Phylogenetic relationships of cone snails endemic to Cabo Verde based on mitochondrial genomes

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Abstract

Background: Due to their great species and ecological diversity as well as their capacity to produce hundreds of different toxins, cone snails are of interest to evolutionary biologists, pharmacologists and amateur naturalists alike. Taxonomic identification of cone snails still relies mostly on the shape, color, and banding patterns of the shell. However, these phenotypic traits are prone to homoplasy. Therefore, the consistent use of genetic data for species delimitation and phylogenetic inference in this apparently hyperdiverse group is largely wanting. Here, we reconstruct the phylogeny of the cones endemic to Cabo Verde archipelago, a well-known radiation of the group, using mitochondrial (mt) genomes.

Results: The reconstructed phylogeny grouped the analyzed species into two main clades, one including Kalloconus from West Africa sister to Trovaconus from Cabo Verde and the other with a paraphyletic Lautoconus due to the sister group relationship of Africonus from Cabo Verde and Lautoconus ventricosus from Mediterranean Sea and neighboring Atlantic Ocean to the exclusion of Lautoconus endemic to Senegal (plus Lautoconus guanche from Mauritania, Morocco, and Canary Islands). Within Trovaconus, up to three main lineages could be distinguished. The clade of Africonus included four main lineages (named I to M), each further subdivided into two monophyletic groups. The reconstructed phylogeny allowed inferring the evolution of the radula in the studied lineages as well as biogeographic patterns. The number of cone species endemic to Cabo Verde was revised under the light of sequence divergence data and the inferred phylogenetic relationships.

Conclusions: The sequence divergence between continental members of the genus Kalloconus and island endemics ascribed to the genus Trovaconus is low, prompting for synonymization of the latter. The genus Lautoconus is paraphyletic. Lautoconus ventricosus is the closest living sister group of genus Africonus. Diversification of Africonus was in allopatry due to the direct development nature of their larvae and mainly triggered by eustatic sea level changes during the Miocene-Pliocene. Our study confirms the diversity of cone endemic to Cabo Verde but significantly reduces the number of valid species. Applying a sequence divergence threshold, the number of valid species within the sampled Africonus is reduced to half.

Keywords: Mitochondrial genomes, Africonus, Trovaconus, Kalloconus
Background

The cone snails (Conidae, Gastropoda) endemic to the archipelago of Cabo Verde in West Africa represent one of the few textbook examples of a well-documented insular species radiation involving marine organisms [1–3]. Cone snails, which are found in tropical and subtropical marine waters throughout the world, show a hotspot of species diversity in the Cabo Verde archipelago with up to 95 endemic species (roughly 10% of cone species diversity worldwide) narrowly confined to about 4000 km² [4]. As in other parts of the world, cone snails endemic to Cabo Verde constitute a key component of the intertidal and subtidal ecosystems associated to rocky shores, coral reefs, and sandy bottoms. All cones endemic to Cabo Verde feed on marine annelid worms [1] and use a sophisticated venom apparatus (including a venom gland that produces conotoxins and a specialized harpoon-like radular tooth) to capture their preys [5]. Another interesting biological feature common to all these endemic species is that they have direct development. Their larvae lack a pelagic stage, and thus show a considerably reduced dispersal capacity [1]. Survival rate is higher for this type of larvae since they are less likely to be eaten by predators and are not dependent on plankton for feeding (i.e., non-planktotrophic).

The origin and evolutionary history of cones endemic to Cabo Verde has been the subject of several recent phylogenetic studies [1, 2, 6, 7]. Molecular phylogenies demonstrated that two different ancestors reached the archipelago independently and subsequently diversified following recurrent biogeographic patterns [1, 2, 7]. The existence of two clades led to the classification of cone species endemic to Cabo Verde into two genera, Africonus and Trovaconus [8]. The question of which species are the closest living sister groups to Africonus and Trovaconus remains open [1, 2]. According to a previous study, the ancestor of Africonus colonized the archipelago in the Miocene, about 16.5 million years ago (mya; [1]), and spread to all islands (except Fogo, the youngest, with steep slopes in the coast and ongoing volcanic activity). Most (95%) of the currently described species endemic to Cabo Verde belong to Africonus, and are normally referred to as restricted to a single island and in some cases even to single bays within an island [3]. The ancestor of Trovaconus arrived at Cabo Verde archipelago in the Pliocene, about 4.6 mya, and diversified only in four islands (Sal, Boa Vista, Maio, and possibly Santiago), which are the closest to the continent [1]. These cones are significantly larger in size than those belonging to Africonus and show wider distributions extending in some cases to more than one island. It has been hypothesized that diversification within each genus was in allopatry and followed recurrent eustatic sea level changes during the Neogene that intermittently connected and disconnected the islands [1, 7]. However, sea level fluctuations alone do not fully explain the extraordinary diversity of cones in Cape Verde since nearby archipelagos in the Macaronesia biogeographic region such as the Canary Islands subjected to similar trends since the Miocene do not have endemic cone species [6]. A larger distance to the mainland, which enhances isolation and restricts gene flow combined with a higher mean sea surface temperature and the presence of more suitable habitats may have promoted a significant increase in diversification rates in the Cabo Verde archipelago [6].

The rate of description of new cone species endemic to Cabo Verde has accelerated more than expected during the last years (Fig. 1). After the early descriptions in the eighteenth and nineteenth centuries based on samples brought to Europe by naturalists [9], the main contribution to the cataloguing of cone species endemic to Cabo Verde was due to the work of Emilio Rolán [10], who drew attention to this singular radiation. Hence, around year 2000, there were about 50 species recognized [11] and remarkably this number has almost doubled in the last 2-3 years [12–19]. However, it is important to note that many of the recent species diagnoses in cones are mainly based on the shape, color, and banding patterns of the shell. These phenotypic characters are highly variable at the population level and prone to local adaptation and convergence, making species assignment problematic and sometimes, misleading [7]. In many cases, distinguishing whether different shell morphotypes of cone snails represent valid species or ecotypes of the same species is challenging [20]. Therefore, determination of genetic variation and inference of phylogenetic relationships based on DNA sequence data are timely as part of a multidisciplinary approach [21] to identify and delimit species and to understand evolutionary processes underlying diversification within cones, in general, and within those endemic to Cabo Verde, in particular.

Here, we used nearly complete mitochondrial (mt) genomes, which have proven to successfully reconstruct robust phylogenies of Conidae [22] and of particular groups such as the cones endemic to Senegal [23]. In this study, we sequenced the nearly complete mt genomes of 88 individuals representing different populations and species of Africonus and Trovaconus endemic to Cabo Verde. We aimed to: (1) reconstruct a highly resolved phylogeny of cones endemic to Cabo Verde; (2) determine the closest living sister groups of Africonus and Trovaconus endemic to Cabo Verde; (3) date major cladogenic events and analyze biogeographical patterns; (4) study radular tooth evolution within the two genera; and (5) provide a first genetic hypothesis of species delimitation in the radiation of Cabo Verde endemic cones.

Results

Sequencing, assembly, and genome organization

The nucleotide sequences of the near-complete mt genomes of 75 specimens of Africonus, 13 specimens of
Trovaconus, and one specimen of Lautoconus ventricosus were determined (Table 1). These mt genomes lacked the trnF gene, the control region, and the start of the cox3 gene because the corresponding fragment was not PCR amplified. The number of reads, mean coverage, and length of each mt genome are provided in Table 1. The mt genomes of Africonus boavistensis and Africonus denizi received the minimum (42,021) and maximum (906,765) number of reads, respectively. The same samples received the minimum (412×) and maximum (8,885×) mean coverage, respectively (Table 1).

All sequenced mt genomes encode for 13 protein-coding, 2 rRNA and 21 tRNA genes (but note that the trnF gene could not be determined; see above). They all share the same genome organization: the major strand encodes all genes, except those forming the cluster MYCWQGE (trnM, trnY, trnC, trnW, trnQ, trnG, trnE) and the trnT gene.

Phylogenetic relationships and sequence divergences between clades

Phylogenetic relationships of cones endemic to Cabo Verde were reconstructed based on the nucleotide sequences of the concatenated 13 mt protein-coding and two rRNA genes using probabilistic methods and Chelyconus ermineus as outgroup. The final matrix was 13,572 positions in length. According to the AIC, the best partition scheme for the protein-coding genes was the one combining all these genes but analyzing each codon position separately. The best substitution model for each of the three codon positions was GTR + I + G. For the rRNA genes, the best scheme had both genes combined under the GTR + I + G model. Both, ML (−lnL = 75,600.18) and BI (−lnL = 76,002.71 for run 1; −lnL = 76,288.44 for run 2) arrived at almost identical topology (Figs. 2 and 3). Most nodes received high statistical support and differences in topology were restricted exclusively to three relatively shallow nodes that had low support in ML and were unresolved in BI. Two of these nodes involved almost identical sequences and corresponded to Africonus bernardinoi / Africonus pseudocuneolus and Africonus teodorae / Africonus fiadeiroi, respectively. The third unresolved node corresponded to a trichotomy involving Africonus felitae, Africonus regonae and Africonus longilineus / Africonus cagarralensis / Africonus melissae.

The reconstructed phylogeny (Fig. 2) grouped the analyzed species into two main clades, one including Kalloconus from mainland West Africa sister to Trovaconus from Cabo Verde and the other having paraphyletic Lautoconus due to the sister group relationship of Africonus from Cabo Verde and Lautoconus ventricosus from Mediterranean Sea and neighboring Atlantic Ocean to the exclusion of Lautoconus endemic to Senegal (plus Lautoconus guanche from Mauritania, Morocco, and Canary Islands). Within Trovaconus, up to three main lineages could be distinguished (Fig. 2). The first one included two specimens from Sal initially identified as Trovaconus ateralbus, which were sister to a clade including one lineage with specimens from Maio and Boa Vista identified as Trovaconus venulatus and another lineage having mostly specimens of Trovaconus pseudonivifer from Maio and Boa Vista but also one specimen of Trovaconus trochulus from Boa Vista and one of Trovaconus atlanticoselvagem from Baixo João Valente (Fig. 2).

The clade of Africonus from Cabo Verde included four main lineages (named I to IV), each further subdivided...
Table 1 Mitochondrial (mt) genomes analyzed in this study

| ID CV | Initial species identification | Location | Coordinates | Coverage mean depth | Length (bp) | GenBank Acc. No | Voucher DNA (MNCN/ADN) | Voucher shell (MNCN 15.05/) | New species proposed* |
|-------|--------------------------------|----------|-------------|---------------------|-------------|----------------|------------------------|--------------------------|------------------------|
| 1020  | Africonus antoniaensis         | Água Doce, Boa Vista, Cabo Verde | 16°12'29"N, 22°44'7"W | 151104 | 1476.8 | 15332 | MF491587 | 95072 | 79889 — |
| 0885  | Africonus antonioniomonteiroi | Pedra Lume, Sal, Cabo Verde | 16°45'44"N, 22°53'2"W | 232049 | 2273.4 | 15328 | MF491578 | 95063 | 79794 — |
| 0927  | Africonus bernardinoi         | Pedra Lume, Sal, Cabo Verde | 16°45'44"N, 22°53'2"W | 59799 | 583.3 | 15328 | MF491582 | 95067 | 79835 Africonus cuneolus |
| 0520  | Africonus boavistensis        | Baia do Ervatão (North), Boa Vista, Cabo Verde | 16°12'3"N, 22°54'45"W | 42021 | 412.8 | 15217 | MF491563 | 95045 | 80413 — |
| 1135  | Africonus cabraloi            | Estancinha, Boa Vista, Cabo Verde | 16°13'12"N, 22°55'9"W | 74446 | 730.4 | 15329 | MF491598 | 95083 | 80004 Africonus crotchi |
| 0895  | Africonus caganalensis        | Pedra Lume, Sal, Cabo Verde | 16°45'44"N, 22°53'2"W | 161290 | 1367.2 | 15320 | MF491579 | 95064 | 79804 Africonus longilineus |
| 0173  | Africonus calhetae            | Praia da Soca, Maio, Cabo Verde | 15°15'8"N, 23°13'4"W | 55433 | 544.7 | 15242 | MF491534 | 95016 | 78798 — |
| 0920  | Africonus cf. anthonyi        | Ilheus do Chano, Sal, Cabo Verde | 16°41'37"N, 22°52'47"W | 172336 | 1678.6 | 15315 | MF491581 | 95066 | 79828 Africonus cuneolus |
| 0162  | Africonus cf. claudiae        | Praia da Soca, Maio, Cabo Verde | 15°15'8"N, 23°13'4"W | 87407 | 858.6 | 15326 | MF491533 | 95015 | 78787 Africonus calhetae |
| 0465  | Africonus cf. delanoaya       | Ponta Antónia, Boa Vista, Cabo Verde | 16°13'24"N, 22°46'59"W | 382817 | 3736.1 | 15335 | MF491559 | 95041 | 80409 Africonus fuscoflavus |
| 0207  | Africonus cf. galeao          | Ponta Pipa, Maio, Cabo Verde | 15°19'30"N, 23°94'8"W | 81447 | 797.3 | 15325 | MF491536 | 95018 | 78832 Africonus galeao |
| 0135  | Africonus cf. gonsaloi        | Praia Gonzalo, Maio, Cabo Verde | 15°16'13"N, 23°6'15"W | 148032 | 1455.5 | 15250 | MF491529 | 95011 | 78760 Africonus gonsalo |
| 0380  | Africonus cf. miguelfdaderoi  | Jorrita, Baia da Gata, Boa Vista, Cabo Verde | 16°12'9"N, 22°22'2"W | 358342 | 3507.9 | 15328 | MF491548 | 95030 | 80398 Africonus vulcanus |
| 1400  | Africonus cf. miruchae        | Calhau, São Vicente, Cabo Verde | 16°51'7"N, 24°51'59"W | 523002 | 5104.9 | 15321 | MF491601 | 95088 | 78562 Africonus sp. nov. 1 |
| 0223  | Africonus claudiae            | Ponta Pipa, Maio, Cabo Verde | 15°19'30"N, 23°94'8"W | 148508 | 1434.5 | 15337 | MF491537 | 95019 | 78848 Africonus galeao |
| 0303  | Africonus condei              | Baia Grande, Derrubado, Boa Vista, Cabo Verde | 16°13'31"N, 22°47'1"W | 253863 | 2472.2 | 15248 | MF491542 | 95024 | 80392 Africonus crotchi |
| 0045  | Africonus crioulius           | Praia Santana, Maio, Cabo Verde | 15°18'13"N, 23°11'49"W | 255019 | 2502 | 15247 | MF491521 | 95003 | 78670 Africonus malaeensis |
| 1075  | Africonus crochti             | Morro de Areia, Boa Vista, Cabo Verde | 16°52'44"N, 22°57'7"W | 332865 | 3232.6 | 15329 | MF491591 | 95076 | 79944 — |
| 0803  | Africonus cuneolus            | Calheta Funda, Sal, Cabo Verde | 16°39'6"N, 22°56'33"W | 184181 | 1791.6 | 15329 | MF491569 | 95053 | 79712 — |
| 0936  | Africonus cuneolus            | Santa Maria, Sal, Cabo Verde | 16°35'38"N, 22°53'36"W | 80472 | 787.4 | 15328 | MF491583 | 95068 | 79844 — |
| 1420  | Africonus cuneolensis         | Praia de Palmo Tostão, Santa Luzia, Cabo Verde | 16°45'19"N, 24°45'24"W | 857123 | 8358.6 | 15329 | MF491602 | 95089 | 78581 — |
| 1017  | Africonus damiai              | Água Doce, Boa Vista, Cabo Verde | 16°12'29"N, 22°44'7"W | 76477 | 745.5 | 15326 | MF491586 | 95071 | 79886 Africonus roeckeli |
| 0405  | Africonus damatti             | Baia da Gata (center), Boa Vista, Cabo Verde | 16°11'50"N, 22°42'32"W | 315488 | 2914.3 | 15358 | MF491551 | 95033 | 80401 — |
| 1428  | Africonus decoratus           | Curral, Santa Luzia, Cabo Verde | 16°46'23"N, 24°47'13"W | 566822 | 5540.2 | 15326 | MF491603 | 95090 | 78589 — |
| 0370  | Africonus deyanoyae           | Jorrita, Baia da Gata, Boa Vista, Cabo Verde | 16°12'9"N, 22°42'22"W | 158489 | 1543.7 | 15323 | MF491547 | 95029 | 80397 — |
| 1471  | Africonus deniai              | Praia Grande, São Vicente, Cabo Verde | 16°51'40"N, 24°52'30"W | 906765 | 8885.2 | 15326 | MF491605 | 95092 | 78621 — |
| 0315  |                                |                                |          | 214173 | 2089.2 | 15243 | MF491543 | 95025 | 80393 — |
Table 1 Mitochondrial (mt) genomes analyzed in this study (Continued)

| Africonus | Location | Coordinates | Accession | Identity | Length | E-value |
|-----------|----------|-------------|-----------|----------|--------|---------|
| derrubado | Baia Grande, Derrubado, Boa Vista, Cabo Verde | 16°13′31″N, 22°47′17″W | MF491566 | 95049 | 80416 |
| 0055      | Africonus diminutus | Ilhéu de Sal Rei, Boa Vista, Cabo Verde | 16°9′50″N, 22°55′31″W | 84042 | 8204.1 | 15330 |
| 1025      | Africonus docensis | Aguas Doce, Boa Vista, Cabo Verde | 16°12′29″N, 22°44′7″W | 47313 | 464.8 | 15329 |
| 0385      | Africonus evarai | Zebraca (near Ilhéu do Galeão), Boa Vista, Cabo Verde | 16°16′26″N, 22°42′40″W | 226416 | 2218 | 15243 |
| 0070      | Africonus fontanalis | Porto Cais, Maio, Cabo Verde | 15°19′15″N, 23°11′0″W | 97527 | 954.7 | 15330 |
| 0835      | Africonus feliae | Rabo de Junco, Sal, Cabo Verde | 16°4′14″N, 22°58′35″W | 344190 | 3343.9 | 15404 |
| 1437      | Africonus fernandesii | Porto Novo, Santo Antão, Cabo Verde | 17°1′4″N, 25°3′22″W | 742414 | 7244.2 | 15324 |
| 0332      | Africonus fiadeiroi | Derrubado (bay West), Boa Vista, Cabo Verde | 16°13′22″N, 22°47′41″W | 205910 | 2016.5 | 15243 |
| 0855      | Africonus fontanum | Baia da Fontona, Sal, Cabo Verde | 16°4′22″N, 22°58′46″W | 156259 | 1523.9 | 15328 |
| 0945      | Africonus fontanae | Regonda, Sal, Cabo Verde | 16°48′57″N, 22°59′33″W | 56310 | 549.8 | 15327 |
| 0450      | Africonus fuscoflavus | Derrubado (bay East), Boa Vista, Cabo Verde | 16°13′31″N, 22°47′3″W | 151904 | 1478.6 | 15331 |
| 0052      | Africonus galaeo | Navio Quebrado, Terras Salgadas, Maio, Cabo Verde | 15°18′34″N, 23°11′2″W | 117940 | 1139.2 | 15326 |
| 0134      | Africonus gonorai | Praia Gonçalo, Maio, Cabo Verde | 15°16′13″N, 23°6′15″W | 188174 | 1835.9 | 15339 |
| 1390      | Africonus grahami | Calhau, São Vicente, Cabo Verde | 16°5′17″N, 24°5′15″W | 464704 | 4536.1 | 15325 |
| 0140      | Africonus irregularis | Porto Cais (North), Maio, Cabo Verde | 15°19′45″N, 23°10′3″W | 202254 | 1937.9 | 15321 |
| 0317      | Africonus irregularis | Baia Grande, Derrubado, Boa Vista, Cabo Verde | 16°13′31″N, 22°47′17″W | 170523 | 1668.2 | 15331 |
| 0392      | Africonus irregularis | Baia da Gata, Boa Vista, Cabo Verde | 16°11′50″N, 22°42′32″W | 252126 | 2454.3 | 15324 |
| 1084      | Africonus irregularis | Morro de Areia, Boa Vista, Cabo Verde | 16°5′24″N, 22°5′7″W | 125264 | 1225.1 | 15330 |
| 1128      | Africonus irregularis | Estancinha, Ponta do Sol, Boa Vista, Cabo Verde | 16°13′12″N, 22°5′59″W | 469101 | 4597.1 | 15313 |
| 0225      | Africonus isabelarum | Ponta do Pau Seco, Maio, Cabo Verde | 15°15′26″N, 23°13′16″W | 247567 | 2431.7 | 15244 |
| 0085      | Africonus josephinae | Lage Branca, Maio, Cabo Verde | 15°18′32″N, 23°8′17″W | 224495 | 2204.6 | 15239 |
| 0555      | Africonus josephinae | Ilhéu de Sal Rei, Boa Vista, Cabo Verde | 16°9′50″N, 22°55′31″W | 169723 | 1611.8 | 15330 |
| 0830      | Africonus langilinus | Serra Negra, Sal, Cabo Verde | 16°3′81″N, 22°5′35″W | 148726 | 1453 | 15316 |
| 0847      | Africonus langilinus | Rabo de Junco, Sal, Cabo Verde | 16°4′14″N, 22°5′35″W | 308057 | 3000.9 | 15333 |
| 0410      | Africonus luquei | Praia Canto, Boa Vista, Cabo Verde | 16°11′10″N, 22°42′28″W | 83198 | 815.1 | 15244 |
| 0064      | Africonus luquensis | Porto Cais, Maio, Cabo Verde | 15°19′15″N, 23°11′10″W | 143797 | 1402.9 | 15327 |
| 0510      | Africonus markeppensi | Ervato Norte, Boa Vista, Cabo Verde | 16°12′3″N, 22°54′43″W | 254030 | 2480.3 | 15330 |
| 0102      | Africonus markeppensi | — | — | — |
| 0565      | Africonus dimiditatis | — | — | — |
| 1025      | Africonus docenisi | — | — | — |
| 0385      | Africonus evaroi | — | — | — |
| 0070      | Africonus fontanalis | — | — | — |
| 0835      | Africonus feliae | — | — | — |
| 1437      | Africonus fernandesii | — | — | — |
| 0332      | Africonus fiadeiroi | — | — | — |
| 0855      | Africonus fontanum | — | — | — |
| 0945      | Africonus fontanae | — | — | — |
| 0450      | Africonus fuscoflavus | — | — | — |
| 0052      | Africonus galaeo | — | — | — |
| 0134      | Africonus gonorai | — | — | — |
| 1390      | Africonus grahami | — | — | — |
| 0140      | Africonus irregularis | — | — | — |
| 0317      | Africonus irregularis | — | — | — |
| 0392      | Africonus irregularis | — | — | — |
| 1084      | Africonus irregularis | — | — | — |
| 1128      | Africonus irregularis | — | — | — |
| 0225      | Africonus isabelarum | — | — | — |
| 0085      | Africonus josephinae | — | — | — |
| 0555      | Africonus josephinae | — | — | — |
| 0830      | Africonus langilinus | — | — | — |
| 0847      | Africonus langilinus | — | — | — |
| 0410      | Africonus luquei | — | — | — |
| 0064      | Africonus luquensis | — | — | — |
| 0510      | Africonus markeppensi | — | — | — |
| 0102      | Africonus markeppensi | — | — | — |

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| Table 1 Mitochondrial (mt) genomes analyzed in this study (Continued) |
|---------------------------------------------------------------|
| **Africonus marccastelluzzii** |
| Lage Branca, Maio, Cabo Verde | 15°18′32″N, 23°8′17″W | Africonus marccastelluzzii |
| 0870 |
| **Africonus melissae** |
| Baia da Parda, Sal, Cabo Verde | 16°45′7″N, 22°53′56″W | Africonus melissae |
| 0455 |
| **Africonus messiasi** |
| Demubado (bay East), Boa Vista, Cabo Verde | 16°1′33″N, 22°47′3″W | Africonus messiasi |
| 0426 |
| **Africonus miguelialidoi** |
| Praia Canto, Boa Vista, Cabo Verde | 16°11′10″N, 22°42′28″W | Africonus miguelialidoi |
| 0905 |
| **Africonus mordax** |
| Baia do Rucamento, Sal, Cabo Verde | 16°41′20″N, 22°56′24″W | Africonus mordax |
| 1091 |
| **Africonus messiasi** |
| Morro de Areia, Boa Vista, Cabo Verde | 16°5′24″N, 22°57′7″W | Africonus messiasi |
| 1395 |
| **Africonus nelsonii** |
| Calhau, São Vicente, Cabo Verde | 16°51′57″N, 24°5′15″W | Africonus nelsonii |
| 0250 |
| **Africonus pseudocuneolus** |
| Serra Negra, Sal, Cabo Verde | 16°38′17″N, 22°53′56″W | Africonus pseudocuneolus |
| 0820 |
| **Africonus raulishai** |
| Praia da Soca, Maio, Cabo Verde | 15°15′8″N, 23′13′4″W | Africonus raulishai |
| 0036 |
| **Africonus regoneae** |
| Baia da Fontona, Sal, Cabo Verde | 16°44′22″N, 22°58′46″W | Africonus regoneae |
| 0865 |
| **Africonus regoneae** |
| Regona, Sal, Cabo Verde | 16°48′5″N, 22°59′33″W | Africonus regoneae |
| 0950 |
| **Africonus roeckeli** |
| Praia Canto, Boa Vista, Cabo Verde | 16°11′10″N, 22°42′28″W | Africonus roeckeli |
| 0586 |
| **Africonus saleiensis** |
| Ilhéus do Sal Rei, Boa Vista, Cabo Verde | 16°5′24″N, 22°57′7″W | Africonus saleiensis |
| 0549 |
| **Africonus saranegrae** |
| Serra Negra, Sal, Cabo Verde | 16°38′17″N, 22°53′56″W | Africonus saranegrae |
| 0810 |
| **Africonus silviae** |
| Morro de Areia, Boa Vista, Cabo Verde | 16°5′24″N, 22°57′7″W | Africonus silviae |
| 1078 |
| **Africonus swinneni** |
| Porto Ferreira, Boa Vista, Cabo Verde | 16°47′5″N, 22°40′17″W | Africonus swinneni |
| 0445 |
| **Africonus teodorae** |
| Estancinha, Ponta do Sol, Boa Vista, Cabo Verde | 16°13′12″N, 22°55′9″W | Africonus teodorae |
| 1125 |
| **Africonus umbilinæae** |
| Espingueira, Boa Vista, Cabo Verde | 16°12′55″N, 22°47′49″W | Africonus umbilinæae |
| 1035 |
| **Africonus verdensis** |
| Tarrafal, Santiago, Cabo Verde | 15°16′50″N, 23′45′15″W | Africonus verdensis |
| 0240 |
| **Africonus vulcanus** |
| Porto Ferreira, Boa Vista, Cabo Verde | 16°7′45″N, 22°40′17″W | Africonus vulcanus |
| 0435 |
| **Africonus zinhasi** |
| Curral Velho, Boa Vista, Cabo Verde | 15°5′8″N, 22°47′42″W | Africonus zinhasi |
| 1110 |
| **Trovacoconus atlanticaselvagem** |
| Baiao João Valente, Boa Vista, Cabo Verde | 15°44′2″N, 23′56′2″W | Trovacoconus atlanticaselvagem |
| 7036 |
| **Trovacoconus cf. atralbus** |
| Serra Negra, Sal, Cabo Verde | 16°38′17″N, 22°53′56″W | Trovacoconus cf. atralbus |
| 0616 |
| **Trovacoconus pseudonivifer** |
| Ponta do Pau Seco, Maio, Cabo Verde | 15°15′26″N, 23′13′16″W | Trovacoconus pseudonivifer |
| 0010 |
| **Trovacoconus pseudonivifer** |
| Lage Branca, Maio, Cabo Verde | 15°18′32″N, 23′8′17″W | Trovacoconus pseudonivifer |
| 0094 |
| **Trovacoconus pseudonivifer** |
| Porto Caí (north), Maio, Cabo Verde | 15°19′45″N, 23′10′57″W | Trovacoconus pseudonivifer |
| 0154 |
| **Trovacoconus pseudonivifer** |
| Praia Canto, Boa Vista, Cabo Verde | 16°11′10″N, 22°42′28″W | Trovacoconus pseudonivifer |
| 0420 |
| **Trovacoconus pseudonivifer** |
| Porto Ferreira, Boa Vista, Cabo Verde | 16°7′45″N, 22°40′17″W | Trovacoconus pseudonivifer |
| 0435 |
| **Trovacoconus pseudonivifer** |
| Porto Ferreira, Boa Vista, Cabo Verde | 16°47′5″N, 22°40′17″W | Trovacoconus pseudonivifer |
| 0445 |
| **Trovacoconus pseudonivifer** |
| Porto Ferreira, Boa Vista, Cabo Verde | 16°5′24″N, 22°57′7″W | Trovacoconus pseudonivifer |
| 0586 |
| **Trovacoconus pseudonivifer** |
| Porto Ferreira, Boa Vista, Cabo Verde | 16°47′5″N, 22°40′17″W | Trovacoconus pseudonivifer |
| 0445 |
Table 1 Mitochondrial (mt) genomes analyzed in this study (Continued)

| Species | Location | Coordinates | Reference | Length (bp) | GenBank Acc. No | Voucher (MNCN/ADN) | Voucher shell (MNCN 15.05/) | New species proposed* |
|---------|----------|-------------|-----------|-------------|----------------|-------------------|---------------------------|------------------------|
| **Lautoconus trochulus** | Baia do Ervatão (North), Boa Vista, Cabo Verde | 16°12.33’N, 22°54.43’W | Cunha et al., (2009) | 15356 | NC_013243 | 6990 | — | — |
| **Lautoconus venulatus** | Praia Real, Maio, Cabo Verde | 15°19.45’N, 23°10.40’W | Abalde et al., (in prep.) | 15276 | MF491531 | 95013 | 78774 | Kalloconus venulatus |
| **Lautoconus venulatus** | Ponta do Pau Seco, Maio, Cabo Verde | 15°15.26’N, 23°13.17’W | Abalde et al., (in prep.) | 15320 | MF491539 | 95021 | 78859 | Kalloconus venulatus |
| **Lautoconus venulatus** | Demrubado (bay West), Boa Vista, Cabo Verde | 16°13.27’N, 22°47.61’W | Abalde et al., (in prep.) | 15326 | MF491546 | 95028 | 80396 | Kalloconus venulatus |
| **Lautoconus venulatus** | Ponta Antoniá, Boa Vista, Cabo Verde | 16°13.24’N, 22°46.59’W | Abalde et al., (in prep.) | 15340 | MF491560 | 95042 | 80410 | Kalloconus venulatus |
| **Kalloconus venulatus** | Kalloconus | 16°11.10’N, 22°42.28’W | Abalde et al., (in prep.) | 15336 | MF491590 | 95075 | 79007 | — |
| **Kalloconus venulatus** | Estani des Peix, Balearic Islands, Spain | 38°43.46’N, 1°24.42’E | Abalde et al., (in prep.) | 15341 | MF491607 | 95094 | 80426 | Lautoconus sp. nov 1 |

GenBank mt genomes

| ID | Species | Location | Coordinates | Reference | Length (bp) | GenBank Acc. No | Voucher (MNCN/ADN) | Voucher shell (MNCN 15.05/) | New species proposed* |
|----|---------|----------|-------------|-----------|-------------|----------------|-------------------|---------------------------|------------------------|
| 6990 | *Afironus* borgesii | Porto Ferreira, Boa Vista, Cabo Verde | 16°74.57’N, 22°40.17’W | Abalde et al., (2017) | 15323 | NC_013234 | 6990 | — | — |
| 6025 | *Afironus* infinitus | Ponta do Pau Seco, Maio, Cabo Verde | 15°15.26’N, 23°13.17’W | Abalde et al., (in prep.) | 15522 | KY864967 | 95001 | 78650 | — |
| 6075 | *Afironus* miruchae | Terrinha Fina, Palhona, Sal, Cabo Verde | 16°49.12’N, 22°59.12’W | Abalde et al., (in prep.) | 15336 | KY864971 | 95062 | 79784 | — |
| 6034 | *Trovaoconus* pseudonivifer | Estancinha, Ponta do Sol, Boa Vista, Cabo Verde | 16°13.12’N, 22°55.59’W | Abalde et al., (in prep.) | 15351 | KY864969 | 95046 | 80418 | Kalloconus trochulus |
| 6050 | *Trovaoconus* venulatus | Ilhéu de Sal Rei, Boa Vista, Cabo Verde | 16°9.56’N, 22°55.23’W | Abalde et al., (in prep.) | 15524 | KX263250 | 86741 | 80419 | Kalloconus venulatus |
| 6061 | *Trovaoconus* aterrailbus | Calheta Funda, Sal, Cabo Verde | 16°39.96’N, 22°56.53’W | Abalde et al., (in prep.) | 15327 | KY864970 | 95051 | 79649 | Kalloconus aterrailbus |
| 1375 | *Kalloconus* cf. byssinus | North Senegal | unknown | Abalde et al., (in prep.) | 15348 | KY864973 | 95085 | 78536 | — |
| 1253 | *Kalloconus* pulcher | Les Alamadies, Dakar, Senegal | 14°44.40’N, 17°31.44’W | Abalde et al., (in prep.) | 15332 | KY864972 | 95084 | 78414 | — |
| 1343 | *Lautoconus* belairensis | Terrou-Bi, Dakar, Senegal | 14°40.20’N, 17°28.12’W | Abalde et al., (2017) | 15321 | KY801849 | 91293 | 78504 | Gen. nov. belairensis |
| 1338 | *Lautoconus* bruguieri | Île de Gorée, Dakar, Senegal | 14°40.16’N, 17°23.58’W | Abalde et al., (2017) | 15340 | KY801851 | 91291 | 78499 | Gen. nov. bruguieri |
| 1296 | *Lautoconus* cloveri | Ndayane, Senegal | 14°33.45’N, 17°7.34’W | Abalde et al., (2017) | 15323 | KY801859 | 91283 | 78457 | Gen. nov. cloveri |
| CG13 | *Lautoconus* guanche | Lanzarote, Canary Islands, Spain | 28°57.16’N, 13°34.22’W | Abalde et al., (2017) | 15506 | KY801847 | 91295 | — | Gen. nov. guanche |
| 1266 | *Lautoconus* hybridus | NGor, Dakar, Senegal | 14°45.67’N, 17°30.36.33’W | Abalde et al., (2017) | 15507 | KY801863 | 91279 | 78427 | — |
| 1278 | *Lautoconus* mercator | NGor, Dakar, Senegal | 14°45.67’N, 17°30.36.33’W | Abalde et al., (2017) | 15329 | KY801862 | 91280 | 78439 | Gen. nov. mercator |
| CV13 | *Lautoconus* ventricosus | Ria Formosa, Faro, Portugal | 36°58.07’N, 7°53.22’W | Uribe et al., (2017) | 15334 | KX263251 | 86742 | — | — |
| CVERM1 | *Chelyconus* ermineus | Praia Gonçalo, Maio, Cabo Verde | 15°16.13’N, 23°6.15’W | Abalde et al., (in prep.) | 15365 | KY864977 | 95095 | 78876 | — |

*Hyphen indicates that original species name is maintained and considered valid
into two monophyletic groups (Figs. 2 and 3). Lineage I was the sister group of the remaining *Africonus* and its two lineages had each species from Maio sister to species from Boa Vista (Fig. 3a). Lineage II included species from Santiago and Maio sister to species endemic to the westernmost islands (Santo Antão, São Vicente and Santa Luzia). These latter species could be grouped into three main lineages, one containing species endemic to São Vicente, another containing species distributed both in Santa Luzia and São Vicente, and the third one including species from the three islands (Fig. 3a). Lineage III included species from Maio sister to species from Boa Vista (Fig. 3a). Lineage IV contained specimens representing most of the described species of *Africonus*. One monophyletic group included species endemic to Sal whereas the other clade included *Africonus isabelarum* from Maio as sister to four lineages, two containing exclusively species from Boa Vista, one having species from Maio sister to *Africonus irregularis* from Boa Vista, and one having species from Boa Vista and *Africonus fantasmalis* from Maio (Fig. 3b).

Pairwise uncorrected sequence divergences were estimated based on the alignment including the nucleotide sequences of the 13 mt protein-coding and two rRNA genes. Pairwise uncorrected sequence divergences between *C. ermineus* and ingroup taxa averaged 18%. The average pairwise uncorrected sequence divergence between the two main ingroup clades (genera *Kalloconus* + *Trovaoconus* versus genera *Lautoconus* + *Africonus*) was 16%. Pairwise uncorrected sequence divergences between *Lautoconus* endemic to Senegal (plus *L. guanche* from Canary Islands) and *Africonus* averaged 11%. The average pairwise uncorrected sequence divergence between *Lautoconus ventricosus* and *Africonus* was 10% whereas between *Kalloconus* and *Trovaoconus*, it was 5%. The pairwise uncorrected sequence divergences between the four main lineages within *Africonus* averaged 6%. The corresponding values for the pairwise divergences between the two major clades defined within each of the lineages I-IV were 4%, 6%, 3%, and 3%, respectively. Pairwise uncorrected sequence divergence comparisons between sister species level were distributed into two different ranges, one closer to 1% (0.5-
Fig. 3 (See legend on next page.)
1.5%) and the other closer to 0% (0-0.5%). The latter divergences were particularly abundant among sister species comparisons within Maio, Boa Vista and Sal. Several mt genomes of different species were almost identical (<0.05%) in sequence including (1) *Africonus delanoyae* and *Africonus luquei*, (2) *Africonus fuscoflavus*, *Africonus* cf. *delanoyae*, and *Africonus messiasi*, (3) *Africonus irregularis* (#1128) and *Africonus cabraloi*, (4) *Africonus verdensis* and *Africonus nelsontiagoi*, and (5) *Africonus gonsaloi* and *Africonus* cf. *gonsaloi*.

**Evolution of radular types**

The different lineages within *Africonus* exhibit distinct radular types (Fig. 3). Most lineages and species showed the “robust” type, which is of medium relative size, with a short, pointed barb and a basal spur (see Additional file 1). The anterior section of the tooth is equal or slightly shorter than the posterior section, and the blade covers most of the anterior section (80% – 85%). There are usually 19 to 30 denticles in the serration, arranged in one row (occasionally two). Several species within lineage IV (*Africonus delanoyae*, *Africonus luquei*, *Africonus swinnen*, *Africonus fuscoflavus*, *Africonus messiasi*, *Africonus silvae* and *Africonus* cf. *delanoyae* from Boa Vista island, and *Africonus fantamalis* from Maio island) exhibited radular teeth of the “elongated” type, similar to the “robust” type but characterized by an anterior section which is longer than the posterior section, a blade covering 40 to 50% of the anterior section, and more numerous denticles in the serration (usually more than 30) often arranged in two rows. Several species (*Africonus borgesi*, *Africonus josephinae* and *Africonus marckeppensi* in lineage I, *Africonus navarroii* in lineage II), all species in lineage III, plus *Africonus vulcanus*, *Africonus miguelfiadeiroi* and *Africonus* cf. *miquelfiadeiroi* in lineage IV) displayed radular tooth of the “broad” type, which is characterized by a medium-sized (Shell Length/Tooth Length = 32-45) and very broad radular tooth (Shell Length/Anterior section Width = 7-12), with an anterior section which is shorter than the posterior section (Tooth Length/Anterior section Length = 2.1-2.9), a blade covering most of the anterior section, and with a variable number of denticles (8 to 30) in the serration arranged in two or more rows. The radular morphology of *Africonus felitae* may represent a special case with a small relative size (Shell Length/Tooth Length = 63-67), narrow (Tooth Length/Anterior section Width = 20-23), the anterior section shorter than the posterior section (Tooth Length/Anterior section Length = 2.2-2.4), and characterized by the total absence of denticles in the serration. The base of this tooth is relatively large and broad.

The species of *Kalloconus* and *Trovaoconus* exhibit essentially two kinds of radular morphologies (Additional file 1). The teeth in *K. pulcher*, and also in *Trovaoconus trochulus*, *T. pseudonivifer* and *Trovaoconus atlanticoselvagem* are narrow and elongated; the blade is moderately short being about one third to almost one-half the length of the anterior section of the tooth, which is distinctly longer than the posterior section of the tooth. There are many denticles (25 to 45 or more) in the long serration, arranged usually in multiple rows with a major row flanked by numerous smaller serrations. In the case of *T. venulatus*, *Trovaoconus aterbus*, and *Trovaoconus cf. aterbus* the teeth are broader, and the anterior and posterior sections are almost equal in length. There are 16 to 33 denticles in the serration, often coarse and hook-shaped in the middle portion, arranged initially in one row becoming two rows below.

**Dating of major cladogenetic events**

Major cladogenetic events within the reconstructed phylogeny were dated using an uncorrelated relaxed molecular clock model, which was calibrated using the age of Sal (28 mya; the oldest island of the archipelago) for the node separating *Africonus* from its sister group, *L. ventricosus*, and the age of the origins of São Vicente, Santo Antão, and Santa Luzia (7.5 mya) for the node splitting the lineage including the endemics to these islands from its sister group lineage including endemics to Maio and Santiago islands [24]. The first divergence event involving *Kalloconus + Trovaoconus* versus (paraphyletic) *Lautoconus + Africonus* was dated at 34 mya (Fig. 4; note that genera and species labels in the chronogram take into account proposed synonymizations, see Discussion). The divergence between the clade containing cones endemic to Senegal (+ *L. guanche*) and the clade including *L. ventricosus* plus *Africonus* was dated at 26 mya. The split between the latter two lineages was dated at 23 mya. The diversification of the crown group of *Africonus* into its four main lineages (I-IV) was estimated to have occurred between 9.4 - 6.9 mya (Fig. 4). The separation of *Kalloconus* and *Trovaoconus* was dated...
9 mya and the diversification of the crown group of *Trovaoncus* was established at 4 mya (Fig. 4).

### Diversification rates through time

Variations in the diversification rates through time were estimated for *Africonus* and the hypothesis of a radiation during the evolutionary history of the clade was tested. The gamma-statistic, which measures departures from a constant rate of diversification, had values of 7.19 ($p < 0.05$) and 3.12 ($p < 0.05$) when considering the currently named (based on phenotypic traits) or only the here-proposed (considering genetic evidence) species for the genus, respectively. In both cases, the hypothesis of a radiation is accepted. According to the lineage through time plots (Fig. 5), the initial rate of increase in the number of species slowed down between six and one and a half million years ago regardless of the species delimitation hypothesis tested. Afterwards, the diversification rate accelerated considerably, and the increase in number of species either continued or abandoned a normal Yule process of speciation when considering the species delimitation hypothesis here proposed or the currently number of named species, respectively.

### Discussion

Cone snails are marine gastropods well known to evolutionary biologists due to their extraordinary species and ecological diversity [25], but also to molecular biologists and pharmacologists due to their sophisticated venom cocktails [26], as well as to amateur naturalists due to their brightly colored and highly appreciated shells [27]. Therefore, they are the subject of intensive research across disciplines and additionally have received wide attention from the general public. There are more than 800 described species and this number increases steadily.
Thus far, species description and identification of cones heavily relies on shell form, color and banding patterns, which may show great variety at local scale leading to important levels of synonymy within the family Conidae [7, 20]. In this regard, species delimitation could greatly improve with the aid of robust molecular phylogenies, which could be used in addition as framework to uncover the evolutionary patterns and processes underlying the diversification of the group. While reconstructing a robust phylogeny for all described cone species worldwide is cumbersome and at present unrealistic within the framework of a single study, it is possible, however, to accomplish a proof-of-concept study in a particular region [23].

We have here reconstructed a molecular phylogeny of cones endemic to Cabo Verde and allied species in the Macaronesian region, continental West Africa, and the Mediterranean region. These cones are particularly interesting from an evolutionary perspective as they have radiated in an oceanic archipelago and constitute a natural experiment to gain insights onto the processes governing diversification and adaptation [29]. Phylogenetic analyses were based on nearly complete mt genomes (only missing the control region and neighboring sequences) and included 105 specimens comprising most of the cone species diversity of the analyzed regions. Probabilistic methods of phylogenetic inference arrived at a robust and highly resolved phylogeny (virtually all nodes received high statistical support, which in most cases was maximal). To our knowledge, this is the first wide application of mt genomes to the resolution of a phylogeny within mollusks (but see [30, 31] for comparable examples in fish and insects, respectively). Previous studies in gastropods were restricted in the number of taxa analyzed (e.g., [22] for the family Conidae) but here we were able to achieve a lineage representation of the reference group (in this case, Cabo Verde cones) only previously attained by studies using few concatenated partial gene sequences (see e.g., [25] for the family Conidae or [1] for the cones of Cabo Verde). Previous phylogenetic studies using complete mt genomes have demonstrated that the level of resolution of these molecular markers is compromised above the superfamily level due to saturation, base composition biases, and among-lineage rate heterogeneity [32, 33]. Here, we show that phylogenetic performance of mt genomes achieves best results when analyzing closely related genera (and their corresponding species). Moreover, results were particularly promising taking into account that Africonus diversity in Cabo Verde was originated through radiation processes, which normally lead to relatively short tree nodes (often difficult to disentangle).

A thorough sampling of closely-related outgroup taxa allowed us to tackle key questions on the origin of the cones endemic to Cabo Verde and on their closest living sister groups. As previously reported, there are two independent origins of Cabo Verde cones, leading to the genera Africonus and Trovaconus, respectively [1]. The closest living sister group of Africonus is L. ventricosus from the Mediterranean Sea and neighboring Atlantic Ocean. Therefore, the origin of this clade is clearly Macaronesian/Mediterranean and these cones are only distantly related to the geographically closer cones endemic to Senegal. These latter cones were ascribed to the genus Lautoconus (as was the case of L. guanche from Canary islands, deeply nested within the clade of Senegal cones; [23]). However, the closest sister group relationship of Africonus and L. ventricosus requires formal description of a different genus for Senegal cones (plus L. guanche), which will be done elsewhere. We could not include any representative of cones endemic to Angola (genus Varioconus) but a recent phylogeny based on partial cox1 gene
sequences recovered all these cones (including Varioconus jourdani from Saint Helena Island) as a monophyletic group sister to Senegal cones [34]. Alternatively, all previously mentioned genera could be merged into genus Lautoconus [35]. However, the relatively high levels of sequence divergence (using the sequence divergence of genus Chelyconus as reference) and the restricted (endemic) distribution of the clades fit better with the former taxonomic proposal. The closest living sister group of Trovaconus is genus Kalloconus from West Africa. Therefore, the origin of this clade is clearly related to neighboring regions of the continent. Actually, the sequence divergence between Trovaconus and Kalloconus is much lower than that estimated between Lautoconus and Africonus. This observation argues against maintaining the generic status of Trovaconus, and supports the inclusion of their species within genus Kalloconus, as some authors have proposed [35]. Hence, Kalloconus would be a genus that is present throughout the coast of West Africa from Morocco to Angola as well as in Canary Islands and Cabo Verde.

Altogether, the two main clades in the reconstructed phylogeny show very distinct patterns of distribution. One clade includes a single genus with widespread distribution in Macaronesia and West Africa whereas the other clade, which occupies the same geographical regions, is divided into several valid genera (Africonus, Lautoconus, Varioconus, Gen. nov. for Senegal endemics). These distinct patterns could be explained partly taking into account differences in larval dispersal capabilities between the two clades [1]. According to the phylogeny, the ancestor of the Kalloconus clade was inferred to have planktotrophic larvae, capable of long dispersals whereas the ancestor of the other clade would have non-planktotrophic larvae, and thus a limited dispersal capability leading to restricted gene flow and higher rates of diversification [36, 37]. Interestingly, the ancestor of Kalloconus species endemic to Cabo Verde (former Trovaconus species) lost planktotrophy, which is a common evolutionary pattern in insular species [38].

According to the reconstructed phylogeny, cones belonging to genus Africonus are divided into four main lineages (I-IV; with each further subdivided into two distinct clades). Species endemic to Maio and Boa Vista are found in all four lineages whereas species endemic to Sal form a clade within lineage IV, species from the westernmost islands (Santo Antão, São Vicente and Santa Luzia) form a clade within lineage II, and the single species from Santiago is recovered within lineage II. Unfortunately, we could not sample specimens of Africonus farnae from Brava and Africonus kersteni from São Nicolau, and cannot determine whether they could be ascribed to any of the above-mentioned four lineages or form their own independent lineages. The single origin of cones endemic to Sal, Santiago, and westernmost islands could be explained by the deep slopes separating these islands whereas the multiple origins of the cones found in Maio and Boa Vista could be associated to the relatively shallow seamount (Baixo João Valente) connecting both islands [1]. These differences in bathymetry in connection with past eustatic sea level changes could be determinant in preventing or promoting dispersal in Africonus species, whose larvae are all non-planktotrophic.

Diversification events among main lineages were concentrated in three major periods. The first one, around the Oligocene-Miocene boundary (23 mya), includes the divergence of cones endemic to Senegal (and Angola) from their sister clade, and the posterior separation within this sister clade of cones endemic to Cabo Verde and those endemic to the Mediterranean Sea and neighboring Atlantic Ocean. During Oligocene-Miocene transition, there was a global cooling event [39, 40], the ice sheet of Antarctica greatly expanded, and a sea level drop of ~50 m occurred [41]. The second period corresponds to a sustained global cooling in the Late Miocene starting 12 mya [42] that produced an eustatic sea level drop between ~10 and ~30 m from 6.26 to 5.50 Mya [43] and culminated with the Messinian Salinity crisis and the desiccation of the Mediterranean Sea at the end of the Miocene from 5.96 to 5.33 Mya [44]. During this period, the divergence of the main lineages within Africonus (I-IV), the cones endemic to Senegal, and Kalloconus occurred. Finally, a burst of speciation events is inferred during the Pleistocene when another cooling period characterized by extreme climate oscillations and drastic eustatic sea level changes concurring with glacial-interglacial periods [45]. Global cooling has been recently proposed to be a driver of diversification of marine species [46] in agreement with our results. The reconstructed phylogeny, the chronogram, and the current geographical distribution of the species altogether support that allopatry is the main mode of speciation for cone snails with non-planktotrophic larvae, as previously suggested [1]. The complex geology of the island of Boa Vista with several eruptions at >16, 15-12.5, and 9.5-4.5 mya [47], involving different parts of the island may have also contributed to creating additional niches along the coast and could explain that this island harbors the highest number of endemic cones.

The reconstructed phylogeny also allows inferring the evolution of the radula in the studied lineages [8, 48]. All analyzed ingroup taxa are vermivorous [8]. Studies documenting potential specialization of the vermivore radular type to prey on specific worm species are scarce and restricted thus far to cone species preying on amphimorphids [49]. Here, we show that most Africonus species show a “robust” radular type, which is shared also with L. ventricosus and a lineage of Senegal cones represented by Lautoconus cloveri and Lautoconus bruguieresii in the phylogeny [23]. Therefore, the common ancestor of cones endemic to Senegal (plus L. guanche), Africonus, and L.
ventricosus was inferred to have a “robust” type radula. The “elongated” type of radular tooth, which was found in several species within lineage IV of Africonus, also appears in a lineage of Senegal cones that is represented by Lautoconus hybridus and L. guanche in the phylogeny [23]. The radular tooth of A. felitae resembles the “small” type observed in a lineage of Senegal cones represented by Lautoconus reticulatus and Lautoconus belairensis in the phylogeny [23]. The “broad” type of radular tooth that appeared independently in several lineages of Africonus has not been observed in any cone from Senegal. While shifts in radular type could be correlated with early cladogenesis in cones endemic to Senegal [23], the evolution of different types of radular tooth within Africonus was restricted to specific cases. Thus, future studies are needed to determine whether in such cases there has been a dietary shift to prey on specific worms. The radula teeth identified in Kalloconus resemble the types “elongated” and “robust” observed within Lautoconus and Africonus, although are clearly distinct. This might indicate instances of convergence, and that only a discrete number of different main types of radula could be found in a given clade.

During the last few years, the number of new cone species described from Cabo Verde has increased at an astonishing rate (e.g., [12]). These new species are identified based on differences (often subtle) in shell shape and color, and their status needs to be contrasted with genetic data to uncover cases of local phenotypic variation within species due to either genetic polymorphism or phenotypic plasticity that may be producing overestimations of the number of species in the group [21]. In addition, genetic data could help identify cases of phenotypic convergence due to adaptation of genetically distinct populations (ecotypes) or species (sibling or cryptic) to similar environments [50–52], also affecting the total number of valid species. Comparative analyses of pairwise uncorrected sequence divergences taking into account the reconstructed phylogeny showed that some described species shared almost identical mt genomes with levels of sequence divergence normally considered to be associated to genetic variation at the population level. Clades comprising these sets of closely related sequences indicate that an uncorrected sequence divergence threshold around 1% could be associated to the species status. This threshold lies well within the so-called grey zone of speciation between 0.5-2% [53]. Of course, these results need to be further confirmed with genomic nuclear data that discard potential events of incomplete lineage sorting and hybridization [54]. In addition, the present study could be further improved in the future by increasing the number of individuals analyzed per original species. Importantly, the comparative analyses on variation of diversification rates through time support the here proposed hypothesis of species delimitation as it concurs with a Yule process of speciation whereas the number of currently named species clearly exceed expectations and would imply an extraordinary recent acceleration of speciation rates.

Our study confirms the diversity of cone endemic to Cabo Verde but significantly reduces the number of valid species. Applying the threshold in a conservative manner (i.e., maintaining described species as valid in case of doubt due to closeness to the threshold) to cones endemic to Cabo Verde would reduce the number of valid species within the sampled Africonus from 65 to 32 (see Table 1 and Fig. 4). The proposed nomenclatural changes follow standard ICZN recommendations maintaining the most senior (oldest) name. Among the species not sampled, two correspond to São Nicolau and Brava islands, four of them are from the islands of São Vicente and Santa Luzia, and most likely represent valid species (Africonus bellulus, Africonus lugubris, Africonus saragasea, Africonus santaluziensis, A. kersteni and A. furnae) given the relative high sequence divergences found among species endemic to these islands. The 19 remaining ones were recently described, mostly from Boa Vista, and are expected to fall in most cases into some of the clades already discussed in the present work, and therefore may correspond to morphs of other described species. A direct consequence of synonymization is that some previously described species of rather restricted distribution are merged as populations into the new species, which considerably increase their range of distribution (Additional file 1). For instance, A. crotchii, which was reported as endemic from Southwest Boa Vista, would be now distributed also in the whole north half of the island. This increase in range of distribution of several species has important effects on their IUCN conservation status [3]. In the case of Kalloconus, some morphotypes attributed to Kalloconus pseudoniviifer are now assigned to Kalloconus trochulus, and Kalloconus atlanticoselvagem is synonymized with K. trochulus. Our specimen of Kalloconus cf. byssinus is from North Senegal and has little sequence divergence compared to Kalloconus pulcher. In this case, it would be important to study K. byssinus from Mauritania or Morocco before considering synonymization. In the opposite direction, there are three clear instances of morphological convergence and thus, of the existence of cryptic species. Those are the cases of Africonus josephi-nae from Maio, Africonus cf. miruchae from São Vicente, and Kalloconus cf. aterralbus from Sal, which will be described as new species in due course.

Conclusions
We reconstructed a robust phylogeny based on mitochondrial genomes of cone snails endemic to Cabo Verde, which provides the necessary framework for future
evolutionary studies focused on this radiation. The double origin of Cabo Verde endemic cones was supported. The ancestor of *Africonus* separated from *L. ventricosus* during the Oligocene-Miocene boundary (about 23 mya) and diversified into four main lineages (I to IV) in the Late Miocene (about 9.4-6.9 mya). The divergence of the ancestor of *Kalloconus* endemic to Cabo Verde from those inhabiting mainland occurred also in the Late Miocene whereas its diversification into three main lineages was dated in the Pliocene (4 mya). Main cladogenetic events within cones endemic to Cabo Verde coincide with global cooling periods, which were characterized by radical climate oscillations and eustatic sea level changes. Recurrent cycles of island connection/ disconnection likely favored speciation in allopatry in these cones, which lack a pelagic larval stage, and thus have limited dispersal capacity. Direct development evolved in the ancestor of *Kalloconus* endemic to Cabo Verde, likely associated to the colonization of the archipelago by a cone with a planktotrophic larval stage. However, in the case of *Africonus*, the ancestor that arrived to Cabo Verde was already non-planktotrophic as the corresponding independent evolutionary shift to direct development predated the separation of cones endemic to Senegal (and Canary Islands) from *L. ventricosus* plus *Africonus*. Radular types were modified during the diversification of *Africonus* from an ancestral “robust” type, although correlation with diet specializations await better knowledge of the specific worm species preyed by the different species of cones. Sequence divergence comparisons and reconstructed phylogenies supported the diversity of cone species endemic to Cabo Verde but significantly reduced its number, which was likely overestimated in the past due to important homoplasy in shell morphology, the, thus far, main discriminant character used for species description and identification.

**Methods**

### Samples and DNA extraction

The complete list of specimens analyzed in this study corresponding to different populations and species of *Africonus* and *Trovaoconus* from Cabo Verde is shown in Table 1, as well as details on the respective sampling localities and museum vouchers. As outgroup taxa, we also sampled and analyzed one specimen of *L. ventricosus* from Formentera Island (Spain). Specimens were collected by snorkel at 1-3 m depth, or picked by hand at low tide. All samples were stored in 100% ethanol. The initial species identification (see corresponding column in Table 1) was based on comparison with type material (mostly deposited in the MNCN) or consulting the original publications. Total DNA was isolated from 5 to 10 mg of foot tissue following a standard phenol-chloroform extraction [55].

### Radular tooth preparation

The radular sac was dissected from the main body and soft parts were digested in concentrated aqueous potassium hydroxide for 24 h. The resulting mixture was then placed in a petri dish and examined with a binocular microscope. The entire radula was removed with fine tweezers and rinsed with distilled water, then mounted on a slide using Aquatex (Merck, Germany) mounting medium, and observed under a compound microscope. Photographs were taken with a charge-coupled device (CCD) camera attached to the microscope. Terminology for radular morphology follows [8], with abbreviations following [48]. Names of radular types follow [23].

### PCR amplification and sequencing

Near-complete (without the control region) mt genomes were amplified through a combination of standard and long PCRs using the primers and following the protocols of [22]. Standard-PCR products were sequenced using Sanger technology. Long-PCR products were subjected to next-generation sequencing. Briefly, PCR amplified fragments from the same mt genome were pooled together in equimolar concentrations. For each cone mt genome a separate indexed library was constructed using the NEXTERA XT DNA library prep kit (Illumina, San Diego, CA, USA). The average size of the Nextera libraries varied between 307 and 345 bp. Libraries were pooled and run in an Illumina MiSeq platform (v.2 chemistry; 2 × 150 paired-end) at Sistemas Genómicos (Valencia, Spain).

### Genome assembly and annotation

The reads corresponding to each mt genome were sorted using the corresponding library indices, and read assembly was performed in the TRUFA webserver [56]. Briefly, adapters were removed using SeqPrep [57], quality of the reads was checked using FastQC v.0.10.1 [58], and raw sequences were trimmed and filtered out according to their quality scores using PRINSEQ v.0.20.3 [59]. Filtered reads were used for de novo assembly of each mt genome using default settings (minimum contig length: 200; sequence identity threshold: 0.95) of Trinity r2012-06-08 [60] in TRUFA, and only retaining contigs with a minimum length of 3 kb. These contigs were used as starting point to assemble the mt genomes using Geneious® 8.0.3. First, the (raw) reads with a minimum identity of 99% were mapped against the contigs to correct possible sequence errors. Then, successive mapping iterations using a 100% identity as threshold were performed to elongate the contigs.

The mt genomes were annotated with the option “Annotate from Database” in Geneious® 8.0.3, using published mt genomes of Conidae as references. Annotations of the 13mt protein-coding genes were refined manually...
identifying the corresponding open reading frames using the invertebrate mitochondrial code. The transfer RNA (tRNA) genes were further identified with tRNAscan-SE 1.21 [61], which infer cloverleaf secondary structures (with a few exceptions that were determined manually). The ribosomal RNA (rRNA) genes were identified by sequence comparison with other Conidae mt genomes [22], and assumed to extend to the boundaries of adjacent genes [62]. GenBank accession numbers of each mt genome are provided in Table 1.

Sequence alignment and phylogenetic analyses
The newly sequenced mt genomes were aligned with the mt genomes of A. borgesi, Africonus infinitus, Africonus miruchae, T. ateralbus, T. pseudonivifer, T. venulatus, and C. ermineus from Cabo Verde, L. hybridus, L. mercator, L. belairensis, L. cloveri, L. bruguieresi, K. pulcher, and K. cf. byssinus from Senegal, L. guanche from Canary Islands, and L. ventricosus from Portugal, which were downloaded from GenBank (Table 1). A sequence data set was constructed concatenating the nucleotide sequences of the 13 mt protein-coding and two rRNA genes. The deduced amino acid sequences of the 13 mt protein-coding genes were aligned separately and used to guide the alignment of the corresponding nucleotide sequences with Translator X [65]. Nucleotide sequences of the mt rRNA genes were aligned separately using MAFFT v7 [64] with default parameters. Ambiguously aligned positions were removed using Gblocks, v.0.91b [65] with the following settings: minimum sequence for flanking positions: 85%; maximum contiguous non-conserved positions: 8; minimum block length: 10; gaps in final blocks: no. Finally, the different single alignments were concatenated using Geneious® 8.0.3. Sequences where format converted for further analyses using the ALTER webserver [66]. The concatenated alignment is available at http://pur.l.org/phylo/treebase/phylobs/study/TB2:S21557. Phylogenetic relationships were inferred using maximum likelihood (ML), [67] and Bayesian inference (BI, [68]). For ML, we used RAxML v8.1.16 [69] with the rapid hill-climbing algorithm and 10,000 bootstrap pseudoreplicates (BP). BI analyses were conducted with MrBayes v3.1.2 [70], running four simultaneous Markov chains for 10 million generation, sampling every 1000 generations, and discarding the first 25% generations as burn-in (as judged by plots of ML scores and low SD of split frequencies) to prevent sampling before reaching stationarity. Two independent Bayesian inference runs were performed to increase the chance of adequate mixing of the Markov chains and to increase the chance of detecting failure to converge, as determined using Tracer v1.6 [71]. The effective sample size (ESS) of all parameters was checked to be above 200. Node support was assessed based on Bayesian Posterior Probabilities (BPP). A node was considered highly supported with BP and BPP values above 70% and 0.95, respectively. The ML and BI phylogenetic trees are available at http://pur.l.org/phylo/treebase/phylobs/study/TB2:S21557.

The best partition schemes and best-fit models of substitution for the data set were identified using PartitionFinder2 [72] with the Akaike information criterion [73]. For the protein-coding genes, the partitions tested were: all genes grouped; all genes separated (except atp6-atp8 and nad4-nad4L); and genes grouped by subunits (atp, cob, cox, and nad). In addition, these three partitions schemes were tested taking into account separately the three codon positions. The rRNA genes were tested with two different schemes, genes separated or combined.

Estimation of divergence times
The program BEAST v.1.8.0 [74] was used to perform a Bayesian estimation of divergence times. An uncorrelated relaxed molecular clock was used to infer branch lengths and nodal ages. The tree topology was fixed using the one recovered by the ML analysis. For the clock model, the lognormal relaxed-clock model was selected, which allows rates to vary among branches without any a priori assumption of correlation between adjacent branches. For the tree prior, a Yule process of speciation was employed. Concatenated protein coding plus rRNA genes were analyzed at the nucleotide level. The partitions and models selected by PartitionFinder2 were applied (see results). The final Markov chain was run twice for 100 million generations, sampling every 10,000 generations, and the first 1000 trees were discarded as part of the burn-in process, according to the convergence of chains checked with Tracer v.1.5. [71]. The ESS of all parameters was above 200.

Despite the fact that there are many fossils of Conidae, it is difficult in many instances to be certain about species identifications given the important levels of homoplasy in shell shape [75]. Hence, although there are fossils attributed to L. ventricosus [76] and L. mercator [77], which could be applied to the reconstructed phylogeny, we opted to calibrate the clock using biogeographical events (i.e., the age of the islands of Cabo Verde). We run a preliminary analysis in which the posterior distribution of the estimated divergence times was obtained by specifying one calibration point as prior for the divergence time of the split between L. ventricosus and the genus Africonus. This genus is endemic to Cabo Verde, and we used the age of formation of the oldest island, Sal (28 Mya; [24]), as biogeographical calibration point. We applied a log-normal distribution as the prior model for the calibration and enforced the median
divergence time to equal 25 (s.d. = 0.05, offset = 0.7). According to the results of the preliminary analysis, we found that only in the case of São Vicente, Santo Antão, and Santa Luzia, the early divergence of living cone endemic lineages followed the origin of the corresponding island, and therefore, we used a second calibration point corresponding to the origin of these islands about 7.5 mya [24]. We applied a log-normal distribution as the prior model for the calibration and enforced the median divergence time to equal 7.5 (s.d. = 0.03, offset = 0). The BEAST tree is available at http://purl.org/phylo/treebase/phylows/study/TB2:S21557.

Diversification rate through time
The chronogram was used to determine diversification rate through time of genus Africonus under alternative (phenotypic versus genetic) species delimitation hypotheses. A lineage through time (LTT) plot analysis was conducted using the APE 4.1 R package [78]. A random sample of 100 trees was selected and mapped over a simulation of 1000 trees following a Yule process of speciation (net diversification rate = 0.4). The phytools R package [79] was used to calculate the Gamma-Statistic [80].

Additional file

Additional file 1: Maps showing sampling localities; diversity of Kalloconus radular teeth. (ZIP 3996 kb)

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Availability of data and materials
Sequence data is available in GenBank.

Authors’ contributions
MJT, CMLA, and RZ collected the material. MJT prepared the radula. SA, JEU, and AIME generated the molecular data. SA analyzed the data. RZ wrote the first draft of the manuscript and all authors contributed to writing the final version. All authors read and approved the final manuscript.

Ethics approval and consent to participate
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Competing interests
The authors declare that they have no competing interests.

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