F (CTT TTA TCA TTT CAC TAA TAC TAG	This study
CONUS nuc-R (AAA AAC GAC TTC CCA CAA ACA GG	This study

After PCR purification using ethanol/sodium acetate precipitation, samples were sequenced directly using the corresponding PCR primers. Samples were cycle-sequenced with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (V3.0) in 10-μL reactions, and following manufacturer's instructions (Applied Biosystems), with 3.25 pmol of primer, 3 μL of Terminator Ready Reaction Mix, and 5% DMSO. The cycling profile for the sequencing reaction consisted of 25 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. Cycle sequencing products were purified using MultiScreen plates (Millipore), and were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). Sequences were deposited in GenBank under the accession numbers given in Table 1.

Phylogenetic Analysis

DNA sequences were aligned using CLUSTAL X version 1.81 (Thompson et al., 1997) with default settings, and alignments were further optimized by eye. Ambiguously aligned (gap-rich) regions were identified using the criteria of Lutzoni et al. (2000). Parsimony analyses

(see running conditions below) performed with these regions (weighted with a step matrix estimated using INAASE 2.4b (Lutzoni et al., 2000) and without them produced identical topologies. Therefore, these regions were excluded from further phylogenetic analyses.

Preliminary phylogenetic analyses based on partial mitochondrial 16S rRNA gene sequences (444 bp) of all the species included in our study, and additional 69 Indo-Pacific *Conus* species retrieved from GenBank were used to establish the most appropriate outgroup taxa for our study. To this end, a maximum likelihood (ML) analysis using Phyml (Guindon and Gascuel, 2003), and the HKY+I+Γ model (the best-fit model as selected by Modeltest 3.6 (Posada and Crandall, 1998); Ti:Tv = 4.09, α = 0.49, Pinvar = 0.53) was performed. All taxa included in the present study were recovered in a monophyletic group except the Cape Verde nonendemics *Conus ermineus*, *Conus tabidus*, and *C*. sp. 3 (not shown). These species were therefore selected as outgroup taxa for further phylogenetic analyses.

Three distinct nucleotide sequence data sets were analyzed: (1) all mitochondrial genes combined; (2) the nuclear gene; and (3) the mitochondrial and nuclear sequence data combined.

Maximum parsimony (MP) analyses.—Gaps were treated as missing data. Step matrices that weighted each nucleotide substitution by their relative frequencies were estimated for the mitochondrial data set using STMatrix 2.2 (Lutzoni and Zoller, 2001). The weights of the step matrix were as follows: A-C 2.55, A-G 1.22, A-T, 1.89, C-G 3.08, C-T 1.26, T-G 2.34. No weighting scheme was applied to the nuclear data set because nucleotide substitutions showed equal relative frequencies. The same weighting schemes were applied to the mitochondrial and nuclear sequences in the combined data set. Heuristic searches for the MP trees were conducted in PAUP* 4.0 b10 (Swofford, 1998) using the TBR branch-swapping algorithm, MULTREES option in effect, and 10 random stepwise additions of taxa. Robustness of the inferred trees was tested using nonparametric bootstrapping proportions (BP) (Felsenstein, 1985) with 500 pseudoreplicates.

Maximum likelihood (ML) analyses.—Phyml (Guindon and Gascuel, 2003) was used to estimate the ML tree, and to test by nonparametric bootstrapping the robustness of the inferred trees using 500 pseudoreplicates. The Akaike information criterion (Akaike, 1974) implemented in ModelTest 3.6 (Posada and Crandall, 1998) selected TVM+I+Γ as the evolutionary model that best fit the mitochondrial and combined data sets. Because this model is not available in Phyml, the GTR+I+Γ (the second best-fit model) was used in the ML analyses of those data sets. TVM was selected as the model that best fit the nuclear data set, and GTR (the second best-fit model) was used in the ML analyses with Phyml. Inferred model parameters that were used in the ML analyses are shown in Table 3.

Bayesian analysis.—Bayesian inferences (BI) were conducted using MrBayes v3.0b3 (Huelsenbeck and Ronquist, 2000) by Metropolis-coupled Markov chain Monte Carlo (MCMCMC) sampling for 10^6 generations (four simultaneous MC chains; sample frequency 100; chain temperature 0.2). Five independent runs were performed with each data set. The mitochondrial data set was analyzed under the GTR+I+Γ model, and the burnin was 40,000 generations. The nuclear data set was analyzed under the GTR model, and the burnin was 50,000 generations. The mitochondrial and nuclear data partitions of the combined data set were analyzed with the GTR+I+Γ and GTR models, respectively. Model parameters were estimated independently for the two data partitions using the "unlink" command in MrBayes. The burnin in this analysis was 50,000 generations. Robustness of the inferred trees was evaluated using Bayesian posterior probabilities (BPPs).

TABLE 3. Evolutionary models and estimated parameters.

Estimated parameters	Mitochondrial data*		Nuclear data*		Combined data*	
No. species used in this study	53	53	46	46	46	46
No. bases sequenced	2048	2048	452	452	2500	2500
No. bases parsimony informative	477 (23%)	477 (23%)	21 (4.6%)	21 (4.6%)	394 (16%)	394 (16%)
Best-fit model	TVM+I+G	GTR+I+G	TVM	GTR	TVM+I+G	GTR+I+G
Nucleotide frequency						
A	0.346	0.349	0.299	0.300	0.335	0.335
C	0.139	0.135	0.246	0.245	0.157	0.157
G	0.160	0.163	0.158	0.158	0.169	0.169
T	0.356	0.353	0.297	0.296	0.339	0.339
Gamma shape (G)	0.800	0.784	—	—	0.839	0.837
Prop. invariable sites (I)	0.503	0.500	—	—	0.597	0.597
R-matrix						
[A-C]	1.618	1.721	0.000	0.000	1.337	1.347
[A-G]	16.321	15.650	3.795	3.487	14.649	14.495
[A-T]	0.855	0.874	0.000	0.000	0.520	0.522
[C-G]	2.216	2.309	1.202	1.202	1.825	1.833
[C-T]	16.321	18.222	3.795	4.008	14.649	14.926
[G-T]	1.000	1.000	1.000	1.000	1.000	1.000

*First column indicates best-fit model inferred from Modeltest 3.6. Second column indicates model used in ML inferences with Phyml 2.4.3.

Correlation between Shell Size and Cape Verde Conus *Phylogeny*

In order to evaluate whether there is a significant clustering of Cape Verde *Conus* species according to shell size, we used MacClade v. 4.03 (Maddison and Maddison, 2001) to trace this morphological character considering two character states ("small" and "large") onto the BI phylogeny that was inferred based on the mitochondrial data set (because it maximizes the number of analyzed taxa, and it is almost identical to the BI topology recovered based on the combined data set). The character state "small" was associated to species with an average individual size between 10 and 29 mm whereas the character state "large" was attributed to species with an average individual size between 35 and 75 mm. Tree lengths were compared with the mean number of steps of the null distribution obtained with 1000 random trees generated in MacClade.

Rates of Evolution and Divergence Time Estimation

To evaluate whether our sequence data met the assumptions of a constant rate of evolution, a likelihood ratio test (LRT; Huelsenbeck and Crandall, 1997) was performed using PAUP* 4.0b10, and the inferred ML tree based on the mitochondrial data set with and without a molecular clock constraint.

In order to date cladogenetic events within *Conus* upon colonization of Cape Verde, and since the LRT rejected the molecular clock hypothesis, we used a Bayesian methodology that incorporates variation of rates of evolution among genes and among lineages (Kishino et al., 2001). We used the ML topology that was inferred based on the mitochondrial data set as the starting phylogeny. Following Thorne and Kishino (2002), PAML v.3.14 (Yang, 1997) was employed to estimate ML parameters using a discrete gamma distribution with five rate categories (Yang, 1994), and the F84 model of nucleotide substitution. This model was selected because of computational tractability (Wiegmann et al., 2003). Branch lengths of the inferred topology were estimated separately based on each mitochondrial gene using the ESTBRANCHES program (Kishino et al., 2001; Thorne and Kishino, 2002). Subsequently, the MULTIDIVTIME program was used to estimate divergence times by combining the four genes. This strategy assumes that the distributions of evolutionary rates among genes are uncorrelated. Bayesian method requires also the specification of prior distributions for parameters. The prior assumption for the mean and standard deviation of the time of the ingroup root node (rttm) was set to 5.5 time units, where 1 time unit in this analysis represents 10 million years (My). This value was obtained based on the earliest *bona fide* fossil record of *Conus*: *C. rouaulti* (France) and *C. concinnus* (England) Sowerby, 1821, from the Lower Eocene (55 My) (Kohn, 1990). The standard deviation of the prior distribution was set to its maximum value (equal to the mean) to avoid violation of the definition of a prior. *Conus* fossils are common in the Cenozoic, but only two of the species in our phylogeny are known from the fossil record. These were used as calibration points: *Conus (Lithoconus) pulcher* Lightfoot, 1786, dated from the Lower Pliocene (5.32 to 3.2 My) of Cuenca de Siena (Italy) (Spadini, 1990); Velerín (Estepona, Spain) (Muñiz Solís, 1999) and *Conus (Chelyconus) ventricosus* Gmelin, 1791, an extant species that occurs in Algarve (South of Portugal) reported from the Middle-Lower Miocene (16.4 to 20.5 My) of Cuenca de Piemonte (Italy) (Sacco, 1893). The MCMC method was employed to approximate both prior and posterior distributions (Kishino et al., 2001). Initial parameter values were randomly selected to initialize the Markov chain and then, a burn-in period of 100,000 cycles was completed before parameters were sampled from the MCMC chain. Afterwards, the state of the Markov chain was sampled every 100 cycles until a total of 10,000 samples were collected.

Testing Diversification Rate Through Time

We perform tests of diversification rates based upon the Bayesian-inferred linearized tree taking incomplete taxon sampling into account. To this end, we followed the procedures proposed by Pybus and Harvey (2000) using the CR (constant-rate) and MCCR (Monte Carlo constant-rate) tests. First, End-Epi 1.0 (Rambaut et al., 1997) was used for generating a semilogarithmic lineage through time (LTT) plot. Then, the value of statistic γ was calculated using Genie v3.0 (Pybus and Rambaut, 2002). Finally, the MCCR test was conducted by calculating the γ distribution with 10,000 replicates using MCCRTest (Pybus, 2000).

RESULTS

Mitochondrial Data Set

The partial sequences of the mitochondrial cytochrome *b* and 12S and 16S rRNA genes, and the complete sequences of the tRNA-Val gene were combined into a single data set that produced an alignment of 2048 positions. Of these, 97 were excluded from the analyses because of uncertainty in positional homology, 1335 were invariant, and 477 were parsimony informative. Ingroup sequence divergences (measured as uncorrected *p* distances) varied between 11% (*Conus raulsilvai* versus *Conus venulatus "nivifer"*) and 0.05% (*C*. sp 2 versus *Conus trochulus "nivifer"*). Intraspecific sequence divergence was checked among five individuals of *Conus diminutus* (0.32% ± 0.16). The sequence divergence between *C. diminutus* and its closest sister group species, *Conus boavistensis*, was 0.56% ± 0.09. Mean pairwise sequence divergence between "small" and "large" shelled species was 10.5% ± 0.003%. Mean pairwise sequence divergences within "small shell" and "large shell" groups were 4.2% ± 0.026% and 1.7% ± 0.05%, respectively. No evidence of saturation was observed in a plot of pairwise transitions and transversions against ML distances (not shown).

BI based on mitochondrial sequence data recovered the tree ($-\ln L = 9558.36$) shown in Figure 2. Two main clades that included "large" and "small" shelled species,

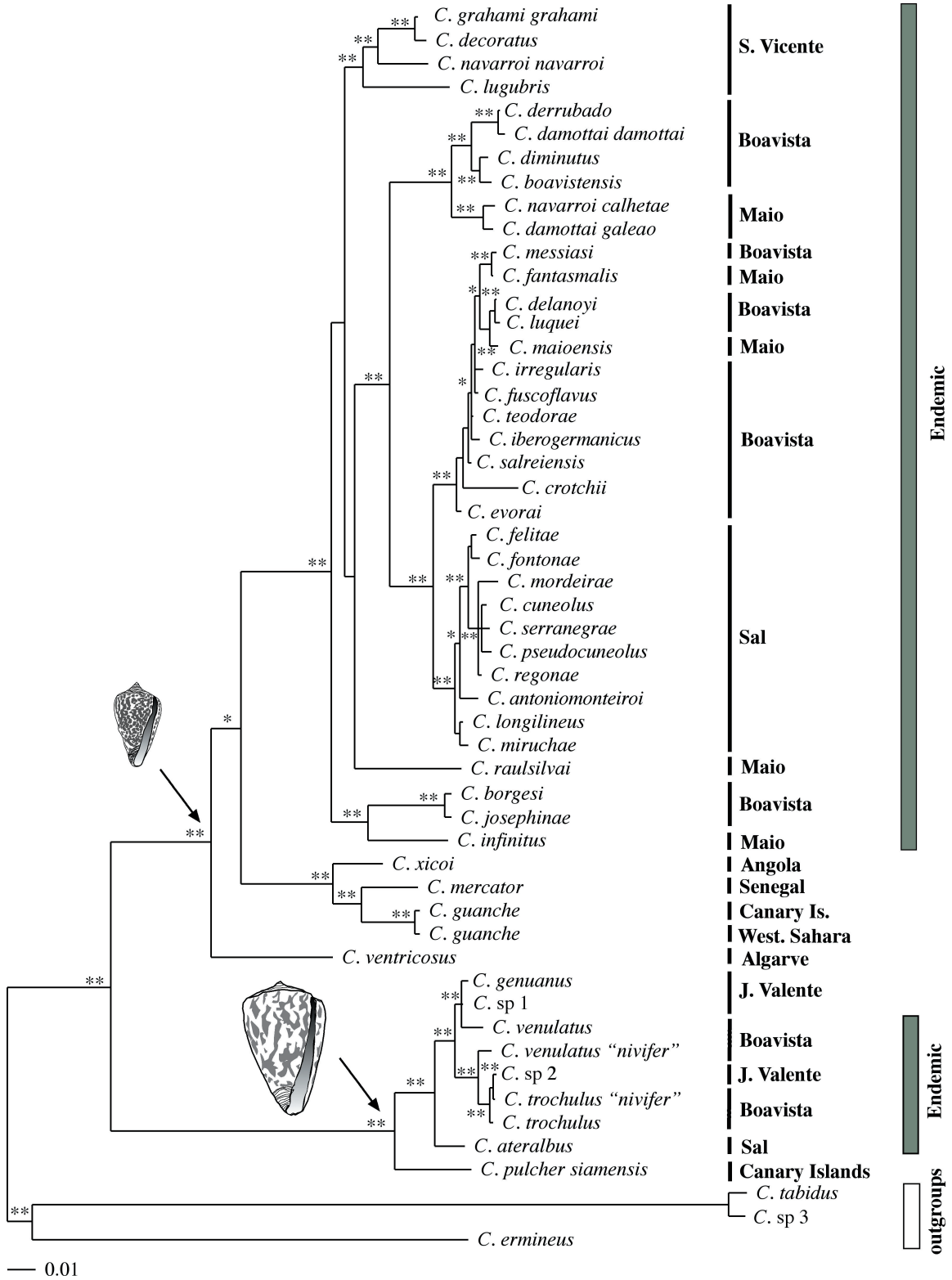

FIGURE 2. Phylogenetic relationships of *Conus* from Cape Verde based on a Bayesian inference analysis of mitochondrial 12S rRNA, tRNA-Val, 16S rRNA, and cyt*b* gene sequence data using the GTR+I+Γ evolutionary model. Statistical support of the nodes is indicated above branches. One asterisk indicates MP and ML bootstrap values between 50% and 70% and posterior probabilities between 90% and 95%; two asterisks indicate MP and ML bootstrap values above 70% and posterior probabilities above 95%. The "small" and "large" shelled clades, origins of each species, and endemic species are indicated.

respectively, were recovered within the studied taxa, both supported by a BPP of 100%. The "large shell" clade comprised Cape Verde endemic *Conus* of the "*venulatus*" group as well as the nonendemic *C. genuanus* whose distribution extends to Senegal, and *C. pulcher siamensis* from Canary Islands (Fig. 2). The latter species was recovered as the most basal lineage within this group. The "small shell" clade included Cape Verde endemic *Conus* species as well as species from West Africa (Angola, Senegal, Western Sahara), *C. guanche* from Canary Islands, and *C. ventricosus* from Algarve (Fig. 2). The latter lineages were recovered in a basal position with respect to Cape Verde endemic species. Furthermore, island structure both within the "large shell" and the "small shell" clades was supported by high BPPs (Fig. 2). The only "large" shelled species from Sal was recovered as sister group of species from Boavista and the nearby submarine bank. Within the "small shell" clade, species from Sal and São Vicente islands were recovered as reciprocal monophyletic groups whereas species from Maio and Boavista were polyphyletic.

The ML analysis yielded the same topology ($-\ln L = 9521.39$) as the BI analysis. The weighted parsimony analysis resulted in six MP trees of 1,921.32 steps (CI = 0.58; RI = 0.85). The 50% majority-rule consensus tree arrived at essentially the same topology as the BI tree. Exceptions were: (1) *C. lugubris* that was not recovered as sister group to other São Vicente species (Fig. 2), but as sister group of all Cape Verde endemic "small shells"; and (2) the clade comprised of two species from Boavista (*C. borgesi*, *C. josephinae*), and one from Maio (*C. infinitus*) is not recovered as the most basal lineage of Cape Verde "small" shelled endemics (Fig. 2) but as sister group of *C. raulsilvai* from Maio (not shown). In both cases, the conflicting nodes had low statistical support.

Nuclear Data Set

Partial megalin-like gene sequences produced an alignment of 452 positions, and no gaps were postulated. One heterozygous site was observed in the nuclear sequence of the species from Algarve, and coded as polymorphism for the subsequent phylogenetic analyses (S = C or G). The analyzed data set included 429 invariant sites and 21 parsimony informative sites. Six different haplotypes were found among the 46 sequenced taxa. Sequence divergence between haplotypes (measured as uncorrected *p* distances) varied between 3% (*C. ventricosus*, Western Sahara versus *C. navarroi navarroi*) and 0.22% (*C. navarroi navarroi* versus *C. grahami grahami*). Mean pairwise sequence divergence between "large shell" and "small shell" groups was 3% ± 0.002%. Divergences within "small shell" and "large shell" groups were 1.4% ± 0.012% and 0.88% ± 0.0%, respectively.

BI based on nuclear sequence data recovered the tree ($-\ln L = 805.47$) shown in Figure 3. *Conus* species were grouped into two distinct clades according to shell size. Phylogenetic relationships within each clade were unresolved due to lack of variation among sequences. However, within the "small shell" clade, species from West Africa and the Canary Islands were recovered as sister group of the remaining taxa. Four species, namely *C. navarroi calhetae*, *C. damottai galeao*, *C. damottai damottai*, and *C. maioensis*, also formed a distinct clade. Interestingly, *C. ventricosus* from Algarve, which in the phylogenetic analyses based on mitochondrial sequence data was resolved in a basal position with respect to all other "small" shelled species, in the nuclear tree was recovered within Cape Verde endemic "small" shelled species. ML and MP analyses rendered identical results.

Combined Data Set

The mitochondrial and nuclear sequences were combined into a single data set that was subjected to BI, ML, and MP. The reconstructed BI tree ($-\ln L = 8752.91$) is depicted in Figure 4. The resulting topology showed the split into two major clades in agreement with phylogenetic analyses based on separate mitochondrial and nuclear sequence data sets, and also revealed island structure in agreement with the recovered tree based on the mitochondrial sequence data set. However, it differed from the mitochondrial-based trees on the relative phylogenetic position of *C. ventricosus* from Algarve that was placed within the "small shell" clade between West African/Canary Island and Cape Verde *Conus* lineages (Fig. 4). This alternative phylogenetic position is in agreement with the nuclear-based trees, but not statistically supported. The weighted parsimony analysis arrived at six MP trees of 1381.33 steps; CI = 0.60; RI = 0.87). The 50% majority-rule consensus topology was identical to the one based only on mitochondrial sequence data. The ML analysis ($-\ln L = 8686.66$) resulted in an almost identical topology to the ones recovered by the BI and MP. The only difference was the relative position of *C. raulsilvai* that was placed as sister group of species of São Vicente (not shown).

Correlation between Shell Size and Conus *Evolution in Cape Verde*

The tracing of the two-character states "small" and "large" shell size in the Cape Verde *Conus* phylogeny resulted in a 1-step tree, which was not in the 95% confidence interval of the null distribution. This result indicates that shell size is significantly correlated with phylogeny.

Rates of Evolution and Divergence Time Estimates

Nuclear sequences showed at least a 3 to 4 times slower rate of evolution with respect to mitochondrial sequences (by measuring the range of ratios of pairwise uncorrected *p* distances derived from the two data sets without considering nuclear sequence divergences below 0.01). Likelihood ratio tests between ML trees inferred with ($-\ln L = 10{,}077.62$) and without ($-\ln L = 9{,}517.04$) molecular clock enforced based on the mitochondrial data set rejected overall constancy of rates of evolution in the studied taxa ($\delta = 1121.16$, $df = 51$, $P \leq 0.01$).

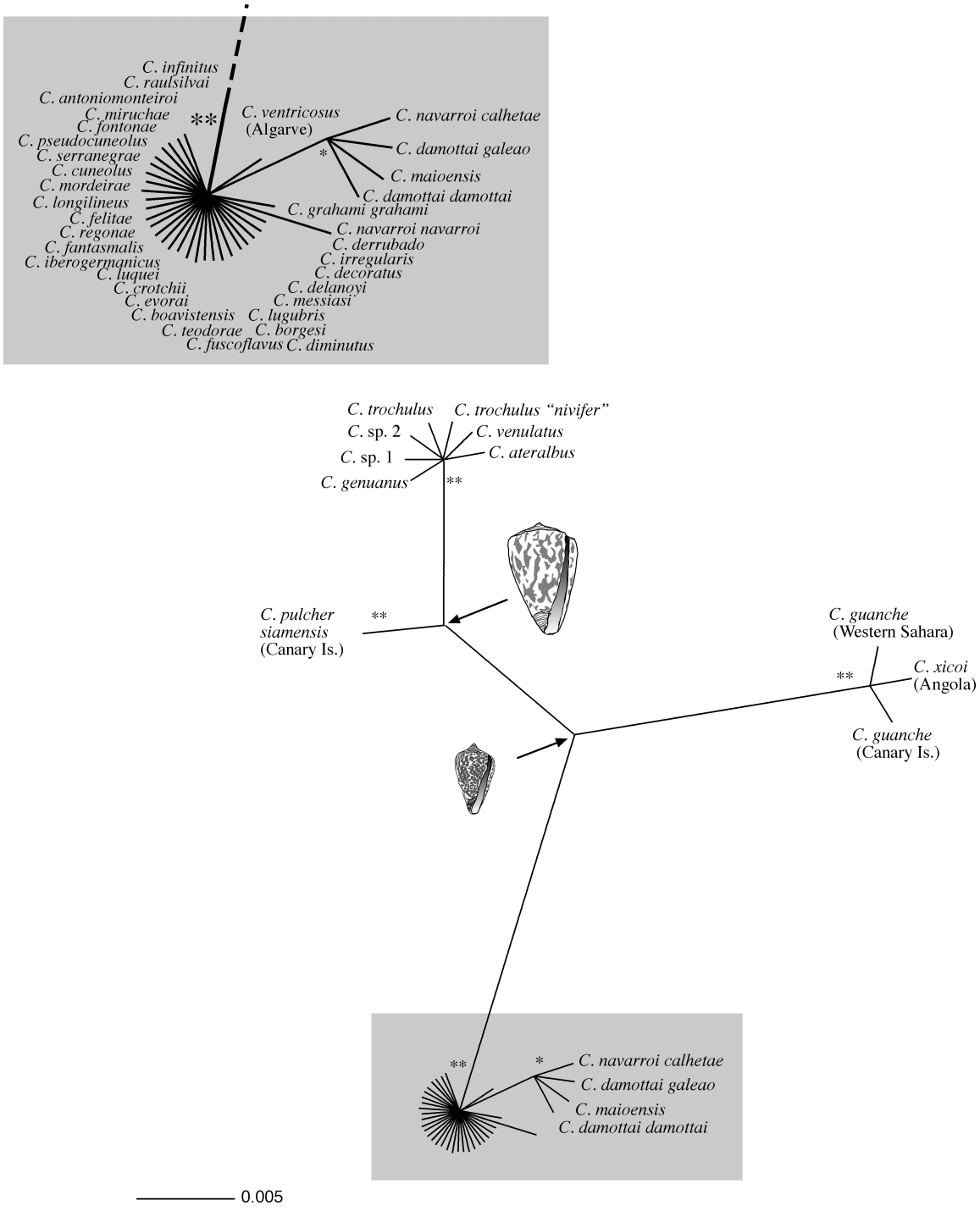

FIGURE 3. Phylogenetic relationships of *Conus* from Cape Verde based on a Bayesian inference analysis of nuclear megalin-like gene sequence data using the GTR evolutionary model. One asterisk indicates MP and ML bootstrap values between 50% and 70% and posterior probabilities between 90% and 95%; two asterisks indicate MP and ML bootstrap values above 70% and posterior probabilities above 95%. The "small" and "large" shelled clades are shown.

No rate heterogeneity among lineages was observed for the nuclear data set because of its reduced sequence variation.

The estimated time for the divergence obtained with MULTIDIVTIME between "small shell" and "large shell" clades was 21.5 (17.5–27) Mya (Fig. 5). The ages of the most recent common ancestors of endemic "small" and "large" shelled species were estimated at 16.5 (14–19) Mya and 4.6 (3.4–5.3) Mya, respectively. The estimated origins of "small" shelled species from São Vicente and

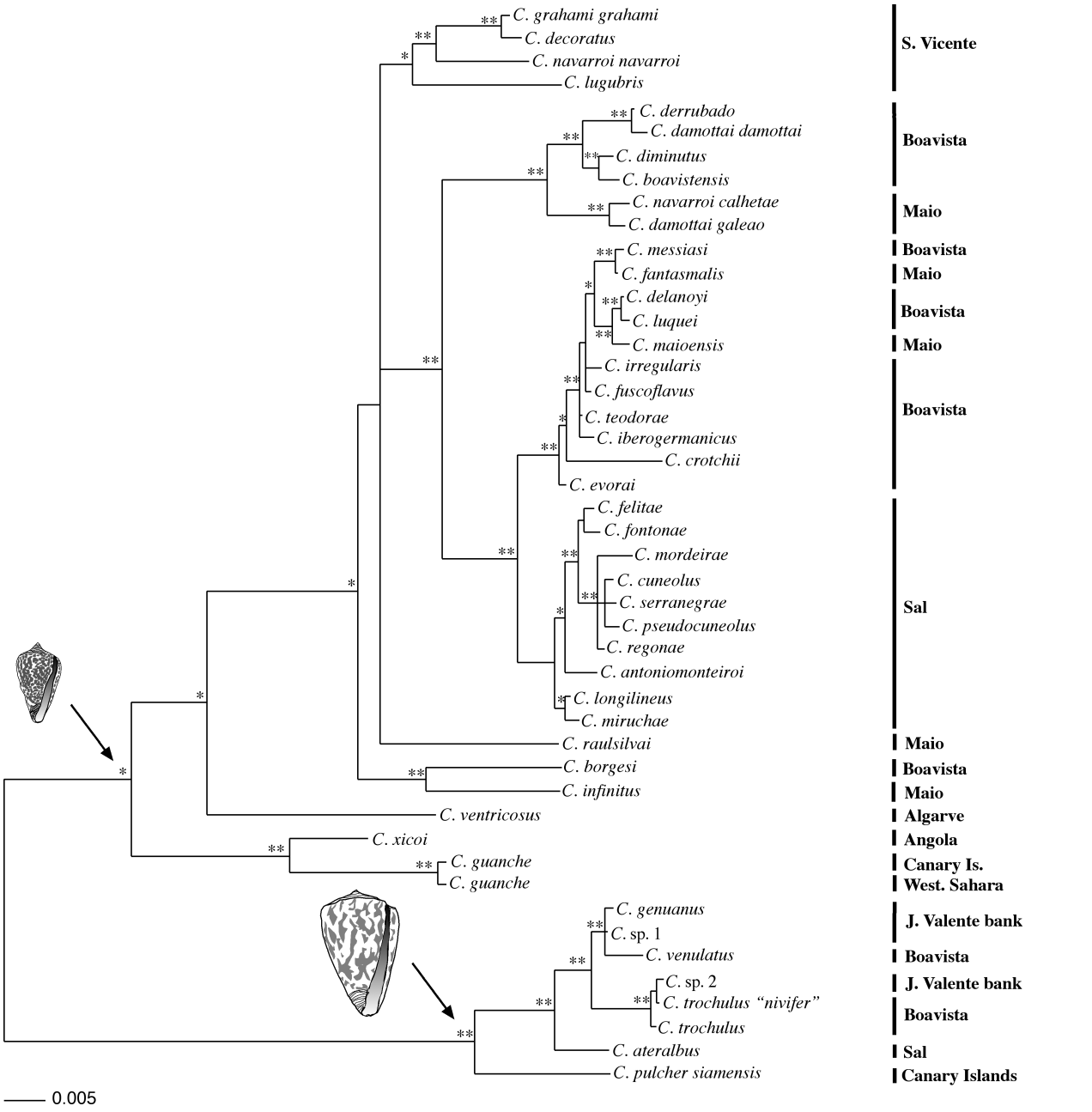

FIGURE 4. Phylogenetic relationships of *Conus* from Cape Verde based on a Bayesian inference analysis of a combined mitochondrial and nuclear data set using the GTR+I+Γ evolutionary model. One asterisk indicates MP and ML bootstrap values between 50% and 70% and posterior probabilities between 90% and 95%; two asterisks indicate MP and ML bootstrap values above 70% and posterior probabilities above 95%. The "small" and "large" shelled clades and the origins of each species are indicated.

Sal were 10.6 (8–14) Mya and 3.8 (1.8–7) Mya, respectively (Fig. 5).

DISCUSSION

Phylogenetic studies of closely related species need to be based on several loci (mitochondrial and nuclear) in order to enhance robustness of the inferences and avoid misleading hypotheses (Moore, 1995). In addition to well-known mitochondrial genes, we used in this study a new molecular marker. A BLAST search shows that this marker is a fragment of a nuclear gene that belongs to the low-density lipoprotein (LDL) receptor gene family (Willnow et al., 1999). Members of this family have been sequenced in all main lineages of vertebrates, but only in few invertebrates (fruit flies, honey bees, and cockroaches). Here, we describe the first megalin-like LDL receptor sequences from mollusks. The

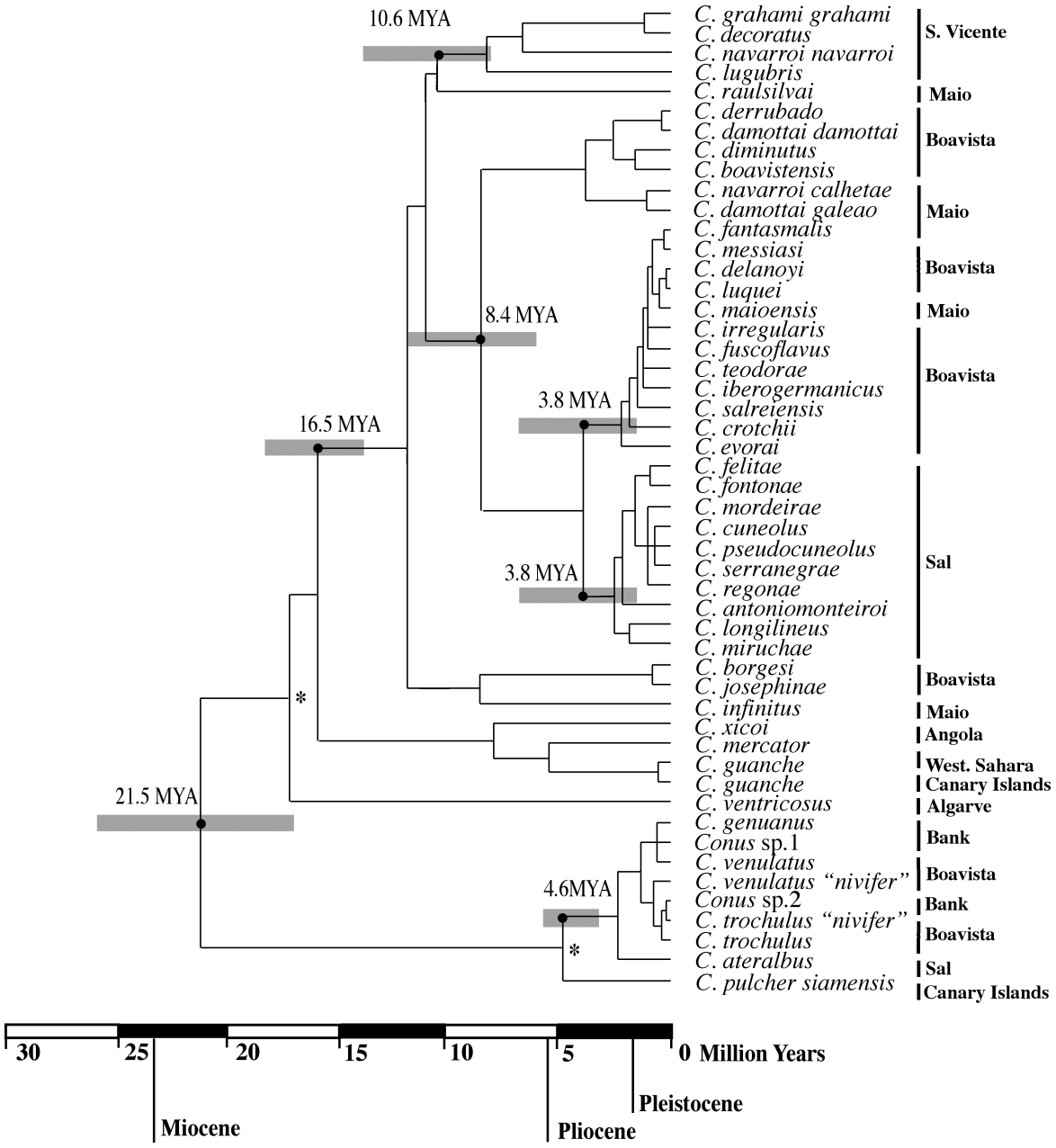

FIGURE 5. Bayesian divergence dating analysis obtained with MULTIDIVTIME. Divergence dates were estimated on the ML topology derived from the combined mitochondrial data set. Asterisks represent minimum age constraints obtained from the fossil record. Grey bars represent the 95% confidence intervals.

mitochondrial-based phylogeny of *Conus* was found to be more resolved than that derived from the nuclear gene, likely due to the increased rates of evolution in the mitochondrial genome. Some sister species of *Conus* showed low levels of genetic divergence that were in the range of those found for *C. diminutus* at the intraspecific level. In all cases, these sister species exhibit distinct egg, larval, and adult morphologies without intermediate forms (Rolán, 1992). Such pattern of little genetic variation coupled with discrete morphological differentiation is likely associated with recent speciation processes (Schluter, 2000). Nevertheless, more detailed studies at the population genetic level, and on mechanisms of reproductive isolation in these taxa are needed in order to fully discard the alternative hypothesis that some of the described species of *Conus* from Cape Verde could represent ecomorphs.

The great number of endemic species of *Conus* in Cape Verde may represent a remarkable example of marine insular radiation. Tree shape methods that take into account incomplete taxon sampling like the CR/MCCR

tests can be used to statistically distinguish bursts of cladogenesis from stochastic background rates. The CR/MCCR tests are based on the statistic γ that measures the relative position of internal nodes in a linearized phylogeny. Under a constant speciation rate model, γ values of complete reconstructed phylogenies follow a standard normal distribution. Positive or negative values of γ indicate that tree internal nodes are closer to the tips (apparent increase in diversification rates towards the present) or to the root (apparent deceleration in diversification rates towards the present) than expected under the null model, respectively. Using a LTT plot (not shown) and the MCCR/CR tests, we are able to detect statistically significant changes in rates of speciation through time in the "small shell" clade (sampled species = 36; total number of species = 44; $\gamma = 1.55$; confidence interval between −1.92 and 1.28). The positive value of the γ statistic may indicate either a recent burst of cladogenesis or high background extinction rates. Because of the intrinsic particularities of insular modes of speciation, it is not easy to distinguish between both competing hypotheses. However, as mentioned above, the small genetic divergence between morphologically distinct sister species seems to favor a recent radiation of *Conus* in Cape Verde. In fact, the LTT plot of the "small shell" clade indicated a marked increase in the rate of cladogenesis throughout the Pleistocene (not shown).

Double Origin of Endemic Cape Verde Conus

The reconstructed phylogenies based on mitochondrial and nuclear data sets agree on the main separation of the studied taxa into two distinct monophyletic groups. According to statistical tests, there is a strong correlation between shell size and the recovered clades. A dendrogram based on morphological characters (Rolan, 1991) also recovered this basal split. Both the "small shell" and "large shell" clades include Cape Verde endemic species as well as species with a wider distribution. The latter are always recovered in a more basal position with respect to the endemics. Therefore, our results are consistent with an independent colonization event of Cape Verde by "small shell" and "large shell" *Conus* lineages, respectively. The ancestor of each lineage diversified upon its arrival to the archipelago, and lead to the current species richness. It is noteworthy that the "small shell" clade includes five times more species, and exhibits almost three times more sequence divergence than the "large shell" clade.

Main Cladogenetic Events in Cape Verde Conus *Consistent with Geological Dates and Eustatic Sea Level Events*

The known geological origins of Cape Verde islands range between 5.8 and 26.6 Mya (Griffiths et al., 1975; Mitchell-Thomé, 1976; Stillman et al., 1982; Carracedo, 1999; Torres et al., 2002). According to our age estimates, the ancestor of the "small shell" clade colonized the archipelago around 16.5 Mya. Estimated dates for major cladogenetic events within the "small shell" clade suggest that there was some delay between the origin of the islands and their colonization by "small" shelled *Conus*. Our dating analysis indicates that this delay is always relatively small when compared with the arrival of terrestrial organisms such as lizards around 4 to 1 Mya (Carranza et al., 2000; Hille et al., 2003). The estimated arrival of the ancestor of "large" shelled *Conus* to Cape Verde was around 4.6 Mya. The later arrival of "large" shelled species to Cape Verde could be related with the recent observation that range limits of large-bodied marine bivalve species are more unstable than small-bodied ones in response to climatic fluctuations (Roy et al., 2001). According to our results, the successful colonization of Cape Verde islands by "large" shelled *Conus*, once "small" shelled *Conus* had already started diverging, can be explained by size assortment and competitive exclusion, as is the case of *Anolis* lizards in the Lesser Antilles (Losos, 1990), rather than by character displacement in sympatry.

Overall, the main cladogenetic events of *Conus* in Cape Verde fairly match with episodes of remarkably low sea levels of −80 m at 10.5 Mya, −50 m at 5.5 Mya, and −30 m at 3.8 Mya (Haq et al., 1987). The interaction between eustatic sea-level fluctuations and bathymetry modifies coastline geomorphology as well as horizontal distances among islands (Graham et al., 2003). During periods of falling sea level, islands increase in area, many currently submerged offshore banks become accessible, and the increased velocity of runoff waters creates a rocky shore. However, as sea level slowly rises extensive beaches are ultimately formed (Graham et al., 2003). Because the preferred habitats of *Conus* communities are rocky reef platforms (Kohn, 1959), the transition between rocky and sandy habitats may be key in understanding diversification of the genus among and within islands.

Nonplanktonic Lecithotrophy and Species Diversification of Conus *in Cape Verde*

Local larval retention is emerging as a major factor promoting speciation in marine organisms (Robertson, 2001; Mora and Sale, 2002; Swearer et al., 2002; Taylor and Hellberg, 2003). Evolutionary shifts from planktotrophy to lecithotrophy have been proposed to be particularly effective in promoting accelerated diversification rates in marine invertebrates (Jablonski and Lutz, 1983; Jablonski, 1986). Planktotrophic development is associated with species that produce very large numbers of small eggs; their planktonic larvae feed on plankton, and have high dispersal abilities that allow them to have wide continuous geographic ranges. Thus, gene flow is maintained among populations, and genetic divergences remain low. In contrast, nonplanktonic lecithotrophy is associated with small numbers of large eggs; larvae are dependant on reserves in the egg, reach metamorphosis without feeding on plankton, and usually have reduced dispersal abilities that lead to more restricted distributions and genetic isolation (Jablonski

and Lutz, 1983; Jablonski, 1986; Scheltema, 1989). Because oceanic archipelagos are isolated, larval dispersal of many marine taxa is necessarily limited to ensure successful settlement into suitable habitats (Swearer et al., 2002). In such instances, the transition between feeding to non-feeding larval development is favored by natural selection. In these cases, reversal to the planktonic stage is highly unlikely (Strathmann, 1985).

Conus have separate sexes, and internal fertilization (Kohn, 1959). The egg capsule is usually attached to a hard substratum. Most *Conus* larvae are obligate planktotrophic, and free-swimming veligers may survive up to 39 days (Kohn, 1959; Kohn and Perron, 1994). In contrast, all Cape Verde endemic species exhibit a nonplanktonic lecithotrophic larval stage (Rolán, 1992; Kohn and Perron, 1994). The observed phylogenetic pattern with species generally clustered into monophyletic island assemblages (Fig. 4) is consistent with a direct effect of nonplanktonic lecithotrophy into the diversification process of *Conus* in Cape Verde. Recruitment may result from the retention of locally produced nondispersing larvae. This process reduces gene flow of *Conus* populations among Cape Verde islands, and enhances within-island speciation over time. The only exception to this general pattern is the case of Maio and Boavista (see polyphyly in trees of Figs. 2 and 4). However, the channel between both islands is relatively shallow (−100 m; Carta Hidrográfica do Arquipélago de Cabo Verde, 1971; Instituto hidrográfico de Lisboa). Hence, these islands were almost certainly connected during periods of lowered sea level at the peak of Pleistocene glaciations, and likely allowed successful larval dispersal and settlement.

It is not possible from our data to determine whether the ancestors of Cape Verde *Conus* "small shell" and "large shell" clades had nonplanktonic lecithotrophy or planktotrophy. However, most isolated oceanic islands support endemics that are closely related to more widespread species with extended planktonic larval periods capable of long-distance dispersal (Scheltema and Williams, 1983; Vermeij et al., 1984; but see Moore (1977) for an extreme example of dispersal of nonplanktonic lecithotrophic larvae over 400 km by rafting). Hence, it is likely that the first colonizers of Cape Verde were species with planktotrophic developmental mode that subsequently lost their structures and capacities for dispersal (e.g., the loss of feeding structures, such as mouth and elaborate ciliated bands that capture food (Strathmann, 1985), and became nonplanktotrophs. In any case, rafting of egg capsules could have also been an important source of founders.

Comparing Marine and Terrestrial Modes of Insular Speciation

There are important differences in the life cycles of terrestrial and marine fauna that likely impose distinct modes of island colonization, speciation, and diversification to both types of organisms (Palumbi, 1994; Swearer et al., 2002). Terrestrial organisms usually reach an oceanic island by accidental long-distance dispersal overcoming a marine barrier. For the founders, island ecosystems are self-contained, unaffected by competition, and provide a potential large number of habitats and empty niches (Hubbell, 1968). Hence, they offer ample opportunities for diversification (either adaptive or nonadaptive). In fact, oceanic archipelagos such as Hawaii, Galápagos, Antilles, and Canary Islands exhibit great levels of endemism, and many instances of within-island speciation bursts of terrestrial invertebrates, reptiles, birds and plants (Emerson, 2002).

Pelagic eggs and/or larvae of most demersal marine species are pervasive, and can be transported by ocean currents within the plankton over long distances (Swearer et al., 2002). Hence, the vast stretch of open sea that isolates any oceanic island is not such an evident barrier to the dispersal of marine organisms as it is for terrestrial ones. High connectivity hinders isolation of genetically distinct demes, and likely results in slower speciation rates (Palumbi, 1994). As a result, the levels of insular marine endemisms are expected to be lower unless selection against dispersal occurs. In Hawaii, marine invertebrate endemic species typically occur throughout the archipelago, and only few are restricted to single islands (Kay and Palumbi, 1987). The levels of marine invertebrate endemisms are substantial (32%), but considerably lower than those (>90%) detected for terrestrial fauna and flora (Kay and Palumbi, 1987). Similar levels of endemism (18% to 42%) were reported for marine mollusks on other tropical Pacific archipelagos (Rehder, 1980; Kay, 1991; Brook, 1998). Interestingly, up to 31% of the marine endemic mollusks of Easter Island, and the Hawaiian and Galapagos archipelagos are likely to have obligate pelagic larvae (Swearer et al., 2002). These results suggest that pelagic larval development may facilitate colonization of remote ecosystems, and differentiation of endemics but not necessarily bursts of speciation.

Cape Verde archipelago is rather small in area, relatively old, and highly eroded in comparison with islands such as Hawaii. Thus, its islands offer fewer habitats for diversification of terrestrial endemic organisms, and explosive radiation events of founding populations into array of species are not reported. In contrast, levels of endemism for the benthic venomous genus *Conus* are extremely high (94%). This genus diversified in numerous endemic species that are restricted to single islands and in some cases even to single bays within an island. Moreover, endemic species have lost the capability for larvae dispersal. These patterns resemble those described for terrestrial fauna and flora that underwent bursts of speciation in other oceanic archipelagos. Hence, *Conus* of Cape Verde may represent a textbook example of a marine insular radiation. We hypothesize that, upon colonization of the archipelago, there was a strong selection in *Conus* endemics for life history traits that facilitate self-recruitment and reduce the risk of dispersal in such isolated habitats. In particular, a shift to a direct mode of development would enhance local larval retention and would ensure successful settlement into

appropriate habitats (Swearer et al., 2002). As a result of this evolutionary shift, limited gene flow among islands (as suggested by the reconstructed phylogeny) would set the stage to undergo explosive speciation by allopatry (Kay and Palumbi, 1987; see Glor et al. [2004] for an example of an insular radiation in terrestrial habitats triggered by allopatric speciation). The niche specialization observed in other archipelagos (Losos et al., 1998) that usually follows radiation within-islands is not clearly observed in *Conus* of Cape Verde. The patchy rocky habitats occupied by *Conus* are apparently identical in all sampled islands, and all endemic species are generally vermivorous. However, more inclusive studies on ecological, dietary, and mating/reproductive specializations are needed to determine the role (if any) of adaptation as contributing factor for higher speciation rates in endemic *Conus* of Cape Verde.

After this paper was submitted, Duda and Rolán (2005) reported a study directly related to ours. The recovered phylogenetic relationships based on partial mitochondrial COI gene sequences agree with those here presented.

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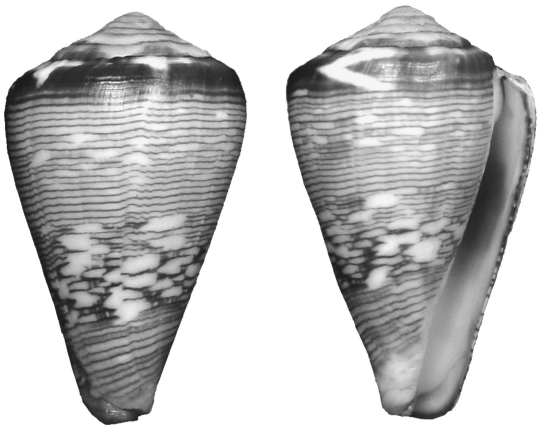

Conus crotchii. Drawing by M. Tenorio, 2005.