

Explosive radiation of Cape Verde *Conus*, a marine species flock

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Abstract

Nearly 50 species of the marine gastropod genus *Conus* are restricted to the Cape Verde archipelago. This unusual concentration of endemics within a single set of oceanic islands is extremely uncharacteristic of marine taxa. Here we used phylogenetic analyses of 90 *Conus* species, including 30 endemics from Cape Verde, to reveal the relationships and origins of the endemic Cape Verde *Conus*. Results show that these species group in two distinct clades and represent a marine species flock that is restricted to a very narrowly confined geographical area. Species' originations occurred in exceptionally limited parts of the archipelago and in some cases radiations took place solely within single islands. Finally, comparison of levels of divergence between Cape Verde endemics and other *Conus* species suggests that the radiation of *Conus* in Cape Verde occurred during the last few million years.

Keywords: Cape Verde, *Conus*, endemic, marine, phylogeny, species flock

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Introduction

Species flocks or species swarms are endemic and monophyletic assemblages of taxa that arose rapidly in small geographical areas (Mayr 1963; Greenwood 1984; Ribbink 1984). In nearly all cited cases, terrestrial species flocks occur on islands or archipelagos (e.g. Darwin's finches (Grant 1986)) and aquatic ones within lake systems (e.g. African rift lake cichlids (Meyer *et al.* 1990)), but species flocks are noticeably unreported from marine environments. The few reported marine species flocks, rockfish in the north-western Pacific (John & Avise 1998), Antarctic fishes (Eastman & McCune 2000) and coral reef fishes in the Caribbean (McCartney *et al.* 2003), have broad distributions and so designating them as species flocks is not entirely consistent with a rigid interpretation of the term's definition.

The marine gastropod fauna of Cape Verde contains 47 endemic and just three nonendemic *Conus* species; most of the endemic species are restricted to single islands or even to particular bays on single islands (Röckel *et al.* 1980; Rolán 1980, 1986a, 1987, 1992; Trovão & Rolán 1986). For comparison, only about 30 other *Conus* species occur elsewhere

throughout the eastern Atlantic (Pin & Leung Tack 1995) and we know of no other oceanic islands that contain so many endemic marine taxa that are members of the same genus. If the endemic *Conus* species of Cape Verde are each other's closest relatives, this species-rich endemic fauna is a marine species flock that, unlike other marine species flocks, occurs in a narrowly circumscribed geographical area.

To investigate the phylogenetic relationships and origins of the endemic Cape Verde *Conus*, we sequenced a region of the mitochondrial cytochrome oxidase subunit I (COI) gene from 89 *Conus* species, including 30 endemic species from Cape Verde; a COI sequence from an additional *Conus* species was obtained from GenBank. We used these data to reconstruct the phylogeny of these species and examined the resultant tree to interpret the evolutionary history of the endemic *Conus* fauna of Cape Verde.

Materials and methods

We extracted DNA from the foot tissue of *Conus* specimens as described previously (Duda & Palumbi 1999). We amplified COI sequences with universal primers (Folmer *et al.* 1994) and directly sequenced the amplification products with an ABI 377 automated sequencer. Sequences were determined from more than one individual per species for

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several taxa. We aligned sequences by eye and combined them with COI sequences of *Conus floridanus* and *Nucella lapillus* from GenBank.

We determined maximum likelihood parameters for reconstructing phylogeny with MODELTEST (Posad & Crandall 1998) and built a neighbour-joining tree with maximum likelihood distances with PAUP* (Swofford 2002). The maximum likelihood settings used correspond to the general time-reversible model (Lanave *et al.* 1984; Rodriguez *et al.* 1990) (base frequencies: A = 0.4874, C = 0.1638, G = 0.0929 and T = 0.2559; substitution rate matrix: A to C = 1.4477, A to G = 28.0163, A to T = 0.8052, C to G = 2.0230, C to T = 30.9184 and G to T = 1.000) with rates assumed to follow a gamma distribution with shape parameter alpha of 0.5615 and proportion of invariable sites of 0.5055. Support for nodes was determined by resampling the data 1000 times with bootstrapping methods in PAUP. The tree was rooted to the COI sequence of *N. lapillus*.

We used the Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999) as implemented in PAUP to test whether the endemic species of Cape Verde are derived from a single ancestral lineage. This test specifically compared the likelihood scores of a tree constrained to represent a single origin of endemic Cape Verde *Conus* and an unconstrained tree. We also compared log likelihood scores of clocked and unclocked trees to determine if a molecular clock could be applied to our data to date species' divergences.

Results and discussion

We obtained COI sequences from 90 *Conus* species including 30 that are endemic to the Cape Verde Archipelago and reconstructed the phylogeny of these taxa. Endemic *Conus* species of Cape Verde group in two clades in the COI tree (Fig. 1). This result implies that these taxa were derived from two ancestral species that invaded the archipelago and subsequently diversified. Furthermore, the topology of one of the endemic clades reveals that in some cases species' radiations occurred solely within single islands. Cape Verde endemic *Conus* represents a marine species flock that arose recently in a narrowly circumscribed geographical area.

Several of the endemic Cape Verde *Conus* possess identical or very similar COI sequences (see Fig. 1) indicating that many species' divergences occurred quite recently. The alternative interpretation, that some of the described endemic species of Cape Verde are variants of a few polymorphic ones, is unlikely. Different species possess unique and invariable characters, including morphology and colouration of larval and adult shells (Röckel *et al.* 1980; Rolán 1986a, 1990, 1992), size and shape of radular teeth (Rolán 1980, 1986a, 1990, 1992) and morphology of egg capsules (Rolán 1986a, 1990, 1992; Trovão & Rolán 1986), and no intermediate morphs have been observed in the field (Röckel *et al.* 1980; Rolán 1992). Distinct species also

fail to interbreed in aquaria (Rolán 1985, 1990, 1992). Very recently diverged taxa may show no fixed differences at genes that were not involved with speciation (Mayr 1984) and so lack of differentiation at COI is not cause to invalidate the taxonomic status of endemic Cape Verde *Conus* species. Furthermore, intraspecific COI diversity is low as determined by examination of sequences from more than one individual of several of the endemic taxa and it seems unlikely that the apparently nonrandom geographical segregation of endemic species in the COI tree (see succeeding discussion and Fig. 2) is the result of the stochastic sorting of ancestral polymorphisms or intraspecific variation.

Dual origin of endemic Conus of Cape Verde

Endemic *Conus* of Cape Verde group in two clades within the phylogram (clades 1 and 2, Fig. 1) and preliminary results from analyses of mitochondrial 16S and nuclear calmodulin intron sequences show the same outcome. Also, a COI tree constrained to represent a single origin of these endemics is significantly worse ($P = 0.014$) than the unconstrained tree. These results plus the presence of two eastern Atlantic species that do not occur in Cape Verde, *Conus mercator* and *Conus ventricosus*, at the base of clade 1 indicate that two ancestral *Conus* species invaded Cape Verde and subsequently diversified. In addition, clade 1 contains more than six times as many species as clade 2. The members of clade 1 also show more divergence at COI (average genetic distance = 3.6%, range = 0–8.1%) than do members of clade 2 (average = 1.2%, range = 0.6–1.6%). We thus conclude that the ancestor of clade 1 invaded Cape Verde earlier than the ancestor of clade 2, clade 1 is older than clade 2, members of clade 1 suffered less extinction than members of clade 2 or members of clade 1 possess unique biological attributes that enhanced their rates of diversification.

The species of clade 1 were collected from sites at Sal, Boavista and Maio, islands that occur in a roughly north-to-south line in the eastern part of the archipelago (Fig. 2). Members of this clade largely segregate in the phylogram relative to the island they inhabit (Fig. 2). All members of clade 1 from Sal group together uniquely while the other four major subclades contain species from Boavista and/or Maio. This illustrates that species' radiations, especially for species from Sal, took place in exceptionally limited geographical regions in the archipelago, a very unusual pattern for marine taxa. Moreover, species from Boavista and Maio are genetically more similar than they are to species from Sal, and migration of species between Boavista and Maio has apparently been more common than between Sal and Boavista. Greater affinity of species and greater presumed migration between Boavista and Maio than for Sal and Boavista is not surprising. Although the geographical distance between Sal and Boavista (about 43 km) is less than

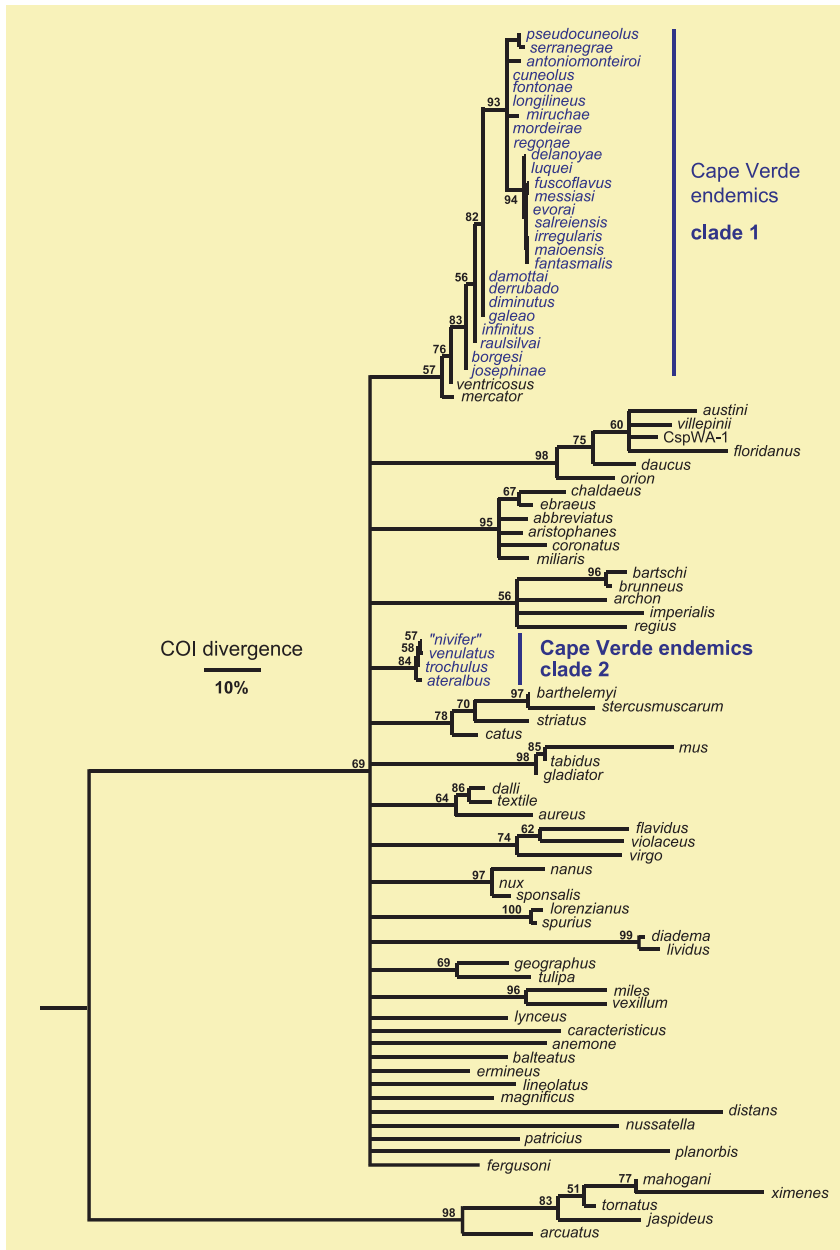


Fig. 1 Phylogeny of 90 *Conus* species from throughout the world's tropical oceans constructed with neighbour-joining methods of maximum likelihood distances of approximately 600 base pairs of the mitochondrial COI gene. Endemic *Conus* from Cape Verde are indicated with blue type. Bootstrap values are shown at nodes; bootstrap values of branches of Cape Verde endemics Clade 1 are only partially indicated here as a result of space limitations but are completely indicated in Fig. 2. Branches with bootstrap support less than 50% were collapsed.

the distance between Boavista and Maio (about 78 km) (Fig. 2), the region between Sal and Boavista has a nearly continuous depth of about 600 m while an underwater platform exists between Boavista and Maio that shows a maximum depth less than 200 m and on average is less than 100 m deep (Carta Hidrográfica del Archipiélago de Cabo Verde 1979).

Marine species flocks and Cape Verde Conus

The remarkable radiation of *Conus* in the Cape Verde Archipelago is the first marine example of a species flock that is consistent with a strict definition of the term, but how

should the term species flock apply to marine taxa? In reference to species flocks of freshwater lakes, Mayr (1984) proposed not to define the term species flock too rigidly and suggested that lacustrine species flocks should only show 'common descent and coexistence in the same lake'. Hodges & Arnold (1994) overlooked any reference to a restricted distribution of species in the definition of species flock in their description of 'a geographically widespread species flock' of nonaquatic plants. Also, as argued by Eastman & McCune (2000) in their proposal that Antarctic fishes represent a marine species flock, the term species flock was conceived with lacustrine faunas in mind and the size of the area that species flocks occur is arbitrary as long

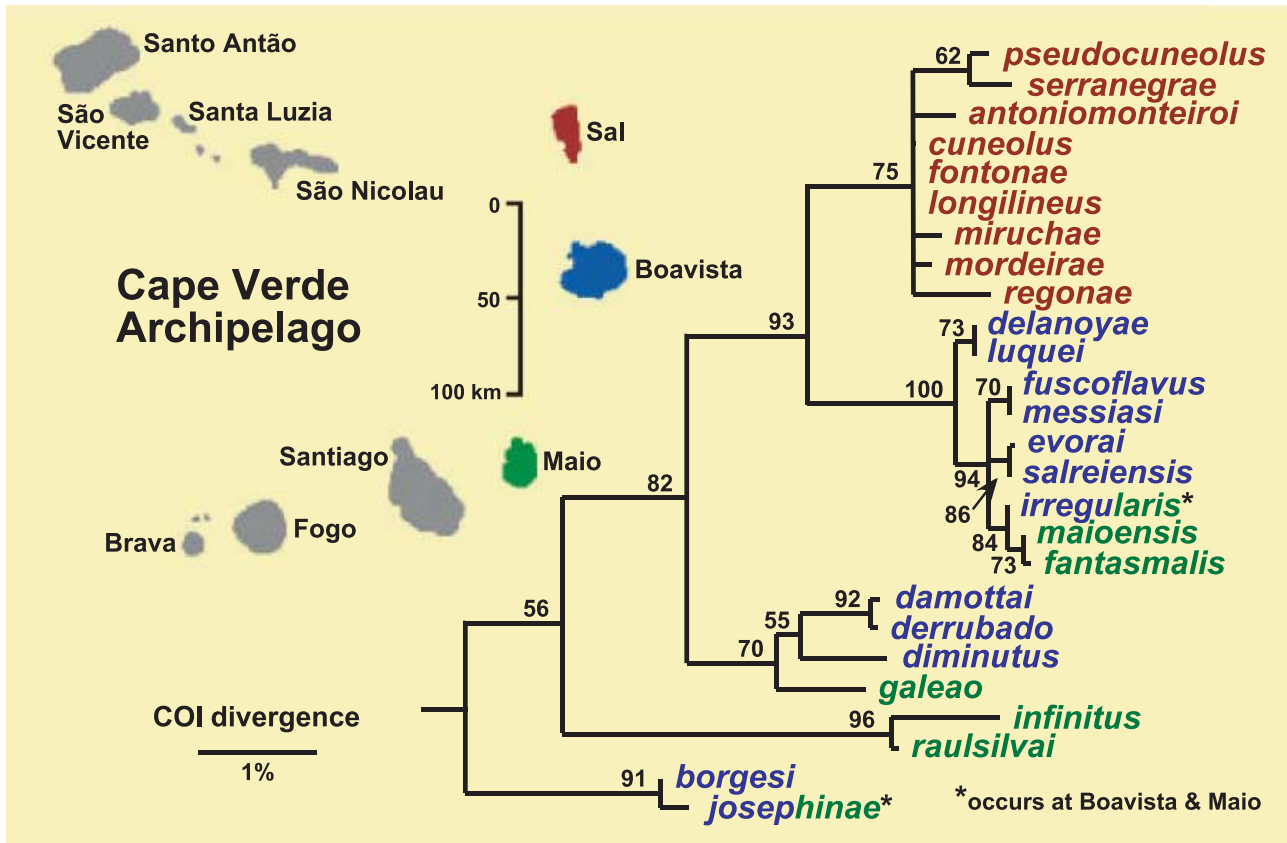


Fig. 2 Magnified topology of Cape Verde endemics Clade 1 from Fig. 1 and map of the Cape Verde Archipelago. Geographic distributions of species are indicated with colours of Sal, Boavista and Maio Islands in map. Bootstrap values are shown at nodes.

as the area is isolated. Thus, a more universal definition of species flock is a group of species that radiated rapidly in an area with no obvious barriers to dispersal. This definition applies to all previously described species flocks including marine ones, but Cape Verde *Conus* differ dramatically from other proposed marine species flocks in that they are restricted to a very much smaller geographical range.

Why are marine species flocks apparently so rare and why are Cape Verde *Conus* so unusual in being so narrowly distributed? Species flocks occur in relatively closed systems (e.g. within lakes or on islands) and colonization of an isolated area is presumably a prerequisite for their establishment. Isolation of marine faunas is very difficult to achieve because of the lack of barriers at fine geographical scales within oceans (Palumbi 1994), but local barriers can be enhanced by reduced dispersal abilities (Mayr 1963). Most *Conus* species (about 75%) whose mode of development is known have an obligate planktonic, feeding larval stage in their life history (Kohn & Perron 1994). However, nearly all eastern Atlantic species, including all of the Cape Verde endemics, are direct developers; they have no dispersing larval phase and juveniles hatch directly from attached egg

capsules (Knudsen 1950; Fioroni 1966; Bandel 1975; Bandel & Wils 1977; Rolán 1985, 1986a, 1986b, 1990, 1992; Trovão & Rolán 1986; Trovão *et al.* 1990). Distances between shallow water environments of the Cape Verde Archipelago and suitable habitats elsewhere and low gene flow attributable to lack of a dispersal stage may have effectively isolated Cape Verde *Conus* and facilitated their radiation.

Species' radiations and island age

More endemic *Conus* species occur on the older islands of Boavista, Maio and Sal than on younger islands. This association of species diversity and island age implies that *Conus* have had more time to diversify on older islands, but estimated ages of islands and dates of species' radiations suggest a different hypothesis. The Cape Verde Archipelago was formed by hotspot volcanism (Carracedo *et al.* 1998) and the islands are distributed as northern and southern islands chains (Fig. 2). Although the easternmost islands of these chains (Boavista, Maio and Sal) are older than the westernmost ones (Brava, Fogo and Santa Antão) based on levels of terrestrial erosion, the geological history of the Cape Verde Archipelago is poorly understood and volcanism

of only two of the 10 main islands has been investigated. The island of Maio, the easternmost island of the southern chain, shows evidence of volcanism as early as 12 million years (Mya) ago (Mitchell *et al.* 1983), but older islands like Maio may date to the Mesozoic (Mitchell-Thomé 1976). The earliest volcanic eruptions of Santa Antão, the westernmost island of the northern chain and a relatively young island, occurred approximately 7.6 Mya ago (Plesner *et al.* 2002). Ages of the remainder of the islands are unknown, but they are probably similar to those of the Canaries which show subaerial volcanism from two to 20 Mya ago (Carrecedo *et al.* 1998).

Although a molecular clock cannot be applied to the COI tree because the likelihood score of a tree built with an enforced molecular clock is significantly greater than that of an unconstrained tree ($P < 0.001$), we can make some inferences about dates of radiations of the Cape Verde endemic *Conus*. The transisthmian sister species pairs *Conus brunneus* and *Conus regius* and *Conus gladiator* and *Conus mus* (see Fig. 1) likely diverged prior to the emergence of the Isthmus of Panamá 3 Mya ago (Coates & Obando 1996); recent analyses suggest that *C. gladiator* and *C. mus* diverged approximately 6.9 Mya ago while *C. brunneus* and *C. regius* diverged 14.3 Mya ago (Duda & Kohn, unpublished). Unless rates of divergence are comparatively low among the Cape Verde endemics, levels of COI divergence within clades 1 and 2 are much less than those among the transisthmian pairs (see Fig. 1) and so radiations of Cape Verde endemics likely occurred sometime during the past few Myr. Thus, most of the Cape Verde Islands were well established, likely for many millions of years prior to the initial colonization and radiation of *Conus*. If estimates of the ages of islands and dates of species' radiations are reasonable, greater diversity of endemic *Conus* on older islands of the archipelago is not the result of the greater amount of time that species have had to diversify on these islands, but may instead be related to other factors including greater complexity of habitats, more diverse niche space or higher prevalence of barriers to dispersal on older vs. younger islands.

Conclusion

We postulate that the two ancestral *Conus* species that gave rise to the modern endemics colonized the Cape Archipelago sometime during the past few million years. Because of the prevalence of direct development among the endemics and other eastern Atlantic taxa, colonization likely occurred as a result of the arrival of rafting egg capsules from source populations in the eastern Atlantic. Low levels of gene flow and patchy distributions of suitable habitats or prey facilitated allopatric divergence of species as suggested by relationships of endemic *Conus* (Fig. 2). Low sea levels or chance colonization among islands, also presumably via rafting of egg capsules, expanded the ranges of species

within the archipelago and caused further isolation and diversification of species. Greater diversity of species on older vs. younger islands resulted because of some factor related to island age, but not because species had more time to radiate on the older islands. Based on the tremendous dietary specializations *Conus* species show elsewhere (see Kohn 1959) and preliminary examinations of diets and preferred habitats of endemic taxa, the diversification of so many endemic species was likely trophic in nature and reflects specialization for different prey species or habitats, but we cannot reject hypotheses that assortative mating or sexual selection played roles in the explosive radiation of Cape Verde *Conus*.

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Thomas Duda investigates the evolutionary history and origins of adaptations of marine molluscs as well as the evolution of conotoxin gene families of *Conus*. Emilio Rolán has long been interested in the biology of Cape Verde *Conus*, but also studies a variety of other marine molluscs of the eastern Atlantic.
