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The imprint of volcanism on within-island diversification of woodlouse-hunter spiders (Araneae, Dysderidae) in the Canary Islands

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1 **ORIGINAL ARTICLE**

2 **The imprint of volcanism on within-island diversification of woodlouse-hunter**

3 **spiders (Araneae, Dysderidae) in the Canary Islands**

4

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24 **Running title:** Phylogeography of *Dysdera verneaui* in Tenerife

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1 **ABSTRACT**

2 Geological processes and ecological adaptation are major drivers of diversification on

3 oceanic islands. Although diversification in these islands is often interpreted as resulting

4 from dispersal or island hopping rather than vicariance, this may not be the case in

5 islands with complex geological histories. The island of Tenerife, in the Canary Islands,

6 emerged in the late Miocene as three precursor islands that were subsequently

7 connected and re-isolated by volcanic cycles. The spider *Dysdera verneaui* is endemic

8 to the island of Tenerife, where it is widely distributed throughout most island habitats,

9 providing an excellent model to investigate the role of physical barriers and ecological

10 adaptation in shaping within-island diversity. Here, we present evidence that the

11 phylogeographic patterns of this species trace back to the independent emergence of the

12 protoislands. Molecular markers identify two distinct evolutionary lineages that

13 correspond to two precursor islands, each with diagnostic genital characters indicative

14 of separate species status. Episodic introgression events between these two main

15 evolutionary lineages explain the observed incongruence between mitochondrial and

16 nuclear markers, probably as a result of the homogenisation of their *ITS-2* sequence

17 types. The most widespread lineage exhibits a complex population structure, which is

18 compatible with either secondary contact, following connection of deeply divergent

19 lineages or, alternatively, a back colonization from one precursor island to another.

20

21 **Keywords:** cryptic species - island vicariance - molecular dating - multispecies

22 coalescents - phylogeography.

23

1   **INTRODUCTION**

2   Oceanic archipelagos serve as natural model systems for studying patterns and

3   processes related to diversification (Gillespie 2004; Emerson and Kolm 2005; Emerson

4   and Oromí 2005; Losos and Ricklefs 2009). Although island hopping accounts for a

5   large proportion of the speciation events on oceanic islands, emerging evidence

6   highlights the contribution of within-island diversification to oceanic island biodiversity

7   (Juan et al. 2000; Gillespie and Roderick 2002). Historical geological factors such as

8   sea-level changes, sea-floor uplifts or volcanic activity have played a key role in

9   generating within-island biogeographic boundaries and, ultimately, in promoting

10   diversification (Carson et al. 1990; Pestano and Brown 1999; Beheregaray et al. 2003;

11   Vandergast et al. 2004; Bloor et al. 2008). However, molecular phylogenetic and

12   phylogeographic studies have identified a growing number of island biogeographic

13   boundaries that do not correspond with obvious geological barriers, but are better

14   explained by divergent selection (Ogden and Thorpe 2002; De Busschere et al. 2010;

15   Thorpe et al. 2010).

16

17   **Case study: the woodlouse hunter spiders on Tenerife**

18   The spider genus *Dysdera* has undergone major diversification in the Canary Islands,

19   where more than 50 endemic species have been reported (Arnedo et al. 2001; Macías-

20   Hernández et al. 2010). Most species are the result of local diversification processes

21   (Macías-Hernández et al. 2008), and within-island speciation events may account for

22   about two thirds of the extant species (Arnedo 1998). Canarian *Dysdera* species

23   richness is positively correlated with the area, elevation, geological age and ecological

24   complexity of the islands (Arnedo and Ribera 1999; Arnedo et al. 2000; Cardoso et al.

1   2010). There is evidence that lava flows and eustatic sea level changes have influenced

2   phylogeographic patterns in the oldest islands of the archipelago (Bidegaray-Batista et

3   al. 2007) and similar vicariant events may also explain the restricted geographic range

4   of many species on younger islands. However, several lines of evidence suggest that

5   natural selection has also played an important role in the diversification of the group.

6   Parapatric speciation through colonisation and adaptation to caves, for instance, has

7   been a major source of species differentiation in Tenerife (Arnedo et al. 2007), and the

8   intertidal zone in pebble beaches that is occupied by a recently described species of

9   *Dysdera* (Macías-Hernández et al. 2010) represents the only case of habitat shift within

10   the radiation of the genus. Although published data and circumstantial evidence suggest

11   the involvement of both geological barriers and ecological adaptation in the generation

12   of the diversity of *Dysdera* spiders in the Canaries, to date there has been little effort to

13   evaluate the relative importance of both factors in shaping genetic diversity at the

14   population level, which may ultimately lead to speciation.

15   The species *Dysdera verneaui* Simon, 1883 is an excellent candidate to

16   investigate factors promoting within-island diversification on Tenerife, the largest and

17   highest island of the Canary archipelago. It is the most widespread *Dysdera* species on

18   the island, and can be found in a wide range of habitats, including caves, along an

19   altitudinal gradient from lowland areas (200 m a.s.l.) to high elevations on the El Teide

20   volcano (>3100 m), and shows considerable intraspecific variability in body size and

21   subtle differences in genitalic structures (Arnedo & Ribera, 1999).

22   Tenerife originated in the late Miocene as three independent volcanic islands,

23   approximately corresponding to the present-day Anaga (NE), Teno (NW) and Roque

24   del Conde-Adeje (SW) massifs, which are dated to approximately 4.9-3.9 Ma, 6.2-5.1

1   Ma and 11.9-8.9 Ma, respectively (Guillou et al. 2004). The large, central Cañadas

2   volcanic edifice, which united the former islands together, began to form approximately

3   3.5 Ma and underwent three cycles of volcanic activity, ending 0.2 Ma (Ancochea et al.

4   1999; Cantagrel et al. 1999). Additionally, the present day geomorphology of Tenerife

5   has been shaped by six major debris avalanches that occurred on both slopes of the

6   island over the past million years (Ancochea et al. 1990; Watts and Masson 1995;

7   Cantagrel et al. 1999; Watts and Masson 2001) (see Fig. 1 for more details). Tenerife is

8   also the most habitat-rich island in the Macaronesian region due to the joint effects of

9   trade winds and elevation. As is the case for most oceanic islands, the main ecological

10   zones and habitats of Tenerife are defined along altitudinal clines and windward-

11   leeward orientations (see Fig. 1).

12   The complex geological history of Tenerife, including precursor islands and

13   recurrent volcanic activity, and its high habitat diversity have provided ample

14   opportunities for local species diversification. Evidence for phylogeographic structure

15   tracing back to the precursor islands of Tenerife has been identified in endemic reptiles

16   (Thorpe et al. 1996; Brown et al. 2000; Gübitz et al. 2000) and insects (Juan et al.

17   1996). In addition, population extinction and fragmentation by the effect of lava flows

18   has been well documented in other islands of the archipelago (Bidegaray-Batista et al.

19   2007). Ecological heterogeneity on the other hand, has been invoked to explain island

20   intraspecific morphological variation. For example, colour pattern in sexually mature

21   males of Tenerife lizard *Gallotia galloti* correlates with different habitat types on

22   northern and southern slopes (Thorpe et al. 1996). Furthermore, a growing body of

23   evidence suggests that divergent selection for habitat type may actually reduce gene

24   flow, paving the way to adaptive speciation. Patterns of gene flow inferred from

1   microsatellite data suggest assortative mattings among ecotypes (Thorpe and Richard

2   2001), and similar findings have also been reported in Lesser Antillean *Anolis* (Thorpe

3   et al. 2010).

4   In the present study, we evaluate the roles of geology and habitat types in

5   structuring phylogeography and gene flow within *D. verneaui* in Tenerife. Based on

6   available information from other endemic organisms we evaluate two hypotheses: (1)

7   phylogeographic patterns within *D. verneaui* correspond to the precursor islands that

8   subsequently became united to form Tenerife; and (2) ecological barriers associated

9   with different habitat types have promoted genetic isolation among populations. We test

10   these two hypotheses by inferring population structure and estimating the timing of

11   phylogeographic breaks using a combination of mitochondrial and nuclear genes.

12

13   **MATERIALS AND METHODS**

14   **Taxonomic sampling**

15   Specimens of *Dysdera verneaui* were collected on Tenerife, with sampling taking place

16   in different habitats (thermo-sclerophyllous woodland, laurel forest, dry subalpine

17   scrub, pine forest and xerophytic shrubs) on the north and south slopes of the island.

18   *Dysdera verneaui* were found in 22 out of 40 localities visited (see Appendix S1). The

19   mainland species *D. inermis* and the Canarian endemics *D. silvatica*, *D. calderensis*,

20   and *D. gomerensis* (the closest relative of *D. verneaui* (Arnedo et al. 2001)) were

21   included in the analyses to provide calibration points for estimating absolute lineage

22   ages (see below). The mainland species *Dysdera adriatica* was used as outgroup to root

23   phylogenetic trees.

24

1   **DNA extractions, PCR amplifications and sequencing**

2   Genomic DNA was extracted from specimens using the DNeasy Tissue Kit (Qiagen)

3   following manufacturer's guidelines. Fragments of mitochondrial cytochrome oxidase I

4   (*cox1*), 16S rRNA (*16S*) and the complete tRNA leu UAG (*L1*), NADH dehydrogenase

5   subunit I (*nad1*), and the nuclear genes internal transcribed spacer 2 (*ITS-2*) and 28S

6   rRNA (*28S*) were amplified and sequenced following Macías-Hernández *et al.* (2008).

7   DNA sequences were assembled and edited using the STADEN software package

8   (http://staden.sourceforge.net/) and managed using the computer program BIOEDIT

9   (Hall 1999).

10   The direct sequencing of the *ITS-2* of some individuals yielded superimposed

11   traces, suggesting heterozygosity for indels. Alleles were individualised by cloning the

12   gel-purified PCR product with pGEM-T Easy Vector cloning kit (Promega). Three to

13   eight colonies per individual were sequenced using bacterial colonies directly as

14   template for PCR amplification with vector primers T7 and SP6, using the following

15   PCR conditions: 94ºC for 5 min, 30 cycles of 94ºC for 1 min, 50ºC for 30 s, and 72ºC

16   for 3 min, followed by a final extension of 72ºC for 5 min. PCR products were purified

17   using MultiScreen PCRµ96 cleanup filter plates from Millipore and cycle-sequenced in

18   both directions.

19

20   **Phylogenetic and phylogeographic analyses**

21   Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI)

22   analyses were conducted to resolve the phylogenetic relationships among *D. verneaui*

23   and its close relatives (see Appendix S1).

1   Ribosomal gene sequences were aligned with the online version of the MAFFT

2   v.5.8 automatic alignment program (Katoh et al. 2002; 2005) (http://align.bmr.kyushu-

3   u.ac.jp/mafft/online/server/) with default options and manual strategy option set to Q-

4   INS-i. Gaps were treated as single mutational events and were scored as

5   presence/absence characters following Simmons & Ochoterena (2000). The program

6   GapCoder (Young and Healy 2003) facilitated the automatic recoding of gaps using the

7   method of Simmons *et al.* (2001). Gene partitions were concatenated with the program

8   WINCLADA v.1.00.08 (Nixon 2002). Parsimony analyses under equal weights were

9   performed with TNT v. 1.1 (Goloboff et al. 2003) using a heuristic search with 1000

10   replicates of random sequence addition, followed by TBR branch swapping (five trees

11   retained per iteration, and final round of TBR branch swapping on all retained trees).

12   Clade support was assessed by means of 1000 jackknife replicates (Farris et al. 1996).

13   Bayesian inference analyses were conducted with MRBAYES v.3.1.2 (Ronquist and

14   Huelsenbeck 2003) and run remotely at the Bioportal computer resources of the

15   University of Oslo (http://www.bioportal.uio.no). Independent substitution models

16   selected by jMODELTEST (Posada 2008) were specified for each gene fragment and a

17   standard discrete model was implemented for the gaps scored as absence/presence data.

18   The substitution estimates were allowed to vary independently between each partition.

19   Two independent runs with eight simultaneous MCMC (Markov Chain Monte Carlo)

20   chains (one cold and seven heated), each starting with random starting trees, were

21   performed for 10 million iterations. Decreasing temperature to 0.15 facilitated the

22   convergence of the chains. Analyses were run for 4 million generations, discarding the

23   first 10% as burn-in. The standard deviation of the split frequencies between runs (<

24   0.01) and the effective sample size (ESS, as measured by the program TRACER version

1   1.4 (Rambaut and Drummond 2007), were monitored to ensure stationarity,

2   convergence and correct mixing of the chains. Maximum likelihood analyses were

3   conducted with the software RAxML v. 7.0.4 (Stamatakis 2006). Independent

4   GTR+G+I substitution models were set for each data fragment. The highest likelihood

5   tree was selected from 10 iterations of random addition of taxa and clade support

6   assessed by 100 bootstraped matrices. Uncorrected genetic distances (p-distance)

7   among lineages were calculated for the *cox1* mitochondrial and *ITS-2* nuclear gene

8   fragments with the software MEGA v. 4.0. Haplotype networks of the *cox1* and *ITS-2*

9   genes were estimated using TCS v. 1.21 software (Clement et al. 2000).

10

11   **Lineage age and population divergence times**

12   Divergence times and substitution rates for gene trees and the species tree were

13   estimated with BEAST v.1.5.3 (Drummond and Rambaut 2007) using three different

14   strategies. First, clade ages were estimated based on a concatenated data matrix of all

15   genes, except *ITS-2*, and representatives of all sampled species, including 11 *D.*

16   *verneaui* haplotypes belonging to independent mitochondrial networks. Bayes Factors

17   provided strong support for choosing the uncorrelated lognormal relaxed clock as the

18   best clock model for the data (BF=2.59 and 2.52 vs. exponential and strict clock

19   models, respectively) (Suchard et al. 2001), and it was used for all subsequent analyses,

20   except the *multispecies coalescent* analyses (see below). The tree prior was set to the

21   speciation Birth-Death process, and calibration points were incorporated as node priors.

22   Second, we applied a *multi-demographic coalescent model* (Ho et al. 2008) to the *cox1*

23   haplotype matrix, constraining those clades that received high support (PP>0.95, MP

24   jackknife or ML bootstrap > 70%) in the concatenated analysis. This method combined

1 a Yule tree prior model with a coalescent demographic model of exponential growth,

2 allowing the joint estimation of divergence times and substitution rates in trees that

3 include species and population level divergences. Absolute divergence times for these

4 two strategies were inferred by including five calibration points. The the divergence

5 between the Iberian and Moroccan lineages of *Dysdera* cf *inermis* was set to 5.3 Ma (1),

6 which corresponds to the opening of the Strait of Gibraltar (Krijgsman et al. 1999). This

7 geological event has been frequently used to date phylogenies of Mediterranean and

8 Macaronesian taxa (eg. (Bidegaray-Batista et al. 2007); (Carranza and Arnold 2003);

9 (Gómez-Zurita 2004), and has been shown to be compatible with calibrations points

10 provided by the subaearial stages of the Canaries (Bidegaray-Batista and Arnedo 2011).

11 The oldest subaerial datation of La Palma (2 Ma) (Carracedo and Day 2002) provided a

12 maximum age estimate for the divergence between La Palma and La Gomera

13 populations of (2) *D. calderensis* and (3) *D. silvatica*, while that of El Hierro (1.2 Ma)

14 (Carracedo and Day 2002) provided a maximum age estimate of the divergence

15 between El Hierro and La Gomera populations of (4) *D. gomerensis* and (5) *D.*

16 *silvatica*. The third strategy used the *multispecies coalescent model* (Heled and

17 Drummond 2010) implemented in BEAST v.1.5.3 (*BEAST), which co-estimates

18 multiple gene trees embedded in a shared species tree using multi-locus data from

19 multiple individuals per species. This approach allowed the *ITS-2* gene to be included in

20 the species tree estimation and its substitution rate inferred. Analyses were rooted by

21 assuming *D. silvatica* as the sister group of the remaining taxa, as recovered in all

22 previous analyses, since *ITS-2* was only available for Canarian taxa. The substitution

23 rates and clock models were unlinked for each gene, with models corresponding to

24 those selected by jMODELTEST. Trees were linked for the mitochondrial genes and

1   unlinked for the *28S* and *ITS-2* genes. Individuals were assigned to each nominal

2   species, except the *D. verneaui* populations from Teno and the rest of the island, which

3   were defined as two different taxa (see results). A relaxed lognormal clock was

4   specified for all genes except *ITS-2*, to which a strict clock was assigned (preliminary

5   runs under *ITS-2* clock set to the relaxed lognormal reported infinite values for the

6   posterior and likelihood scores). The species tree prior was set to the Yule process, and

7   the "share the same tree prior" option was checked. To simplify calculations, absolute

8   ages were obtained by setting ucld.mean priors to include the 95% highest posterior

9   density substitution rate values obtained in the first strategy. All analyses were run for

10   50 million generations (10^8 and $5*10^7$ generations for the *multispecies coalescent model*

11   analysis), sampling every 1000 generations and removing the first 10% of samples as

12   burn-in. Two independent runs were carried out for each analysis to assess convergence.

13   The results of the two MCMC runs were analysed in TRACER v. 1.4 (Drummond and

14   Rambaut 2007), and ESS values were monitored to assess the correct mixing of the

15   chains. The accompanying programs LogCombiner and Tree Annotator were used to

16   combine the parameter values and trees of each run in a single data set and to

17   summarise tree information, respectively.

18   Coalescent-based methods, as implemented in MDIV (Nielsen and Wakeley

19   2001), were used to estimate the time of population divergence and the time of the most

20   recent common ancestor (TMRCA) between pairs of populations based on the mtDNA

21   *cox1* and nDNA *ITS-2* genes. The following population pairs were analysed: Centre vs.

22   Anaga and Anaga E vs. Anaga W. MDIV assumes that there is no recombination within

23   loci. A preliminary Hudson and Kaplan's (Hudson and Kaplan 1985) four-gamete test

24   applied to the mitochondrial and nuclear data sets using 1000 replicates in DNAsp

1 (Rozas et al. 2003) revealed non-significant values (P= 1.000) and confirms prior

2 expectations of low intra-sequence recombination in population level comparisons.

3 MDIV analyses were run at Cornell's CBSU computer cluster

4 (http://cbsuapps.tc.cornell.edu/mdiv.aspx). Three independent simulations were run to

5 ensure convergence of the results (see Bidegaray-Batista et al. 2007) for more details).

6 Values for T and TMRCA were calculated using the lineage-specific substitution rates,

7 with confidence intervals estimated in the present study (see below) and a generation

8 time of 1.5 years (Cooke 1965).

9

10 **Genetic diversity and population genetic structure**

11 Standard diversity indices, including nucleotide diversity (π), number of haplotypes

12 (*Nh*) and haplotypic diversity (*h*) for the complete *cox1* and *ITS-2* matrices, were

13 calculated using ARLEQUIN 3.01 (Excoffier et al. 2005). The F_{ST} sequence-based

14 statistic (Wright 1951) between populations for the *cox1* gene were used to assess

15 population structure, as implemented in ARLEQUIN 3.01, and their significance

16 (P<0.01) was assessed by carrying out 10000 permutations.

17 The computer program SAMOVA 1.0 (Dupanloup et al. 2002) was used to

18 identify geographically homogeneous populations that maximise the genetic variance

19 between groups of populations. Analyses were performed for *k* values ranging from 2 to

20 13 groups for *cox1* and from 2 to 6 for *ITS-2*, using 100 simulated annealing

21 procedures.

22

23 **RESULTS**

24 **Sequence variation**

1   Specimens and sequences analysed in the present study, with their corresponding

2   GenBank accession numbers, are listed in Appendix S1. Approximately 1 kb of the

3   mitochondrial *cox1* gene was obtained from 85 specimens of *D. verneaui* from 22

4   localities, yielding 57 haplotypes, including 249 polymorphic sites, 226 of which were

5   parsimoniously informative. The A-T bias was 64.29%, and 67.58% of nucleotide

6   substitutions were transitions. Among the 57 haplotypes, the average pairwise

7   difference was 7.5% ± 0.4%, and the maximum sequence divergence was 14.4%.

8   The complete nuclear *ITS-2* sequence (442 bp and 13 additional gap characters)

9   was obtained from 43 specimens, including 32 *D. verneaui* individuals from 16

10   localities and 6 individuals of the closely related species *D. gomerensis*. Five

11   individuals were cloned, three of which showed two different alleles of different

12   lengths. The polymorphisms due to singleton mutations among sequenced colonies

13   were assumed as errors of the *Taq* polymerase and cloning artefacts (see Pääbo and

14   Wilson 1988; Villablanca et al. 1998; Calderón et al. 2009). The final alignment of *ITS-*

15   *2* yielded 10 polymorphic sites, the G-C content was 53.1%, and 40% of nucleotide

16   substitutions were transitions. A total of 9 sequences types were detected in *D.*

17   *verneaui*, and 5 were found in *D. gomerensis*. The average pairwise difference among

18   the five *ITS-2* sequence types of *D. gomerensis* and among the nine *ITS-2* sequence

19   types of *D. verneaui* was the same (0.8% ± 0.3%). The mean number of pairwise

20   differences among the 9 sequence types was 3.1488 ± 1.6526.

21

22   **Phylogenetic analyses**

23   Two matrices with contrasting levels of missing data were assembled for phylogenetic

24   analyses. The first matrix (M1, low proportion of missing data) included 2725 bp from

1   four genes (*cox1* = 1008 bp, *16S* = 571 bp, *nad*1 = 343 bp and *28S* = 803 bp) for 37

2   taxa corresponding to a geographically diverse subsample of 27 *D. verneaui* specimens

3   with unique mtDNA haplotypes, along with 10 specimens of five additional species

4   (outgroups and calibration points). Separate analyses of the mtDNA and nDNA

5   partitions of M1 were conducted to investigate incongruence between the data sets. A

6   second matrix (M2, large proportion of missing data) was obtained by adding 31 unique

7   *cox1* haplotypes of *D. verneaui* and 6 of *D. gomerensis* to M1.

8   Parsimony analyses of M1 and M2 yielded 2 trees of 2355 and 2478 steps,

9   respectively. Maximum likelihood analyses yielded single trees of logL -13875.306008

10   and logL -14687.607514 for M1 and M2, respectively. The topologies recovered for M1

11   (not shown) and M2 (Fig. 2) reveal paraphyly of *D. verneaui*, which includes two

12   divergent, lineages (13.2% *cox1* pairwise divergence): the Teno lineage, which includes

13   haplotypes exclusively from the Teno region, and the Anaga-Centre lineage, which

14   includes haplotypes from the rest of the island, and one from Teno. The Anaga-Centre

15   lineage is the sister group of *Dysdera gomerensis* (M1= 64% MP jackknife, 47% ML

16   bootstrap and 0.98 PP support; M2= 70% MP jackknife, 62% ML bootstrap and 0.99 PP

17   support) while the position of the Teno lineage remains unresolved.

18   The Anaga-Centre lineage is further divided into clades A and B. Clade A is

19   comprised of two well-supported lineages, one of which includes all individuals from

20   eastern Anaga (localities 21 and 22, see Fig. 2), whereas the second lineage includes

21   individuals from two central localities close to Anaga (16 and 20) and individuals from

22   other localities in central Tenerife (6, 8 and 13). Clade B includes four well-supported

23   lineages that approximately match particular geographical areas: western Anaga (grey

24   circles), the northern slope, and the southern and central ridges (see Fig. 1 and 2).

1   Individuals from clades A and B co-occur in several localities along the central ridge

2   (e.g., 6, 8, and 13). One individual collected in Teno (N186) was included in one of the

3   clade B lineages. Separate analyses of the mtDNA and nDNA partitions revealed that

4   N186 is a putative case of introgression, as it combines a Teno nuclear sequence type

5   with an Anaga-Centre clade B mtDNA haplotype (53MAT).

6

7   **Lineage age, population divergences and species tree estimation**

8   The lineage age estimations obtained with the concatenated (M1) and the *cox1*-only

9   matrices were approximately similar when confidence intervals were taken into account.

10   The resulting chronogram of the *cox1*-only matrix is shown in Fig. 4. The substitution

11   rate estimated for *cox1* for the *cox1*-only matrix (0.034 substitutions per lineage/million

12   years, 95%HPD= 0.024-0.045) was lower than for the concatenated matrix (0.049,

13   95%HPD= 0.034-0.067). Therefore, we chose to use the geometric mean of two

14   substitution rates (0.0415 per lineage/million years) for subsequent analyses.

15   The species tree inferred under the *multispecies coalescent model* (see Fig. 5),

16   recovered *D. gomerensis* as the sister group of the *D. verneaui* Anaga-Centre lineage

17   although with low posterior probability (0.88), and the Teno lineage as sister group to

18   the *D. gomerensis*+Anaga-Centre lineage with an even lower support. The substitution

19   rate of the *ITS-2* was estimated at 0.00171 per lineage/million years (95%HPD=

20   0.00086-0.0027).

21   The divergence time between the Anaga and Centre populations for *cox1* was

22   0.72 Ma (1.02-0.5 Ma), and the TMRCA was estimated at 1.4 Ma (2.01-1.04 Ma), while

23   for *ITS-2* these values were 0.16 Ma (0.37-0.09 Ma) and 1.4 Ma (3.38-0.88 Ma),

24   respectively. The eastern and western Anaga divergence time for *cox1* was 0.76 Ma

1   (1.09-0.5 Ma), and the TMRCA was estimated at 1.19 Ma (1.7-0.88 Ma). The migration

2   rate between Anaga and Centre populations was low based on both the *cox1* (1.27×10^{-6}

3   migrants per generation) and *ITS-2* (4.18×10^{-6}) sequences, and even lower between

4   the eastern and western populations of Anaga (*cox1*, 7.19×10^{-7}).

5

6   **MtDNA and nDNA networks**

7   The statistical parsimony analysis of the *cox1* haplotypes consisted of 10 unlinked

8   networks plus two single haplotypes (data not shown). Sixty-one steps separated the

9   network of *D. gomerensis* from the *D. verneaui* Anaga-Centre networks, while 76 and

10 137 steps separated the Teno network from the *D. gomerensis* and *D. verneaui* Anaga-

11 Centre networks, respectively.

12   The statistical parsimony analysis of the nuclear *ITS-2* gene yielded two

13 independent networks separated by fourteen steps (see Fig. 3), one for the sequence

14 types of *D. gomerensis* and one for those of *D. verneaui*. All Teno individuals (n=7),

15 including the specimen with the *cox1* haplotype 53MAT, exhibited the same sequence

16 type (9ITS), which is more closely related to the sequence types found in the rest of

17 Tenerife (6 steps) than to the *D. gomerensis* types. The pairwise *ITS-2* genetic

18 divergence between the Teno and Anaga-Centre lineages ranged from 1.4 to 2.2%,

19 whereas the largest divergences within the Anaga-Centre lineage were 0.7%. The

20 pairwise genetic distances between *D. gomerensis* and any of the *D. verneaui* sequence

21 types ranged from 3.6 to 6.1%.

22   The sequence types 3ITS and 4ITS, which were shared by 11 and 10 individuals

23 respectively, were widespread across Tenerife except on Teno. The sequence types 1-

24 2ITS and 5-8ITS were represented by one or two individuals and were mainly found in

1   localities from central and southern Tenerife (localities 3, 5, 7, 8 and 11) as well as one

2   locality in western Anaga (17) (see Fig. 3). The *ITS-2* sequence network analyses were

3   consistent with those of *cox1* in revealing a clear difference between individuals from

4   Teno and Anaga-Centre, although they differed in that the *ITS-2* Teno sequence types

5   were more closely related to those from Anaga-Centre, while the *cox1* haplotypes from

6   Anaga-Centre were more closely related to the *D. gomerensis* types. Unlike for the *cox1*

7   haplotypes, the western and eastern Anaga populations did not show exclusive *ITS-2*

8   sequence types.

9

10   **Genetic diversity and population genetic structure**

11   The nucleotide diversity (π), number of haplotypes (*Nh*) and haplotypic diversity (*h*)

12   within populations for the *cox1* and *ITS-2* genes are summarised in Table 1. All *cox1*

13   haplotypes were exclusive to single localities, except the widespread haplotype 8vCR,

14   which was shared by 9 individuals collected in two close localities on western Anaga

15   (18 and 19) and in central Tenerife (12), and the haplotype v29CPG, found in two close

16   localities on the central Tenerife Dorsal mountain range (12 and 13). The nucleotide

17   diversity within populations ranged from 0 to 0.0547, with the highest value being

18   reported from a high elevation locality on the northern side of the Dorsal range (6).

19   Localities with high *h* values for both the *cox1* and *ITS-2* genes were distributed

20   along the central shield (3, 7, 8, 11 and 13). Other populations distributed across the

21   island show high *h* values for *cox1* but only one *ITS-2* sequence type (e.g., localities 2,

22   16 and 22), while other localities found on western Anaga show low *h*-values for *cox1*

23   but several *ITS-2* sequence types (e.g., localities 17 and 19). All former comparisons

24   were restricted to localities with similar sample sizes.

1   The population pairwise F_{ST} values were generally high for the *cox1* gene (see

2   Table 2), indicating that genetic variation is larger between than within locations. All

3   F_{ST} comparisons between Teno and eastern Anaga and the remaining localities were

4   significant ($P < 0.05$). One locality in southern Tenerife (5) also presented significant

5   F_{ST} values when compared with all other localities. Localities belonging to the same

6   habitat type also showed significant values of F_{ST} among them. For example, more than

7   a half of the 9 pine forest localities showed significant F_{ST} values. Similar patterns were

8   found for the laurel forest localities from Anaga (17-19, 21-22) and the thermo-

9   sclerophyllous vegetation (1 and 20), but not for the dry subalpine scrub localities (6

10   and 7) that showed non-significant differences.

11   Results of SAMOVA analyses are summarized in Table 3. The best grouping,

12   which explained the maximum genetic variance as differences between groups and

13   minimizing the population variance within groups, for *cox1* localities was $K= 7$. Genetic

14   variance among groups was 63.73%, whereas among populations within groups was

15   9.03% and 27.23% within populations ($\Phi_{CT} = 0.637$, $\Phi_{SC} = 0.249$ and $\Phi_{ST} = 0.72$,

16   respectively; all comparisons significant, $P < 0.05$). Φ_{ST} values were high across

17   groupings (0.8-0.71), indicating high within population genetic variability. The

18   preferred grouping for *ITS-2* sequences was $K= 5$, genetic variance among groups was

19   84.69%, whereas among populations within groups was 2.5% and 12% within

20   populations ($\Phi_{CT} = 0.876$, $\Phi_{SC} = 0.0053$ and $\Phi_{ST} = 0.877$, respectively, all comparisons

21   significant, $P < 0.05$). The two markers only agree in grouping the Teno localities part

22   from the others. Mitochondrial groups show a clear geographic signal, while nuclear

23   groups are show less geographic structure. All groups included more than one habitat

type, except two cases of neighbouring populations of similar geological history (W and

E Anaga).

DISCUSSION

The phylogeographic signature of past geological events

The phylogeographic structure observed in *D. verneaui* has a strong geographical

signal: divergent lineages are grossly circumscribed to particular areas of Tenerife.

Conversely, habitat types do not seem to have had a relevant impact on the population

genetic structure of the species. The patterns of population differentiation, as suggested

by high and significant F_{ST} values among localities of the same habitat type, and the

locality grouping suggested by SAMOVA analyses, among which only two cases were

found for undifferentiated populations with the same habitat type (W and E Anaga),

provide further supported for the lack of association between habitat types and the

partitioning of genetic variability.

One of the main findings of the present study was the identification of an old,

mostly isolated lineage of *D. verneaui* in the Teno region. According to our different

estimates, the divergence of this lineage most likely preceded the volcanic phase that

gave rise to the Cañadas edifice, which began to form approximately 3.5 Ma and

eventually joined the three island volcanoes that constitute present-day Tenerife.

Individuals sampled from the rest of the island form a lineage that may have originated

before or after the connection of the precursor islands, depending on the preferred time

estimate. In contrast to the close relationships among Teno populations, some Anaga

populations show close genetic affinities to central Tenerife. The eastern and western

populations from Anaga are clearly distinct, at least based on mtDNA data (9.2%

1 pairwise genetic divergence of *cox1* gene), but they are not differentiated at the nuclear

2 level, suggesting the occurrence of either ongoing male-mediated gene flow or, more

3 likely, recent mitochondrial divergence among formerly continuous populations.

4 Furthermore, nuclear data suggest close links between Anaga and Central populations.

5 Genetic differentiation among Anaga populations has also been reported in the ground

6 beetles *Eutrichopus canariensis* (Moya et al. 2004) and *Calathus abaxoides* (Emerson

7 et al. 1999), and this has been explained as the result of habitat discontinuity (Moya et

8 al. 2004). Additionally, landslides have been invoked to explain phylogeographic breaks

9 in Tenerife's Güímar region observed for both the lizard *Gallotia galloti* (Thorpe et al.

10 1996; Brown et al. 2006) and the gecko *Tarentola delalandii* (Gübitz et al. 2000). The

11 occurrence of a major landslide 1-0.5 Ma (Watts and Masson 2001) has been

12 documented in the central Anaga area of Taganana, approximately corresponding to the

13 estimated age of Anaga's western and eastern populations (~0.76 Ma, 1.09-0.5 Ma).

14 Alternatively, Anaga's eastern and western populations may have different origins, with

15 the former populations being formed by the original local individuals and the latter

16 being the result of colonisations from central Tenerife (see below).

17 Evidence for phylogeographic structure associated with Tenerife's precursor

18 islands has been found in the gecko *Tarentola delalandii* (Gübitz et al. 2000), the skink

19 *Chalcides viridanus* (Brown et al. 2000), the lizard *Gallotia galloti* (Thorpe et al. 1996)

20 and a species complex within the darkling beetle *Pimelia* (Juan et al. 1996). For

21 *Gallotia galloti* and *Pimelia*, the Anaga populations are the most divergent, while the

22 Teno and South populations are closely related or undifferentiated. Conversely, the

23 Teno region harbours the most divergent populations of *Tarentola delalandii*, *Chalcides*

24 *viridanus* and, as shown in the present study, *Dysdera verneaui*. Unlike *Tarentola* and

1 *Chalcides*, however, *D. verneaui* shows some evidence of gene flow between Anaga

2 and Central populations. The individuals from Roque Conde, which represents the only

3 current day remnants of the once isolated Central shield, do not form a basal lineage but

4 are closely related to other populations from the southern slope of central Tenerife.

5 Several evolutionary scenarios could account for these results. Anaga could have served

6 as a source for several, independent colonisations that would have repopulated the

7 central part of the island after lava flow-driven extinctions. The fact that Anaga has not

8 experienced volcanic activity since the mid-Pliocene, whereas central Tenerife was

9 almost completely covered by recurrent lava flows until as recently as 0.2 My, supports

10 this scenario. Alternatively, some older populations could have survived in central

11 Tenerife refugia, as suggested by exclusive *ITS-2* sequence types in the area, and hence,

12 Anaga and central Tenerife could have both acted as sources and exchanged migrants.

13 This scenario would account for the observation that each of the two main clades is

14 mostly formed by haplotypes from one of the two areas: Anaga for clade A and central

15 Tenerife for clade B, which suggests that the two lineages might have originated on

16 different precursor islands.

17   Central Tenerife populations show the lowest *Fst* values but the highest levels of

18 haplotype and nucleotide diversity, suggesting ongoing gene flow and admixture among

19 populations. The considerable geological activity endured by the region almost

20 uninterruptedly for the last 3 My, including volcanic eruptions and large debris

21 avalanches, most likely shaped local *D. verneaui* populations by causing extinctions,

22 bottleneck episodes and subsequent recolonisations from different sources.

23

24 **Potential cryptic species**

1   Patterns of deep mitochondrial divergence in allopatric populations are common in

2   many organisms (Avise 2000) and are usually interpreted as the result of long isolation

3   periods due to extrinsic barriers (Hayes and Harrison 1992; Zarza et al. 2008). Our

4   results revealed almost complete isolation of the Teno populations of *D. verneaui* from

5   the rest of the island, a pattern also reported in the endemic beetle *Tarphius canariensis*

6   (Emerson et al. 2000; Emerson and Oromí 2005). Volcanism may explain this isolation

7   pattern, as the Teno massif has been surrounded by recurrent lava flows over the past

8   3.5 My, following the phases of volcanic activity that gave rise to the Las Cañadas

9   edifice (Ancochea et al. 1999). Reasons other than volcanism may have accounted for

10   the deep genetic divergences observed between Teno and the rest of Tenerife. The

11   characteristic steep cliffs of the Teno massif have been singled out as the underlying

12   cause for the limited spatial expansion of the Teno clades of the gecko *T. delalandii*

13   (Gübitz et al. 2000) and the lizard *G. galloti* (Thorpe et al. 1996). Similarly, it has been

14   demonstrated that the rough topography of La Gomera island, which is similar to that of

15   Teno, has restricted gene flow in the ground-beetle *Paraeutrichopus harpaloides* (Moya

16   et al. 2007).

17   Mitochondrial and nuclear data support conflicting phylogenetic positions of the

18   Teno lineage: mitochondrial data suggest that *D. verneaui* is paraphyletic, with *D.*

19   *gomerensis* being the sister-group of the Anaga-Centre lineage, while *ITS-2* supports a

20   sister group relationship of the two main *D. verneaui* lineages. Incongruence between

21   mitochondrial and nuclear genes is commonly found when examining relationships at

22   the population/species interface due to the different effective population sizes (*Ne*),

23   recombination and substitutions rates of the two types of markers. Unlinked genes may

24   support distinct, yet correct topologies that may in turn differ from species trees (Brito

1   and Edwards 2009). In the case of *D. verneaui*, the peculiarities of the *ITS-2* marker

2   used here might underlie the observed incongruence between the investigated

3   mitochondrial and nuclear genes. The closest similarity of the two *D. verneaui* lineages

4   inferred from the *ITS-2* might be the result of the homogenisation of *ITS-2* copies

5   exchanged by the two lineages following sporadic occurrences of gene flow, as

6   suggested by the detection of at least one instance of mtDNA introgression from Anaga-

7   Centre (clade B) into Teno. In contrast, limitations to gene flow between islands would

8   have preserved the distinctiveness of the *D. gomerensis ITS-2* sequence copies.

9   Deep mitochondrial divergences and sorted nuclear differentiation suggest that

10 Teno populations of *D. verneaui* represent an independent evolutionary lineage. The

11 levels of genetic divergence in the mtDNA and *ITS-2* sequences observed between the

12 two lineages of *D. verneaui* are higher, for instance, than those reported for closely

13 related *Dysdera* species on the eastern Canary Islands (Macías-Hernández et al. 2010).

14 Although individuals from the Teno and Anaga-Centre lineages are undistinguishable in

15 their somatic morphology, they appear to differ in small genitalic features. Arnedo *et al.*

16 (1999, see Fig. 167) reported the existence of a well-developed additional lateral fold in

17 the male bulb of some *D. verneaui* individuals, which we have recognized as exclusive

18 to male specimens from Teno. Mating experiments may allow clarification of the role of

19 this feature as a prezygotic barrier, although our data have already shown that this

20 difference did not prevent cross-population mating of Teno males and females from the

21 centre of the island. The observed patterns of congruence between mitochondrial and

22 nuclear genetic divergence, along with allopatric geographic ranges and male genitalic

23 diagnostic characters support the species status of the Teno populations of *D. verneaui*.

24

1   **Concluding remarks**

2   Our findings confirm former suggestions that geological barriers, but not habitat

3   types, have played an important role in generating within-island population structure,

4   and ultimately speciation, in Canarian woodlouse-hunter spiders. Our results also

5   suggest that many cryptic *Dysdera* species, with restricted ranges and subtle

6   morphological diagnostic characters, may await further discovery.

7

22

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7
8

9   **TABLE AND FIGURE LEGENDS**

10   Table 1.- Diversity measures of the *cox1* and *ITS-2* genes for the localities of *Dysdera*

11   *verneaui* sampled in this study. (N) sample size, (*H*) number of *cox1* haplotypes or *ITS-*

12   *2* sequences types, (π) nucleotide diversity, (*h*) haplotype diversity.

13

14   Table 2.- Pairwise F_{ST} values among locations for *cox1* based on the pairwise difference

15   method. Significant comparisons at the $P < 0.05$ are indicated in bold. Populations

16   represented by one sequenced individual were excluded (localities 4 and 9). Habitat

17   type: (T) Thermo-sclerophyllous woodland, (L) Laurel forest, (S) Dry subalpine scrub,

18   (P) Pine forest, (X) Xerophytic shrubs.

19

20   Table 3.- Preferred SAMOVA population grouping for *cox1* (*K*= 7, in columns) and

21   *ITS-2* (*K*= 5, in rows). Groups roughly ordered following an E to W distribution.

22   Locality numbers as in Fig. 1 and Table 1. Locality numbers between brackets were not

23   sampled for *ITS-2*. Habitat type follows locality number. Thermo: Thermo-

24   sclerophyllous woodland, laurel: Laurel forest, subalpine: Dry subalpine scrub, pine:

25   Pine forest, xerophytic: Xerophytic shrubs.

1   Fig. 1. Map of Tenerife, with numbers indicating the localities where specimens of *D.*

2   *verneaui* were collected. The colours of the circles refer to the three main ancient

3   protoislands, and the colours of localities refer to the vegetation type. The main

4   geological events are indicated.

5

6   Fig. 2.- Maximum likelihood tree topology of the combined mitochondrial and nuclear

7   genes (*cox1, 16S, nad*1 and *28S*) plus the 57 *cox1* haplotypes of *D. verneaui.* Bars on

8   branches denote support for each clade with Parsimony, Maximum likelihood and

9   Bayesian inference, respectively (Black: MP jackknife and ML bootstraps>70% and

10 PP>0.95; white: MP jackknife and ML bootstraps <70%, PP <0.95%; X: clade not

11 recovered). Circles refer to localities according to geographical haplotype distribution in

12 Tenerife (see Fig.1). The *cox1* haplotype networks obtained with statistical parsimony

13 are indicated.

14

15 Fig. 3.- Statistical parsimony network of the *ITS-2* sequence types of *D. verneaui* and *D.*

16 *gomerensis*. Circles refer to localities according to the geographical haplotype

17 distribution in Tenerife (see Fig. 1).

18

19 Fig. 4.- Chronogram obtained using the *multi-demographic coalescent model* of the

20 *cox1* haplotype matrix. Numbers on nodes are estimated lineage ages, and bars indicate

21 95% HPD intervals. The TMRCA of the main lineages are indicated into boxes. Open

22 circles and filled circles correspond to maximum and fixed calibration points,

23 respectively (see text for detail). The *x*-axis scale is in million years. (*) Beginning of

24 volcanic activity that joined the three ancient protoislands.

1　Fig. 5.- Chronogram of the species tree obtained with the *multispecies coalescent model*

2　method. The *x*-axis scale is in million years. Bars indicate 95% HPD intervals.

3

4　**SUPPLEMENTARY MATERIAL**

5　Additional Supplementary Material may be found in the online version of this article:

6　**Appendix S1.** Summary of sequences and sampling locations of the *Dysdera* specimens

7　analysed in the study.

1　**TABLES**

2　Table 1

Dysdera verneaui			cox1				ITS-2			
Locality	Habitat types	Latitude/longitude	N	H	π	h	N (Exx.)	H	π	h
1　El Aderno. Buenavista	Thermo-sclerophyllous	28.3582 -16.8644	5	2	0.004762 +/- 0.003267	0.4000 +/- 0.2373	4 (2)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
2　Monte del Agua. Teno	Laurel forest	28.3238 -16.8172	6	5	0.052579 +/- 0.030755	0.9333 +/- 0.1217	10 (5)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
3　Las Lajas. Vilaflor	Pinus forest	28.1903 -16.6691	2	2	0.005964 +/- 0.006442	1.0000 +/- 0.5000	4 (2)	3	0.00123 +/- 0.00152	0.8333 +/- 0.2224
4　Roque del Conde	Xerophytic scrubs	28.0931 -16.6988	1	1	NC	NC			NC	NC
5　Madre del Agua. Vilaflor	Pinus forest	28.1694 -16.6306	5	2	0.000397 +/- 0.000505	0.4000 +/- 0.2373	4 (2)	2	0.00164 +/- 0.00184	0.6667 +/- 0.2041
6　La Fortaleza. Las Cañadas	Subalpine shrubs	28.3167 -16.5912	5	4	0.054762 +/- 0.033567	0.9000 +/- 0.1610	4 (2)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
7　Mña. Chusqueros. Siete Cañadas	Subalpine shrubs	28.2922 -16.5590	3	3	0.029431 +/- 0.022376	1.0000 +/- 0.2722	4 (2)	2	0.00234 +/- 0.00232	0.5000 +/- 0.2652
8　Cumbres de Arico	Pinus forest	28.2492 -16.5287	5	5	0.037897 +/- 0.023354	1.0000 +/- 0.1265	4 (2)	3	0.00236 +/- 0.00233	0.8333 +/- 0.2224
9　Cumbres de Arico. Contador	Pinus forest	28.1977 -16.5312	1	1	NC	NC			NC	NC
10　El Guanche. La Orotava	Pinus forest	28.3472 -16.5140	3	2	0.006614 +/- 0.005355	0.6667 +/- 0.3143			NC	NC
11　Orticosa	Pinus forest	28.3845 -16.4474	5	5	0.025595 +/- 0.015903	1.0000 +/- 0.1265	4 (2)	2	0.00663 +/- 0.00528	0.6667 +/- 0.2041
12　Caldera de Pedro Gil	Pinus forest	28.3484 -16.4717	3	3	0.041997 +/- 0.031744	1.0000 +/- 0.2722	2 (1)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
13　Bco. del Agua. Güímar	Dry-Laurel forest	28.3078 -16.4481	6	5	0.054497 +/- 0.031862	0.9333 +/- 0.1217	4 (2)	2	0.00157 +/- 0.00176	0.6667 +/- 0.2041
14　Torre del Gaitero	Pinus forest	28.3947 -16.4319	3	2	0.032407 +/- 0.024595	0.6667 +/- 0.3143			NC	NC
15　Las Lagunetas	Pinus forest	28.4185 -16.4100	4	3	0.038525 +/- 0.025561	0.8333 +/- 0.2224	2 (1)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
16　Las Raíces	Pinus forest	28.4297 -16.3808	5	3	0.012103 +/- 0.007728	0.8000 +/- 0.1640	6 (3)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
17　Las Hiedras-Carboneras	Laurel forest	28.5400 -16.2737	3	1	0.000000 +/- 0.000001	0.0000 +/- 0.0000	2 (1)	2	0.00707 +/- 0.00817	1.0000 +/- 0.5000
18　Batán-Cruz del Carmen	Laurel forest	28.5353 -16.2968	3	2	0.001323 +/- 0.001359	0.6667 +/- 0.3143			NC	NC
19　Cruz del Carmen	Laurel forest	28.5319 -16.2799	6	1	0.000000 +/- 0.000001	0.0000 +/- 0.0000	6 (3)	2	0.00141 +/- 0.00149	0.6000 +/- 0.1291
20　Monte de las Mesas	Thermo-sclerophyllous	28.4813 -16.2636	2	2	0.008929 +/- 0.009412	1.0000 +/- 0.5000			NC	NC
21　Camino a Ichires	Laurel forest	28.5400 -16.2319	4	1	0.000000 +/- 0.000000	0.0000 +/- 0.0000			NC	NC
22　Ensillada-Chamorga	Laurel forest	28.5562 -16.1798	5	5	0.005357 +/- 0.003630	1.0000 +/- 0.1265	4 (2)	1	0.00000 +/- 0.00000	0.0000 +/- 0.0000
Total			85	57	0.075024 +/- 0.036146	0.9815 +/- 0.0068	32	9	0.00769 +/-0.00448	0.7970 +/- 0.0257

1 Table 2

2

	1 (T)	2 (L)	3 (P)	5 (P)	6 (D)	7 (D)	8 (P)	10 (P)	11 (P)	12 (P)	13 (L)	14 (P)	15 (P)	16 (P)	17 (L)	18 (L)	19 (L)	20 (T)	21 (L)	22 (L)
1 (T)	0.0000																			
2 (L)	0.0446	0.0000																		
3 (P)	**0.9613**	**0.6221**	0.0000																	
5 (P)	**0.9817**	**0.7769**	0.9734	0.0000																
6 (D)	**0.7722**	**0.5564**	0.3310	**0.6017**	0.0000															
7 (D)	**0.9002**	**0.6117**	0.1671	**0.8162**	0.2355	0.0000														
8 (P)	**0.8408**	**0.6302**	**0.4739**	**0.4585**	0.2903	**0.4275**	0.0000													
10 (P)	**0.9581**	**0.6778**	0.8773	**0.9540**	0.1312	0.5557	**0.5766**	0.0000												
11 (P)	**0.8858**	**0.6690**	**0.5870**	**0.5355**	0.3421	**0.4402**	-0.0076	**0.5775**	0.0000											
12 (P)	**0.8680**	**0.5966**	0.4227	**0.5635**	0.2587	0.2987	-0.0105	0.5406	-0.0949	0.0000										
13 (L)	**0.7588**	**0.5655**	0.3507	**0.4822**	0.1042	**0.3470**	0.0415	**0.4738**	0.2222	0.1183	0.0000									
14 (P)	**0.8911**	**0.6149**	0.5274	**0.7220**	0.0476	0.2560	**0.2742**	0.1238	0.1588	0.1390	0.2713	0.0000								
15 (P)	**0.8546**	**0.6133**	0.4311	**0.7058**	0.1598	0.2120	**0.3726**	0.2923	**0.3419**	0.1925	**0.3360**	0.0403	0.0000							
16 (P)	**0.9328**	**0.7130**	**0.8634**	**0.9303**	0.4529	**0.7939**	**0.6537**	**0.8900**	**0.7843**	**0.7409**	0.3317	**0.7825**	**0.7347**	0.0000						
17 (L)	**0.9762**	**0.7072**	0.9638	**0.9961**	0.5034	0.7201	**0.6453**	0.9405	**0.7194**	0.5767	**0.5275**	0.7066	**0.5190**	**0.9119**	0.0000					
18 (L)	**0.9725**	**0.6960**	0.9407	**0.9892**	0.4993	0.6844	**0.6344**	0.9241	**0.7012**	0.4480	**0.5191**	0.6806	**0.4709**	**0.9089**	0.9762	0.0000				
19 (L)	**0.9838**	**0.7779**	**0.9792**	**0.9973**	**0.6358**	**0.8136**	**0.7435**	**0.9630**	**0.7947**	0.6473	**0.6386**	**0.8100**	**0.6356**	**0.9415**	**1.0000**	0.2500	0.0000			
20 (T)	**0.9560**	**0.6426**	0.9032	**0.9766**	0.2846	0.7450	0.5798	0.9182	0.7543	0.6570	0.1761	0.7241	0.6633	0.2019	0.9663	0.9568	**0.9832**	0.0000		
21 (L)	**0.9778**	**0.7306**	**0.9812**	**0.9973**	0.5617	**0.8618**	**0.7144**	**0.9706**	**0.8308**	**0.8005**	0.4821	**0.8499**	**0.7831**	0.8254	**1.0000**	**0.9942**	**1.0000**	0.9386	0.0000	
22 (L)	**0.9509**	**0.7362**	**0.9322**	**0.9664**	**0.5689**	**0.8425**	**0.7136**	**0.9362**	**0.8212**	**0.7914**	0.4954	**0.8332**	**0.7767**	**0.7830**	**0.9620**	**0.9567**	**0.9741**	**0.8652**	-0.0179	0.0000

1   Table 3

2

| *ITS-2*	cox1						
	Teno	Central ridge	S Centre and Dorsal ridge	N Centre and Dorsal ridge	E Centre	W Anaga	E Anaga
Teno	1, thermo 2, laurel						
S Centre and Dorsal ridge		3, pine 7, subalpine	(4), xerophytic 5, pine				
Dorsal ridge 1			(9), pine 12, pine	(10), pine (14), pine 15, pine	16, pine (20), thermo		
Dorsal ridge 2			11, pine				
Centre and Anaga			8, pine 13, laurel	6, subalpine		17, laurel (18), laurel 19, laurel	21, laurel (22), laurel

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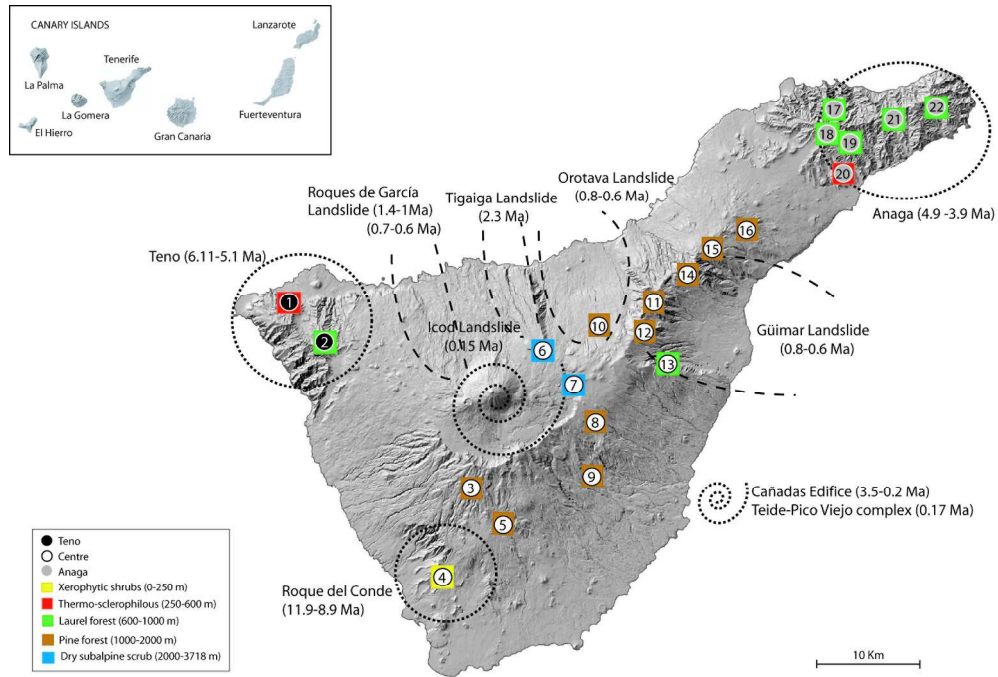
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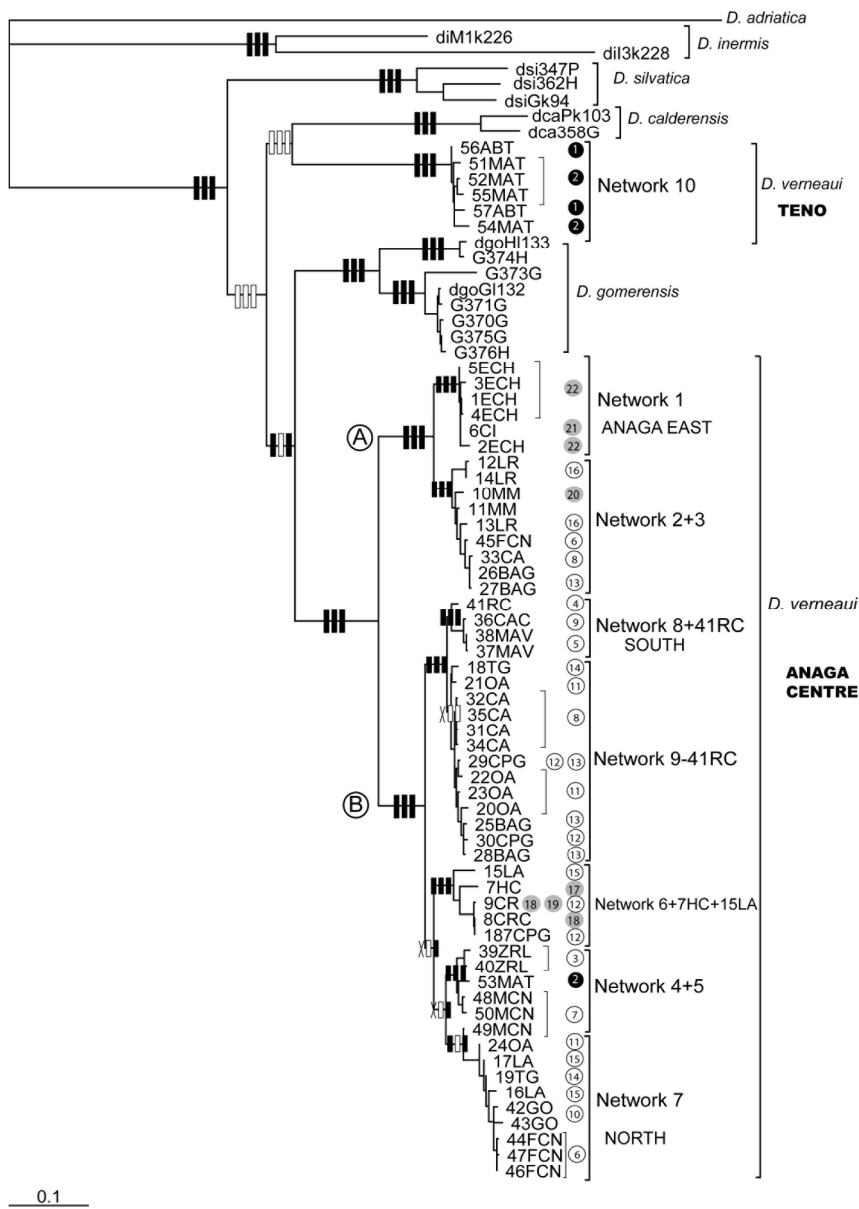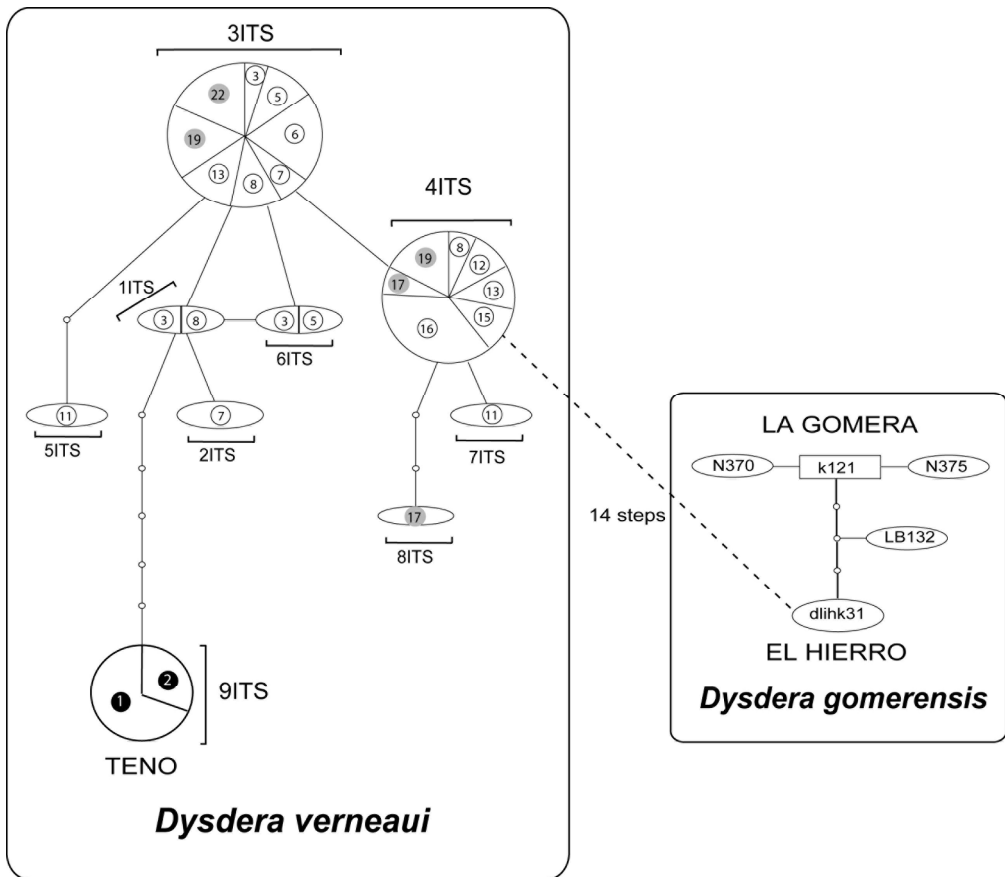41
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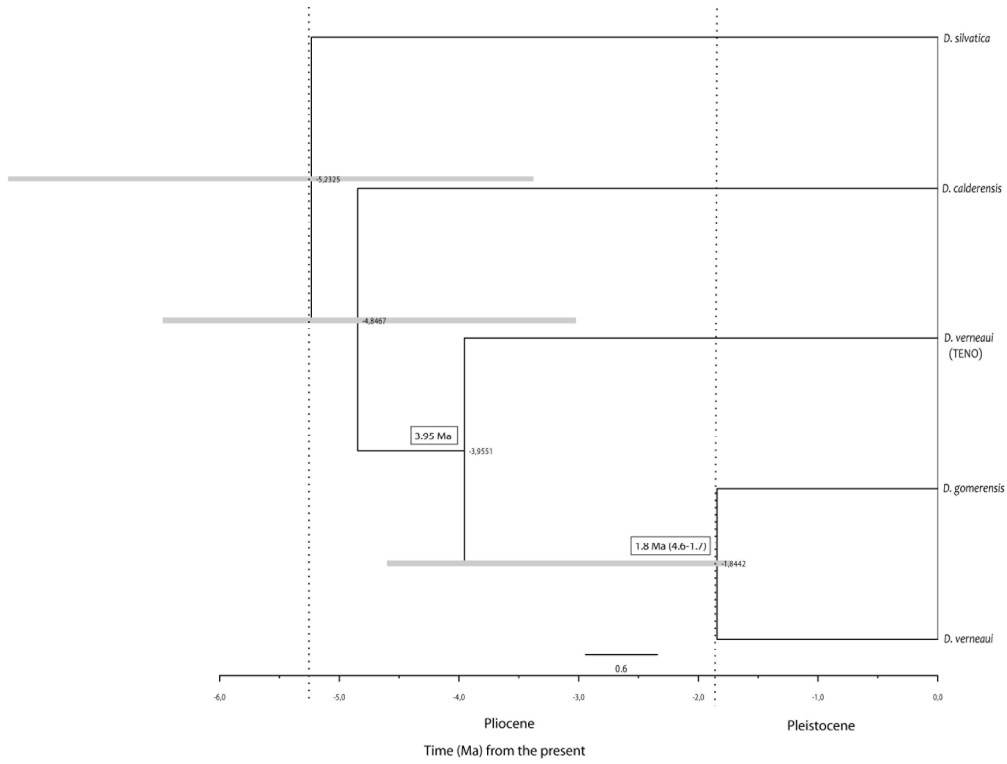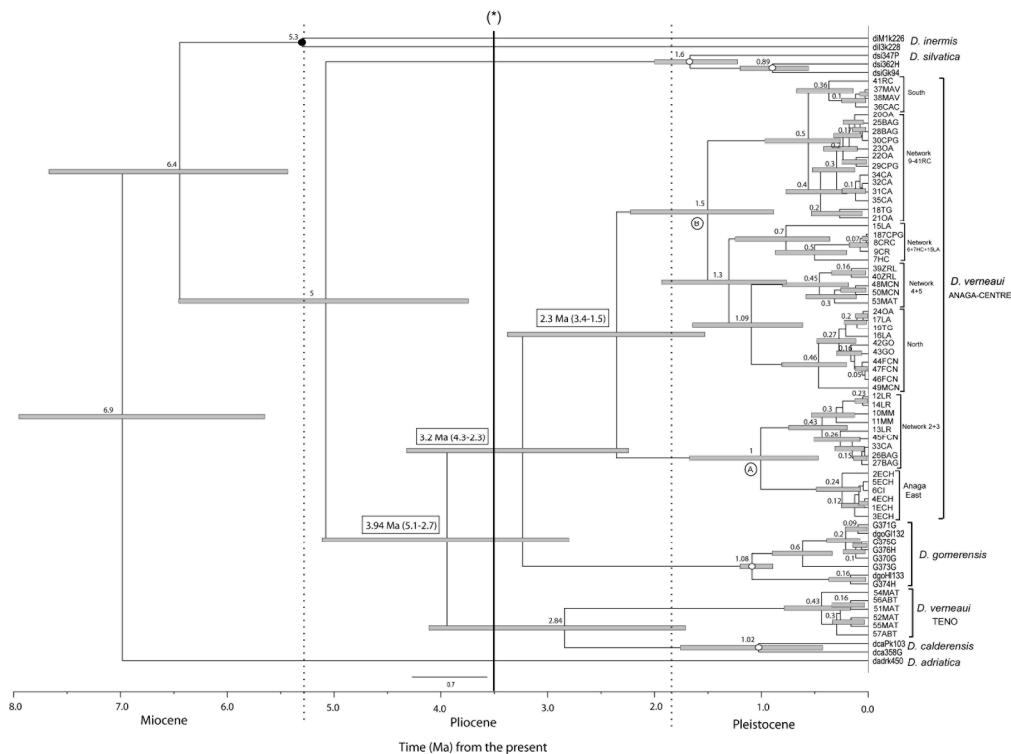45
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Macías-Hernández_SupMat_tableS1. Phylogeography of *Dysdera verneaui* in Tenerife. *Journal of Heredity*.

Appendix S1. Summary of sequences and sampling locations of the *Dysdera* specimens analysed. Code: number of locality used in Fig. 1.; *N*: number of individuals sampled per locality; list of *cox1* haplotypes and *ITS-2* sequences types collected in each locality, with the number of individuals showing the same haplotype in brackets; *cox1, ITS-2, 16S, rrnL/nad1* and *28S* column entries are GenBank accession numbers.

Locality	Code	N	Haplotypes *cox1*	*cox1*	Sequences types. *ITS-2*	*ITS-2*	*16S-L1-nad1*	*28S*
					GeneBank accesion number			
Dysdera verneaui								
El Aderno. Buenavista	1	5	56ABT (4)		9ITS (2)			
			57ABT (1)					
Monte del Agua. Teno	2	6	51MAT (2)		9ITS (5)			
			52MAT (1)					
			53MAT (1)					
			54MAT (1)					
			55MAT (1)					
Las Lajas.Vilaflor	3	2	39ZRL (1)		1ITS (1)			
			40ZRL (1)		3ITS (1)			
					6ITS (1)			
Roque del Conde	4	1	41RC (1)					
Madre del Agua. Vilaflor	5	5	37MAV (4)		3ITS (1)			
			38MAV (1)		6ITS (1)			
La Fortaleza. Las Cañadas	6	5	44FCN (1)		3ITS (2)			
			45FCN (2)					
			46FCN (1)					
			47FCN (1)					
Mña. Chusqueros. 7 Cañadas	7	3	48MCN (1)		2ITS (2)			
			49MCN (1)		3ITS (1)			

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Locality	Code	N	Haplotypes cox1	cox1	Sequences types. ITS-2	ITS-2	16S-L1-nad1	28S
					GeneBank accesion number			
Cumbres de Arico	8	5	50MCN (1)					
			31CA (1)		1ITS (1)			
			32CA (1)		3ITS (1)			
			33CA (1)		4ITS (1)			
			34CA (1)					
			35CA (1)					
Cumbres de Arico. Contador	9	1	36CAC (1)					
El Guanche. La Orotava	10	3	42GO (2)					
			43GO (1)					
Orticosa	11	5	20OA (1)		5ITS (1)			
			21OA (1)		7ITS (1)			
			22OA (1)					
			23OA (1)					
			24OA (1)					
Caldera de Pedro Gil	12	3	8CR (1)		4ITS (1)			
			29CPG (1)					
			30CPG (1)					
Bco. del Agua. Güímar	13	6	25BAG (1)		3ITS (1)			
			26BAG (2)		4ITS (1)			
			27BAG (1)					
			28BAG (1)					
			29CPG (1)					
Torre del Gaitero	14	3	18TG (1)					
			19TG (2)					
Las Lagunetas	15	4	15LA (2)		4ITS (1)			
			16LA (1)					
			17LA (1)					
Las Raíces	16	5	12LR (1)		4ITS (3)			
			13LR (2)					
			14LR (2)					

Locality	Code	N	Haplotypes *cox1*	*cox1*	Sequences types. *ITS-2*	*ITS-2*	*16S-L1-nad1*	*28S*
					GeneBank accesion number			
Las Hiedras-Carboneras	17	3	7HC (3)		4ITS (1)			
					8ITS (1)			
Batán-Cruz del Carmen	18	3	8CR (2)					
			9CR (1)					
Cruz Carmen	19	6	8CR (6)		3ITS (2)			
					4ITS (2)			
Monte de las Mesas	20	2	10MM (1)					
			11MM (1)					
Camino a Ichires	21	4	6CI (4)					
Ensillada-Chamorga	22	5	1ECH (1)		3ITS (2)			
			2ECH (1)					
			3ECH (1)					
			4ECH (1)					
			5ECH (1)					
TOTAL		**85**						
Dysdera gomerensis								
Puntallana. La Gomera		1	G370G (1)		10ITS(1)			
Noruegos. La Gomera		1	G371G (1)					
Mña. Las Pilas. La Mérica. La Gomera		1	G373G (1)					
Enchereda. La Gomera		1	G375G (1)		11ITS (1)			
Cañada de Jorge. La Gomera		1	G132G (1)	>dgoGl132	12ITS (1)			
					14ITS (1)			
Pista Garoé. El Hierro		1	G374H (1)					
Pista Mercader. El Hierro		1	G376H (1)					
Casa Forestal de Frontera. El Hierro		1	G133H (1)	>dgoHl133	13ITS (1)			
Dysdera calderensis								
Juan Adalid, Garafía. La Palma			>dcaPk103	AF244309			AF244218/EU139665	EU139788
Riscos de Alojera. La Gomera			>dca358G					
Dysdera silvatica								

						GeneBank accesion number			
Locality	**Code**	**N**	**Haplotypes** *cox1*	*cox1*	**Sequences types.** *ITS-2*	*ITS-2*	*16S-L1-nad1*	*28S*	
Barranco de Juel, Hermigua. La Gomera			>dsiGk94	AF244273		EU143842	AF244177/EU139674	EU139808	
Pinar Roque Faro. La Palma			>dsi347P						
Mirador de Bascos. El Hierro			>dsi362H						
Dysdera inermis									
Tangier-Tétouan. Morocco			>diM1k226	EF458142		NO	EF458092	EU139795	
Andalucía, Cadiz, Tarifa. Iberian Peninsula			>diI3k228			NO			
Dysdera adriatica									
Kozina. Slovenia			>dadrk450			NO			

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