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

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Abstract

The mite genus *Stegunucurus* 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 Canana 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 Stegunucurus species from La Gomera, morphologically quite distinct from the other Canarian *Stegunucurus*, 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 *Pimelia, Hegeter* and the butterfly *Gonepteryx*) appear to have colonized the Canary Islands from Africa and/or iberia and indicate an east-to-west stepping-stone type of mlonization, 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 mlonization (Thorpe *et al.* 1994; Brown & Pestano 1998).

Because of their lifestyle and low power of dispersion (Bernini 1991; Schatz 1991), soil oribatid mites (Acari: Oribatida), present an opportunity for investigating the processes of mlonization 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 (Avanzatiet*al.* 1994, 1996). The *Steganacarus* 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, *Steganacarus* 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 (Bemini 1991).

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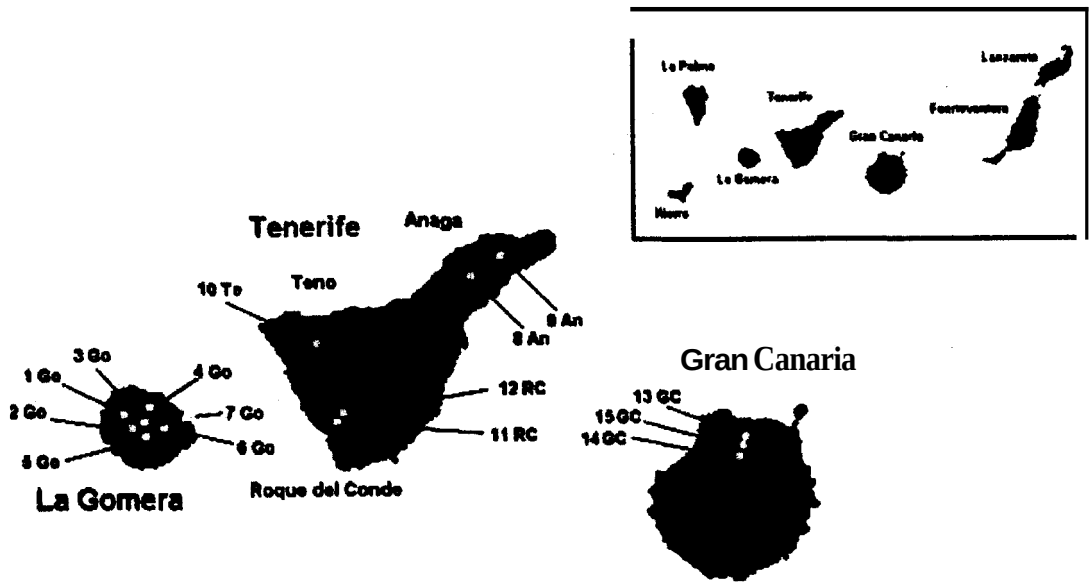

Fig. 1 Map of the Canary Islands showing sampling locations of specimens analysed in the present study. Codes of the collection sites correspond with codes in Table 1.

The genus *Steganacarus* (box-mites) comprises ≈ 50 species and is represented across Europe, Western Asia and Northern Africa including Macaronesia (Madeira, Bernini & Magari **1993;** and the Canary Islands, Pérez-Iñigo 1988; Niedbala 1992; Pérez-Iñigo & Peiia 1996). Fuerteventura and Lanzarote do not support ***Steganacarus* species,** probabiy owing to the absence of suitable habitats for these organisms, which require forest soiis 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*** Niedbaia (1984), widespread on ail 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 weil-defined group not represented in western Europe or in northem Africa, but present in southeastern Europe (Anatolian peninsula and Caucasus) (Avanzati ***et al.* 1996).** Subsequent detaiied and quantitative morphological studies on ***S. carlosi*** and ***S. tenerifensis*** have provided such a wide range of variabiiity **as** to cast doubt on their specific iimits (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** c_3 notogastral seta, modified **as** in ***S. tenerifensis.*** These morphotypes have been referred to **as** 'mono' and their systematic position is stiil uncertain (Avanzati ***etal.* 1996).** A similar morphotype was also found in Gran Canaria **by** Pérez-Iñigo & Peña (1996) and, on the basis of variation in the c_3 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 homogenity 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 (Ye ***et al.* 1999),** providing an ideal template for studying phylogeographical pattern within *Steganacarus* mites. The archipelago is composed of seven mapr 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 posible 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 eastern-most Fuerteventura and Lanzarote (≈ 15–20 Myr) (Coello ***et al.*** 1992), Gran Canaria (≈ ***13.5–14*** Myr) (Ye ***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)**

and Hierro (< 1Myr) islands. The present-day configuration of Tenerife is relatively recent (= 1Myr); before this time there were 2–3 smaller, separate islands represented by the older volcanic massifs of the Roque del Conde (southern Tenerife, 8.5–6.4 Myr), Teno (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 Cañadas later replaced by the emission of the Teide-Pico Viep complex which linked the older eroded edifices (Ancochea *et al.* 1990).

This study addresses the phylogenetic history of *Steganacarus* species fmm the Canary islands using characters 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 fmm 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 *Steganacarus* 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 the specimens were identified and stored at −80 °C. Collection sites and codes of the samples used in the analysis are detailed in Table 1. *Stegunucarus carlosi* 10Te (Teno-Tenerife, collected in March 1992) and *Steganacarus* n. sp. 7Go (P. Liana-La Gomera, collected in December 1987) were obtained from collection material kept in 75% ethanol. A specimen of the same genus, *S. (S) carusoi* from Sardinia (Italy), was also sequenced and used

Tabk 1 List of specimens examined in this study

Code	Island	Sampling Sites	Morphotypes analysed
1 Go	La Gomera	Aparta-caminos	*Steganacarus* carlosi
			S. carlosi/mono
2 Go	La Gomera	Cerro de Araña	S. carlosi
3 Go	La Gomera	La Laguna Grande	*S. carlosi*
4 Go	La Gomera	Mirador de Vallehermoso	*S. carlosi*/mono
5 Go	La Gomera	Alto de Cherelepin	*S. carlosi*
			S. carlosi/mono
6 Go	La Gomera	Bosque de El Cedro	*S. carlosi*
			S. carlosi/mono
7Go	La Gomera	Punta Llana	*Steganacarus* n. sp.
8 An	Tenerife	Anaga	*S. carlosi*/mono-1
		Las Mercedes	*S. carlosi*/mono-2
			S. carlosi/mono-3
9 An	Tenerife	Anaga	*S. tenerifensis*-1
		Las Casas de la Cumbre	*S. tenerifensis*-2
			S. tenerifensis-3
10Te	Tenerife	Teno	*S. carlosi*
		Ruigomez	
11 RC	Tenerife	Roque del Conde	*S. carlosi*
		Barranco dell'Infierno	*S. carlosi*/mono
12 RC	Tenerife	Roque del Conde	*S. carlosi*
		Barranco dell'Infierno	*S. carlosi*/mono
13GC	Gran Canana	Los Tilos de Moya	*S. carlosi*
		Bosque Oscuro	
14GC	Gran Canana	Los Tilos de Moya	S. carlosi
		Barranco de Laurel	
15GC	Gran Canana	Los Tilos de Moya	*S. carlosi*
		Bosque Oscuro	S. cariosi-2
			S. guanarteme

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

DNA extraction

Single individualswere used for amplificationand sequencing. Total genomic DNA was extracted from living, frozen or alcohol-preserved tissues following a modified CTAB method (Boyce *et al.* 1989). In short, specimens were digested with CTAB buffer (0.1 M Tris–HC1 pH 8.0, 1.4 M NaCl, 0.02 M EDTA, 2% CTAB, 02% 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-f-1751 (5'-GGATCACCTGATATAGCATTCCC-3') and C-1-N-2191 (5'-CCCGGTAAAATTAAAATATAAACTTC-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 diiuted in H_2O. 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 profiie 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'-CTTTATGTTAAGAATTGTTGT-3'). Sequencing reactions were performed either manualiy (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 & Beckenbach 1989) and the software package DNASP Version3.14 (Rozas & Rozas 1999) was used to estimate DNA polymorphism. Phylogenetic analyses were performed with PAUP* (Swofford 1998) with *S. carusoi* as the outgroup, using both maximum parsimony (MP) and maximum likelihood (ML). MP analyses were performed using a heuristic search with equal weighüng of ail 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 & Crandali 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 equai base frequenaes, Kimura 3 parameter, SYM, F81, HKY85, Tamura–Nei, Kimura 3-parameter with unequal base frequenaes and the general time reversible (GTR) model (see Posada & Crandali 1998; for detaiis of models). Each of four different rate heterogenity 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 MODELTECT 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 (NJ) 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 speaes *Steganacarus carusoi*. No deletion or insertion of bases was observed. A

total of 150 polymorphic sites (representing 1% 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 pasition, 80.6%) is observed in the outgroup, *S. carusoi*. A previous study on the same COI region for Steganacarus speaes 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 Canarian lineage, the bias to an increased A+T content is lower than in the west-Mediterranean congeneric species.

Molecular differentiation

No pattem 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 intraisland genetic divergence was 7.8% (ML) (Anaga-Roque del Conde). In Tenerife, genetic distance between the two morphologically defined species *S. carlosi* and *S. tenerifensis* range from 9.8 to 15.8%. inter-island genetic differentiation 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. tenerifensis*) 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 MODELTEST supported 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 (ti/tv ratio = 3.7494, gamma shape parameter = 0.261). The ML analysis produced a topology that was largely congruent with the parsimony results (Fig. 3). For both MP and ML analyses Steganacarusn. 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 pattem of genetic differentiation between *S. carlosi* and the 'mono' morphotypes. Haplotypes 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 *tenerifensis* 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

COZ *sequence evolution*

The average sequence divergence among populations and speaes 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 could be evolving intrinsically faster in the Steganacarus belonging to the Canarian lineage than the south-European congeneric 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 anaent 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 speaes and pattems of geographical variation within populations that has not been posible 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 Chelicerata (Avise *et* al. 1994; Navajas et al. 1996; Salomone et al. 1996), tends to be less biased in the Canarian lineage. A similar shift in nucleotide composition between closely related species and even in a single taxon has already been described withii the *Dermaptera* (Wirth *et* al. 1999). Several hypotheses have been put forward to explain biases in nucleotide contents (Moriyama &

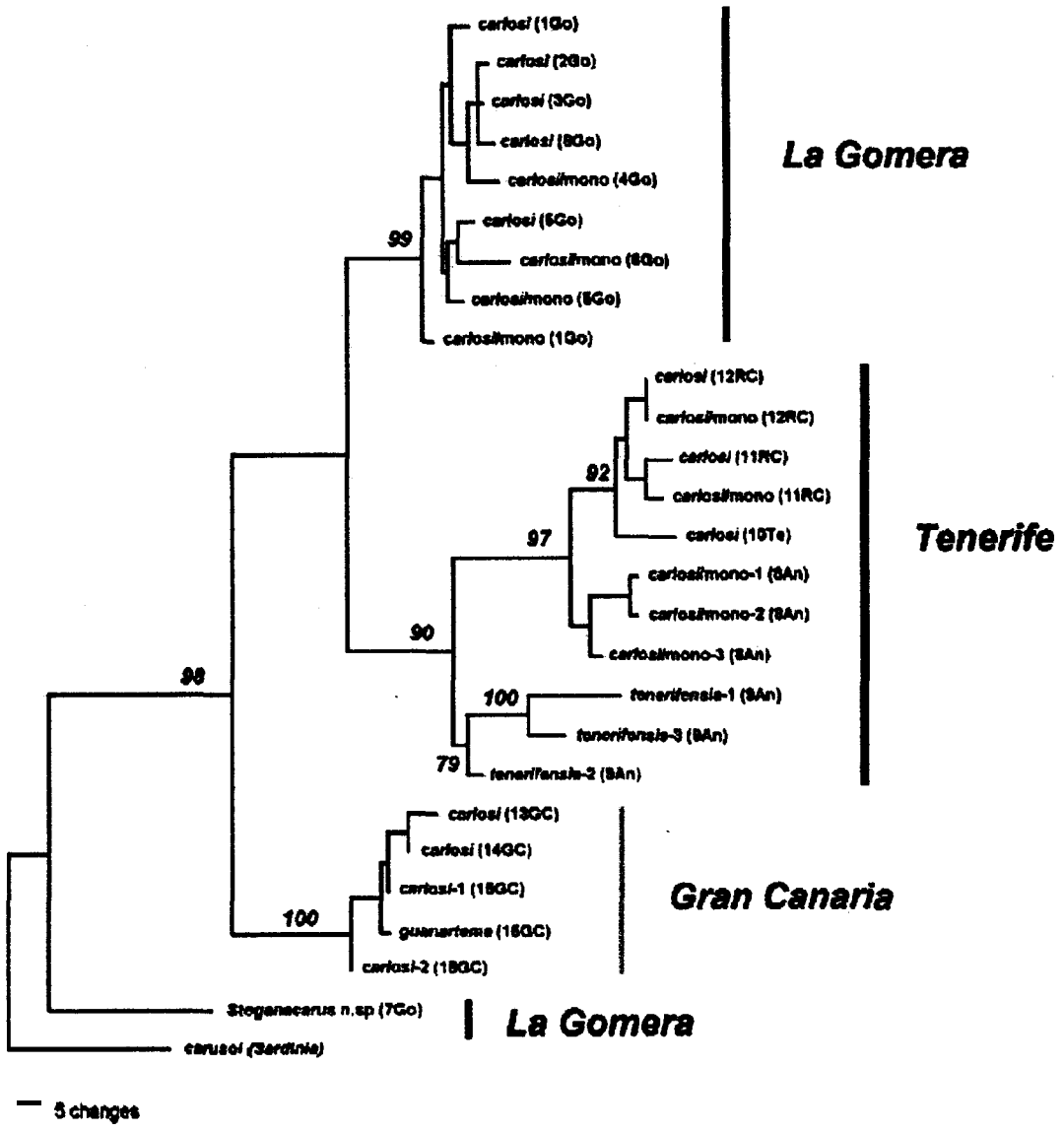

Fig. 2 One of 12 most parsimonious trees for Canarian *Steganacarus* mites calculated from mitochondrial COI sequences. Bootstrap values are indicated for nodes gaining > 70% support (500 replications).

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 *Steganacarus* 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 *Steganacarus* 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. Phyiogenetic reconstructions do not result in the differentiation of the two morphotypes Several

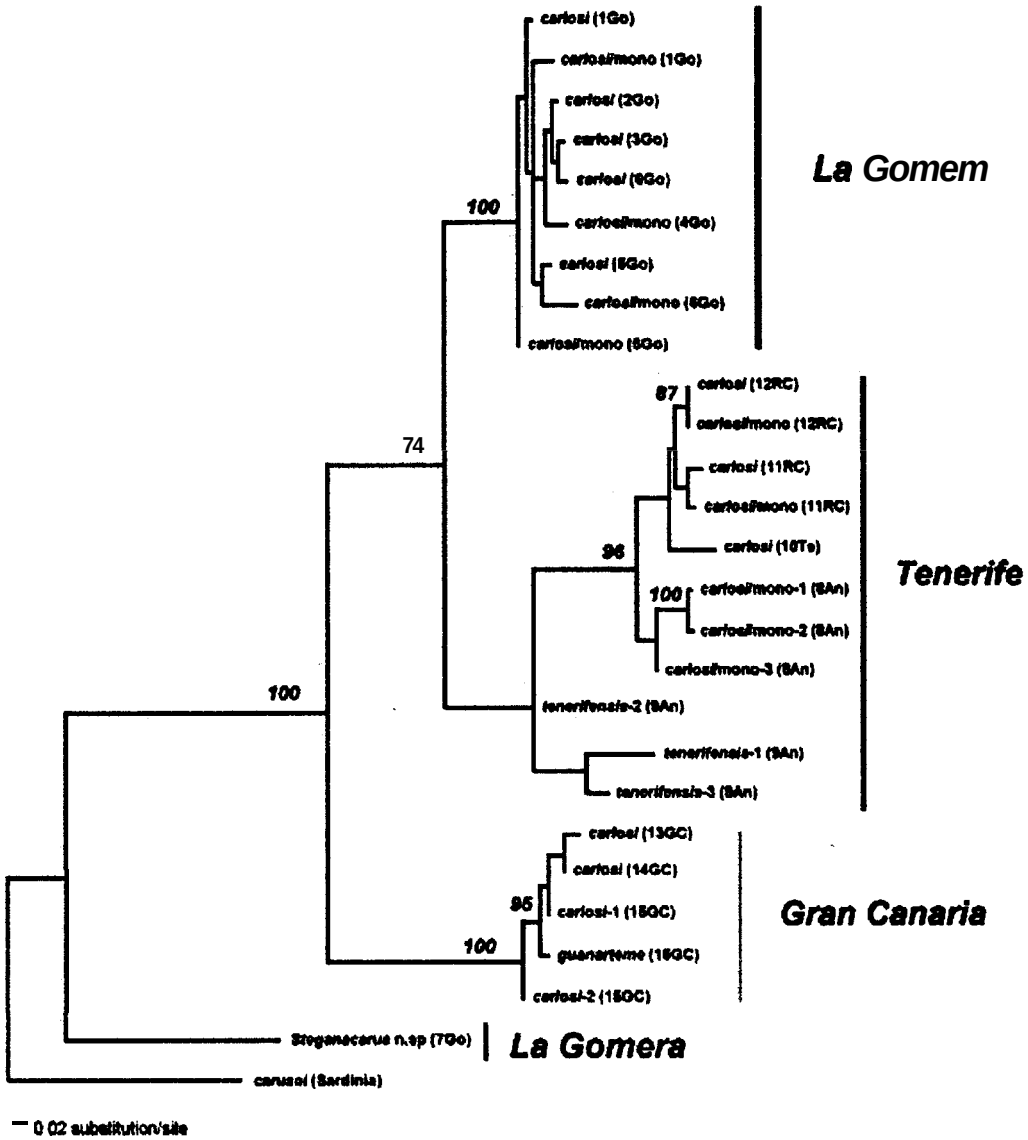

Fig. 3 Maximum likelihood (ML) *tree* for Canarian *Steganacarus* mites calculated from mitochondrial COI sequences. Bootstrap values obtained via neighbourhood pining (using ML distance setting) are indicated at nodes with > 70% support (500 replications).

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 historial 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_3 notogastral setae. This pattern of variabiiity 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 intraspecific polymorphism related to the c_3 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 Phytoseiidae mites a functional view of dorsal chetotaxy suggesting protection in soil micro-environments (as for example in avoiding entrapment) has been suggested (Sabelis & Bakker 1992). However, in the Canarian Steganamrus, 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 six 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 altematives 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 divergentes among populations of *S. carlosi* in the three islands are much higher than those between the two Tenerife species (*S.* carlosi and *S. tenerifensis*), and are comparable with divergence levels found between morphologically well-distinct congeneric 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 experimenb.

The new *Steganacarus* speaes discovered on La Gomera and determined on the basis of morphological distinction is also geneticaiiy distinct. Phylogenetic analyses identify Steganamrus 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 *Steganacarus* and the presence of two geneticaiiy distinct clades, corresponding to *S. tenerifensis* 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 northem and southern part of the island have aiready emerged between closely related species and populations of invertebrates e.g. *Eutrichopus* (Cobolli Sbordoni *et* al. 1991), *Pimelia* (Juaret al. 1996) *Calathus* (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 dock-iike 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 cases our data suggest that colonization events took place before the emergence of the central Cañadas volcanic region. During

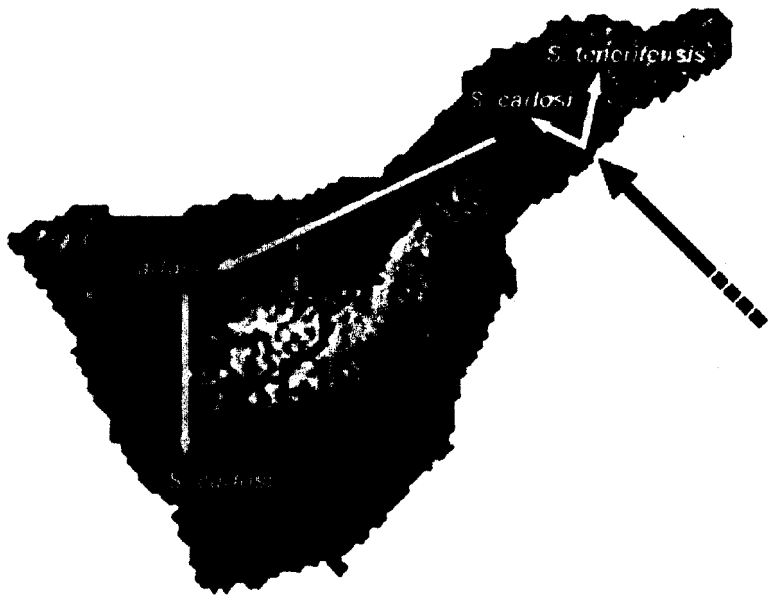

Fig. 4 Map of Tenerife indicating proposed colonization sequence and divergence events for *Steganacarus carlosi* and *Steganacarus tenerifensis*.

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 *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 posible 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 *etal.* 1990) and it may be that this has resulted in the absence of geographical bamers, 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 *carlosi* lineage from the new species found on La Gomera, and the possibility of other continental relatives (see Emerson *etal.* 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 Gomeran endemic, *Steganacarus* n. sp. appears to be adapted to the xeric habitats clase to the coast. The remaining *Steganacarus* iineages 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 iineages. 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 imposible 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 pattems 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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