Phylogenetic relationships among the Canary Island Steganacaridae (Acari, Oribatida) inferred from mitochondrial DNA sequence data

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Abstract

The mite genus Steganacarus is represented in the Canary Islands by three endemic species, one recently discovered species, and several morphotypes of uncertain taxonomic position. We used a fragment of the mitochondrial cytochrome oxidase I gene to reconstruct the phylogenetic relationships among representatives of the different taxa from the three central islands of the archipelago, Tenerife, La Gomera and Gran Canaria. Sequence data were analysed by both maximum parsimony and maximum likelihood methods. The inferred phylogenetic relationships do not correlate well with current morphological taxonomy but reveal four deeply divergent and geographically coherent lineages, one each on Gran Canaria and La Gomera and two on Tenerife. No pattern of molecular differentiation was observed among different morphotypes. Possible explanations for this incongruence are suggested in relation to the ecology and biogeography of the group. A recently discovered Steganacarus species from La Gomera, morphologically quite distinct from the other Canarian Steganacarus, is clearly identified as a taxon distantly related to all the other Canarian samples.

Keywords: Canary islands, COI gene, mitochondrial DNA, morphotypes, oribatid mites, phylogeography

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Introduction

The study of organisms on oceanic islands has greatly contributed to a better understanding of mechanisms responsible for phylogenetic diversification and to the development of ecological and evolutionary theories. The Canary Islands have recently become a focus for evolutionary studies, and many organisms that have colonized the islands have radiated to produce an impressively rich array of endemic species and subspecies (Juan et al. 2000). Several organisms, in particular insects (the beetles Piemelia, Hegeter and the butterfly Cometeryx) appear to have colonized the Canary Islands from Africa and/or Iberia and indicate an east-to-west stepping-stone type of mlanization, compatible with the geological dating of the islands (Juan et al. 1995, 1996; Gonzales et al. 1996; Brunton & Hurst 1998). In some cases, e.g. the reptiles Gallotia and Chalcides, ecological characteristics seem to have strongly influenced this pattern of mlanization (Thorpe et al. 1994; Brown & Pestano 1998).

Because of their lifestyle and low power of dispersal (Bernini 1991; Schatz 1991), soil oribatid mites (Acari: Oribatida), present an opportunity for investigating the processes of mlanization and differentiation. In particular, species of Steganacarus from the Canary Islands offer an intriguing example in which to investigate evolutionary diversification and speciation patterns in insular biota (Avanzati et al. 1994, 1996). The Steganacaridae are macrophytophages with a preference for dead organic matter and occur in greatest numbers in the surface layers of forest soil horizons, where the organic layer is thick. In common with other oribatid mites, Steganacaridae have an extremely limited vagility and even though they may be occasionally transported passively, as for example with nesting material camed by birds (Schatz 1991), long-distance dispersal seems to play a negligible role in their geographical distribution (Bernini 1991).
The genus *Steganacarus* (box-mites) comprises 50 species and is represented across Europe, Western Asia and Northern Africa, including Macaronesia (Madeira, Bermej & Magari 1993) and the Canary Islands, Pérez-Iñigo 1988; Niedbala 1992; Pérez-Iñigo & Peña 1996). Fuerteventura and Lanzarote do not support *Steganacarus* species, probably owing to the absence of suitable habitats for these organisms, which require forest soils rich in organic substances (Rajski 1967). Recent faunistic studies identify three endemic species distributed in the central and western islands of the archipelago: (Pérez-Iñigo 1972, 1988; Niedbala 1984, 1992; Pérez-Iñigo & Peña 1996): *Steganacarus (Steganacarus) tenerifensis* Pérez-Iñigo (1972), endemic to the Anaga Massif (northeastern Tenerife), *S. (S) carlosi* Niedbala (1984), widespread on all five islands of Tenerife, Gran Canaria, La Gomera, La Palma and El Hierro, and *S. (S) guanarteme* Pérez-Iñigo & Peña (1996), inhabiting the Barranco de Los Tilos on Gran Canaria. These three species seem to belong to a well-defined group not represented in western Europe or in northern Africa, but present in southeastern Europe (Anatolian peninsula and Caucasus) (Avanzati et al. 1996). Subsequent detailed and quantitative morphological studies on *S. carlosi* and *S. tenerifensis* have provided such a wide range of variability as to cast doubt on their specific limits (Avanzati et al. 1994). In particular, morphotypes of *S. carlosi* were found in Tenerife and La Gomera, sometimes mixed with the typical *S. carlosi* and showing continuous variation for some morphological characters, such as a c5 notogastral setae, modified as in *S. tenerifensis*. These morphotypes have been referred to as 'mono' and their systematic position is still uncertain (Avanzati et al. 1996). A similar morphotype was also found in Gran Canaria by Pérez-Iñigo & Peña (1996) and, on the basis of variation in the c5 notogastral setae, they described this morphotype as the species *S. guanarteme*, living in sympatry with *S. carlosi*. Because of a paucity of unambiguous morphological characters, molecular genetic tools have been used in an attempt to discern taxonomic relationships. A study of allozyme variation across populations of *S. tenerifensis* and *S. carlosi* did not help to clarify relationships due to the very low level of heterozygosity detected. However, the high genetic homogeneity observed made it difficult to justify the separation of the two species (Avanzati et al. 1994).

From a geological point of view, the Canary Islands represents one of the best studied oceanic island systems (Ya et al. 1999), providing an ideal template for studying phylogeographical pattern within *Steganacarus* mites. The archipelago is composed of seven major oceanic islands and several islets of volcanic origin. Situated 100 km from the northwest African coast, the archipelago was probably never attached to the continent (Fig. 1). From K-Ar and Sr-Nd-Pb isotopic dating of volcanic rocks it is possible to determine the probable age of emergence of several mounts (islands or parts of islands) and to describe the temporal volcanic history of islands (Schmincke 1982; Ancochea et al. 1990). The oldest islands are the easternmost Fuerteventura and Lanzarote (= 15–20 Myr) (Coello et al. 1992), Gran Canaria (= 13.5–14 Myr) (Ya et al. 1999), La Gomera (= 11–12 Myr) (Cantagrel et al. 1984) and Tenerife (= 5–8.5 Myr) (Ancochea et al. 1990). The youngest islands are the westernmost La Palma (2 Myr) (Ancochea et al. 1993).

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and Hierro (< 1 Myr) islands. The present-day configuration of Tenerife is relatively recent (< 1 Myr); before this time there were 2–3 smaller, separate islands represented by the older volcanic masses of the Roque del Conde (southern Tenerife, 8.5–6.4 Myr), Tení (northwestern Tenerife, 6.7–4.5 Myr) and Anaga (northeastern Tenerife, 6.5–3.6 Myr). Most of the island is now covered by the younger (≈2 Myr) central composite volcano of Las Canadas later replaced by the emission of the Teide–Pico Viey complex which linked the older eroded edifices (Ancochea et al. 1990).

This study addresses the phylogenetic history of *Stegunucarus* species from the Canary Islands using characteristic features derived from mitochondrial DNA (mtDNA). We sequenced and analysed a fragment of the mtDNA cytochrome oxidase subunit I (COI) gene for representatives of each recognized taxon collected from the three central islands of the archipelago (Gran Canaria, Tenerife and La Gomera). A recently discovered, and as yet undescribed, *Stegunucarus* species is also included in this analysis. This specimen, found in the preserved material kindly supplied by the Centre for Land and Biological Resources Research (Ottawa, Canada), was collected by A. Fjellberg in the coastal areas of La Gomera and is morphologically quite distinct from the *carlosi* group. We evaluate genetic diversity among species and morphotypes of Canarian *Stegunucarus* and compare genetic findings with current morphological taxonomy. Molecular results provide a preliminary insight into the process that have shaped intra- and inter-island differentiation with regard to the ecology of the group and to the volcanic history of the archipelago.

**Materials and methods**

**Sample collection**

Most of the Canarian *Stegunucarus* samples were collected in May 1997 from the three central islands of Tenerife, Gran Canaria and La Gomera. Live samples were sent to the laboratory where they were identified and stored at −80 °C. Collection sites and codes of the samples used in the analysis are detailed in Table 1.

Tab. 1. List of specimens examined in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Island</th>
<th>Sampling Sites</th>
<th>Morphotypes analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Go</td>
<td>La Gomera</td>
<td>Aparta-caminos</td>
<td><em>Stegunucarus</em> carlosi</td>
</tr>
<tr>
<td>2 Go</td>
<td>La Gomera</td>
<td>Cerro de Araña</td>
<td>S. carlosi/mono</td>
</tr>
<tr>
<td>3 Go</td>
<td>La Gomera</td>
<td>La Laguna Grande</td>
<td>S. carlosi</td>
</tr>
<tr>
<td>4 Go</td>
<td>La Gomera</td>
<td>Mirador de Vallehermoso</td>
<td>S. carlosi/mono</td>
</tr>
<tr>
<td>5 Go</td>
<td>La Gomera</td>
<td>Alto de Cherelepín</td>
<td>S. carlosi</td>
</tr>
<tr>
<td>6 Go</td>
<td>La Gomera</td>
<td>Bosque El Cedro</td>
<td>S. carlosi</td>
</tr>
<tr>
<td>7 Go</td>
<td>La Gomera</td>
<td>Punta Llana</td>
<td><em>Stegunucarus</em> n. sp.</td>
</tr>
<tr>
<td>8 An</td>
<td>Tenerife</td>
<td>Anaga</td>
<td>S. carlosi/mono-1</td>
</tr>
<tr>
<td>9 An</td>
<td>Tenerife</td>
<td>Las Mercedes</td>
<td>S. carlosi/mono-2</td>
</tr>
<tr>
<td>10 Te</td>
<td>Tenerife</td>
<td>Anaga</td>
<td>S. carlosi/mono-3</td>
</tr>
<tr>
<td>11 RC</td>
<td>Tenerife</td>
<td>Teno</td>
<td><em>S. teneriffensis</em>-1</td>
</tr>
<tr>
<td>12 RC</td>
<td>Tenerife</td>
<td>Roque del Conde</td>
<td>S. teneriffensis-2</td>
</tr>
<tr>
<td>13 GC</td>
<td>Gran Canaria</td>
<td>Barranco del Infierno</td>
<td>S. teneriffensis-3</td>
</tr>
<tr>
<td>14 GC</td>
<td>Gran Canaria</td>
<td>Los Tilos de Moya</td>
<td>S. carlosi</td>
</tr>
<tr>
<td>15 GC</td>
<td>Gran Canaria</td>
<td>Los Tilos de Moya</td>
<td>S. carlosi</td>
</tr>
</tbody>
</table>

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as outgroup in the phylogenetic analysis. This species is widely distributed in southwest Europe and northwest Africa (Maghreb) (Bernini & Avanzati 1989, Gill-Jaun et al. 1992). An ongoing study indicates that S. carnosi is the only Steganacarus species inhabiting the westernmost corner of the African continent inferring it to be the probable closest relative to the Canary Island Steganacarus. However, it should be acknowledged that potential historical differences from contemporary geographical distributions may mean that the closest relative to the Canary island Steganacarus is geographically distant (Emerson et al. 2000a).

**DNA extraction**

Single individuals were used for amplification and sequencing. Total genomic DNA was extracted from living, frozen or alcohol-preserved tissues following a modified CTAB method (Boye et al. 1989). In short, specimens were digested with CTAB buffer (0.1 M Tris-Cl pH 8.0, 1.4 M NaCl, 0.02 M EDTA, 2% CTAB, 0.2% mercaptoethanol) for 6-12 h, purified with phenol-chloroform and chloroform, then desalted and concentrated by ethanol precipitation.

**Polymerase chain reaction and DNA sequencing**

A 488-bp fragment of the COI gene was amplified by the polymerase chain reaction (PCR) using primer pairs C1-f21751 (5'-GGATCAGCTGATGACACTCCC-3') and C-1-N-2191 (5'-CCCCTAGAAAATGTTAAAACACTT-3') (Simon et al. 1994). PCR amplification was performed in 25 μL of reaction volume following a touch-down profile consisting of 25 cycles with temperatures of 95 °C for 20 s, 44 to 40 °C for 30 s and 72 °C for 30 s. Reaction products were run on a 1.5% agarose gel and the appropriate band was cut and digested in H2O. This product was used as template for a second PCR to generate double-stranded template suitable for DNA sequencing. The second amplification was carried out in 100 μL reaction volume for 25 cycles, with a temperature profile of 95 °C for 20 s, 60 to 58 °C for 20s and 72 °C for 20s followed by a final extension step of 72 °C for 5 min. After the elimination of excess nucleotides and primers by gel separation and purification with concert Rapid gel extraction systems columns, the final products were sequenced using the same primers plus an internal primer designed for Steganacarus (5'-CTTATAGTTAAAGATTGTGT-3'). Sequencing reactions were performed either manually (Sanger et al. 1977) or with an automated sequencer at the core facility of ENEA, Rome, using the dye terminator technology combined with a Perkin-Elmer 373A Stretch. Most of the samples were sequenced more than once from independent PCR amplifications.

**Phylogenetic analysis**

Sequences were aligned by eye using ESEE (Cabot & Beekenbach 1989) and the software package dnasp Version 3.14 (Rozas & Rozas 1999) was used to estimate DNA polymorphism. Phylogenetic analyses were performed with PAUP* (Swofford 1998) with S. carnosi as the outgroup, using both maximum parsimony (MP) and maximum likelihood (ML). MP analyses were performed using a heuristic search with equal weighting of all characters (TBR branch swapping; Multrees option in effect). Ten replicates were performed within each heuristic search using random taxon addition. The ML analysis requires a specific model of evolution to be specified a priori. In order to select the substitution model that best describes our data, sequences were analysed with Modeltest (Posada & Crandall 1998). This program allows comparison of different models of DNA substitution to be tested in a hierarchical hypothesis testing framework. The models tested are Jukes-Cantor, Kimura 2-parameter, Tamura–Nei with equal base frequencies, Kimura 3 parameter, SYM, F81, HKY 85, Tamura–Nei, Kimura 3-parameter with unequal base frequencies and the general time reversible (GTR) model (see Posada & Crandall 1998; for details of models). Each of four different rate heterogeneity categories are applied to each of the above models: (i) equal rates; (ii) estimating the number of invariant sites; (iii) estimating the gamma shape parameter across all sites; and (iv) estimating both the number of invariant sites and the gamma shape parameter for variable sites. Parameter estimates and likelihood values were obtained for each of the models using PAUP*. The likelihood values are then analysed with Modeltest to determine which of the models best describes the data (log likelihood tests were used for nested hypotheses, Akaike information criterion (Akaike 1974) for nonnested hypotheses). The optimal model defined by Modeltest was then selected for a ML analysis. To assess the robustness of relationships, 500 bootstrap replications were performed for MP, whereas owing of prohibitive computer time, the bootstrapping method could not be used in the ML analysis. Instead, for the ML tree we report bootstrap values for 500 replicates obtained from a neighbour-joining (N) analysis of ML distances obtained using the parameter estimates derived from Modeltest.

**Results**

**DNA polymorphism**

A total of 404 nucleotide positions (134 codons) of the COI gene has been examined in 26 sequences from the Canary Islands, and from the outgroup species Steganacarus carosi. No deletion or insertion of bases was observed.
total of 150 polymorphic sites (representing 11 mutations) was detected, resulting in 125 parsimony informative sites. As the protein coding genes evolve under functional constraints, most of the substitutions are, as expected, in third positions and produce primarily silent substitutions.

Across all sites the average A+T content for the Canarian Steganacarus was 62.4%, increasing to = 68.0% at third codon positions. Comparatively higher A+T content (total, 67.4% and third position, 80.6%) is observed in the outgroup, S. carlosi. A previous study on the same COI region for Steganacarus species occurring in Italy resulted in even higher A+T contents (total, 69.5% and third position, 84.2%) (Salomone et al. 1996). It appears that in the Canary lineage, the bias to an increased A+T content is lower than in the West Mediterranean congenic species.

Molecular differentiation

No pattern of nucleotide differentiation distinguishes S. carlosi from S. guanarteme on Gran Canaria, or S. carlosi from S. carlosi/'mono' on Tenerife and La Gomera. Within haplotypes of S. carlosi the maximum intraspeciand genetic divergence was 7.8% (ML) (Anaga-Roque del Conde). In Tenerife, genetic distance between the two morphologically defined species S. carlosi and S. tenerensis range from 98 to 15.8% inter-island genetic divergence among populations of S. carlosi is surprisingly high, with values ranging from 16.8 to 23.4%. The divergence of the carlosi lineages (including S. tenerensis) from the morphologically well distinct Steganacarus n. sp. varies from 21.5 to 28.6%.

Phylogenetic analysis

MP analysis resulted in 12 equally parsimonious trees (tree length = 375, CI = 0.573, RI = 0.828). Trees differed in minor branch arrangements within the La Gomera clade. One of the 12 most parsimonious trees is shown as a phylogram with bootstrap values in Fig. 2. Sequence analysis using MODEST supports two best fit models of DNA substitution: HKY+G for log likelihood ratio test and TVM+G for Akaike information criterion. Of these the HKY+G was chosen and used to parameterize likelihood distances (ln/lv ratio = 3.7849, gamma shape parameter = 0.266). The ML analysis produced a topology that was largely congruent with the parsimony results (Fig. 3). For both MP and ML analyses Steganacarus sp. is divergent from the remainder of the Canary Island taxa. The remainder of the Canary Island taxa are divided into three strongly supported monophyletic island clades. In both analyses, the Gran Canarian clade is supported by high bootstrap values (MP = 100; NJ = 100) and is divergent from the remaining two island clades of Tenerife and La Gomera. Within the Gran Canarian clade the S. guanarteme haplotype falls within the haplotypes of S. carlosi. Similarly, in La Gomera and Tenerife there is no pattern of genetic differentiation between S. carlosi and the 'mono' morphotypes. Haplotype from La Gomera form a monophyletic group strongly supported by bootstrap values (MP = 99; NJ = 100). Within Tenerife, phylogenetic analyses define two well-supported clades, one for tenerensis haplotypes (MP bootstrap values for this node = 79), and the other for haplotypes of S. carlosi and 'mono'. Within the carlosi/'mono' clade a clear relationship between phylogenetic differentiation and geographical distribution is evident. All sequences from Roque del Conde (sites 11RC and 12RC) are clustered together and are distinct, but closely related to the sample from Teno (site 10Te). The S. carlosi collected from Anaga cluster together and are divergent from the other S. carlosi of Tenerife.

Discussion

COI sequence evolution

The average sequence divergence among populations and species of Canarian Steganacarus is surprisingly high. A previous study on Italian populations of Steganacarus magnus found much lower values for the same fragment of the COI gene (Salomone et al. 1996). This high divergence is an interesting feature with two possible explanations. The COI gene can be evolving intrinsically faster in the Steganacarus belonging to the Canary lineage than the south-European congenic species. However, increased mutational rate seems unlikely because factors generally associated with mutational rate, such as generation time and metabolic rate, are presumably not that dissimilar among species belonging to the same genus. Assuming a similar rate of mtDNA evolution, the high levels of divergence observed in the Canary Islands would appear to infer a more ancient origin for this group with respect to the European lineage, as previously suggested (Avanzati et al. 1996). The level of divergence observed within the fragment of the mtDNA COI gene allows for the examination of variability within species and patterns of geographical variation within populations that has not been possible in earlier studies of allozymes (Avanzati et al. 1994).

It is interesting to note that the A+T content, highly biased in the COI gene of the European Steganacarus and in other studied Chelicera (Avise et al. 1994; Navajas et al. 1996; Salomone et al. 1996), tends to be less biased in the Canary lineage. A similar shift in nucleotide composition between closely related species and even in a single taxon has already been described within the Dermoptera (Wirth et al. 1999). Several hypotheses have been put forward to explain biases in nucleotide contents (Moriyama &
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Fig. 2 One of 12 most parsimonious trees for Canarian Stegancarus mites calculated from mitochondrial COI sequences. Bootstrap values are indicated for nodes gaining >70% support (500 replications).

Gojobori 1992; Jermiin & Crozier 1994; Wirth et al. 1999), but a consistent explanation for this high variability in nucleotide frequencies has not yet been provided, although the presence of a higher shift at the third codon position suggests a correlation between base content and rates of synonymous substitution in the sites under low functional constraint. A reduced A+T bias observed in the Canarian Stegancarus could be a return to some equilibrium under more relaxed constraints. Clearly, in order to determine the mechanisms governing evolutionary rates and the base composition in Stegancarus more detailed analysis on the pattern and/or direction of mutation in the mitochondrial genome of this taxon are necessary.

Phylogenetic relationships

The pattern of genetic variation does not correlate with the current taxonomic status of S. carlosi and S. guanarteme (Pérez-Iñigo & Peña 1996). The analysis of mtDNA sequences from Gran Canaria indicate that the two entities are not genetically distinct (the smallest observed distance is only 0.7%). There is considerably higher genetic diversity among the haplotypes of S. carlosi than between S. carlosi and S. guanarteme. Similar results are obtained when comparing S. carlosi with S. carlosi 'mono' from Tenerife and La Gomera. Phylogenetic reconstructions do not result in the differentiation of the two morphotypes Several

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mechanisms can be invoked to explain this discrepancy between the molecular phylogeny and classical taxonomy. If one assumes that the current taxonomy is a true representation of the phylogenetic relationship between *S. carlosi* and *S. guanarteme*, one may argue that the lack of correspondence for the mtDNA phylogeny is due to stochastic lineage sorting. However, the purely random sorting of mtDNA polymorphisms should not produce any sort of biologically meaningful pattern among different evolutionary lineages. If lineage sorting were the only cause of discrepancy between species taxonomy and phylogenetic positioning based on genetic data of populations within a species, there should be no correlation between geographical location and the distribution of sequence variations. This is clearly not the case. For example, on Tenerife, all the sequences of *S. carlosi* (including the phenotypes determined as 'mono') are clustered together on the basis of their geographical distribution. Such biogeographical pattern suggests a nonstochastic microevolutionary process is influencing the pattern of mtDNA variation.

If one assumes that the mtDNA phylogeny accurately represents the historical relationships among sampled populations, the current systematic distinction between *S. carlosi* and *S. guanarteme* may not reflect their proper taxonomic status. The presence of both morphotypes in the same natural environment leads one to exclude any mapr
ecological factor as a possible explanation for differentiation of the two morphotypes. Furthermore, morphological differences between them are not marked, and essentially concern the shape of the $c_i$ notogastral setae. This pattern of variability is not limited to a single island, but the same morphological variants are found in all the three islands examined ('mono' on Tenerife and La Gomera) suggesting that the two entities may belong to the same taxon which is characterized by an infraspecific polymorphism related to the $c_i$ setae.

The presence of both setal morphotypes on three islands is an interesting feature. It seems reasonable to assume that setal patterns are phylogenetically constrained. For Phytosceliidae mites a functional view of dorsalgchelae suggests protection in soil micro-environments (as for example in avoiding entrapment) has been suggested (Sabelis & Bakker 1992). However, in the Canarian Steganamus, whether the observed polymorphism related to a single trait could be moulded by natural selection is not immediately obvious. The occurrence of identical setal morphotypes on multiple islands can be explained by at least two different mechanisms. It may represent an ancestral polymorphism that has been retained within newly colonized populations. Another perhaps more remote possibility is that the two morphotypes on the each of the three islands represent biological species, and that on each island that two species occur sympatrically. This would imply a recent origin for the two morphs independently on each of the three islands and incomplete mtDNA lineage sorting within each clade confounding the phylogenetic delimitation of species boundaries. Both scenarios of either (i) the persistence of a discrete morphological polymorphism after island colonization; or (ii) the convergent evolution of morphotypes on the three islands imply a role for natural selection and are interesting alternatives open to further testing with a population genetic marker approach using either microsatellite markers or amplified fragment length polymorphisms.

Perhaps one of the more surprising results of this study is that, despite a lack morphological differentiation (Avanzati et al. 1994) among specimens of S. carlosi collected across the three islands (including the presence of similar morphotypes in all the three studied islands), molecular analysis identifies three well-differentiated clades with a high level of interisland genetic differentiation. Sequence divergences among populations of S. carlosi in the three islands are much higher than those between the two Tenerife species (S. carlosi and S. teneriffensis), and are comparable with divergence levels found between morphologically well-distinct congenic species in other terrestrial arthropods. This strongly suggests that populations of S. carlosi from different islands have had long independent evolutionary histories, and are in fact cryptic species. Cryptic groups provide a challenge in determining the genetic boundaries among taxa and that can only be by a molecular approach. Wilcox et al. (1997) used mtDNA sequence data to uncover cryptic species further to those already uncovered by molecular and reproductive data (Zeh et al. 1992; Zeh & Zeh 1994) with the pseudoscorpion species Cordylochernes scorpioides. More recently Kobayashi et al. (2000) described cryptic species within the ladybird beetle Epilachna vigintioctopunctata using mtDNA together with information from karyotypes, and crossing experiments.

The new Steganamus speaeae discovered on La Gomera and determined on the basis of morphological distinction is also genetically distinct. Phylogenetic analyses identify Steganamus n. sp. as a taxon distantly related to all the other Canarian samples. A more detailed morphological description of this taxon will be presented in a subsequent paper.

Intra-island evolution

Owing to the high levels of genetic divergence between haplotypes from different islands and the long independent history of these islands, the genetic differentiation of specimens on the three islands are discussed separately.

Evolution on Tenerife. Phylogenetic analyses support a monophyletic origin for the Tenerife Steganamus and the presence of two genetically distinct clades, corresponding to S. teneriffensis and S. carlosi; a result consistent with the morphological taxonomic determinations within Tenerife of Niedbala (1984). The three populations of S. carlosi exhibit clear geographical structuring, with haplotypes from Roque del Conde genetically distinct but closely related to the sample from Teno, and both divergent from Anaga specimens. Based on genetic evidence, similar geographical breaks between the northern and southern part of the island have already emerged between closely related species and populations of invertebrates e.g. Eustichopus (Cobolli-Sordoni et al. 1991), Pimelia (Juanet et al. 1996) Catulus (Emerson et al. 1999) Tarphius (Emerson et al. 2000b) and there is a probable relationship with the disjunct volcanic evolution of the island. Studies by DeSalle et al. (1987) and Brower (1994) suggest that mtDNA in arthropods evolves in an approximately clock-like manner, at least when divergence is low. These authors propose very similar divergence rate estimates (2 and 2.3%/Myr, respectively) and we apply an average of these rates (2.15%/Myr) to our data. Using this calibration the mtDNA diversity among S. carlosi populations dates back to 3.2 Myr between Anaga and Teno and from 2.5 to 3.6 Myr between Anaga and Roque del Conde (Fig. 4). Although we acknowledge that these estimates of divergence times must be regarded with some caution, in both case our data suggest that colonization events took place before the emergence of the central Cañadas volcanic region. During
the eruptive period of the large Cañadas volcano over the last 2 Myr (Ancochea et al. 1990), continued volcanic and erosional activity would have acted to limit migration events between the three massifs.

**Evolution on La Gomera.** High levels of intraspecific genetic variability characterize the *S. carlosi* lineage of La Gomera. However, phylogenetic analyses reveal no significant relationship of the *S. carlosi* haplotypes with geographical location, contrasting with the geographical differentiation that characterizes Tenerife populations. A possible explanation lies in the different geological histories of the two islands. Evolution on Tenerife occurred in the relative small pre-Teide massifs, and was characterized by intense volcanic activity until recent geological history. In contrast, La Gomera, except for some minor eruptions, has experienced no volcanic activity in the last 4–4.6 Myr (Ancochea et al. 1990) and it may be that this has resulted in the absence of geographical barriers, thus favouring intra-island dispersion and a reduced geographical genetic structuring of populations.

**Colonization**

Our results suggest monophyly for all the Canarian *Steganacarus* analysed in this study and thus a single continental origin for the group. However, the high genetic divergence that divides the *S. carlosi* lineage from the new species found on La Gomera, and the possibility of other continental relatives (see Emerson et al. 2000a for an example) means we are unable to conclusively determine whether colonization of the Canary Islands originated from a single common ancestor or from more than one.

The La Gomera endemic, *Steganacarus* n. sp. appears to be adapted to the xeric habitats close to the coast. The remaining *Steganacarus* lineages have colonized the laurel forest habitat of higher altitudes. The phylogeography of this second group suggests that there was a somewhat ancient colonization of the archipelago followed by local diversification events within confined geographical areas, one each on Gran Canaria and La Gomera and two on Tenerife, where, on the Anaga Massif, the single colonizing population gave rise to two descendant lineages. One of these lineages, *S. carlosi* subsequently colonized the two massifs of Teno and Roque del Conde, probably over an ocean barrier before the emergence of Las Cañadas. The phylogenetic analyses suggest a basal position for Gran Canaria, but it is impossible to convincingly infer the sequence of island colonization events from our data. However, an origin on Gran Canaria is compatible with geological and ecological evidence. Gran Canaria is the oldest and closest island to the mainland to support appropriate habitat for *S. carlosi*, although the older islands of Lanzarote and Fuerteventura, currently lacking suitable habitat for *Steganacarus*, may have played a role in the past when conditions were less xeric.

The utility of species that are facultatively associated with a particular forest habitat as a tool for indirectly inferring the history of that habitat has recently been demonstrated for the pine forest ecosystem of the archipelago (Emerson et al. 2000a). The ecology of *Steganacarus* provides a model system for future work to examine population structuring and genetic diversity within the fauna of the laurel forest and it will be interesting to examine patterns within the extensive laurel forest domains of La Palma and El Hierro.
DNA sequences have been deposited in the EMBL Nucleotide Sequence Database under Accession nos AJ414175–AJ414201.

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References


This work is part of Nicola Salomone’s PhD thesis. His research interests centre around a combined molecular, morphological and ecological approach to speciation studies. Brent Emerson is a lecturer in Evolutionary Biology at the University of East Anglia with interests in the application of molecular data to interpret phylogenetic history and population dynamics. Professor Godfrey Hewitt’s research focuses on molecular evolutionary genetics with special emphasis on hybrid zones, speciation, and phylogeography. Fabio Bernini is Professor in Zoology at the University of Siena with interests in systematics and biogeography of orbibatid mites.