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Multiple independent colonization of the Canary Islands by the winged grasshopper genus *Sphingonotus* Fieber, 1852

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ABSTRACT

Volcanic archipelagos represent ideal systems to study processes of colonization, differentiation and speciation. The Canary Islands are one of the best studied archipelagos, being composed of seven main islands with a well-known geological history. Most taxa have colonized these islands stepwise from the African or Iberian mainland from east to west, following their geological origin as well as the predominating wind direction and ocean currents. Furthermore, within-island radiations have been reported for several taxa. The grasshopper genus *Sphingonotus* is species-rich and occurs with nine fully winged species on the Canary Islands, seven of which are endemic to single or few islands. We inferred a phylogeny of these species and their North African and Iberian relatives based upon sequences of three mitochondrial genes and one nuclear gene of 136 specimens. Surprisingly, our results suggest that almost all *Sphingonotus* species colonized the archipelago independently from the mainland and nearly no inter-island colonization occurred. Despite their strong flight capabilities, only one pair of endemic species are closely related (*S. sublaevis* from Gran Canary and *S. pachecoi* from Lanzarote). Moreover, no within-island speciation events were detected. We hypothesize that passive wind dispersal from the African mainland was the main driver of the colonization process and that most *Sphingonotus* species are not able to cover inter-island distances by active flight. This, together with strong intrageneric niche overlap might explain the lack of within-island speciation in this taxon.

1. Introduction

Oceanic archipelagos represent ideal settings to study colonization and differentiation processes (MacArthur and Wilson, 1967; Gilbert, 1980; Emerson, 2002; Whittaker et al., 2008). As such the Galapagos, the Caribbean, Hawaii and the Canary Islands have become model systems for biogeographic and evolutionary research (Gillespie et al., 1994a; Emerson et al., 1999, 2000a; Grant and Grant, 2002; Shaw, 2002; Losos, 2009). The main processes determining speciation on islands are colonization, extinction and speciation as a result of geographical barriers or ecological niche differentiation (MacArthur and Wilson, 1967; Emerson, 2008; Losos, 2009). The geographical setting and the geological history of an archipelago have strong effects on the relative importance of each of these processes. Hence, a good knowledge on the origin and history of islands is crucial to understand the evolution of island radiations.

The Canary Islands are one of the best studied volcanic archipelagos, concerning both their geological history (e.g. Ancochea et al., 1990; Acosta et al., 2003) and phylogeography (reviewed in Juan et al., 2000). Due to their geographic position, the African mainland is the predominating colonization source (Juan et al., 2000). Many taxa have undergone radiations within the archipelago subsequent to a single colonization event. Most of these radiations follow a simple stepping stone model from east to west following the prevailing ocean currents and wind directions (e.g. Díaz-Pérez et al., 2008; Hochkirch and Görzig, 2009). However, multiple variants of this pattern have been found, including back-colonization, within-island speciation or reverse colonization from the Canary Islands to the African mainland (Juan et al., 2000). In many cases, the successful colonization of one island is followed by subsequent radiation on the complete archipelago. This has been reported for darkling beetles of the genus *Pimelia* (Contreras-Díaz et al., 2003) and *Hegeter* (Juan et al., 1996), ground beetles of the genus *Calathus*

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(Emerson et al., 2000a), bark beetles of the genus *Tarphius* (Emerson et al., 2000b), *Gallotia* lizards (Cox et al., 2010), *Chalcides* skinks (Brown and Pestano, 1998), and plants of the genus *Echium* (Böhle et al., 1996). However, the individual patterns and timing of colonization differ depending on the taxon and its intrinsic characteristics (Juan et al., 2000).

Orthoptera represent interesting model systems for the study of island biogeography as they include both mobile as well as rather sessile taxa, which allows a comparison of the colonization mechanisms in organisms with different mobility. The phylogenies of several Canarian Orthoptera have already been reconstructed (López et al., 2007; Arnedo et al., 2008; Hochkirch and Görzig, 2009). However, most of these taxa have rather limited dispersal abilities. For example, a phylogenetic study of the flightless genus *Arminda* has revealed a stepwise colonization pattern which has led to single island endemics (Hochkirch and Görzig, 2009). Similarly, the very sedentary genus complex *Acrostira/Purpuraria* consists mainly of single island endemics, albeit with very close genetic relationships (López et al., 2007). In the katydid genus *Calliphona*, which has variable wing lengths, phylogenetic analyses suggested an inter-island taxon cycling process (Arnedo et al., 2008).

Here, we analyze the island biogeography of a grasshopper genus with strong flight abilities, which nevertheless has a high number of endemic species on the Canary Islands. The genus *Sphingonotus* Fieber, 1852 is one of the most species-rich grasshopper genera worldwide, with currently 145 described species in three subgenera (*Neosphingonotus* 14 species, *Parasphingonotus* 3 species, *Sphingonotus* s. s. 128 species) (Eades et al., 2014). Nine *Sphingonotus* species occur on the Canary Islands, seven of which are endemic to single or two islands: (1) *S. pachecoi* (Bolívar, 1908) from Lanzarote; (2) *S. fuerteventurae* Husemann, 2008 from Fuerteventura; (3) *S. rugosus* (Bland, 1998) from Lanzarote and Fuerteventura; (4) *S. sublaevis* (Bolívar, 1908) from Gran Canaria; (5) *S. guanchus* (Johnsen, 1985) from Gran Canaria; (6) *S. picteti* (Krauss, 1892) from coastal Tenerife; and (7) *S. willemsei* Mistshenko, 1937 from subalpine Tenerife (Cañadas). Two further species, *S. savignyi* (Saussure 1884) and *S. rubescens* (Walker, 1870) are widely distributed from north-western Africa to Central Asia (Hochkirch and Husemann, 2008). In the Mediterranean, the genus *Sphingonotus* is known to comprise two recent radiations, which differ in their stridulatory organs (Husemann et al., 2013b). The *Sphingonotus caerulans*-group possesses the plesiomorphic Oedipodinae type of stridulatory mechanisms (a serrated intercalary vein on the elytra), while the *Sphingonotus azurescens*-group belongs to the subgenus *Neosphingonotus*, which has an apomorphic stridulatory organ (thickened cross-veinlets between radius and media). *Sphingonotus* species typically inhabit xeric habitats with scarce vegetation and a high cover of bare ground, such as deserts, semi-deserts, river gravel, dunes or rocky habitats. All *Sphingonotus* species are fully winged and known to be good flyers (Nachtigall, 1998). They have colonized several oceanic islands, including the Galapagos archipelago, the Caribbean and all Mediterranean islands (Husemann et al., 2012, 2013b; Eades et al., 2014).

Due to the high species richness and the highly variable courtship displays, including melodious songs, hind leg flagging, mid leg drumming, courtship flights with wing crepitation (García et al., 1997; Hochkirch, 2003; Husemann and Hochkirch, 2007; Larrosa et al., 2010), *Sphingonotus* represents a particularly interesting taxon for phylogenetic studies. Here, we investigate the phylogeography of Canarian *Sphingonotus* species and relatives on the European and African mainland in order to reconstruct the colonization history and speciation processes of the genus. We hypothesized that the endemic *Sphingonotus* species of the Canary Islands differ in their colonization history from flightless species due to their high dispersal abilities. We thus expected to reveal a complex intra- and inter-island colonization history with a strong influence of the prevailing wind direction and ocean currents and the geological history of the islands.

2. Methods

2.1. DNA sequencing

A total of 136 specimens of Canarian, NW African and European *Sphingonotus* species as well as some other Oedipodinae have been collected from 2002 to 2012 (Table S1). The specimens were stored either in 99% ethanol p.a. or frozen. We chose the *Schistocerca gregaria* Forskål, 1775, *Oedipoda caerulescens* (Linnaeus, 1758), *Vosseleriana arabica* (Mishchenko, 1937), *Helioscirtus capsitanus* (Bonnet, 1884) and three species of the genus *Thalpomena* (*T. coerulescens* (Uvarov, 1923), *T. rungsi* (Dirsh, 1949), *T. algeriana* (Lucas, 1849)) and *Sphingoderus carinatus* (Saussure, 1888) as outgroups. These taxa represent different divergence levels to *Sphingonotus* as has been demonstrated in previous studies (Fries et al., 2007; Chapco and Contreras, 2011; Husemann et al., 2012, 2013b). The DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was used for DNA extraction from femoral or thoracic muscle tissue following the manufacturer's protocol. Four gene fragments (three mitochondrial, one nuclear) were amplified: (1) NADH dehydrogenase subunit 5 (ND5, 1050 bp), (2) 12s rRNA (12s, 350 bp), (3) a fragment containing parts of 16s rRNA, tRNA-Leu and NADH dehydrogenase subunit 1 (NDS, 537 bp), (4) internal transcribed spacer 2 (ITS2, 308 bp). Primer information is given in Table 1. PCR conditions are provided elsewhere (Husemann et al., 2012; Husemann et al., 2013a,b). The 5Prime HotMasterMix (5Prime, Hilden, Germany) and the HotStarTaq Master Mix Kit (Qiagen) were used for amplification. The PCR product was purified using either the Qiaex II Gel Extraction Kit (Qiagen) or the Roche PCR product purification kit (Roche, Risch, Switzerland) according to the manufacturer's protocols. Sequencing was performed with the Big Dye sequencing kit (Perkin Elmer, Cheshire, UK) for sequencing reactions on a Perkin–Elmer ABI automated sequencer or with the DYEnamic ET Terminator Cycle Sequencing Premixkit (GE Healthcare, Munich, Germany) for the MEGAbace 1000 automated sequencer (GE Healthcare). Sequences, which have been published before (Hochkirch and Husemann, 2008; Husemann et al., 2012, 2013b) were included in the analysis. All sequences were aligned with the MAFFT v.7.017 (Katoh et al., 2002) algorithm as included in Geneious v.6.1.7 (Kearse et al., 2012), trimmed and polymorphisms were additionally checked by eye. For coding genes (ND1, ND5) we further checked for reading frame errors with Geneious. Sequences were deposited in GenBank (accession numbers in Table S1).

2.2. Statistical analysis

Sequence statistics, i.e. the sequence length, number of polymorphic sites, number of gaps and the number of parsimony informative sites were calculated with DNAsp v. 5.10 (Librado and Rozas, 2009). In a next step we used the partition homogeneity test in PAUP v.4.0b10 (Swofford, 2002) to test for congruence of the genes. We ran the analyses with 100 homogeneity replicates and 10 addition-sequence replicates. We then used PartitionFinder v.1.1.1 (Lanfear et al., 2012) to test for the best partitioning scheme and estimated the appropriate models of evolution; the NDS gene was subdivided in its 16s, the tRNA for Leucin (trnL) and ND1 components; coding genes (ND1, ND5) were further subdivided in codon positions. The program divided the data into four partitions: the HKY + I + G model was chosen for ITS2 which represented the first partition; the second partition was the 3rd codon positions of ND1 as well as the 3rd codon positions for ND5 for which the

Table 1
Details of the primers used in this study; provided are the primer names, the amplified gene, the primer sequence and the reference for the first publication of the primer sequences.

Name	Gene	Sequence (5′ – 3′)	Reference
ITS3	ITS2	GCA TCG ATG AAG AAC GCA GC	Tautz et al. (1988)
ITS4	ITS2	TCC TCC GCT TAT TGA TAT GC	Tautz et al. (1988)
12sai	12s rRNA	AAA CTA GGA TTA GAT ACC CTA TTA T	Kocher et al. (1989)
12sbi	12s rRNA	AAG AGC GAC GGG CGA TGT GT	Kocher et al. (1989)
NDII	16s rRNA	ACA TGA TCT GAG TTC AAA CCG G	Vogler and DeSalle (1993)
NDS	ND1	TAG AAT TAG AAG ATC AAC CAG C	Pashley and Ke (1992)
NDV – His	ND5	CCT GTT TCT GCT TTA GTT CA	Su et al. (1998)
NDV – Phe	ND5	GTC ATA CTC TAA ATA TAA GCT A	Su et al. (1998)
NDV – 270N	ND5	TAT GTG TGC GGG ATC TAT AAT TC	Hochkirch unpubl.
NDV – 270r	ND5	GAA TAA TAG CTC CAG CAC ATA TA	Düring and Brückner (2000)
NDV – 400N	ND5	AGC AGG GTT TTA TTC TAA GG	Hochkirch unpubl.
NDV – 400rN	ND5	ATC CTT AGA ATA AAA CCC AG	Hochkirch unpubl.
NDV – 850N	ND5	GAT TTA TAC CTT TTC TTT CAA C	Hochkirch unpubl.
NDV – 850rN	ND5	GTT GAA AGA AAA GGT ATA AAT C	Hochkirch unpubl.

same model (HKY + I + G) was chosen; the program chose this model also for the third partition which consisted of 12s, 16s, and the 1st codon positions of ND1 and ND5, The last partition was the tRNA for Leucin, and the 2nd codon positions of ND1 and ND5 for which the TrN + I + G model was chosen (Table 2).

We used the partitioning scheme for the following phylogenetic analyses: (1) we generated a Maximum Likelihood tree with RAxML (Stamatakis, 2006, 2014) on the Trex Web Server of the Université du Québec à Montréal. We used the hill-climbing algorithm and obtained branch support from 1000 bootstrap replicates. (2) A Bayesian analysis with MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003). The analysis was run for 50 million generations sampling every 5000 generations for a total of 10,000 trees. A standard burn-in of 25% was discarded and a consensus tree was build before visualization with FigTree v. 1.3.1 (Rambaut, 2009). (3) A second Bayesian analyses was run with BEAST v.2.1.2 (Bouckaert et al., 2014). For this we tried three different settings from which the best model was subsequently chosen based on likelihoods with Tracer v1.5 (Rambaut and Drummond, 2009). The tested models differed in the tree priors: coalescent with constant or exponential population priors or the Yule speciation prior. All analyses were run with the same settings by linking the substitution models of the previously defined partitions and linking the clock models for the mitochondrial genes; the tree model was linked for all genes. We ran all analyses for 100 million generations sampling trees every 10,000 generations. The resulting log-files were imported into Tracer v1.5 (Rambaut and Drummond, 2009) which was used to determine the best of the three models. Tracer determined the coalescent exponential population prior to be most suitable, however the chain did not converge after the 100 million generations and therefore we performed another BEAST run with the coalescent exponential prior for 300 million generations sampling every 30,000 generations. The tree was again checked with Tracer for convergence and a burn-in of 10% of all samples was discarded before constructing a consensus tree in TreeAnnotator which was visualized in FigTree.

A separate BEAST run with the coalescent exponential population prior was performed to obtain rough divergence time estimates. For this we only used the ND5 gene with a relaxed exponential clock as a likelihood ratio test in TreePuzzle v.5.2 (Schmidt et al., 2002) rejected a clock like evolution of our data. The substitution model used was the GTR + G (closest model supported by BEAST to TIM2 + G as determined by JModeltest v. 2.1.1 (Posada, 2008)); the substitution rate for the ND5 gene was set to 0.0113 according to Husemann et al. (2013a). We ran the analysis for 100 million generations sampling trees every 10,000 generations. A consensus tree was build with TreeAnnotator and visualized in FigTree showing the 95% HPD intervals of divergence times to provide confidence levels.

To investigate the geographic origin of the island radiation and each island endemic we performed a statistical DIVA analysis in RASP v.3.0 (Yu et al., 2011). We used the 10,000 trees from our best BEAST run as input and discarded the first 1000 trees as burn-in. Four separate areas were predefined: A – Canary Islands, B – Northern Africa, C – Europe (including Mediterranean islands), D – Turkey, Egypt, Oman. The maximum number of possible areas was set to two for each node.

3. Results

We obtained an alignment containing a total of 2245 bp: the mitochondrial gene fragments 12s and ND5 were 350 bp and 1050 bp in length, NDS was 537 bp in total, but subdivided in 16s (108 bp), trnL (71 bp) and ND1 (358 bp). The nuclear gene frag-

Table 2
Alignment statistics, partitioning scheme, and substitution models for each gene fragment.

Locus	Location	Nr of seq.	Max. length (bp)	Indels	Polymorphic sites (excluding gaps)	Parsimony informative sites	% Variability (excluding gaps)	Partition	Substitution model
ITS2	Autosomal	136	308	20	58	24	15.3	1	HKY + I + G
12s	Mitochondrial	136	350	9	83	39	23.7	2	HKY + I + G
NDs-16s	Mitochondrial	136	108	9	27	15	25.0	2	HKY + I + G
NDs-trnL	Mitochondrial	136	71	5	5	3	7.0	3	TrN + I + G
NDs-ND1	Mitochondrial	136	358	0	126	82	35.2	1st – 2 2nd – 3 3rd – 4	1st, 3rd – HKY + I + G., 2nd – TrN + I + G
ND5	Mitochondrial	136	1050	0	378	257	36.0	1st – 2 2nd – 3 3rd – 4	1st, 3rd – HKY + I + G, 2nd – TrN + I + G
Total			2245						

ment ITS2 was 308 bp (Table 2). Due to the larger amount of data we acknowledge that the phylogeny is strongly driven by the mitochondrial sequences. Indels were found in most genes (ITS2 – 20, 12s – 9, 16s – 9, trnL – 5) besides the two coding genes (ND1, ND5). Most indels were found between *Schistocerca gregaria* and the other taxa. Translation of the coding genes did not show any internal stop codons suggesting that no pseudo-genes were sequenced. The most variable gene fragments were ND5 (36%, 378 polymorphic sites) and ND1 (35.2%, 126 polymorphic sites), whereas trnL was the least variable (7%, 5 polymorphic sites). The other genes ranged in between with 15.3% variable sites for ITS2 (58 polymorphic sites), and 23.7% (9 sites) and 25% (27 sites) for 12s and 16s, respectively (Table 2). The partition homogeneity test yielded no significant difference between the partitions suggesting that they could be combined for subsequent analyses ($p = 0.82$). The likelihood ratio test rejected clock-likeness of our data set ($p < 0.05$).

The phylogenetic analyses were largely consistent across the three methods. All analyses split *Sphingonotus* in two large groups: the first group contained mainly the taxa belonging to the *Sphingonotus azurescens*-group (sensu Husemann et al., 2013b) as well as some sister taxa to this group (Fig. 1). The following species belonged to this clade: the widespread species *S. azurescens*, *S. savignyi, S. canariensis*, and *S. cf. morini*, the North African endemic *S. tricinctus*, the Canarian endemics *S. pachecoi* (Lanzarote), *S. sublaevis* (Gran Canaria), and *S. fuerteventurae* (Fuerteventura), as well as the Iberian endemics *S. nodulosus*, *S. imitans* and *S. almeriense*. In addition, several taxa belonged to this clade which split basally from the *S. azurescens*-group: the Canarian endemic *S. rugosus* (Lanzarote, Fuerteventura), the Northern African endemics *S. vosseleri*, *S. lucasii* and *S. radioserratus* and the Sahelian *S. femoralis*.

The second group contained all members of the *S. caerulans*-group (sensu Husemann et al., 2013b): the two widespread species *S. rubescens* and *S. caerulans*, the Corsican endemic *S. corsicus*, the Canarian endemic *S. willemsei* (Tenerife: Cañadas), and *S. llucipomaresi* from the Iberian Peninsula (Fig. 1). *S. uvarovi* and *S. candidus* branched off as the sister clade to this group (both are endemic to Corse and Sardinia). The Canarian *S. picteti* (Tenerife) and *S. guanchus* (Gran Canary) split basally to this clade. The only close relationship of Canarian endemics was found between *S. pachecoi* and *S. sublaevis* which occur on Lanzarote and Gran Canaria, respectively. The other endemics, including those found on the same island, were not closely related or even belonged to different clades.

The rate dated tree suggested that the split into the two major clades occurred about 4.6 mya (Fig. 2). The major radiation events within the two groups occurred within the last 1.1 my, whereas some of the island endemics appear to be relatively old. The Canarian *S. guanchus* was one of the oldest species in the group with an age of ~3.5 my. Similarly, *S. picteti* with ~2.5 my and *S. rugosus* with almost 1.4 my represent rather old taxa compared to the young age of many of the more widespread species like *S. azurescens*, *S. savignyi* and *S. caerulans*, which likely split within the last 1 my from their sister clades. The ancestral area reconstruction revealed a high likelihood for an African origin of the *S. azurescens*-group (Fig. 1). For the *S. caerulans* group the situation was less clear; the RASP analysis suggested the greatest likelihood for a Canarian or European origin of the group depending on the inclusion of the basal Canarian taxa (Fig. 1).

4. Discussion

4.1. Colonization pattern

Our results are in conflict with the hypothesis of a step-wise colonization pattern with subsequent within-island radiations. Except for the species pair *S. pachecoi* (Lanzarote)/*S. sublaevis* (Gran Canaria), no endemic species pair from the Canary Islands had a close genetic relationship. Even morphologically very similar species, such as *S. picteti* from coastal Tenerife, *S. guanchus* from Gran Canaria and *S. rugosus* from Fuerteventura and Lanzarote (which have formerly been placed in a separate genus *Wernerella* Karny, 1907 based upon their similar appearance) do not seem to have a close genetic relationship. In fact, they even represent rather old lineages without any closely related species. Other endemics are nested within recent radiations, such as *S. willemsei* (subalpine Tenerife) which belongs to the poorly differentiated *S. caerulans*-group, or *S. pachecoi* (Lanzarote), *S. sublaevis* (Gran Canary) and *S. fuerteventurae* (Fuerteventura) which belong to the *S. azurescens*-group. The most parsimonious explanation for this pattern is a process of multiple independent colonization without subsequent radiation. The lack of radiations within or between the islands suggests that *Sphingonotus* grasshoppers were able to reach the island group several times, but were not able to subsequently colonize adjacent islands. On the other hand, the dispersal capability seems to have been strong enough to maintain gene flow within each island, so that within-island differentiation did not occur. This lack of within-island differentiation might have been further promoted by niche conservatism with relatively broad niches: all *Sphingonotus* species prefer habitats with a large cover of bare ground (Bland and Gangwere, 1998; Bland, 2001; Hochkirch, 2003; Husemann and Hochkirch, 2007), which are abundant and well-connected on most islands, particularly in the coastal zone. Only on Tenerife a second desert-like situation is found in the subalpine zone, which has recently been colonized by *S. willemsei*, which is a sister species to *S. caerulans*, which occurs also at high altitudes in Morocco (Atlas Mountains).

The lack of inter-island colonization events is more difficult to explain, particularly as *Sphingonotus* grasshoppers are believed to be very good flyers (Nachtigall, 1998). However, this interpretation is mainly based upon the escape flights of a few well-studied species (e.g. *S. caerulans*, *S. savignyi*) and the overall wide distribution of the genus *Sphingonotus*, which represents one of the most widespread grasshopper genera. In fact, the two most widespread Canarian *Sphingonotus* species (*S. savignyi*, *S. rubescens*) are good flyers, whereas smaller *Sphingonotus* species (e.g. *S. picteti, S. guanchus, S. rugosus*) usually fly only very short distances (AH personal observation). It might thus be that passive wind dispersal was more important for the original colonization of the Canary Islands (e.g. by storms from the African continent), whereas active dispersal capabilities of most species are too low for subsequent secondary between-island colonization events. Multiple passive wind dispersal from the African continent may thus explain the unusual phylogenetic relationships of the Canarian endemics.

4.2. Taxon ages and range extension

Another interesting finding of our analyses is that the widespread species on the Canary Islands belong to species-rich radiations, whereas many of the endemics split rather basally and do not have any closely related sister species. The widespread *S. rubescens* and *S. savignyi* both represent rather young taxa and must have colonized their vast range in a very short period of time, while the oldest Canarian taxa (*S. guanchus*, *S. picteti* and *S. rugosus*) represent rather old *Sphingonotus* lineages and must have survived on the islands for a very long time. Some younger endemics have colonized the Canary Islands later, but apparently show a similar pattern (no closely related sister species on the Canaries). This suggests that the strong dispersal ability of the two widespread taxa is a trait that has evolved recently in both lineages.

Interestingly, the molecular clock analysis suggests a fairly recent age of all endemics, much later than the emergence of the

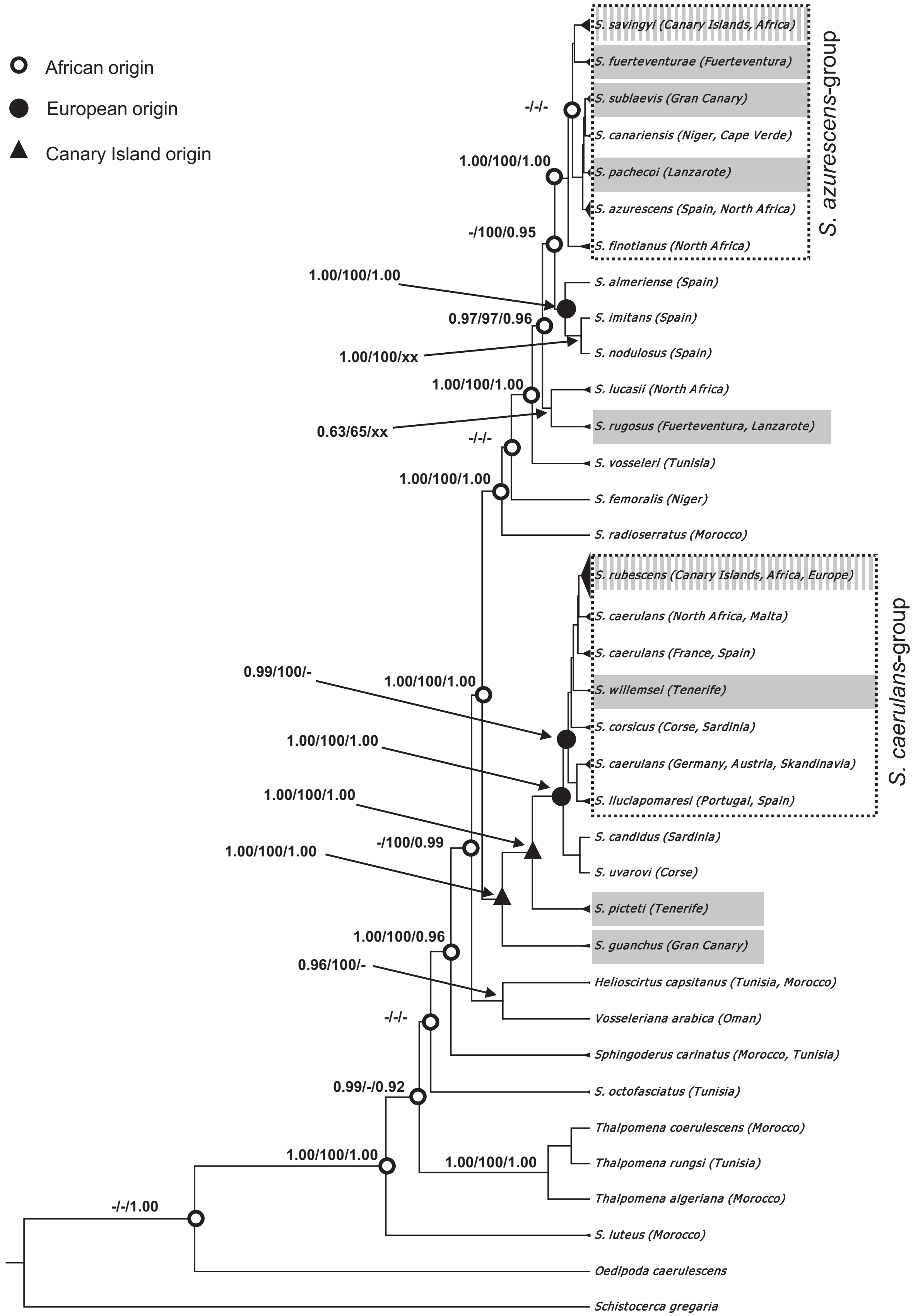

Fig. 1. Phylogenetic reconstruction of the genus *Sphingonotus* using two Bayesian approaches and a Maximum Likelihood approach. The tree represents the 50% majority rule consensus tree from the BEAST analyses; posterior probabilities and bootstrap support are given as branch support (pp from BEAST/bootstrap values from RaxML/pp from MrBayes). The branch for *Sphingonotus azurescens* includes the close relatives *S. tricinctus* and *S. morini*. Endemic species are shaded in grey, widespread species occurring on the Canary Islands in striped grey. The circles at internal nodes represent the ancestral distribution with the highest likelihood from S-DIVA analysis in RASP.

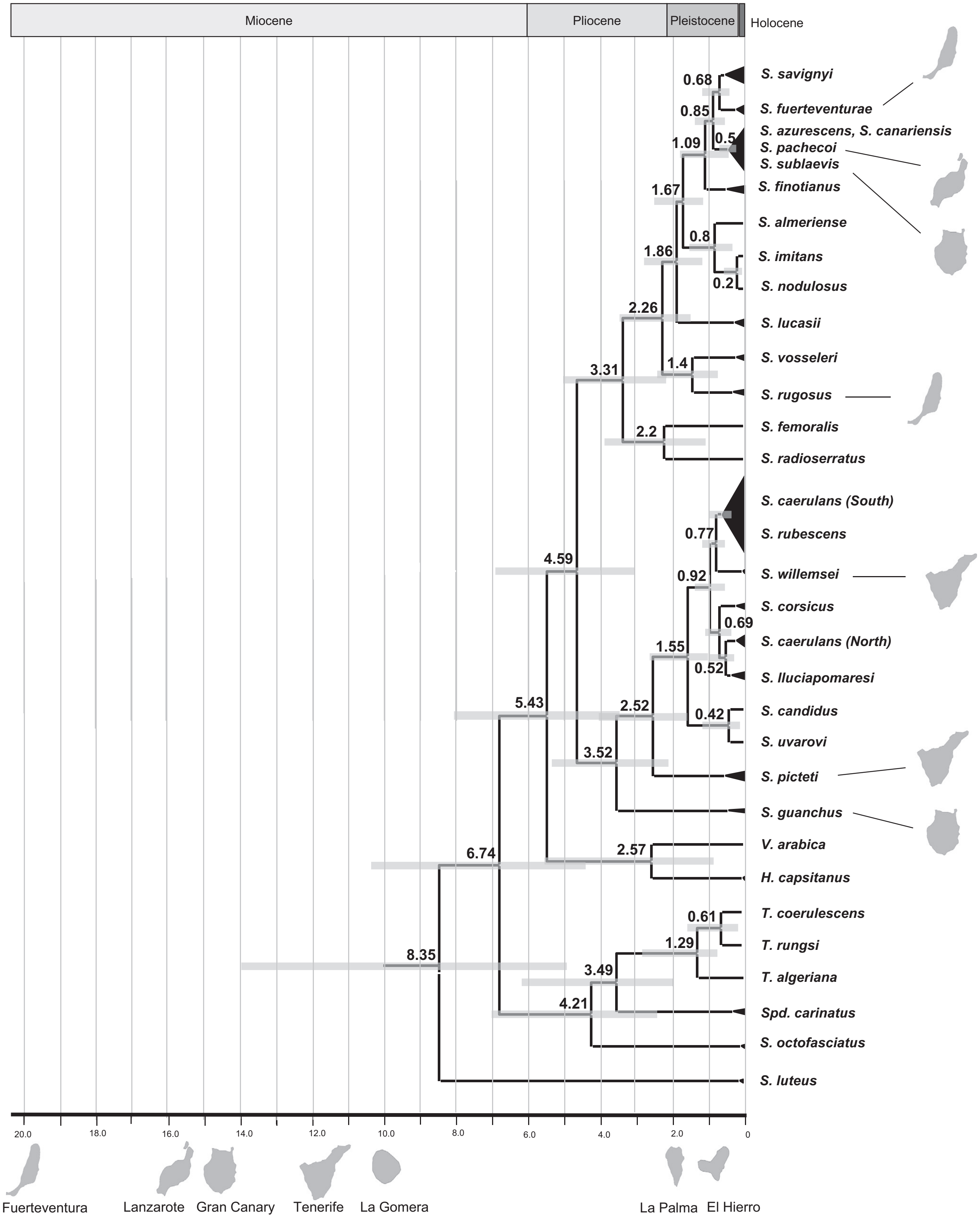

Fig. 2. Chronogram of the genus *Sphingonotus* based on the ND5 gene and a substitution rate of 0.0113 estimated with a coalescent prior in BEAST. Values represent the mean ages and the grey bars indicate the 95% HPD intervals. Island ages are displayed on the lower axis, whereas geological epochs are displayed on the upper axis. The island shapes next to the taxa indicate the islands the respective species are endemic to. The branch for *Sphingonotus azurescens* includes the close relatives *S. tricinctus* and *S. morini*.

islands (Fig. 2). While we have to acknowledge that these dating estimates, in the absence of good fossil calibration points, are very crude estimates, the estimates are much younger than the ages of the islands and the confidence intervals do not overlap with island origins. These young ages support the hypothesis that passive colonization events are scarce and probably rarely successful. Furthermore, the volcanic activity of the islands might have led to extinctions, which remain a major obstacle to phylogenetic reconstructions (Juan et al., 2000; Hochkirch and Görzig, 2009). The lack of information on extinctions probably also influences the reconstruction of refuges. While, the RASP analysis revealed a North African origin of the *S. azurescens*-group, it also suggested a Canarian origin of the extended *S. caerulans* group. We hypothesize that this is an artifact of the different speciation/extinction dynamics on the archipelago compared to the mainland. The rather stable climatic history of the Canary Islands (Meco et al., 2002) might have promoted a higher survival of taxa, whereas on the climatically more dynamic mainland, old *Sphingonotus* lineages might have become extinct, so that the true sister species of ancient Canarian species may be missing.

5. Conclusions

Colonization histories of archipelagos have frequently been studied in the past (Gillespie et al., 1994b; Emerson, 2002, 2008; Shaw, 2002) and many island radiations stem from single or few colonization events with subsequent inter- and intra-island radiation (Emerson et al., 1999, 2000a; Contreras-Díaz et al., 2003; Hochkirch and Görzig, 2009). The case of the winged grasshopper genus *Sphingonotus* on the Canary Islands deviates from this classic island radiation pattern. Except for one species group (*S. pachecoi*/*S. sublaevis*) all taxa colonized the Canary Islands independently without any intra- or inter-island speciation and only two widespread species (*S. rubescens*/*S. savignyi*) subsequently colonized the complete archipelago. Our study shows that despite superficially resembling a typical island radiation species groups might differ in their evolutionary history and only detailed phylogeographic studies can reveal these new patterns.

Data accessibility

DNA sequences: Genbank accessions Table S1.

Sampling locations and final DNA sequence assembly uploaded as online supplemental material.

Author contributions

AH and MH designed the study. AH, JD and MH generated the genetic data. AH and MH analyzed the data and wrote the paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.09.017.

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