Syst. Biol. 0(0):1–17, 2014

DOI:10.1093/sysbio/syu059

Evolution at a Different Pace: Distinctive Phylogenetic Patterns of Cone Snails from Two Ancient Oceanic Archipelagos

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Received 20 August 2013; reviews returned 15 July 2014; accepted 5 August 2014
Associate Editor: Adrian Paterson

Abstract.—Ancient oceanic archipelagos of similar geological age are expected to accrue comparable numbers of endemic lineages with identical life history strategies, especially if the islands exhibit analogous habitats. We tested this hypothesis using marine snails of the genus *Conus* from the Atlantic archipelagos of Cape Verde and Canary Islands. Together with Azores and Madeira, these archipelagos comprise the Macaronesia biogeographic region and differ remarkably in the diversity of this group. More than 50 endemic *Conus* species have been described from Cape Verde, whereas prior to this study, only two nonendemic species, including a putative species complex, were thought to occur in the Canary Islands. We combined molecular phylogenetic data and geometric morphometrics with bathymetric and paleoclimatic reconstructions to understand the contrasting diversification patterns found in these regions. Our results suggest that species diversity is even lower than previously thought in the Canary Islands, with the putative species complex corresponding to a single species, *Conus guanche*. One explanation for the enormous disparity in *Conus* diversity is that the amount of available habitat may differ, or may have differed in the past due to eustatic (global) sea level changes. Historical bathymetric data, however, indicated that sea level fluctuations since the Miocene have had a similar impact on the available habitat area in both Cape Verde and Canary archipelagos and therefore do not explain this disparity. We suggest that recurrent gene flow between the Canary Islands and West Africa, habitat losses due to intense volcanic activity in combination with unsuccessful colonization of new *Conus* species from more diverse regions, were all determinant in shaping diversity patterns within the Canarian archipelago. Worldwide *Conus* species diversity follows the well-established pattern of latitudinal increase of species richness from the poles towards the tropics. However, the eastern Atlantic revealed a striking pattern with two main peaks of *Conus* species richness in the subtropical area and decreasing diversities toward the tropical western African coast. A Random Forests model using 12 oceanographic variables suggested that sea surface temperature is the main determinant of *Conus* diversity either at continental scales (eastern Atlantic coast) or in a broader context (worldwide). Other factors such as availability of suitable habitat and reduced salinity due to the influx of large rivers in the tropical area also play an important role in shaping *Conus* diversity patterns in the western coast of Africa. [*Conus*; eustatic sea level changes; latitudinal gradient of species diversity; oceanic islands; RF models; species diversity; SST.]

On oceanic islands, the area of available shallow-marine habitat is strongly related to changes in the eustatic sea level throughout evolutionary time scales. Episodes of falling sea level are often associated with extinction, whereas sea level rise is thought to promote marine species radiation (Jablonski and Flessa 1986). This view has however been contradicted by several studies that either found no relationship between sea-level changes, shallow marine habitat availability and diversity (Raup 1976; Valentine and Jablonski 1991), or showed that "sea level rise does not consistently generate an increase in shelf area, […] suggesting that the diversity response to sea level change will be largely idiosyncratic" (Holland 2012). Other studies showed that periods of remarkably low sea level coincided with speciation or population expansions of coastal marine mollusks from oceanic islands (Cunha et al. 2005; 2011).

The latitudinal increase of species richness from the poles toward the tropics has been recognized by biogeographers since the 18th century (Hillebrand 2004). Nevertheless, there is a lack of consensus regarding the mechanisms driving this diversity gradient and numerous hypotheses have been proposed to explain this pervasive pattern (Turner 2004; Jablonski et al. 2006;. An extensive body of literature has suggested sea surface temperature (SST), habitat area, and changes in primary productivity, among others, as major factors shaping broad-scale richness gradients (Wright et al. 1993; Hawkins et al. 2003). For instance, a strong correlation between species diversity and SST was found in the western Atlantic and eastern Pacific oceans (coastal marine gastropods [Roy et al. 1998] and in fiddler crabs [Levinton and Mackie 2013]).

Two Atlantic oceanic archipelagos, Cape Verde and Canary Islands, provide an excellent model system for studying gradients of species diversity and phylogenetic patterns over evolutionary time scales, given their similar geological age and the availability of paleoclimatic and bathymetric data for both. These two archipelagos, along with Madeira and Azores, belong to the Macaronesian region (Fig. 1). This biogeographic region is defined on the basis of shared flora and faunas and high levels of endemism especially in the southern islands (Fernández-Palacios et al. 2011). Cape Verde has

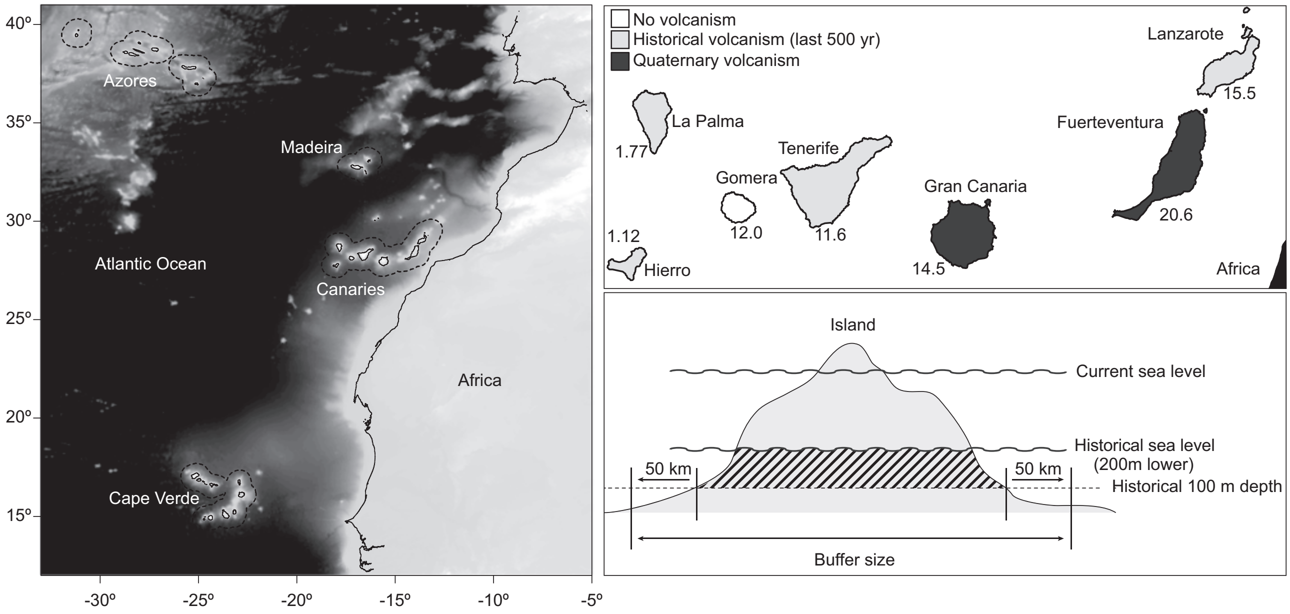

FIGURE 1. Map of the biogeographic region of Macaronesia showing the oceanic archipelagos of the Azores, Madeira, Canaries, and Cape Verde. The dashed line shows the maximum area used to estimate the shallow-marine area around each archipelago. The schematic representation of the 50-km perimeter around the depth contour, enough to encompass the 0–100 m of the shallow-marine habitat of each archipelago even when the global sea level was 200 m lower than its current position, is also shown. The volcanism of each island of the Canarian archipelago is represented by different colors (Quaternary - dark gray; in the last 500 years—light gray; no volcanism—white). Numbers represent the geological age, in million years, of the Canary Islands.

a volcanic origin and none of the islands has ever been in contact with a continental landmass. The shortest distance between the archipelago and the African continent is about 450 km (Cap Blanc: Senegal, West Africa). Geochronological data places the age of Cape Verde Islands between 5.9 (Brava) and 25.6 (Sal) million years (Griffiths et al. 1975; Mitchell-Thomé 1976; Torres et al. 2002). The seven islands included in the Canary archipelago were formed in an east-to-west progression, as a result of volcanic activity (Coello et al. 1992). The Canary Islands are located <100 km off the coast of West Africa. The oldest island, Fuerteventura, arose 20.7 Ma (Carracedo 1999) whereas the most recent, El Hierro, has an estimated age of 1.12 myr (Guillou et al. 2004).

A major radiation of more than 50 endemic species in Cape Verde was described for marine snails from the genus *Conus* (Rolán 1992). Molecular analyses of specimens from Cape Verde (Cunha et al. 2005; Duda and Rolán 2005) showed the existence of two groups with distinct shell sizes (small and large shell clades), and established a correspondence between cladogenetic events and periods of low sea level (Cunha et al. 2005). The diversification patterns showed that species clustered into monophyletic island assemblages, probably as a consequence of their non-planktonic larvae (Cunha et al. 2005).

On the Canary Islands, this genus is represented by two nonendemic species: *Conus pulcher siamensis* Hwass in Bruguière, 1792, which occurs throughout the entire archipelago and also in Madeira, and *Conus guanche* Lauer, 1993, which occurs on the islands of Lanzarote, Tenerife, Gran Canaria, Fuerteventura, and on the islets of Graciosa and Lobos. *Conus guanche* can also be found along the West coast of Africa (from Mauritania to south of Morocco, including all Western Sahara). Based on variation in shell morphology, Lauer (1993) described a subspecies (*C. guanche nitens*) distributed across the islands of Lanzarote, Lobos, Graciosa, and Fuerteventura. Given the plasticity of shell banding patterns in *Conus* (Röckel et al. 1995), independent sources of data (molecular and geometric morphometrics of radula and shell) are needed to test whether these populations are in fact, different taxa. There are no aquarium observations regarding the larval development of *C. guanche*, but the paucispiral form (shell with few whorls) of its protoconch suggests that this species has non-planktonic larvae (E. Rolán, personal communication), like all *Conus* species endemic to Cape Verde Islands. Benthic invertebrates whose life histories lack a planktonic larva often exhibit reduced dispersal abilities (Scheltema 1989). Therefore, low levels of gene flow and differentiation might be predicted among *C. guanche* populations, supporting the idea that cryptic species may occur.

Here, we used an integrative approach combining molecular phylogenetics with geochronological and paleoclimatic reconstructions to further understand speciation patterns in a group of marine snails from two oceanic archipelagos (Cape Verde and Canary Islands) with similar geological ages but remarkably different *Conus* species richness. Our objectives were: (i) to compare phylogenetic patterns within the genus *Conus* between Cape Verde and Canary Islands; (ii) to investigate whether *C. guanche* is a single polymorphic species or represents a cryptic species complex using molecular and geometric morphometric data; (iii) to determine whether the number of Cape Verde *Conus* lineages through time is consistent with a constant rate of speciation or if there were shifts in the diversification rate that explain the striking species richness in this archipelago; (iv) to evaluate the effect of available habitat area according to sea level fluctuations over geological time on *Conus* diversity in the main islands of Macaronesia; (v) to test whether the irregularity of the coastline promotes speciation in this group; and (vi) given the similarity of habitats and geological ages, to propose an explanation for the observed disparity in *Conus* species richness between the two archipelagos.

Material and Methods

Specimen Collection, DNA Extraction, Amplification, and Sequencing

A total of 201 specimens of *C. guanche* (Supplementary material S1, available from http://dx.doi.org/10.5061/dryad.48s53) were collected from five out of six islands/islets of the Canarian archipelago (30 sample locations on Gran Canaria, Fuerteventura, Graciosa, Tenerife, and Lanzarote) and also from Tarfaya (Morocco) and Cap Blanc (Western Sahara/Mauritania). All specimens were preserved in 70–96% ethanol, and total genomic DNA was isolated from foot muscle tissue using the cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). Specimens were gathered over several years from private collections. As most of the samples had been stored for long periods in ethanol 70%, DNA extractions were frequently unsuccessful. Although the genus *Conus* has been recently divided into many different genera (Tucker and Tenorio 2009; 2013), the taxonomy of the family Conidae in the present work follows (Bouchet and Rocroi 2005) for convenience and simplicity.

Two partial mitochondrial genes obtained through polymerase chain reaction (PCR) amplification were used in the phylogenetic analyses. One fragment obtained with the universal primers 16Sar-L and 16Sbr-H (Palumbi 1996), amplified a 472 bp of the 16S rRNA gene in 115 specimens. The other fragment was obtained using *C. guanche*-specific primers CGnad4 – F (GATACTTGCTAGTCAAAGATCGG) and CGnad4 – R (GAGGAGCAGCTATATTAATAATGC) and amplified a 777-bp portion of the NADH dehydrogenase subunit 4 (NADH4) mitochondrial genes in 143 specimens. Additionally, a partial fragment (505 bp) of the internal transcribed spacer (ITS1) of nuclear-encoded ribosomal DNA was amplified in 129 specimens using *C. guanche*-specific primers CGITS1 – F – (GTGCGCAGTACAGCAGACGCTCG) and CGITS1 – R – (GTGTCCTGCAATTCACATTAG).

PCR amplifications of the two mitochondrial fragments (NADH4 and 16S rRNA) and the nuclear ITS1 were carried out in 25 µl reactions containing 1X PCR buffer, 0.2 mM of each dNTP, 0.2 µM of each primer, 1 µl of template DNA, and Taq Advantage®2 Polymerase mix DNA polymerase (1 U; CLONTECH-Takara), using the following program: one cycle of 1 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 56 °C (NADH4), 50 °C (16S rRNA), 54 °C (ITS1), 50–90 s at 68 °C, and finally, one cycle of 5 min at 68 ° C.

PCR amplicons were purified using ethanol/sodium acetate precipitation and directly sequenced with the corresponding PCR primers. Sequencing was performed in an automated sequencer (ABI PRISM 3700) using the BigDye Deoxy Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and following the manufacturer's instructions. All new sequences were deposited in GenBank (accession numbers: 16S rRNA - KC748581 - KC748695; ITS1 - KC748696 - KC748824; NADH4 - KC748825 - KC748967; sequences of *C. guanche* used in dating analyses: cyt *b* - KF049928 - KF049938; 12S rRNA + tRNA Val + 16S rRNA - KF049928-KF049949).

Morphometric Characterization of Shell and Radula Variability in C. guanche

A geometric morphometric analysis of the shell shape was performed on 92 specimens of *C. guanche* collected from six locations (Fuerteventura, Lanzarote, Graciosa, Gran Canaria, Tenerife, and Western Sahara). To avoid the effects of allometry, only adult specimens with lengths between 20 and 47 mm and well-preserved spires were selected. To photograph the shell of each specimen, we followed the procedures described by Cunha et al. (2008). A total of 17 points were selected to capture shell shape (Supplementary material S2 and S3). Points 1–11 were treated as landmarks, and the remaining as semilandmarks. These points cover most of the ratios used for classical morphometric characterization of cone shell morphometry (Kohn and Riggs 1975).

Radular tooth morphology was examined in a total of 42 specimens from six locations. From each location, 6–8 specimens were selected. Landmark positioning was performed using two teeth per specimen. To obtain the radular teeth and the digital images of each tooth, we followed procedures described by Cunha et al. (2008). All radular teeth included in this study were obtained from adult specimens, and all teeth were between 0.49 and 0.89 mm in length. A total of 84 teeth were digitized using tpsDig2 (Rohlf 2005), and 16 points were selected for capturing the shape of each tooth (Supplementary material S2 and S3). These points cover most of the ratios used for classical morphometric characterization of the vermivorous radular tooth (Rolán and Raybaudi-Massilia 1994; Kohn et al. 1999). Points 1–4, 6–8, and 13–16 were treated as landmarks, and the remaining as semilandmarks.

Shell and radular tooth semilandmarks were allowed to slide (Zelditch et al. 2012) to the position that minimizes the Procrustes distance between the form of the shell (or the form of the radular tooth) of a given specimen, and the consensus reference form for all analyzed shells (or radular teeth), using Semiland6 (Sheets 2003-2005). Landmarks were aligned by a standard generalized least squares (GLS) procedure. The within-population Procrustes distances were calculated as the mean ± standard deviation (SD) of the shell or radular tooth pairwise Procrustes distance matrices, respectively, as generated by CoordGen6h (Sheets 2003-2005). The pairwise Procrustes distances between locations based upon either shell or radular tooth morphometry were obtained with TwoGroup6h (Sheets 2003-2005) using resampling methods (500 bootstraps) in order to circumvent problems related to the adjustment of the correct number of degrees of freedom when semilandmarks are used. The significance of the Goodall's *F*-test was assessed by a bootstrapped *F*-test. The 95% probability interval and standard error were also determined by bootstrapping. Partial warps and their principal components (relative warps) were computed using PCAGen6p (Sheets 2003-2005) included in the IMP software suite. This program was also used for generating the two-dimension (2D) scatter plots and the corresponding deformation grids and vectors. Canonical Variate Analysis (CVA) was performed using CVAGen6n (Sheets 2003-2005). Allometry and Multivariate Analysis of Variance (MANOVA) tests were performed with Regress6k and MANOVA board, respectively (Sheets 2003-2005).

Sequence Analysis and Phylogenetic Reconstruction

All DNA sequences were aligned using MAFFT version 6.0 (Multiple alignment using Fast Fourier Transform) (Katoh and Toh 2010) using the auto option that automatically selects the appropriate strategy according to data size. The alignment of mitochondrial sequence data required no insertions and amino acid translations were checked using MacClade v. 408 (Maddison and Maddison 2005). Several sequences for the nuclear ITS1 gene showed ambiguities that were coded with International Union of Pure and Applied Chemistry (IUPAC) codes.

To analyze phylogenetic patterns within *C. guanche*, we performed maximum likelihood (ML) and Bayesian Inference (BI) analyses using PhyML v.3.0 (Guindon and Gascuel 2003) and MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001), respectively. The data set used in the ML analysis comprised partial sequences of the mitochondrial NADH4 (777 bp) and 16S rRNA (472 bp) genes, and the nuclear ITS1 (508 bp) from 80 specimens of *C. guanche* that were concatenated into a single data set of 1757 bp. The Akaike information criterion (Akaike 1974) implemented in Modeltest (Posada and Crandall 1998) selected the GTR+I as the evolutionary model that best fits the combined data set. Inferred model parameters were used in the ML analysis.

The BI analysis was performed using the same data set and considering three data partitions: NADH4, 16S rRNA, and ITS1. Each partition was analyzed according to the best-fit model selected by Modeltest (NADH4: HKY; 16S rRNA: TVM; ITS1: TIM+I). Model and model parameters were estimated independently for each of the data partitions using the unlink command in MrBayes. Analyses accommodated among-partition rate variation through use of the "prset applyto = (all) ratepr = variable;" command in MrBayes. Metropolis-coupled Markov chain Monte Carlo analyses were run for 20 000 000 generations with a sample frequency of 1000. Length of burn-in was determined by visual examination of traces in Tracer v.1.6 (Drummond and Rambaut 2008). The first 20 000 generations were discarded as burn-in, and robustness of the inferred trees was evaluated using Bayesian posterior probabilities. The data set was analyzed in two independent runs and the final tree was obtained from the combination of the accepted trees from each run. Convergence between the two runs was assessed by examining the potential scale reduction factors (PSRF) and effective sample size (ESS). We also used Awty (Nylander et al. 2008) to confirm that the standard deviation of split frequencies approached zero, which also indicates convergence of the results.

To further analyze phylogenetic relationships within *C. guanche*, haplotype genealogies based on the concatenated data set and on each gene separately (16S rRNA, NADH4, and ITS1) were reconstructed with Network v4.6 ([Bandelt et al. 1999] available at fluxus-engineering.com). Median-joining networks (Bandelt et al. 1999) that contained all possible equally short trees were simplified by running the maximum parsimony calculation option to eliminate superfluous nodes and links (Polzin and Daneschmand 2003).

Bayesian Evolutionary Analysis by Sampling Trees

We used Bayesian evolutionary analysis by sampling trees (Beast) v.1.7.4 (Drummond et al. 2012) to obtain an ultrametric tree to be used in the estimation of the number of Cape Verde *Conus* lineages through time. This analysis was based on published sequence data from 51 species of *Conus*, including species from Cape Verde, Senegal, Portugal, Angola, and Canary Islands of a mitochondrial fragment of 2044 bp. This fragment includes the 3′-end of the 12S rRNA, the complete tRNA-Valine (Val), the 5′ portion of the 16S rRNA, and a partial sequence of the cytochrome *b* gene—cyt *b* (GenBank accession numbers retrieved from Cunha et al. [2005]). We amplified this fragment in 11 new specimens of *C. guanche*, which encompasses almost the entire geographic distribution of this species, following the methods of Cunha et al. (2005).

We used a Yule tree prior, which assumes a constant rate of speciation among lineages (Yule 1925; Gernhard 2008). The Yule tree prior does not include a model of coalescence and rate estimation for closely related sequences can be overestimated (Ho et al. 2005; McCormack et al. 2011), but in this analysis we were interested in dating the age of the crown group of *C. guanche*, therefore, we used a total of 13 specimens, 11 from this study and sequences from two specimens retrieved from Cunha et al. (2005). The analysis was performed considering two partitions, one including the partial sequence of cyt *b* and the other, the two ribosomal RNAs (12S and 16S) together with the complete sequence of the tRNA-Val. Sequence variation was partitioned among partitions and specific models selected by the AIC criterion implemented in Modeltest were used (cyt *b*: GTR+I+Γ; tRNA-Val+12S+16S: HKY+I+Γ).

We used two calibration points based on the paleontological record. One refers to *Conus pulcher* Lightfoot, 1786, an extant species dated from the Lower Pliocene (5.32–3.2 myr) of Cuenca de Siena (Italy) (Spadini 1990) and Velerín (Estepona, Spain) (Muñiz Solís 1999). The second calibration corresponded to *Conus ventricosus* Gmelin, 1791, an extant species that occurs in the Mediterranean and neighboring Atlantic including Algarve (South of Portugal) reported from the Middle Miocene (16.4–20.5 myr) of Cuenca de Piemonte (Italy) (Sacco 1893). Calibrations using the two fossils were modeled with a lognormal distribution, where 95% of the prior weight fell within the geological interval in which each fossil was discovered. For *C. pulcher* [5.32–3.2 myr], the parameters of the lognormal calibration prior were: hard minimum bound 3.2, mean in real space 0.49 and SD1.0. For *C. ventricosus* [20.5–16.4 myr], the parameters of the lognormal calibration prior were: hard minimum bound 16.4, mean in real space 0.953 and SD 1.0. A lognormal distribution was used because evolutionary rates change along the branches instead of converging at the nodes as occurs in the exponential distribution (Drummond et al. 2006).

The starting tree for the Beast analysis was an ultrametric tree obtained from a short run (1 000 000 generations and a sample frequency of 100) using the same parameters as above but without fossil calibrations. Markov chain Monte Carlo (MCMC) analyses were run four times (each run with 20 000 000 generations and a sample frequency of 1000) following a discarded burn-in of 2 000 000 steps. Tree and log output files from the four runs were combined in LogCombiner v.1.7.4 (Drummond et al. 2012), and the final tree was produced by TreeAnnotator v.1.7.4 (Drummond et al. 2012) using the "maximum clade creditability" option and mean node height. The convergence to the stationary distribution was confirmed by inspection of the MCMC samples and of effective sample sizes (ESS should be >200) using Tracer v1.6 (Drummond and Rambaut 2008).

BI and ML analyses were performed on the CCMAR Computational Cluster Facility (http://gyra.ualg.pt) at the University of Algarve, and on the web server Mobyle (http://mobyle.pasteur.fr), respectively.

Number of Lineages Through Time and Diversification Rates

To determine whether the diversification of Cape Verde endemic *Conus* was constant through time, a lineage through time (LTT) plot was produced using R 3.0.2 (R Development Core Team 2013) and the package Laser v.2.3 (Rabosky 2006a; 2006b). Based on Tenorio (2004) and Rolán (2005) we considered a total of 56 endemic Cape Verde *Conus* species. LTT plots with a convex shape indicate early radiation events, whereas concave shapes suggest recent bursts of speciation (Nee et al. 1994). We also used the constant-rate (CR) test with the γ statistic (Pybus and Harvey 2000) to evaluate whether the LTT plots were consistent with a constant net rate of diversification through time. Significant positive γ values indicate an increase of the diversification rate toward present (recent burst of cladogenesis), whereas negative values specify a slowdown in the rate of diversification through time (Pybus and Harvey 2000). In phylogenies with incomplete taxon sampling, such as the *Conus* phylogeny, the γ statistic has to be obtained from simulations using the Monte Carlo constant-rate (MCCR) test (Pybus 2000). We further examined rates of diversification across the reconstructed Bayesian chronogram using the relative cladogenesis statistic as implemented in the R package Laser v.2.3 that identifies branches in the tree that have higher rates than expected under a constant-rate birth–death model.

Shallow-Marine Habitat Area in the Macaronesia Versus Sea Level Changes

To test whether historical variations in habitat availability could explain the disparity in the diversity of *Conus* species among the Macaronesia archipelagos, we calculated the evolution of the relative shallow-marine habitat area as a function of the eustatic sea level changes in the last 25 myr. We defined the shallow-marine habitat as the sea floor area between 0 and 100 m depth, which is the depth range where *Conus* species are most frequently found (Kohn and Perron 1994).

Seafloor bathymetry data at 1 arc-min resolution were acquired from the ETOPO1 data set (Amante and Eakins 2009) available at NOAA's National Geophysical Data Center (http://www.ngdc.noaa.gov/mgg/global/global.html). Eustatic sea level curves for the last 25 myr, varying between ∼−150 and 150 m, were obtained from Miller et al. (2005). Variability could appear higher in periods where the sea level data had higher resolution; hence, we used R (R Development Core Team 2013) to aggregate Miller et al. (2005) data, creating a regular time series with a regular frequency (time increments) of 0.1 myr. Following Holland (2012), we used GMT5 (generic mapping tools; Wessel and Smith [1998]) to convert the binary bathymetry data files into ASCII xyz format, which were subsequently processed in R 3.0.2 (R Development Core Team 2013). Then, for each archipelago, we computed the total area between 0 and 100 m depth as a function of time. We limited the area section to a 50-km perimeter around the 300 m depth contour, enough to encompass the 0–100 m habitat even when the global sea level was 200 m lower than its current level (Fig. 1). This step was necessary to exclude spurious data from areas not related to the main archipelago islands (e.g., the Desertas Islands or the African continental shelf were not considered when computing historical changes in the relative shallow-marine habitat area for the Canary Islands).

Fractal Dimension of Cape Verde and Canary Islands Coastlines

To test the hypothesis that islands with more convoluted coastlines (with a larger number of isolated bays) would maximize the opportunities for speciation within *Conus* due to its reduced dispersal ability, we measured the island contour jaggedness using fractal dimension analysis. The spatial dimension of natural lines (e.g., mountain outlines) is called "fractal dimension" (Mandelbrot 1983). Fractal dimension has been successfully applied to estimate the contour of coastlines, and the "box-counting" dimension (Hilborn 2000) is the most frequently used method for measurements due to its simplicity and automatic computability (Peitgen et al. 2004).

We used the software Fractal Dimension Calculator (FDC) V1.01 (http://paulbourke.net/fractals/fracdim/). The method involves subdividing the image space into square boxes of equal size (s), and counting the number of boxes that contain at least one pixel of the image (n). The process continues by splitting the image space into smaller and smaller boxes. When the series of log [n(s)] is plotted against log (1/s), the least squares regression line obtained is the fractal dimension of the object, which ranges between 1 and 2, and because the fractal index is represented in a logarithmic scale, each increase of 0.1 in the fractal dimension represents a two-fold increase on the structural complexity.

Because image treatment can affect the box-counting calculations, it is important to treat images exactly the same way to obtain comparable results. All island coastlines were obtained from GSHHG—a global self-consistent, hierarchical, high-resolution geography database (http://www.ngdc.noaa.gov/mgg/shorelines/gshhs.html) as PDF files, and processed in ImageJ (Abràmoff et al. 2004), obtaining just the coastal contour with a binary image file size of 1600 × 1600 pixels.

Two important aspects of fractal dimension calculation are the choice of the box size and the positioning of the grid over an object. FDC offers a maximum of 12 box sizes within a single run. We used the largest box size, automatically determined according to an algorithm based on the size of the image, and the smallest box size equal to 1 pixel. FDC also allows the user to select the number of "offsets" for each box size to allow for placement of the grid at different starting positions, and to carry out an exhaustive count with every possible starting position. Because there is no preferred origin for the boxes that initiates the positioning of the grid, fractal dimension measures are the average computed from 100 different box origins.

Conus *Diversity Versus Environmental Variables*

We selected 12 environmental variables from the Bio-ORACLE database (Tyberghein et al. 2012) likely to directly or indirectly influence the diversity of the genus *Conus* (mean chlorophyll A concentration, CHLA; mean calcite concentration, CAL; mean percentage of cloud cover, CC; mean diffuse attenuation coefficient, DA; mean dissolved oxygen concentration, DOX; mean nitrate concentration, NIT; photosynthetically available radiation, PAR; mean pH, PH, mean phosphate concentration, PHOS; mean salinity, SAL; mean silicate concentration, SIL; mean sea surface temperature, SST). A coastal diversity map for the East Atlantic was produced by digitizing the distribution ranges of 111 species of *Conus* based on (Monteiro et al. 2004) and calculating latitudinal species richness by each 1 arc-degree of latitude/longitude using the package "raster" (Hijmans and Etten 2013) on R (R Development Core Team 2013). A similar map was generated for the world (longitudinal range: 180W–180E, latitudinal range: 41S–48N) using data from IUCN (2012). Environmental data were aggregated into 1 arc-degree spatial resolution to match species diversity data. The two geographical distributions of *Conus* diversity obtained from both the eastern Atlantic and the global data sets were then related with the Bio-ORACLE environmental data using used Random Forests (RF) models, with the main objective of ranking the environmental variables in function of their importance for the distribution of this genus. RF models offer very high classification accuracy even when dealing with strong interactions among variables (Cutler et al. 2007; Evans and Cushman 2009), show very low susceptibility to overfitting (Breiman 2001; Prasad et al. 2006) and have successfully been used to model the distribution of coastal species (Hilbish et al. 2012). Models were built using conditional inference trees (Party package for R, (Strobl et al. 2008)), which are specially recommended when variables might be highly correlated (Strobl et al. 2009). Our models were parameterized with 5000 trees per run and included three randomly preselected predictor variables on each split.

RESULTS

Geometric Morphometrics of Shell and Radula Shapes of C. guanche

Results of the one-way MANOVA tests for shell and radular shape data of *C. guanche* using location (Fuerteventura, Lanzarote, Graciosa, Gran Canaria, Tenerife, and Mauritania/Western Sahara) as the grouping criterion are shown in Table 1. Differences

TABLE 1. Results of the one-way MANOVA from shell and radula shape of C. Guanche using locality as grouping criteria

Grouping factor: Locality	Explained SS	Unexplained SS	% Explained	F^a	P^a
Shell	0.0124	0.0412	23.17	5.19	0.002
Radula	0.0189	0.0595	24.13	4.96	0.002

Note: SS, sum of squares.
[a] Significance is assessed by a bootstrapped F-test (500 bootstraps).

in mean shell length between locations (Fuerteventura, 29.4 mm; Graciosa, 38.0 mm; Gran Canaria, 30.7 mm; Lanzarote, 34.1 mm; Western Sahara, 30.7 mm; Tenerife 29.1 mm) were not significant (P >0.05), except for the differences between the length of the shells from Graciosa and the rest of the islands, but excluding Lanzarote. The low percentages of the explained variance (23%; Table 1) suggest low correlation between shell shape and location. The CVA of the shape of 92 shells showed a large overlap among groups (Supplementary material S3a). The CVA using locality as grouping factor and the principal components 1–10 of the partial warp scores as variables (which account for the 92.3% of the variance in shell shape) correctly classified only 54 specimens out of 92 (58.7%) for the location. Pairwise Procrustes distances between different groups were small (between 0.009 and 0.031; Supplementary material S4b). Procrustes distances between and within groups defined by location and based on shell morphometry are of the same order of magnitude (Supplementary material S4a, b) suggesting that there is little variability of shell shape among populations. Nevertheless, some values were statistically significant (for instance, between Fuerteventura and Graciosa; Supplementary material S4b).

The large overlap among clusters corresponding to each island suggests little variability of the radular shape between specimens from different locations (Supplementary material S3b). Differences in radular tooth mean size between locations (Fuerteventura, 0.70 mm; Graciosa, 0.70 mm; Gran Canaria, 0.66 mm; Lanzarote, 0.68 mm; Western Sahara, 0.68 mm; Tenerife 0.61 mm) were not significant (P >0.05), except for the differences between the mean sizes of the radular teeth of specimens from Tenerife and the rest of the islands, but excluding Western Sahara. The low percentages of the explained variance (24%; Table 1) suggest low correlation between radular shape and location. The CVA of the radular shape of the entire data set (84 radular teeth) is shown in Supplementary material S3b. Location was used as grouping factor and the principal components 1–10 of the partial warp scores as variables. Using these CV axes, 27 out of 84 specimens were misclassified (67.8% correct). Pairwise Procrustes distances between different groups were also small (between 0.011 and 0.045) but significant in several cases (e.g., between Lanzarote and Tenerife; Supplementary material S4d).

Phylogenetic Reconstructions

Potential scale reduction factors in the BI analysis were about 1.00 and ESS values were all >200, indicating convergence of runs. Assessment of convergence statistics in Awty indicated that the analyses converged given that the standard deviation of split frequencies approached zero. ML ($-\ln L = 3370.16$) and Bayesian ($-\ln L = 3431.00$) analysis based on the concatenated data set (NADH4, 16S rRNA, and ITS1) arrived at a similar topology showing that phylogenetic relationships within *C. guanche* were generally unresolved. BI analysis yielded the topology shown in Supplementary material S5. The few clades that received statistical support showed no geographic structure (Supplementary material S5).

Haplotype genealogies based on 16S rRNA, NADH4, and ITS1, separately, showed that the central and most common haplotype is shared among all locations (Fig. 2). In the combined data set, the most common haplotype is also shared among all locations with the single exception of Graciosa. Most haplotypes diverged from the central haplotype by only 1–4 mutations.

The ultrametric tree obtained with BEAST showed ESS values >200 in TRACER indicating that chains had reached stationarity. Our age estimates for lineage splitting events within *Conus* (Fig. 3a) differed by <1 myr from the results shown in Cunha et al. (2005).

LTT Plots and Diversification Rates within Cape Verde Conus

According to the relative cladogenesis statistic, one instance of significant increase in the diversification rate of Cape Verde *Conus* was identified within the BEAST tree (represented by an asterisk in Fig. 3a) occurring within the small-shelled clade. The concave shape of the LTT plot (Fig. 3c) and the positive γ value (3.62), both support an increase in the number of lineages toward the present (recent radiation) (Nee et al. 1994; Pybus and Harvey 2000). CR/MCCR tests indicated statistically significant changes in rates of diversification through time (species sampled = 42; estimated total number of endemic species = 56; $\gamma = 3.62$; critical value of γ at $P =$ 0.05, one-tailed test, $\gamma_{0.05} = 1.10$).

Shallow-Marine Habitat Area and Sea Level Change

The available relative shallow-marine habitat area for all Macaronesian archipelagos from the Miocene to the present based on sea level data from (Miller et al. 2005) is shown in Figure 3b. There are substantial differences in the relative shallow-marine area available among archipelagos and within each archipelago, but the effect of the sea level variation (lower or higher sea stands) is identical on each archipelago. The relative shallow-marine area in Madeira and Azores seems to be affected in a lesser extent by variations of the sea level. Cape Verde and Canary Islands show identical variation of the relative shallow-marine area over time, and the area of each archipelago almost double the area of Madeira or Azores. In the last 5 myr, all four archipelagos experienced a reduction in the relative habitat area with Cape Verde and the Canary Islands showing higher losses (Fig. 3b).

Fractal Dimension of the Coastline

The average fractal dimension of the Cape Verde coastline is 1.432 ± 0.075 and the fractal dimension of the Canary Islands is 1.411 ± 0.080 (Fig. 4). Islands that showed more convoluted coastlines (and higher

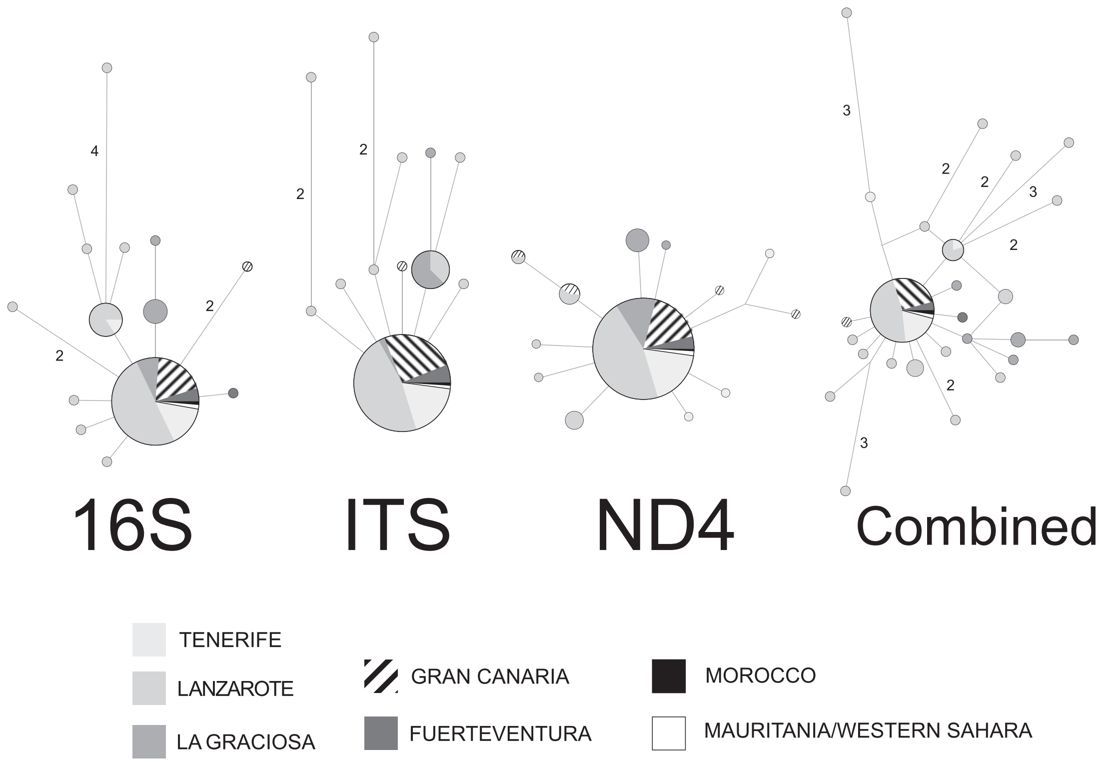

FIGURE 2. Median-joining network of *Conus guanche* based on the concatenated data set and on each gene separately (16S rRNA, NADH4, and ITS1) reconstructed with 'Network'. The area of each circle is proportional to the number of individuals sharing a particular haplotype. Colors refer to sample location, and the size of each slice is proportional to the number of individuals with that haplotype. Haplotypes are connected by branch lengths approximately equal to the inferred mutational steps. Numbers represent mutational steps.

fractal dimensions) were Gomera and Maio whereas Graciosa and Santa Luzia exhibited the most reduced values of fractal dimension (Fig. 4). A linear regression between the fractal dimension of each island and the total number of species revealed a nonsignificant relationship ($R^2 = 0.04$, data not shown).

Relationship between Environmental Variables and Conus *Species Diversity*

We analyzed the relationship between 12 oceanographic variables (CHLA; CAL; CC; DA; DOX; NIT; PAR; PH; PHOS; SAL; SIL; SST) and *Conus* diversity to explain the observed latitudinal gradient of species richness along the West African coast, applying the RF model (Cutler et al. 2007). We also used the RF model and the same 12 parameters to explain worldwide *Conus* diversity.

Results from the RF model indicated that all parameters showed a significant correlation with diversity for both eastern Atlantic and worldwide analyses except CHLA and CAL (eastern Atlantic; Fig. 5a) and CHLA (worldwide; Fig. 5b). The RF model explained 83.2% of the total variance for the worldwide data and 67.5% of the total variance for the eastern Atlantic data. The contribution of each variable for each RF model is shown in Figure 5, and in more detail in Supplementary material S6. In the eastern Atlantic, SST, PAR, SAL, and CC, all explained more than 10% of the model (Fig. 5b). Of all environmental variables tested, SST explained the most variation in *Conus* diversity. The relationship between SST and *Conus* diversity across the West African coast and the biogeographic region of Macaronesia is shown in Figure 6. The number of *Conus* species is higher in the tropics (between ≈20ºS and 20ºN), with the maximum diversity (up to 20 species per 1ºlatitude/longitude) occurring off Senegal and off the Cape Verde archipelago where the poleward North Equatorial Counter Current meets the Canary Current forming a large oceanographic discontinuity (Longhurst 1998), and where average SST is ~24 ºC (Fig. 6). In the worldwide RF model, SAL, NIT, DOX, and SST contributed more than 10% to the model variability, and SST explained the most variation in *Conus* diversity.

DISCUSSION

It is a reasonable expectation that speciation patterns of a given group of organisms be comparable on two ancient oceanic archipelagos sharing a similar setting. Although this premise is valid for several groups of terrestrial organisms, such as the emblematic case of *Anolis* lizards from the Greater Antilles (Losos et al. 1998), where islands with similar resources support nearly identical communities (Case and Cody 1987), its application in the marine environment is yet to be

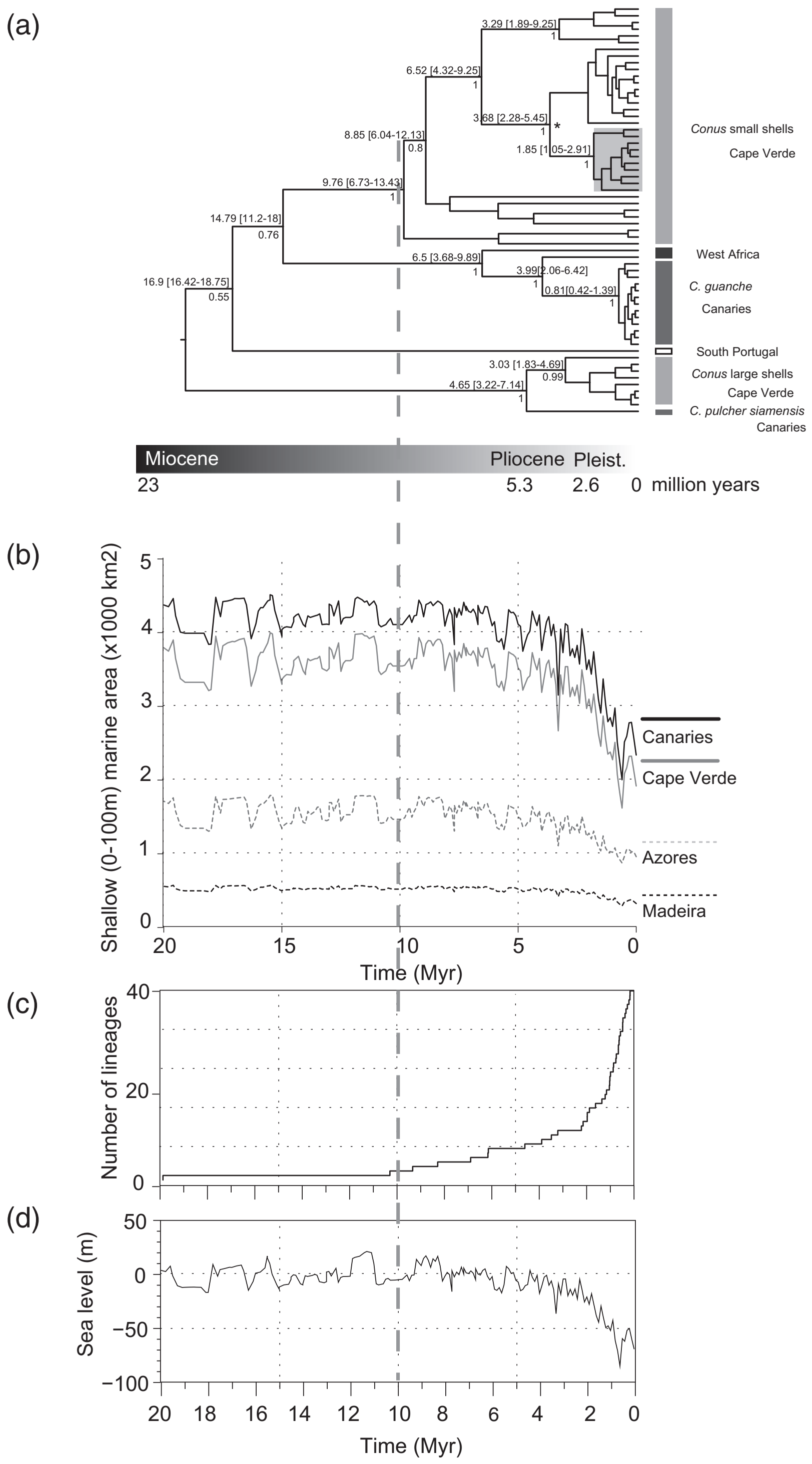

FIGURE 3. **a**) Beast maximum clade credibility chronogram showing main cladogenetic events within *Conus* based on the mitochondrial 2044 bp data set (12S rRNA, tRNA-VAL, 16S rRNA, and cyt.) Numbers above and below nodes represent node heights (ages) and 95% highest posterior density (HPD) intervals and Bayesian posterior probabilities, respectively. The shaded area represents the clade of species from Sal; **b**) Variation of the shallow-marine habitat area of the four Macaronesian archipelagos (Canaries, Cape Verde, Azores, and Madeira) according to sea level changes since the Miocene (based on data from Miller et al. [2005]); **c**) Lineage through time plot for the endemic Cape Verde *Conus* showing an increased number of lineages toward the present. The *x*-axis corresponds to time (in myr); **d**) Eustatic sea level changes during the last 20 myr based on data from Miller et al. (2005). The vertical dashed line represents the upturn in the number of Cape Verde *Conus* lineages that started ≈ 10 Ma.

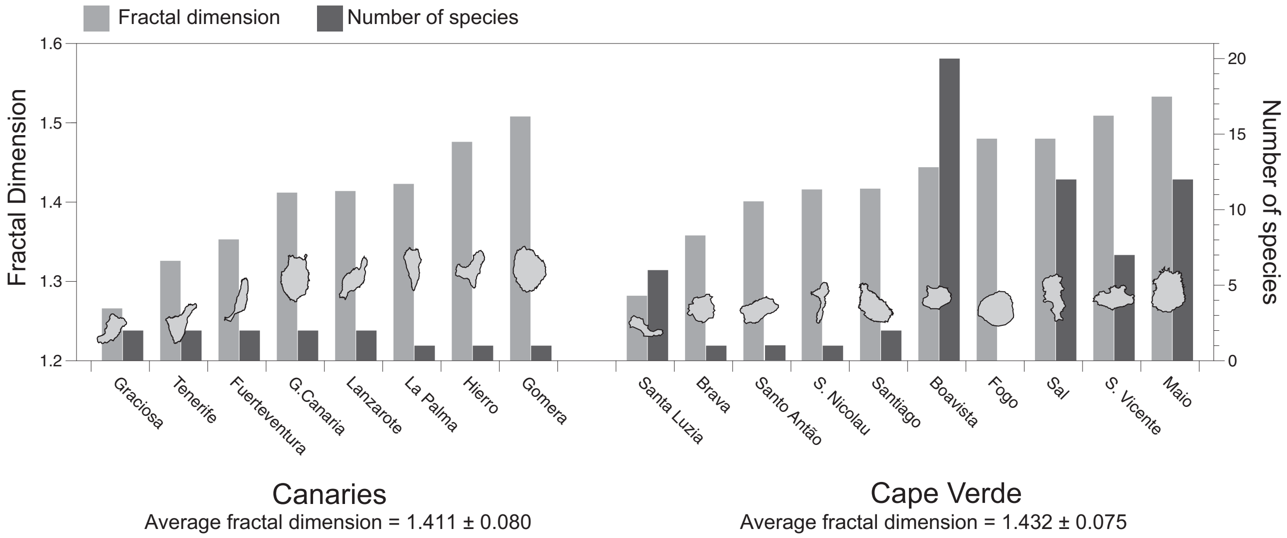

FIGURE 4. Coastline fractal dimension of Cape Verde and Canarian archipelagos using Fractal Dimension Calculator V1.01. Bars represent the fractal dimension of each island (light gray) and the total number of species (dark gray), endemics, and nonendemics. The contour of the coastline of each island is shown.

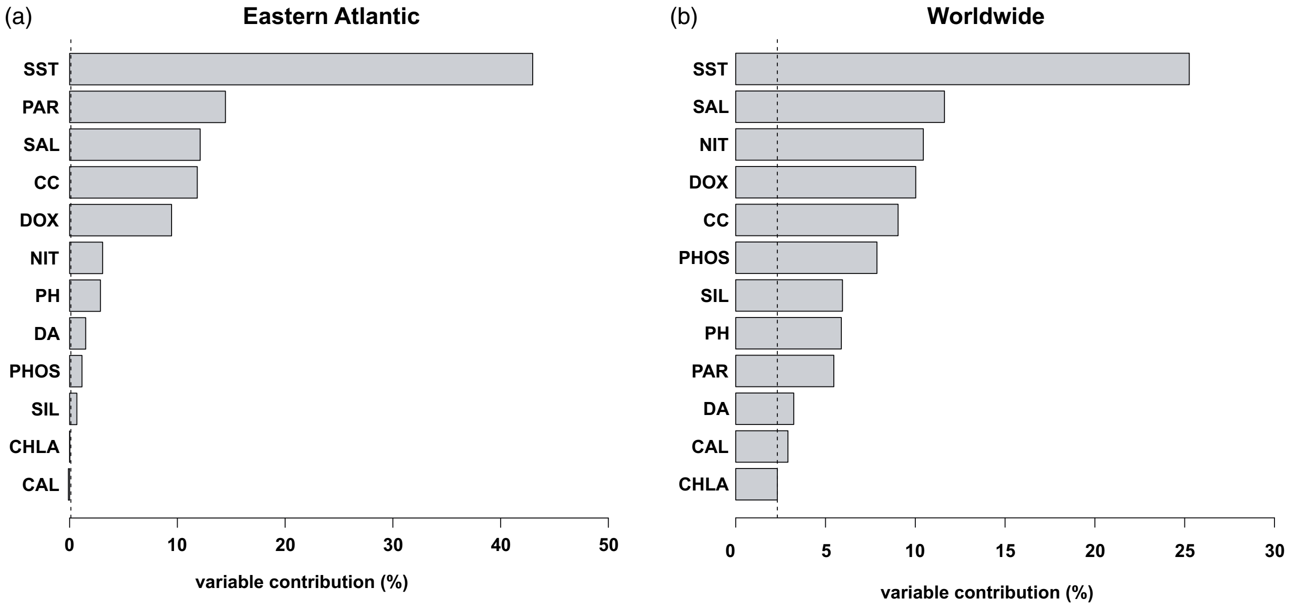

FIGURE 5. Relationship between *Conus* diversity and 12 environmental variables (mean CHLA; mean CAL; mean percentage of CC; mean DA coefficient; mean DOX concentration; mean NIT concentration; PAR; PH; mean PHOS; mean SAL; mean SIL concentration; mean SST). Predictors to right of dashed vertical line are significant. **a**) eastern Atlantic *Conus* diversity; **b**) worldwide *Conus* diversity.

determined. The remarkable marine radiation of the genus *Conus* in the Cape Verde Islands (Rolán 2005) has no correspondence in the Canary archipelago where this genus is represented by only two, nonendemic species. The integrative approach presented here intends to elucidate patterns and processes underlying species diversity in comparable environments. Our findings suggest that species diversity in the Canarian archipelago is even lower than initially anticipated, as no cryptic speciation was detected within *C. guanche*. Moreover, Cape Verde and Canary Islands experienced comparable changes in the amount of available relative shallow-marine area during eustatic sea level fluctuations observed since the Miocene. Hence, we explored alternative hypotheses that could explain the distinctive diversity patterns found in both archipelagos.

Conus guanche: *A Cryptic Species Complex or Recent Colonization?*

Our results show that the genus *Conus* diversified in very different ways on the Canary Islands and in Cape Verde, despite similar geological ages and

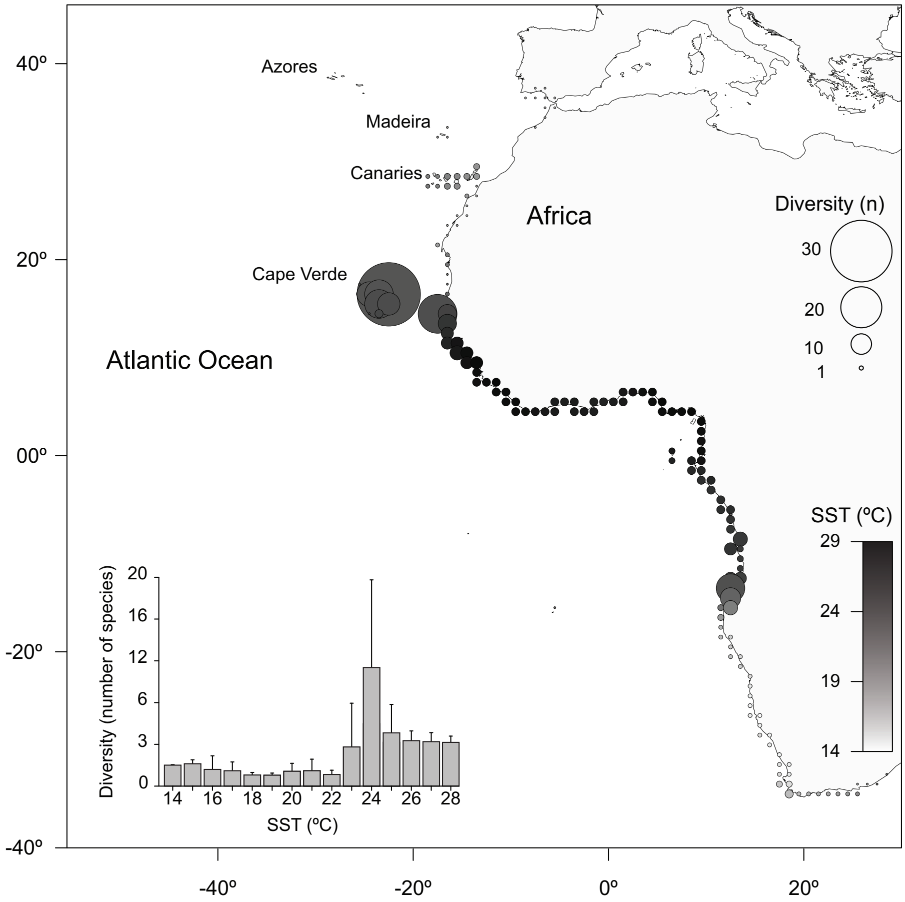

FIGURE 6. Latitudinal variation in species richness of the marine snails belonging to the genus *Conus* along the West African coast and Macaronesian islands. Colors represent the mean SST and the size of the circles the number of species per 1°latitude/longitude. The graph represents the diversity (number of *Conus* species) along the west coast of Africa versus mean surface temperature (in degrees Celsius).

comparable habitats of these archipelagos. Although we predicted reduced gene flow among populations of *C. guanche* due to its non-planktonic larval development, this was not confirmed by our data. BI analyses (Supplementary material S5) showed no differentiation of the species within the Canarian archipelago or between the islands and the West African coast (Western Sahara/Mauritania), with all *C. guanche* specimens clustered in one well-supported clade. CVAs of the shell and radula shapes showed a large overlap between locations, with no inter-island differentiation or significant differences between the Canarian archipelago and the mainland (Supplementary material S3a, b). Both morphometric and molecular data failed to support the hypothesis that this species might represent an example of cryptic speciation.

The Canary Islands have an ancient geological history (20.7 myr) (Carracedo 1999) and according to Bayesian dating analyses, the crown group age of *C. guanche* was estimated at 0.81 (0.42–1.39) myr (Fig. 3a), which implies a recent colonization of the archipelago. The existence of shared haplotypes between *C. guanche* populations from the Canarian archipelago and the West African coast (Fig. 2) suggests recurrent gene flow between these areas. Considering that *C. guanche* has no planktonic veliger larval stage, the existence of gene flow between locations 100 km apart and separated by a 1500-m-deep channel, is an unexpected finding. Nevertheless, species that lack a planktonic larva or exhibiting a short veliger larval stage have other forms of dispersal that may include rafting and adult migration (Scheltema 1989). For example, distances >100 km of open ocean are not an impediment for dispersal of Hawaiian limpets that are competent to settle only 4 days after their release (Bird et al. 2007).

Conus guanche has internal fertilization and nonplanktonic development but no further information is available regarding its life history (i.e., whether larvae persist in the water column for a short period before settling or if crawl-away larvae are released directly from egg masses). For a nonplanktonic species such as *C. guanche*, dispersal between West Africa and Canary Islands may be difficult to explain. Even though there are no records of rafting for this species, there is recent evidence suggesting that more than 80 species including nonplanktonic gastropods, polychaete worms and crabs were able to travel >5000 km by rafting on pumice stone (Bryan et al. 2012). The volcanic nature of the Canarian archipelago may facilitate the rafting of egg masses and/ or juveniles of *C. guanche* on pumice stone. The Canary Current is the most important oceanographic feature affecting the Canarian archipelago. The current has a near-shore branch that flows from the Canary Islands toward Africa (between 30ºN and 10ºN and offshore to 20ºN during summer), promoting contact between the Western Sahara and the islands. However, given its predominant southward direction (Barton 2001; Fedoseev 1970), it may represent an obstacle to colonization of the Canary Islands from more southern locations where *Conus* diversity is higher.

Diversification Patterns and Habitat Structure of the Genus Conus *in the Cape Verde and Canarian Archipelagos*

The phylogeographic pattern of *Conus* from Cape Verde does not follow the "progression rule" (Funk and Wagner 1995) in which older islands are inhabited by older lineages. Instead, the clade comprising the species from the oldest island (Sal, shaded area in Fig. 3a) originated more recently (1.85 myr; Fig. 3a) than others occurring in the younger islands. The Hawaiian limpets genus *Cellana* is another example of marine insular organisms that do not follow the "progression rule" pattern (Bird et al. 2011). Unlike *Conus*, *Cellana* reflects a signature of non-allopatric speciation with the ancestral lineage showing the largest distribution, and range overlapping with more recent lineages (Bird et al. 2011). Apparently, within-island diversification following the "progression rule" is more frequently observed in terrestrial organisms (e.g., Galápagos land snails [Parent and Crespi 2006] or Hawaiian spiders [Gillespie 2004]).

Conus habitats in Cape Verde are not species specific. With the single exception of *Conus derrubado*, which inhabits soft coral (M. Tenorio, personal observation), all endemic species occur in rock crevices, inside empty sea urchin pocket holes, sheltered under rocks (or on top of them) or partially buried in sand (Rolán 1985; 1992; Tenorio and Afonso 2004). The occurrence of several *Conus* species within the same bay or even in syntopy (under the same rock; R. L. Cunha, personal observation), is not restricted to Cape Verde. In total, 36 *Conus* species occurring on a single reef platform in Papua New Guinea showed high overlap in resource utilization and habitat type (Kohn 2001). In the Canarian archipelago, *C. guanche* also occurs in rock crevices or on top of rocks. The only difference in habitat is that in the Canary Islands rocks are usually covered with sea grass (Lauer 1993), whereas in Cape Verde are covered with algae (Rolán 1992).

All Cape Verde *Conus* share the same diet (polychaete worms) with a single known exception of a nonendemic species that may occasionally eat fish (*Conus ermineus*) (Rolán 1985; 1992). Like all Cape Verde endemics, the radular morphology of *C. guanche* is of vermivorous type (Bandel and Wils 1977; Lauer 1993). Field observations also confirm that this species feeds on polychaete worms (M. Tenorio, personal observation). The hypothesis of ecological speciation or niche partitioning seems therefore rather unlikely. When cone snails are abundant, the community composition is reduced to only a few other gastropod species (e.g., *Cantharus viverratus*, *Aplysia senegalensis*, or *Luria lurida*), (M. Tenorio, personal observation), suggesting that this group is able to successfully out-compete other gastropods for food and/or space. Furthermore, given the abundant food resources available for *Conus* in Cape Verde (M. Tenorio, personal observation), there is no apparent need to develop distinct feeding ecologies, as frequently occurs in other species from the Indo-Pacific (Duda et al. 2001).

Despite the habitat structure or geological age similarities between both archipelagos, there is a crucial difference regarding their distance to the mainland; Cape Verde Islands are ≈450 km apart from the mainland, whereas the Canary Islands are located <100 km off the coast of West Africa. Our data suggest that the geographic isolation of the Cape Verde archipelago seems to inhibit gene flow between islands and the mainland, promoting speciation. In contrast, the smaller distance between the West coast of Africa and the Canarian archipelago should allow for repeated migration events, and therefore, gene flow is maintained between these two locations.

The Combined Effects of the Relative Shallow-Marine Area Variation, Coastline Irregularity, and Volcanic Activity on Conus *Diversity*

Early studies on the variation of shallow-marine habitat areas with sea level fluctuations often relate extinctions with marine regressions, whereas transgressions are believed to promote diversification (Jablonski and Flessa 1986; Newell 1967). This prevalent view was based on paleontological records and sequence stratigraphy that provided quantitative data on taxonomic composition, and its variation throughout evolutionary time scales (Brett 1998). Recent studies measuring the change in area during sea level fluctuations based on a global elevation data set contradicted this widespread idea by finding idiosyncratic relationships between species diversity and sea level variation (Holland 2012).

Our results showed that since the Miocene, sea level fluctuations have produced similar effects on the availability of the relative shallow-marine area on the four Macaronesian archipelagos. Cape Verde and Canary Islands have more area available and registered more pronounced losses of habitat area than Madeira or the Azores (Fig. 3b). Variation in the amount of relative shallow-marine habitat area through time follows similar trends for both Canary Islands and Cape Verde (Fig. 3b) and therefore does not explain the observed disparity in species richness.

We analyzed whether the striking number of *Conus* species endemic to Cape Verde could be related to shifts in diversification rates and habitat availability driven by sea level changes. The positive value of γ (3.62), even taking into account the incomplete taxon sampling (critical value of γ, one-tailed test, $\gamma_{0.05} = 1.10$), may indicate either the existence of high background extinction rates or that Cape Verde *Conus* have undergone a recent episode of rapid speciation. It is difficult to choose between the two competing hypotheses but the small genetic divergence between Cape Verde endemic sister species (data not shown) suggests a recent radiation. Moreover, results from the rate of cladogenesis test identified one branch with a significant increase in the rate of diversification in the *Conus* phylogeny (represented by an asterisk; Fig. 3a), further supporting the rapid diversification hypothesis. There is, however, no relationship between the number of lineages and the observed variation of the shallow-marine habitat area of Cape Verde. Although the number of Cape Verde species increased over the last 10 myr (Fig. 3c), the available habitat has shown an overall progressive decrease during the same period (Fig. 3b).

Recurrent periods of low sea level are expected to have caused habitat fragmentation in Cape Verde, creating opportunities for speciation in the poor-disperser endemic *Conus*. The irregular shape of the Cape Verde coastline with enclosed rocky bays, fringing reefs in sandstone and a shelf platform with smooth slopes (Grevemeyer et al. 2010) is thought to have allowed the survival of these marine species during sea regressions. On the contrary, the shelf area of the Canary Islands is quite reduced and there are no fringing reefs in the archipelago (Faerber and Baird 2010; Navarro-Pérez and Barton 2001). We measured the contour irregularity of each island, and results indicated that the average fractal dimension did not differ significantly between the two archipelagos. Nonetheless, there are several islands from the Canarian archipelago (e.g., Gomera or Hierro; Fig. 4) that show quite irregular coastline shapes with high fractal dimensions that could have promoted diversification within *C. guanche* populations. However, only a single species can be found either in Gomera or in Hierro. In contrast, Boavista and Maio (Cape Verde) harbor 20 and 12 species, respectively, and both islands show high fractal dimensions. Against expectations, our results showed that coastline raggedness does not seem to promote speciation (there is no correlation between species diversity and island fractal dimension; $R^2 = 0.04$) and some other factors must be affecting the diversification process, considering the observed differences in species richness between islands.

Violent volcanic activity recorded in the island of Gran Canaria (Canary Islands) between 5.5 and 3 Ma (Carracedo 1994) generated massive extinctions of terrestrial local fauna and flora (Emerson 2003). There are also records of more recent volcanism in the Canarian archipelago (Carracedo 1994), in the islands of Fuerteventura, and again Gran Canaria (Fig. 1). The shape of the coastline suffered major rearrangements during volcanic eruptions and habitat destruction might have severely affected shallow-water marine species such as *Conus*. The paleontological record of this genus from the Canary Islands includes only four species from the Quaternary marine deposits of the Lanzarote island: *C. pulcher siamensis*, *C. guanche*, *C. ermineus* and *Conus genuanus* (Groh 2010), which are representatives of the pre-volcanic fauna. The low number of species currently found in the Canarian archipelago may reflect the loss of species as a result of the intense volcanic activity over the last 500 years (Carracedo 1994), in combination with unsuccessful colonization of new species from more diverse regions. In Porto Santo (Madeira archipelago), there are also records of volcanic activity (Santos et al. 2011) that could have caused habitat disturbance and local disappearance of *Conus* spp. but there is no fossil record available to support this hypothesis.

Factors Underlying Conus *Diversity Patterns along the West African Coast*

Despite the vast number of hypotheses proposed to explain the "peaking in the tropics and tailing off towards the poles" (Roy et al. 1998), the underlying factors causing this gradient are still under debate. There is a growing body of literature showing that latitudinal patterns of species richness are highly correlated with water temperature (Roy et al. 2000; Rutherford et al. 1999; Tittensor et al. 2010; Yasuhara et al. 2012). The RF model used in this study identified SST as the most determinant factor for the observed diversity pattern in the eastern Atlantic (Fig. 5a). Although SST alone explained 43% of data variation in the eastern Atlantic, all other parameters contributed individually with <15% (Fig. 5a and Supplementary material S6). When analyzing the relationship between *Conus* diversity and worldwide SST variation (Fig. 5b and Supplementary material S6), the contribution of this variable decreases to 25.3%, whereas each of the remaining variables explained individually <12% of data variation.

According to our results, the worldwide distribution of *Conus* follows the well-established pattern of latitudinal gradient of species richness with increasing diversities toward the tropics (Colwell and Lees 2000; Roy et al. 2000), particularly in the Indo-West Pacific (IWP) region where this group attains its maximum diversity (Supplementary material S6). However, the eastern Atlantic revealed a different pattern with two main peaks of diversity located in the sub-tropical region (Fig. 6). Maximum *Conus* diversity along the western coast of Africa (by each 1 arc-degree of latitude/longitude) occurs at ≈15ºN, Senegal (Fig. 6), where the SST is around 24 ºC and more than 20 species have been recently described (Monteiro et al. 2004; Nolf and Verstraeten 2008; Boyer and Pelorce 2009; Monnier and Limpalaër 2010). The SST in Cape Verde is ≈24 ºC, whereas in the Canary Islands it decreases to 21 ºC due to seasonal upwelling (Navarro-Pérez and Barton 2001) (Fig. 6). While a large number of endemic *Conus* species has been described in Cape Verde, only two nonendemic species can be found in the Canarian archipelago.

In the tropical West African coast, mean SST reaches 29 ºC and the number of *Conus* species is quite reduced (four species; Fig. 6). We would expect higher diversity in this area, considering that *Conus* species richness reaches maximum values in the IWP (e.g., Coral Triangle, Supplementary material S7) where mean temperatures are higher than 30°C (Supplementary material S8). In contrast to the IWP, tropical West Africa is mostly composed of sandy beaches that are not the preferred habitat for this group, which may explain the lower diversity levels. Additionally, it is likely that *Conus* species richness could be underestimated in some West African locations (between Sierra Leone and Benin) due to undersampling, thus, current numbers may not reflect the true diversity for this area.

There is a correlation between SST and sea level variation, that is, an increase in the water temperature is usually followed by a sea level rise (Webb and Bartlein 1992; Bintanja and Van de Wal 2008). The Pliocene (5.3–2.6 myr) oceans had higher temperatures than today, and it has been suggested that the North Atlantic thermohaline circulation was more intense than at present, enhancing heat transport from the tropics, which also increased SST (Filippelli and Flores 2009). The higher temperatures recorded during this interglacial period and the subsequent sea level rise seem to have promoted a northward displacement of tropical fauna (Yara et al. 2012) by creating additional habitats for coastal organisms. Pleistocene deposits (2.6–0.012 myr) from the island of Santa Maria, Azores) revealed the existence of warm-water gastropod fossils (Avila et al. 2002), which provides evidence for the suggested range expansion. Paleontological studies in the Azores indicated a decrease in the SSTs during the last glacial maximum (18 000–15 000 years ago; [Seidov et al. 1996]) that seemed to have caused a local extinction of gastropods with West African or Caribbean affinities such as *Conus ventricosus*, *Conus venulatus* or *C. ermineus* (Avila et al. 2002, 2008). The present-day existence of a single *Conus* species in Madeira (Monteiro et al. 2004) also fits the temperature-driven *Conus* diversity, as this area is colder (Fig. 6). There is no fossil record for this archipelago, and therefore, it is not possible to conjecture whether this genus may have once been represented by more species.

According to the RF model, salinity is also highly related to *Conus* diversity in the eastern Atlantic (Fig. 5a). Large freshwater inputs (heavy rainfall drains through several major rivers such as the Niger in the Gulf of Guinea) (Spalding et al. 2001) reduce coastal salinity, which may affect these species and contribute to the reduced diversity of *Conus* in the West African tropical area, when compared with the IWP region.

CONCLUSION

Eustatic sea level fluctuations since the Miocene caused similar effects on the relative shallow-marine area in the four studied Macaronesian archipelagos, and as such, cannot fully explain the disparate number of extant endemic *Conus* lineages. The observed differences in *Conus* speciation patterns from both Atlantic archipelagos seem to result from a combination of different factors acting to promote diversification in Cape Verde but preventing divergence in the Canary Islands. The southward direction of the Canary Current may represent an obstacle to colonization from southern mainland locations where *Conus* are more abundant. We also believe that other factors influential to the evolution of *Conus* in the Canarian archipelago include habitat destruction caused by intense volcanism during the Late Quaternary, proximity to the mainland, low SSTs, and the recent colonization of these islands by *C. guanche*.

Results of the RF model showed that SST is clearly identified as the most important predictor of *Conus* diversity either at continental scales (eastern Atlantic coast) or in a broader context (worldwide) and may represent a major factor underlying the worldwide distribution of this group. The latitudinal gradient of *Conus* diversity with increasing species richness toward the tropics, predominantly observed in the IWP region where SSTs are higher, is not reflected in the eastern Atlantic. We found that *Conus* species diversity is higher at subtropical latitudes (≈15ºN, Senegal and ≈15ºS, Angola) significantly decreasing toward the tropics (Fig. 6). The observed distribution of *Conus* species richness in the eastern Atlantic (Fig. 6) is most likely to be due to habitat unsuitability (large freshwater inputs and absence of rocky beaches) of the tropical western African coastline.

SUPPLEMENTARY MATERIAL

Supplementary material can be found in the Dryad data repository at http://dx.doi.org/10.5061/dryad.48s53.

FUNDING

This work was supported by Portuguese National Funding Agency for Science, Research and Technology (FCT - Fundação para a Ciencia e Tecnologia) post-doctoral fellowships [SFRH/BPD/65830/2009 to R.L.C. and SFRH/BPD/34932/2007 to F.P.L.].

ACKNOWLEDGEMENTS

We are very grateful to the following people for providing samples of *C. guanche*: F. Deniz, F. Sicilia, R. Mesa, F. Titselaar, R. Vega and J. Ahuir. We thank Dr. Nicolas Puillandre (Museum National d'Histoire Naturelle de Paris) for the generous offer of samples of *C. guanche* from Western Sahara. We thank Dr. Howard Peters (University of York, UK) for making the *Conus* worldwide distribution shapefile available at the IUCN database. We thank Prof. Frank Anderson, Dr. Adrian Paterson, Dr. Christopher E. Bird and two anonymous reviewers for helpful comments on an earlier version of this manuscript. We also thank Luci Jesus and Rui Candeias for technical help.

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