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Research Article

Integrative taxonomy uncovers hidden species diversity in woodlouse hunter spiders (Araneae, Dysderidae) endemic to the Macaronesian archipelagos

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The development of molecular techniques as a taxonomic tool and their integration with information provided by other disciplines, has enhanced species discovery, facilitated species delimitation and produced invaluable data for inferring species phylogenies. Here, we provide an example of how DNA sequence data, together with morphometric, distributional and ecological information, assist in identifying and diagnosing previously overlooked lineages. The nocturnal, ground-dwelling spider genus *Dysdera* has colonized all the Macaronesian archipelagos, and has undergone a major diversification in the Canary Islands. A recent molecular phylogenetic analysis of *Dysdera* species from the eastern Canary Islands revealed deep genetic divergences among some populations, suggesting the existence of cryptic taxa. Here, we combine data from mitochondrial and nuclear loci with morphological and ecological evidence to delimit and formally describe three previously overlooked species: *D. aneris* sp. nov., endemic to the Salvage Islands; *D. mahan* sp. nov., distributed along coastal habitats of Lanzarote, north of Fuerteventura and adjacent islets; and *D. simbeque* sp. nov., restricted to two valleys in northern Lanzarote. Molecular markers provide key information that allows apparent morphological polymorphisms to be used as diagnostic features of evolutionarily independent lineages. *Dysdera mahan* sp. nov. is unique among the Canarian *Dysdera* in that it is found in the intertidal zone on pebbled beaches. Low levels of genetic variability and genital differentiation associated with relatively high somatic divergence suggest that speciation in *D. mahan* sp. nov. was driven by a selection of phenotypic traits that are adaptive to this rare environment. Separate analyses and statistical tests revealed phylogenetic incongruence between mitochondrial and nuclear genes, probably as a result of incomplete lineage sorting. The temporal framework for the origin and diversification of the new species inferred from the molecular data corroborates former hypotheses on the late Pliocene origin of the present-day biota of the Salvage Islands.

Key words: Canary Islands, cryptic species, data set incongruence, Fuerteventura, incomplete lineage sorting, Lanzarote, molecular phylogeny, Salvage Islands

Introduction

Species are the basic units of taxonomy and the subject of evolution (Wiley, 1981; Ereshefsky, 1992). However, few ideas in biology have revealed themselves as more elusive to define or have sparked hotter debates than the species concept (Ereshefsky, 1992). The plethora of species definitions available in the literature (Harrison, 1998) has been grouped into those that consider species as reproductive communities and those referring to species as evolutionary lineages (Templeton, 1994). The reproductive and evolutionary perspectives on species have been reconciled by considering the lineage-based evolutionary species concept as a general theoretical definition of species and treating other concepts as operational tools for species recognition and delimitation (Mayden, 1997; de Queiroz, 1998). Some authors, however, go further and suggest that species should not be defined on the basis of specific necessary properties, as implied by most definitions, but that such properties should instead be used as a line of evidence to infer species limits (Sites & Marshall, 2003, 2004). Consequently, the focus on species concepts has recently shifted towards the development of methods to detect species boundaries (Wiens & Penkrot, 2002 and references therein; Morando *et al.*, 2003; Sites & Marshall, 2003, 2004).

It has been suggested that traditional species delimitation based on gross morphological features underestimates and simplifies biodiversity (Mayden, 1997; Bickford *et al.*,

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2007). The development and popularization of molecular techniques have favoured the use of DNA sequence data to test traditional morphology-based taxonomies (Vogler & Monaghan, 2007 and references therein). The inclusion of molecular data in taxonomy has aided species delimitation and diagnosis (Sites & Marshall, 2004; DeSalle *et al.*, 2005), enhanced the discovery of cryptic species (Bickford *et al.*, 2007), and extended species identification beyond complete adult specimens (Hebert *et al.*, 2003). However, the definition of species boundaries requires an integrative method that includes multiple lines of evidence, such as those provided by classical morphology-based taxonomy in addition to molecular, ecological, behavioural and geographical information (Stockman & Bond, 2007; Bond & Stockman, 2008).

The Macaronesian biogeographic region is included within the Mediterranean biodiversity hotspot and is one of the most important areas worldwide for conservation (Myers *et al.*, 2000). Arthropods are among the most diverse and highly endemic organisms in Macaronesian terrestrial ecosystems. Despite a 150-year-old tradition of carrying out taxonomy and biotic surveys, new species of arthropods continue to be found in Macaronesia at a rate of approximately 25 to 200 new taxa per decade (Izquierdo *et al.*, 2004; Borges *et al.*, 2005, 2008). Although increases in human resources and funding for bio-inventories have enhanced species discovery within the region, the use of DNA-based techniques has also contributed to increase the number of species identified. In the Canaries, genetic distinctiveness has been used to describe a new endemic species of the grasshopper genus *Arminda* (Hochkirch & Gözig, 2009), to detect cryptic species among the *Palmorchestia* landhoppers (Villacorta *et al.*, 2008), and to corroborate species delimitation in morphologically similar species of the *Halophiloscia* coastal woodlice (Taiti & López, 2008), to cite just a few examples.

The nocturnal wandering hunter spider genus *Dysdera* Latreille, 1804 is a conspicuous component of the Mediterranean ground-dwelling arthropod fauna that is generally associated with warm and wet ground habitats. *Dysdera* is one of the largest genera in the Mediterranean basin, with nearly 250 described species (Platnick, 2010), a fifth of which are endemic to the Macaronesian archipelagos. The genus is unevenly distributed across the main archipelagos: the Canary Islands harbour almost 50 endemic species (Arnedo *et al.*, 2007), while Madeira has five species (Wunderlich, 1994) and Cape Verde (Berland, 1936), the Azores (Arnedo, unpubl. data) and the Salvage Islands (Arnedo *et al.*, 2000) have one each. Cladistic analyses of morphology and mitochondrial DNA sequence data (Arnedo *et al.*, 2001) suggested a close relationship of the Cape Verde species with those from the Canaries, and an independent colonization of the Azores. Relationships of the Madeiran taxa remain largely unresolved. Canarian endemics, on the other hand, are more likely the result of a single colonization

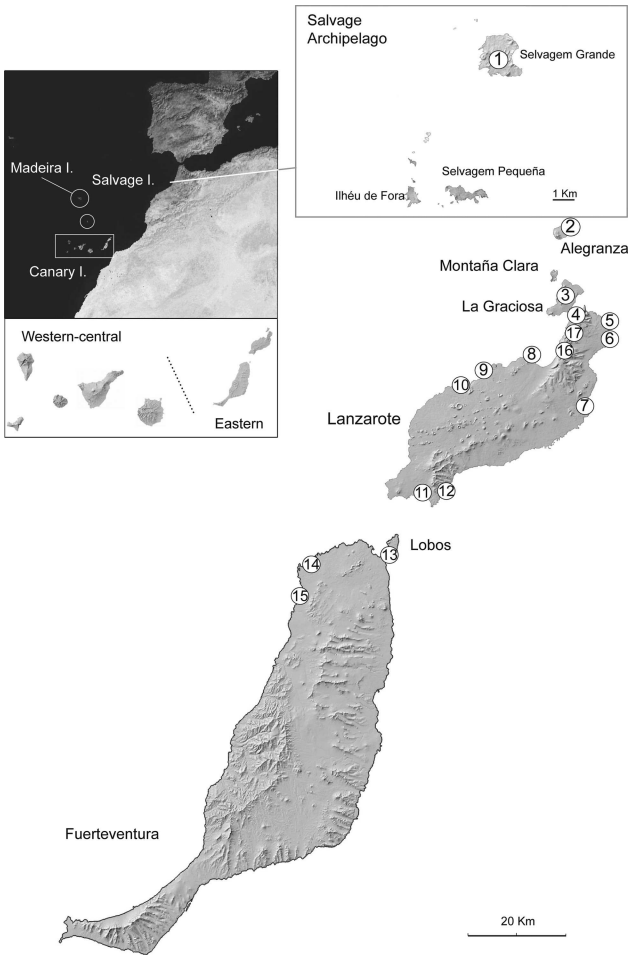


Fig. 1. Map with the sampling localities and the distribution of the three new species described. Code numbers correspond to those in the locality list in Appendix 1 (see supplementary material which is available on the Supplementary tab of the article's Informaworld page at http://www.informaworld.com/mpp/uploads/tsab. . .).

event, with the only exception being *D. lancerotensis* Simon, 1907, which colonized the eastern Canaries independently (Bidegaray-Batista *et al.*, 2007; Macías-Hernández *et al.*, 2008).

The eastern Canary Islands (Fig. 1), including Fuerteventura, Lobos, Lanzarote and the Chinijo islets are home to five endemic *Dysdera* species in addition to the aforementioned *D. lancerotensis*, one of which is also found in the Salvage Islands. A recent study of the phylogeny and evolution of these endemic species (Macías-Hernández *et al.*, 2008) based on DNA sequence data revealed the existence of deeply divergent lineages that had gone unnoticed in previous taxonomic revisions (Arnedo *et al.*, 2000). The eastern Canaries are the oldest islands of the archipelago (Fuerteventura is 22 million years old; Coello *et al.*, 1992) and the closest to the mainland (northwest Africa). These two factors have shaped their terrestrial ecosystems which, compared with the remaining Canary Islands, are

xerophilic, due to long-term erosion resulting in low altitudes (which prevents them from capturing the humid trade winds from the northeast) and to the arid, dusty winds blowing in from the Sahara. The eastern Canaries are an exposed part of a continuous volcanic ridge, and the islands have been connected by land bridges in the past as a result of eustatic sea-level changes. The Salvage archipelago includes two small islands (Selvagem Grande and Selvagem Pequena) and one islet (Ilhéu de Fora) that are located 165 km north of the Canary Islands and 300 km south of Madeira island (Fig. 1). The subaerial phase of the archipelago dates back to 21 million years ago (Mya) and was followed by two post-erosional periods (12–8 and 3.4 Mya) separated by gaps in volcanic activity. The archipelago was beneath sea level during volcanic quiescence periods due to erosion and eustatic sea level changes (Geldmacher *et al.*, 2001). The present-day terrestrial ecosystems probably originated after the last post-erosional volcanic episode.

In the present study, we adopted a unified species concept, i.e. species are independently evolving metapopulations (de Queiroz, 2007), and we used multiple lines of evidence (morphology, DNA sequence data, ecology, distribution) to find and delimit previously overlooked species. We elaborated on the phylogenetic results of Macías-Hernández *et al.* (2008) to re-examine morphological evidence and formally describe three new endemic species. We present new molecular and morphological data of the new species from the Salvage archipelago to delimitate and further investigate its origins.

Materials and methods

Taxonomy

Specimens were examined with Leica MZ95 and Leica MZ16A dissection microscopes, the latter was equipped with a Nikon DXM1200 digital camera. Digital microscope images were edited using the Auto-Montage software package. Digital illustrations were generated following the guidelines provided in Coleman (2003) with the assistance of a WACOM digitizer board and Adobe Illustrator 10 and Photoshop 8.0.1 software. Male palps were detached, cleaned by ultrasound, critical-point dried and then coated for examination using a HITACHI S2300 scanning electron microscope at the Serveis Científico-Tècnics of the Universitat de Barcelona. Female vulvas were removed with the aid of needles, and the muscle tissue was digested with a 35% KOH solution before observation. The final plate layout and editing were conducted with Adobe Illustrator CS3. Measurements were taken using an ocular measuring graticule mounted on the dissection microscope. All characters were recorded in DELTA (DEscription Language for TAxonomy) format (Dallwitz, 1980) using the DELTA editor (Dallwitz *et al.*, 1999). Taxonomic procedures and descriptions follow Arnedo & Ribera (1999) and Arnedo *et al.* (2000).

Table 1. Abbreviations used in text and figures.

Collections	
BMNH	The Natural History Museum, London, UK
CRBA	Centre de Recursos de Biodiversitat Animal, Universitat de Barcelona, Barcelona, Spain
GBIF	Global Biodiversity Information Facility
MHNP	Muséum National d'Histoire Naturelle, Paris, France
OXUM	Hope Entomological Collections, University Museum, Oxford, UK
ULL	Departamento de Biología Animal, Universidad de La Laguna, Tenerife, Canary Islands, Spain
Eyes	
AME	anterior medial eyes
PLE	posterior lateral eyes
PME	posterior medial eyes
Cheliceral teeth	
B	basal tooth
D	distal tooth
M	medial tooth
Male copulatory bulb	
AC	additional crest
AL	additional lateral sheet at the internal border
AR	arch-like ridge
C	Crest
DD	distal division
DH	distal haematodoca
ES	external sclerite
F	Flagellum
IS	internal sclerite
L	lateral sheet
LA	lateral sheet anterior apophysis
LF	lateral fold over lateral sheet between internal and external sclerites
P	posterior apophysis
T	Tegulum
Female genitalia	
DA	dorsal arch
DF	dorsal arch fold
MF	major fold
S	spermatheca
TB	transversal bar
VA	ventral arch
VS	ventral sclerotization

The terminology used to describe the male palp and female vulva structures is based on Deeleman-Reinhold & Deeleman (1988) and Arnedo *et al*. (2000), and was illustrated in Arnedo & Ribera (1999) and Arnedo *et al.* (2000). Leg spination was recorded using the codification method fully described in Arnedo *et al*. (1999). The abbreviations used in text and figures are listed in Table 1.

Phylogenetics

Molecular analyses were based on the data matrix of Macías-Hernández *et al.* (2008) for the eastern Canarian

Table 2. Taxa examined and GenBank accession numbers (asterisks indicate that only the *16S* fragment could be amplified).

Voucher #	DNA #	Species	Locality	*cox1*	*16S-L1-nad1*	*28S*	*H3*	*ITS-2*
Eastern Canaries								
NMH429	N47	*D.alegranzaensis*	Montaña de Lobos, Alegranza	EF458132	EF458087	EU139759	EU139688	EU143814
NMH364	N49	*D. alegranzaensis*	Montaña Clara	EU139610	EU139637	–	EU139689	EU143815
NMH424	N55	*D. alegranzaensis*	Valle Fenauco, Yaiza, Lanzarote	EU139611	EU139638	EU139760	EU139690	EU143816
NMH449	N75	*D. alegranzaensis*	Montaña de las Agujas, La Graciosa	EU139612	EU139639	EU139761	EU139691	EU143817
NMH73	N77	*D. alegranzaensis*	Mirador del Río, Haría, Lanzarote	EU139609	EU139640	EU139762	EU139692	EU143818
NMH462	N79	*D. alegranzaensis*	Montaña de Tinache, Tinajo, Lanzarote	EU139613	EU139641	EU139763	EU139693	EU143819
NMH59	N37	*D. simbeque* sp. n	Bco. Elvira Sánchez, Valle de Malpaso, Haría, Lanzarote	EU139614	EU139659	EU139783	EU139712	EU143838
NMH60	N39	*D. simbeque* sp. n	Bco. Elvira Sánchez, Valle de Malpaso, Haría, Lanzarote	EU139631	EU139660	EU139784	EU139713	EU143839
NMH55	N40	*D. simbeque* sp. n	Bco. Elvira Sánchez, Valle de Malpaso, Haría, Lanzarote	EU139632	EU139661	EU139785	EU139714	–
NMH163	N94	*D. lancerotensis*	Morro del Cavadero, Jandía, Fuerteventura	EF458120	EF458086	EU139758	EU139687	EU143813
NMH441	LB2	*D. lancerotensis*	Caldera, Alegranza	EF458127	EF458080	EU139757	EU139686	EU143812
NMH168	N91	*D. longa*	Morro del Cavadero, Jandía, Fuerteventura	EF458134	EF458090	EU139781	EU139710	EU143836
NMH169	N92	*D. longa*	Morro del Cavadero, Jandía, Fuerteventura		EU139658	EU139782	EU139711	–
NMH358	N57	*D. mahan* sp. n	Playa del Trillo, Alegranza	EU139620	EU139647	EU139769	EU139700	EU143826
NMH451	N58	*D. mahan* sp. n	Caleta de Arriba, La Graciosa	EU139621	EU139648	EU139770	–	EU143827
NMH356	N59	*D. mahan* sp. n	Playa Catalina Cabrera, Famara, Lanzarote	EU139622	EU139649	EU139771	EU139701	–
NMH447	N65	*D. mahan* sp. n	Playa del Congrio, Papagayo, Lanzarote	EU139623	EU139650	EU139772	EU139702	EU143828
NMH490	N66	*D. mahan* sp. n	Playa de Majanicho, Fuerteventura	EU139624	EU139651	EU139773	EU139703	EU143829
NMH572	N76	*D. mahan* sp. n	Las Salinas, Lobos	EU139625	EU139652	EU139774	EU139704	–
NMH57	N43	*D. nesiotes*	Bco. Elvira Sánchez, Valle de Malpaso, Haría, Lanzarote	EU139615	EU139642	EU139764	EU139695	EU143820
NMH428	N48	*D. nesiotes*	Montaña de Lobos, Alegranza	EU139616	EU139643	EU139765	EU139694	EU143821
NMH369	N50	*D. nesiotes*	Montaña Clara	EF458133	EF458088	EU139766	EU139696	EU143822
NMH425	N56	*D. nesiotes*	Valle Fenauco, Yaiza, Lanzarote	EU139617	EU139644	EU139767	EU139697	EU143823
NMH398	N78	*D. nesiotes*	Mirador del Río, Haría, Lanzarote	EU139618	EU139645	–	EU139698	EU143824
NMH476	N80	*D. nesiotes*	Montaña de Tinache, Tinajo, Lanzarote	EU139619	EU139646	EU139768	EU139699	EU143825
NMH50	N85	*D. sanborodon*	Morro Tabaiba, Vallebrón, Fuerteventura	EF458135	EF458089	EU139775	EU139705	EU143830
NMH506	N86	*D. sanborodon*	Montaña de la Cruz, Betancu-ria,Fuerteventura	EU139626	EU139653	EU139776	EU139706	EU143831
NMH49	N87	*D. spinidorsum*	Morro Tabaiba, Vallebrón, Fuerteventura	EU139627	EU139654	EU139777	EU139707	EU143832

(Continued on next page.)

Table 2. Taxa examined and GenBank accession numbers (asterisks indicate that only the *16S* fragment could be amplified)*(Continued)*.

Voucher #	DNA #	Species	Locality	*cox1*	*16S-L1-nad1*	*28S*	*H3*	*ITS-2*
NMH494	N88	*D. spinidorsum*	Montaña de la Cruz, Betancuria, Fuerteventura	EU139628	EU139655	EU139778	EU139708	EU143833
NMH78	N89	*D. spinidorsum*	Cuchillete Montaña Peños, Fuerteventura	EU139629	EU139656	EU139779		EU143834
NMH114	N90	*D. spinidorsum*	Morro del Peñón, Fuerteventura	EU139630	EU139657	EU139780	EU139709	EU143835
NMH 290	X125	*D. aneris* sp. n	Selvagem Grande, Salvage Islands	EU139634	EU139683*	–	–	–
NMH608	K510	*D. aneris* sp. n	Selvagem Grande, Salvage Islands	HQ396319	HQ396277	HQ396308	HQ396298	HQ396288
NMH609	K511	*D. aneris* sp. n	Selvagem Grande, Salvage Islands	HQ396320	HQ396278	HQ396309	HQ396299	HQ396289
NMH610	K512	*D. aneris* sp. n	Selvagem Grande, Salvage Islands	HQ396321	HQ396279	HQ396310	HQ396300	HQ396290
NMH611	K513	*D. aneris* sp. n	Selvagem Grande, Salvage Islands	HQ396322	HQ396280	HQ396311	HQ396301	HQ396291
NMH612	K514	*D. aneris* sp. n	Selvagem Grande, Salvage Islands	HQ396323	HQ396281	HQ396312	HQ396302	–
NMH613	K515	*D. aneris* sp. n	Selvagem Grande, Salvage Islands	HQ396324	HQ396282	HQ396313	HQ396303	–
Western Canaries								
UB4013	K103	*D. calderensis*	Juan Adalid, Garafía, La Palma	AF244309	AF244218/ EU139665	EU139788	EU139718	HQ396292
NMH1438	N358	*D. calderensis*	Riscos de Alojera, La Gomera	HQ396325	HQ396283	HQ396314	HQ396304	HQ396293
CRBA1393	LB132	*D. gomerensis*	Cañada de Jorge, La Gomera	HQ396326	HQ396284	HQ396315	HQ396305	HQ396294
CRBA1395	LB133	*D. gomerensis*	Casa Forestal de Frontera, El Hierro	HQ396327	HQ396285	HQ396316	HQ396306	HQ396295
UB4155	K94	*D. silvatica*	Barranco de Juel, Hermigua, La Gomera	AF244273	AF244177/ EU139674	EU139808	EU139739	EU143842
NMH 1395	N347	*D. silvatica*	Pinar Roque Faro, La Palma	HQ396328	HQ396286	HQ396317	HQ396307	HQ396296
NMH 1439	N362	*D. silvatica*	Mirador de Bascos, El Hierro	HQ396329	HQ396287	HQ396318	–	HQ396297
Continental								
NHM255	K226	*D.* cf. *inermis*	4 km S Tanger, Morocco	EF458142	EF458092	EU139795	EU139726	–
NHM075	K228	*D.* cf. *inermis*	Mirador del Estrecho, Tarifa, Iberian Peninsula	EF458141	EF458091	HQ407381	HQ407382	–
UB-ery105	K105	*D. erythrina*	Sant Llorenç del Munt, Barcelona, Iberian Peninsula	AF244252	AF244162*	EU139790	EU139720	EU143840
CRBA590	K294	*D. scabricula*	Desert de les Palmes, València, Iberian Peninsula	EU068046	EU068078	EU139809	EU139740	–

endemics, with the addition of 12 new individuals: six specimens from Selvagem Grande, five specimens from three western Canarian endemisms (*D. calderensis*, *D. silvatica* and *D. gomerensis*) and one from Morocco (*D.* cf. *inermis*). Specimens from the western Canaries provided additional calibration points for lineage age estimation (see below). The complete set of species, specimens and genes analysed is listed in Table 2, and the sampling localities in the eastern Canaries and Salvage Islands are indicated in Fig. 1 and Appendix 1 (see supplementary material which is available on the Supplementary tab of the article's Informaworld page at http://www.informaworld.com/mpp/uploads/tsab. . .). All analyses were rooted assuming a sister-group relationship of the continental species *Dysdera scabricula* Simon, 1882 to the other species sampled (Arnedo *et al.*, 2007; Macías-Hernández *et al.*, 2008). Samples were stored in absolute ethanol at −20 °C until DNA extractions were performed. All the eastern Canarian *Dysdera* species and additional

specimens from the western Canary Islands and continental species were also sampled and included in the analyses.

Unless stated otherwise, the programs and settings used for our phylogenetic analyses followed those described in Macías-Hernández *et al.* (2008). Ribosomal genes were aligned with MAFFT v. 5.8 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/, manual strategy option set to Q-INS-i). The best substitution model for each gene, as indicated by the Akaike information criterion, was selected with the program jModelTest, version 0.1.1 (Posada, 2008). Maximum likelihood and Bayesian inference analyses were conducted with RAxML v. 7.0.4 (Stamatakis, 2006) and MRBAYES v.3.1.2 (Ronquist & Huelsenbeck, 2003), respectively, defining unlinked evolutionary models (GTR+G fro RAxML) for each gene partition in both cases. Two independent runs were conducted for four million generations. The best likelihood tree was selected out of 10 iterations of random addition of taxa and non-parametric bootstrap support values were drawn from 100 resampled matrices. Uncorrected genetic distances within and between species were calculated with MEGA v. 4.1 software (Tamura *et al.*, 2007).

Divergence time estimation

Divergence times were estimated with the computer program R8S (Sanderson, 2003). A preliminary cross-validation analysis was conducted to select the clock method that best suited the data (Sanderson, 2002). Clade age was estimated on the Bayesian topology. Each species was only represented by one or two specimens to avoid very short branches that could negatively impact time estimation algorithms (Sanderson, 2003) and to simplify calculations. Only mitochondrial data were included in the divergence time analysis to produce results that were comparable to former studies (Bidegaray-Batista *et al.*, 2007; Macías-Hernández *et al.*, 2008), and the partially sequenced (only mitochondrial data) specimen X125 of *D. aneris* sp. nov. was added to the analyses. Tree editing was performed with the computer program TREEEDIT v. 1.0 a 10. Branch lengths were re-estimated on the preferred topology using the computer program RAxML and defining independent GRT+I+G models for each data partition. The outgroup taxon (*D. scabricula*) was pruned from trees before conducting clock analyses to ensure a dichotomous root node. Clade age confidence intervals were obtained from 100 trees by re-estimating branch lengths after character bootstrapping while keeping the topology constant. The program TreeAnnotator (Drummond & Rambaut, 2007) assisted in summarizing the confidence intervals from the sample of trees with bootstrapped branch lengths analysed in R8S. In these analyses, we have improved divergence time estimation by including four calibration points in addition to the single point used in former analyses, based on the time of divergence of the Iberian and Moroccan populations of *D.* cf. *inermis*, which was fixed to 5.3 Ma by assuming a population split following the opening of the Strait of Gibraltar (Krijgsman *et al.*, 1999). The island populations of *D. gomerensis* and *D. silvatica* from La Gomera and El Hierro, and the island populations of *D. calderensis* and *D. silvatica* from La Palma and La Gomera were set to a maximum time of divergence of 1.2 or 2 Ma respectively, corresponding to the time of origin of the subaerial stages of the youngest islands (Carracedo & Day, 2002).

Analysis of morphological variation

The morphological variation data presented in Macías-Hernández *et al*. (2008) were completed and reanalysed by including 10 specimens of *D. aneris* sp. nov. (no specimens were available in the former analyses) and additional material of *D. longa* (two new male specimens). Analyses were based on the following measurements, obtained from five males and five females of each target species (only two *D. sanborondon* males were available): maximum carapace length (P1), minimum (P2min) and maximum carapace width (P2max), length of the basal segment of the chelicera in lateral view (Q1), maximum width of the basal segment in lateral view (Q2), cheliceral fang length (F), length of the prolateral margin of the basal segment (Esc), femur length of leg 1 (fe1), and metatarsus length of leg 4 (mt4). The assumptions of normality and homogeneity were rejected by the Kolmogorov–Smirnoff test, so we applied a non-parametric Kruskal–Wallis test to detect possible interspecific morphological differences and intraspecific sexual dimorphism. A Pearson test detected high levels of autocorrelation between the morphological variables. Therefore, residual values (calculated by means of an interspecific regression using the Pearson correlation for each variable against P1) were used in subsequent analyses to reduce the effects of the reported correlation with body size (Losos *et al.*, 1998). A species similarity matrix was estimated across a hierarchical agglomerative cluster using the Bray–Curtis distance. Finally, principal components analyses (PCA) of all individuals was conducted to assess the variance explained by each independent axis (Legendre & Legendre, 1998). The analyses were performed using the software packages SPSS v. 15, PRIMER v. 5.2.2 (Clarke & Warwick, 1994), and STATISTICA (StatSoft Inc., 1999), and the results were plotted with SIGMAPLOT version 7.0 (SPSS, 2001).

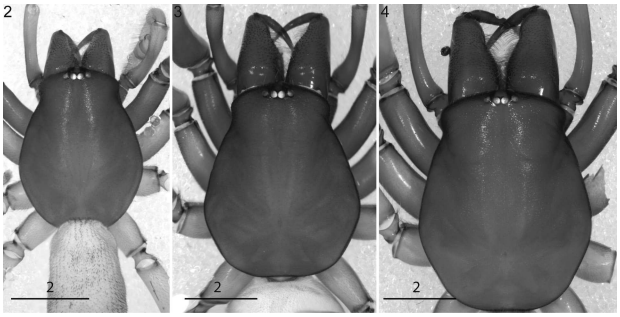
Results

Taxonomy

Dysderidae C. L. Koch, 1837

Dysdera Latreille, 1804

TYPE SPECIES: *Aranea erythrina* Walckenaer, 1802: 224 (unspecified sex) by original designation, unspecified num-



Figs 2–4. Carapace, dorsal view. **2**, *Dysdera aneris* sp. nov. holotype; **3**, *Dysdera mahan* sp. nov. holotype; **4**, *Dysdera simbeque* sp. nov. holotype.

ber of syntype specimens from France, surroundings of Paris (C. A. Walckenaer), repository unknown, supposed lost.

DIAGNOSIS: See Deeleman-Reinhold & Deeleman (1988).

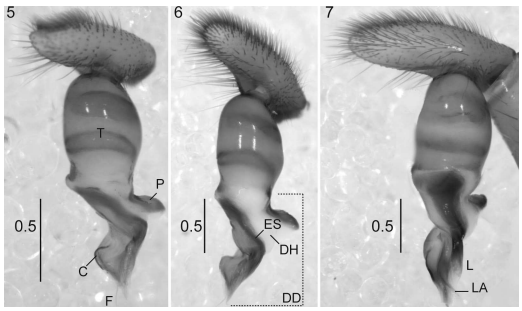
SPECIES INCLUDED: The genus presently includes 248 species (Platnick, 2010).

Dysdera aneris Macías-Hernández & Arnedo, sp. nov.
(Figs 2, 5, 8–12, 13–14, Tables 3–4)

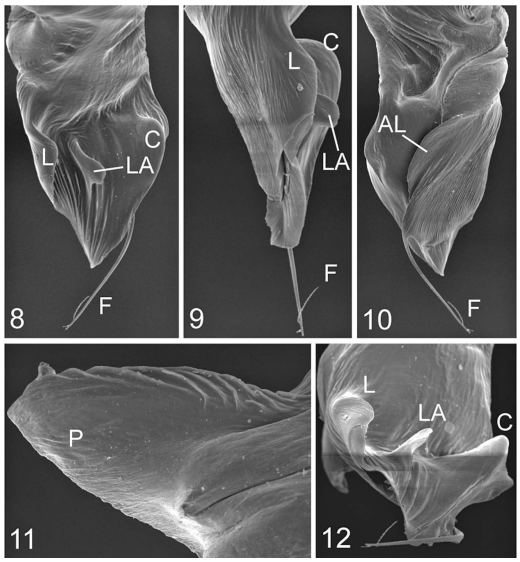
Dysdera wollastoni Kulczyński, 1899: 342, pl. 6, Figs 22 – 24 (3♂, 2♀, 4 juvs, Selvagens; coll. W. Kulczyński; stored at OXUM, examined) (♂, ♀ misidentified).—Simon, 1912: 59 – 60.—Berland & Denis, 1946: 224. – Wunderlich, 1991: 312. Fig. 129 (♂, misidentified). *Dysdera nesiotes* Denis, 1963: 37 – 38.—Rambla, 1978: 132 – 133.—Arnedo *et al*., 2000: 277–281, Figs 59 – 61 (♂,♀ misidentified).—Arnedo, 2003: 145.

HOLOTYPE: ♂ [CRBA-4267], 8 Oct 2005 (I. Silva) (CRBA).

PARATYPES: ♀ [CRBA-4268, CRBA-4273], same data as holotype (CRBA); 1 ♂ [GBIF 21705, right bulb removed for SEM], 2♀ [GBIF 21706–21707], same data as holotype (ULL).



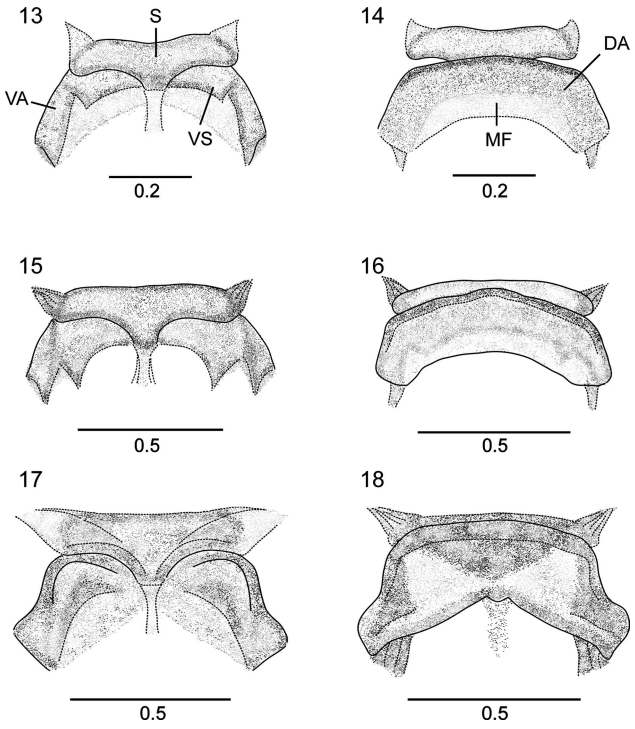
Figs 5–7. Left male palps, retrolateral view. **5**, *Dysdera aneris* sp. nov. holotype; **6**, *Dysdera mahan* sp. nov. holotype; **7**, *Dysdera simbeque* sp. nov. holotype.



Figs 8–12. *Dysdera aneris* sp. nov. right male bulb. **8**, anterior view; **9**, retrolateral view; **10**, posterior view; **11**, P detail, retrolateral view; **12**, distal tip, ventral view.

TYPE LOCALITY: Salvage Islands: Selvagem Grande (N 30.146105 W 15.864975).

ADDITIONAL MATERIAL: Salvage Islands: 3♂, 1 sub♂, 1♀, 1 juv. [BM1897.10.18.41–46], label states; '*Dysdera*



Figs 13–18. Female vulva. **13–14**, *Dysdera aneris* sp. nov. **13**, ventral view; **14**, dorsal view; **15–16**, *Dysdera mahan* sp. nov. **15**, ventral view; **16**, dorsal view; **17–18**, *Dysdera simbeque* sp. nov. **17**, ventral view; **18**, dorsal view.

Table 3. Intraspecific spination variability of *Dysdera aneris* sp. nov.

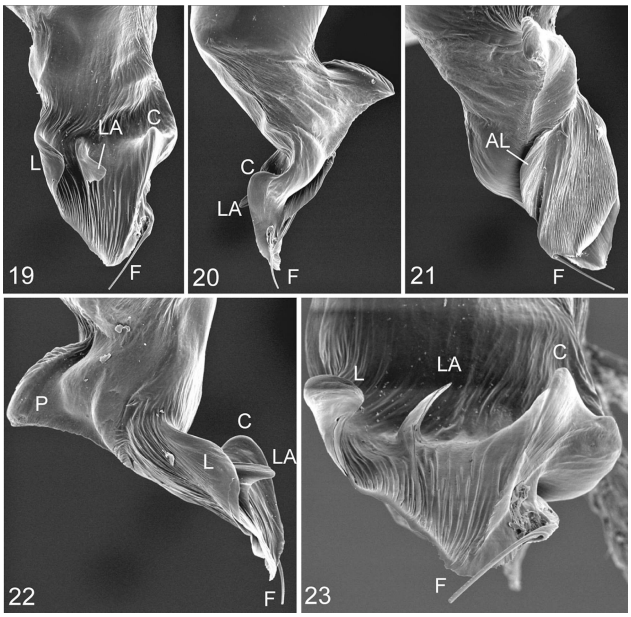
	Proximal	Medial-proximal	Medial-distal	Distal
Tibia 3 dorsal	1.0–1-2.0–1	0	0	0–1.0.0–1
Tibia 3 ventral	0–1.0.0–1	0	0	0–1.0.0–1
Tibia 4 dorsal	0–1.0.0–1	0.0.0–1	0	0–1.0.0–1
Tibia 4 ventral	0–1.0.0–1	0–1.0.0	0	1.0.0–1
		Number of rows		**Number of spines**
Femur 3 dorsal		0–1		0–1
Femur 4 dorsal		2		1–4/4–7

verneaui Simon' (Grant) (BMNH); 1♂, 1♀, 3 juvs [B 536] (Garreta) (MHNP); 3♂, 2♀, 4 juvs labels state: '*Dysdera wollastoni*, Selvagens' 'Ins. Zool. P.A.N. coll. W. Kulczynski' (OXUM); Selvagem Grande 1♂ [NMH608], 3♀ [NMH610, 611, 613], 8 Oct 2005 (I. Silva) (UB); 1♀ [NMH1489], 3♂ [NMH1490–1492], 26 March 2009 (L. García) (ULL).

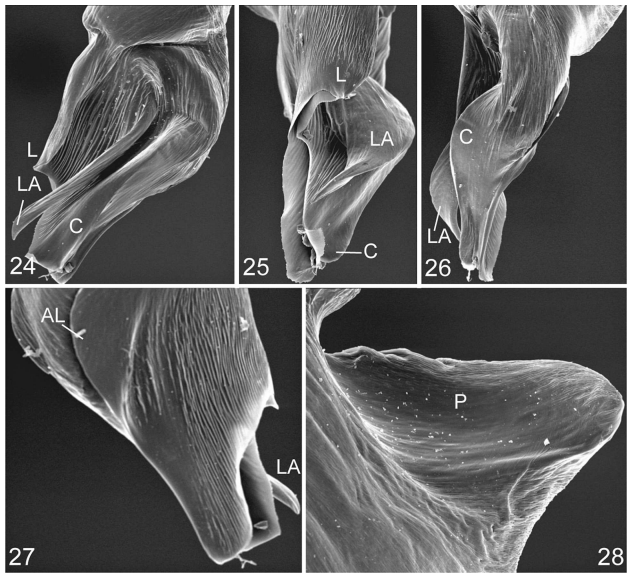
HABITAT AND DISTRIBUTION: This species is found on Selvagem Grande in the Salvage Islands, 165 km north of the Canary Islands (Fig. 1). Denis (1963) also reported the presence of *D. nesiotes* (species to which the Salvage Island specimens had been transferred) also in Selvagem Pequena (Pitão) and Ilhéu de Fora. These last records, however, could not be confirmed.

ETYMOLOGY: The species epithet is a noun in apposition; it is the name of a female character in the book *La Pell Freda* ('Cold Skin') by Albert Sánchez Piñol. Aneris belongs to a strange marine race whose members emerge from the sea when night falls, and wander around a desolate island.

DIAGNOSIS: *Dysdera aneris* sp. nov. closely resembles *D. nesiotes* and *D. mahan* sp. nov. but differs from them in vulval and DNA sequence characters. It can be distinguished from *D. nesiotes* by a rectangle–like DA (width/length ratio range 1.7–2.3, in square-like *D. nesiotes* 1.2–1.6) (Fig. 14). Sclerotization of VA is restricted to the frontal margin (it extends to the halfway point of the lateral margins in *D. nesiotes*; Fig. 13). It can be distinguished from *D. mahan* sp. nov. by tooth-like VA projections shorter than VA lateral margins (as long as lateral margins in *D. mahan* sp. nov.; Figs 13, 15), and smaller body size (average carapace length 4.1 and 5.26 in *D. aneris* sp. nov. and *D. mahan* sp. nov., respectively). The three species can also be diagnosed by fixed nucleotide differences in the DNA barcode of *cox1* as follows: position 146 (G/T/A), 257 (A/G/T) and



Figs 19–23. *Dysdera mahan* sp. nov. right male bulb. **19**, anterior view; **20**, prolateral view; **21**, posterior view; **22**, retrolateral view; **23**, distal tip, ventral view.



Figs 24–28. *Dysdera simbeque* sp. nov. right male bulb (horizontal flipped images of the left bulb). **24**, anterior view; **25**, retrolateral view; **26**, prolateral view; **27**, posterior view; **28**, P detail, prolateral view.

398 (A/T/G), in *D. aneris* sp. nov., *D. nesiotes* and *D. mahan* sp. nov., respectively (alignment positions correspond to a reference alignment deposited in TreeBASE, available at http://purl.org/phylo/treebase/phylows/study/TB2:S10950).

MALE (*holotype*): Figs 2, 5, 8–12. Carapace (Fig. 2) 4.08 mm long; maximum width 3.16 mm; minimum width 2.09 mm. Brownish orange, frontally darker, becoming lighter towards back; smooth. Frontal border roughly triangular, from 1/2 to 3/5 carapace length; anterior lateral borders convergent. AME diameter 0.22 mm; PLE 0.21 mm; PME 0.18 mm; AME separated from each other by approximately 2/3 diameter, PME approximately 1/3 PME diameter from PLE. Sternum orange, uniformly distributed; smooth.

Chelicerae 1.68 mm long, approximately 1/3 of carapace length in dorsal view; fang medium-sized, 1.53 mm; basal segment dorsal, ventral side completely covered with granulations. Chelicera inner groove short, approximately 1/3 cheliceral length; armed with three teeth and lamina at base; B>D>M; D round, located roughly at centre of groove; B close to basal lamina; M at middle of B and D. Legs orange. Lengths of male described above: fe1 3.31 mm (all measurements in mm), pa1 2.29, ti1 3.01, me1 3.01, ta1 0.66, total 12.29; fe2 2.91, pa2 1.99, ti2 2.55, me2 2.60, ta2 0.71, total 10.76; fe3 2.29, pa3 1.38, ti3 1.53, me3 2.29, ta3 0.61, total 8.11; fe4 3.16, pa4 1.73, ti4 2.6, me4 2.96, ta4 0.71, total 11.17; fe Pdp 2.04, pa Pdp 1.12, ti Pdp 0.97, ta Pdp 0.92, total 5.05; relative length: 1>4>2>3. Spination: leg1, 1eg2 spineless; tb3d spines arranged in two bands; proximal 1.0.1; distal 1.0.0; tb3v spines arranged in two bands; proximal 1.0.0; distal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 1–0; backward 5–3; tb4d spines arranged in two bands; proximal 0.0.1; distal 0.0.1; tb4v spines arranged in three bands; proximal 1.0.0–1; medial-proximal 0.0.1; distal 1.0.0; with two terminal spines. Claws with 8 teeth or less; only slightly larger than claw width.

Abdomen 4.9 mm long; whitish; cylindrical. Abdominal dorsal hairs 0.11 mm long; medium-sized, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

Male copulatory bulb (3A) T as long as DD. DD bent approximately 45º in lateral view; internal distal border markedly expanded. ES wider, more sclerotized than IS; IS continuous to tip. DD tip (Figs 8–10, 12) frontal (upper) sheet internal portion markedly projected above posterior (lower) sheet. C present, long; distal end close to DD internal tip; distal border rounded, smooth, markedly expanded, perpendicular to DD. L well developed; external border sclerotized, laterally markedly folded, distally projected; distal border divergent, continuous. LA present, hook-like; shorter than L. F present, tip divided and distally curved to external side; proximally fused to DD. AL present, well-developed; not joined to F; proximal border in posterior view smooth, not fused with DH. P (Fig. 11) fused to T; perpendicular to T in lateral view; lateral length from 1/2 to 2/3 of T width; ridge present, perpendicular to T; distinctly expanded, rounded, upper margin slightly toothed along its extent, mainly on external side; few teeth (4–6); not distally projected; back margin not folded.

FEMALE (*paratype* CRBA-4268): Figs 13, 14. All characters as in male except: carapace 3.88 mm long; maximum width 3.01 mm; minimum width 1.94 mm. Anterior laterally rounded at maximum dorsal width, back lateral borders straight. AME diameter 0.21 mm; PLE 0.195 mm; PME 0.17 mm.

Chelicerae 1.68 mm long; fang medium sized, 1.53 mm. Lengths of female described above: fe1 3.01 mm (all measurements in mm), pa1 2.0, ti1 2.55, me1 2.55, ta1 0.56, total 10.66; fe2 2.55, pa2 1.73, ti2 2.29, me2 2.24, ta2 0.56, total 9.38; fe3 2.0, pa3 1.22, ti3 1.48, me3 2.09, ta3 0.61, total 7.45, fe4 2.8, pa4 1.68, ti4 2.35, me4 2.75, ta4 0.71, total 10.3; fe Pdp 1.78, pa Pdp 0.87, ti Pdp 0.82, ta Pdp 0.97, total 4.44; relative length 4>1>2>3. Spination: leg1, leg2 spineless. Tb3d spines arranged in two bands; proximal 1.0.1; distal 0–1.0.0–1; tb3v spines arranged in two bands; proximal 1.0.0; distal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 1; backward 3; tb4d spines arranged in two bands; proximal 0.0.1; distal 0.0.1; tb4v with two terminal spines.

Abdomen 4.65 mm long; whitish; cylindrical. Abdominal dorsal hairs 0.1424 mm long; thin, roughly straight, compressed, lanceolate; uniformly thickly distributed.

Vulva (Figs 13, 14) DA not distinguishable from VA; rectangular; DA twice as wide as long; DF wide in dorsal view. MF margins fused, sheet-like, well-developed, completely sclerotized, projected backwards, shorter than DA lateral length. VA frontal region completely sclerotized; posterior region sclerotized in most anterior area; tooth-shaped expansion from internal back border; not joined to lateral sclerotization, slightly shorter than DF lateral margins. S attachment not projected under VA; arms as long as DA, m-shaped; ends projected forwards; neck hardly visible.

VARIATION: The male carapace ranges in length from 4–4.8 mm and female from 3.5–4.4 mm (N = 5). The colour of the carapace varies between red orange to brownish orange in some individuals. The vulva shows a different degree of VA sclerotization, and in some females S arms are smaller and rounded. Spination and leg measurement variability are listed in Tables 3 and 4, respectively.

REMARKS: In 1864 the British naturalist John Blackwall described a new *Dysdera* species from the Salvage Islands, *Dysdera wollastoni*. Almost 20 years later, in a taxonomic treatment of spiders from Atlantic Ocean islands the great French arachnologist Eugène Simon expressed his doubts about the validity of the former species, which he considered most likely to be a senior synonym of the cosmopolitan species *D. crocata* C. L. Koch, 1838 (Simon, 1883). In 1899 the Polish arachnologist W. Kulczyński published a redescription with excellent illustrations of *D. wollastoni*, based on newly collected material from the Salvage

Table 4. *Dysdera aneris* sp. nov. Leg measurements variability. Males (N = 5)/Females (N = 5).

	I	II	III	IV
Fe	3.3–3.8/2.6–3.5	2.9–3.4/2.3–3.1	2.2–2.6/1.9–2.6	3.2–3.6/2.8–3.5
Pa	2.3–2.5/1.8–1.9	1.9–2.2/1.6–2.2	1.3–1.5/1.1–1.5	1.7–1.9/1.4–1.9
Ti	3.0–3.4/2.3–3.1	2.5–3.0/1.9–2.8	1.5–1.8/1.4–1.8	2.6–2.7/2.0–2.7
Mt	3.0–3.5/2.1–3.0	2.6–3.0/1.9–2.6	2.3–2.5/1.6–2.5	2.9–3.4/2.5–3.3
Ta	0.6–0.7/0.5–0.6	0.71/0.5–0.6	0.6–0.6/0.5–0.6	0.7–0.7/0.6–0.7
Total	12.3–13.9/9.5–12.7	10.7–12.3/8.5–11.2	8.1–9.1/6.7–9.0	11.1–12.5/9.4–12.3

Islands. Simon examined a new batch of specimens from these islands collected by M.L. Garreta, which, according to him, closely resembled the species *D. nesiotes*, which he had recently described from the Canary Islands, except for their smaller size and fewer spines on fe IV. Therefore, he proposed downgrading the status of the Canarian specimens to subspecies and referring to it as *D. wollastoni nesiotes*, probably after observing Kulczyński's redescription (Simon, 1912). It took more than half a century to confirm Simon's original suggestion that *D. wollastoni* was a junior synonym of *D. crocata*. Jacques Denis (1963) considered Kulczyński's redescription of *D. wollastoni* to be based on a misidentification and, supported by information provided by J.A.L. Cooke, who was based at Oxford where Blackwall types were stored, hence reinstated *D. nesiotes* to full species status and transferred all specimens from the Salvages identified as *D. wollastoni* sensu Kulczyński to this species. Wunderlich (1991) argued against the former synonym, suggesting that a spider specialist such as Blackwall could not have possibly misidentified *D. crocata*. Arnedo *et al*. (2000) examined material from both the Salvage Islands and Lanzarote and concluded that there were not any clear diagnostic differences separating these island populations. We have now had the chance to examine the original material used by Blackwall to describe *D. wollastoni*, and we can confirm that this is a junior synonym of *D. crocata*, and we have examined the material used by Kulczyński for his redescription, which belongs to *D. aneris* sp. nov.

Dysdera mahan Macías-Hernández & Arnedo, sp. nov.
(Figs 3, 6, 15–16, 19–23, Tables 5–6)

Dysdera nesiotes Arnedo *et al.*, 2000: 278–280, Fig. 63 (♀ misidentified).

HOLOTYPE: ♂ [CRBA-4269] (right bulb removed for SEM), 8 Dec 2004 (GIET) (UB).

PARATYPES: Canary Islands: ♀ [CRBA-4270], same data as holotype (CRBA); 1♀ [GBIF 21708], Fuerteventura, La Oliva, Playa de Esquinzo, 31 March 2004 (H. López) (ULL); 1♀ [GBIF 21709], Lanzarote, Yaiza, Playa Caleta del Congrio, Papagayo, 7 Feb 2005, (N. Macías-Hernández) (ULL); 1♀ [GBIF 21710], Lanzarote, Tinajo, Playa de la Madera, Timanfaya, 28 March 2004 (H. López) (ULL); 1♀ [GBIF 21711], 2 ♂ [GBIF 21711–21712], Lanzarote, Haría, Playa Catalina Cabrera, Famara, 28 Nov 2004 (GIET) (ULL); 1♂ [GBIF 21714], Alegranza, Playa de El Trillo; 8 Dec 2004 (GIET) (ULL).

TYPE LOCALITY: Canary Islands: Alegranza, Playa de El Trillo (N 29.404183 W 13.490834).

ADDITIONAL MATERIAL: Canary Islands: La Graciosa 4 juv. [NMH 451], Caleta de Arriba, 31 Jan 2005 (N. Macías-Hernández) (ULL); Lobos: 4 juv. [NMH 572], Playa Las Salinas, Mar 2004 (N. Macías-Hernández & H. López) (ULL); Lanzarote: 1♀ [NMH65], Haría, Punta Pasitos, Mala, 26 March 2004 (A.J. Pérez) (ULL); 1♀ [2887UB], Órzola, Charcos de marea, 25 Feb 1995 (M. Arnedo, C. Ribera & P. Oromí) (CRBA); 1 juv. [NMH 113], Tinajo, Playa Caleta del Mariscadero, Timanfaya, 28 March 2004 (H. López) (ULL); 1 juv. [NMH443], Playa de Teneza, 8 Feb 2005 (N. Macías-Hernández) (ULL); 1 juv. [NMH448], Yaiza, Playa Las Salinas, Puerto Calero, 9 Feb 2005 (N. Macías-Hernández); Fuerteventura: 3 juvs [NMH490–492], Corralejo, Playa Majanicho, 5 Feb 2005 (M. Arnedo & N. Txasco) (CRBA).

HABITAT AND DISTRIBUTION: This species is found in the intertidal zones of pebble beaches on the sea-shores

Table 5. Intraspecific spination variability of *Dysdera mahan* sp. nov.

	Proximal	**Medial-proximal**	**Medial-distal**	**Distal**
Tibia 3 dorsal	1.0.0–1	0	0	0–1.0.1–0
Tibia 3 ventral	0–1.0.0	0	0	0–1.0.0
Tibia 4 dorsal	1–2.0.0	0	0	0–1.0.0–1
Tibia 4 ventral	0–1.0.0	0	0	0
		Number of rows		**Number of spines**
Femur 3 dorsal		0		0
Femur 4 dorsal		2		1–3/4–6

of Lanzarote, the northern islets, Lobos and the north of Fuerteventura (Fig. 1).

ETYMOLOGY: The species epithet is a noun in apposition; the name refers to a giant aborigine that inhabited Fuerteventura, and it is also used to refer to the single island that formed Lanzarote and Fuerteventura during past episodes of marine regression.

DIAGNOSIS: The species closely resembles *D. aneris* sp. nov. and *D. nesiotes*. It can be distinguished from the former species by its larger size, longer legs IV, anterior tooth-like projections of vulva VA as long as DA (Figs 13, 15), rectangle-like DA (Figs 14, 16), and inter-tidal, pebble-beach habitat. The three species differ in DNA sequences (see *D. aneris* sp. nov. diagnosis). It differs from close relative *D. spinidorsum* by copulatory bulb with shorter LA (Fig. 19), attenuated C (Fig. 22), AL folded at its prolateral margin (Fig. 21), vulva with shorter MF backward projections (only slightly longer than DA; Fig. 15), and tooth-like sclerotization of frontal VA (Fig. 15). The two species can also be diagnosed by fixed nucleotide differences in the DNA barcode of *cox1* as follows: position 5 (A/T), 92 (T/A), 155 (C/T), 188 (T/A), 254 (G/A), 314 (A/G), 320 (G/A), 338 (G/A), 416 (A/T), 488 (C/T) and 527 (C/T) in *D. aneris* sp. nov. and *D. spinidorsum*, respectively (reference alignment deposited in TreeBASE, available at http://purl.org/phylo/treebase/phylows/study/TB2:S10950).

MALE (*holotype*): Figs 3, 5, 19–23. Carapace (Fig. 3) 5.3 mm long; maximum width 4.28 mm; minimum width 2.6 mm. Brownish red, frontally darker, becoming lighter towards back; smooth. Frontal border roughly round, from 1/2 to 3/5 carapace length; anterior lateral borders convergent. AME diameter 0.247 mm; PLE 0.234 mm; PME 0.221 mm; AME separated from one another by approximately 2/3 diameter; PME less than 1/4 PME diameter from PLE. Sternum brownish orange, frontally darker, becoming lighter towards back or darkened on borders; very slightly wrinkled, mainly between legs and frontal border.

Chelicerae 2.7 mm long, approximately 1/3 of carapace length in dorsal view; fang medium-sized, 1.785 mm; basal segment dorsal, ventral side completely covered with piligerous granulations. Chelicera inner groove medium-size, approximately 2/5 cheliceral length; armed with three teeth and lamina at base; D = B>M; D trapezoid, located roughly at centre of groove; B close to basal lamina; M close to B. Legs dark orange-coloured. Lengths of male described above: fe1 5.56 mm (all measurements in mm), pa1 3.57, ti1 5.35, me1 5.41, ta1 1.02, total 20.91; fe2 4.7, pa2 3.06, ti2 4.59, me2 4.69, ta2 1.02, total 18.05; fe3 3.82, pa3 2.14, ti3 3.1, me3 3.67, ta3 1.02, total 13.72; fe4 4.84, pa4 2.65, ti4 4.28, me4 5.05, ta4 1.07, total 17.9; fe Pdp 2.86, pa Pdp 1.58, ti Pdp 1.53, ta Pdp 1.33, total 7.29; relative length: 1>2>4>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.0.0–1; distal 1.0.0; tb3v spines arranged in one band; proximal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 3–2; backward 5–6; tb4d spines arranged in two bands; proximal 0–1.0.1; distal 0.0.1; tb4v spines arranged in one band; proximal 1.0.0; with two terminal spines. Claws have 8 teeth or less; hardly larger than claw width.

Abdomen 6.22 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly thickly distributed.

Male copulatory bulb (Figs 6, 19–23) as in *D. aneris* sp. nov.

FEMALE (*paratype* CRBA-4270): Figs 15–16. All characters as in male except: carapace 4.84 mm long; maximum width 3.77 mm; minimum width 2.5 mm. Back lateral borders rounded. AME diameter 0.25 mm; PLE 0.23 mm; PME 0.19 mm.

Chelicerae 2.19 mm long, approximately 1/3 of carapace length in dorsal view; fang medium-sized, 0.34 mm. Legs orange. Lengths of female described above: fe1 4.44 mm (all measurements in mm), pa1 3.01, ti1 4.03, me1 4.03, ta1 0.87, total 16.37; fe2 3.88, pa2 2.65, ti2 3.47, me2 3.52, ta2 0.82, total 14.33; fe3 3.21, pa3 1.99, ti3 2.45, me3 3.01, ta3 0.82, total 11.47; fe4 4.23, pa4 2.5, ti4 3.67, me4 4.23, ta4 1.02, total 15.66; fe Pdp 2.55, pa Pdp 1.27, ti Pdp 1.02, ta Pdp 1.27, total 6.12; relative length 1>4>2>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.0.0–1; distal 1.0.0; tb3v spines arranged in one band; proximal 1.0.0; with two terminal spines. Fe4d spines in two rows; forward 1; backward 5–6; tb4d spines arranged in two bands; proximal 0.0–1.2; distal 0.0.1; tb4v spines arranged in two bands; proximal 0–1.2–0.0; medial-proximal 1.0.0; with two terminal spines.

Abdomen 9.96 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

Vulva (Figs 15–16) as in *D. aneris* sp. nov. except VA posterior region sclerotized in lateral margins; tooth-shaped expansion from internal back border as long as DF lateral margins.

VARIATION: Male cephalothorax ranges in length from 4.9–5.3 mm (N = 4), female from 4.8–6 mm (N = 5). Carapace and leg colours vary from red orange to brownish orange in some specimens. The internal part of the pedipalps presents denser short black hairs in females. Spination and leg measurement variability is listed in Tables 5 and 6, respectively.

REMARKS: A female specimen of the new species had already been studied by Arnedo *et al*. (2000), who rendered its particular vulva DA shape as a case of intraspecific variability in *D. nesiotes*.

Dysdera simbeque Macías-Hernández & Arnedo, sp. nov. (Figs 4, 7, 17–18, 24–28, Tables 7–8)

Table 6. *Dysdera mahan* sp. nov. Leg measurements variability. Males (N = 4)/Females (N = 5).

	I	II	III	IV
Fe	4.8–5.5/4.4–5.2	4.1–4.7/3.8–4.8	3.3–3.8/3.2–3.8	4.5–4.8/4.2–5.1
Pa	2.9–3.6/2.9–3.3	2.6–3.0/2.6–3.2	1.9–2.1/1.9–2.3	2.4–2.7/2.4–2.8
Ti	4.6–5.3/4.0–4.6	4.0–4.6/3.5–4.4	2.3–3.0/2.3–2.9	3.7–4.3/3.6–4.5
Mt	4.8–5.4/4.0–5.1	3.9–4.7/3.5–4.6	3.2–3.7/3.0–3.9	4.3–5.0/4.2–5.39
Ta	0.9–1.0/0.8–0.9	0.8–1.0/0.7–0.9	0.8–1.0/0.8–0.9	1.0–1.0/1.0
Total	18.0–20.9/16.4–19.7	15.7–18.0/14.3–18.0	11.7–13.7/11.3–13.8	16.0–17.9/15.6–18.7

HOLOTYPE: ♂ [CRBA-4271] (right bulb removed for SEM), 29 March 2004 (GIET) (CRBA).

PARATYPE 1♀ [CRBA-4272], same data as holotype (CRBA); 2♂ [GBIF 21715–21716], 3♀ [GBIF 21717–21718-21719], same data as holotype, 29 March 2004 (GIET) (ULL).

TYPE LOCALITY: Canary Islands: Lanzarote, Cabecera del Barranco Elvira Sánchez, Haría (N 29.130723 W 13.516902).

ADDITIONAL MATERIAL: CANARY ISLANDS: Lanzarote: Haría, Fuente Ovejas, Guinate, 1 juv. [NMH 576], 26 Nov 2004 (N. Macías-Hernández) (ULL); 3 juvs [NMH1294–1296], MSS pitfall traps, 13 Jan 2007 (H. López & H. Morales) (ULL).

HABITAT AND DISTRIBUTION: This species is only known from two nearby sites on northern Lanzarote (Fig. 1).

ETYMOLOGY: The species epithet is an adjective in apposition; it means 'big' or 'voluminous' in the language of the aboriginal inhabitants of the Canary Islands.

DIAGNOSIS: *Dysdera simbeque* sp. nov. differs from closely related *D. alegranzaensis* in its larger size (average carapace lengths 6.12 and 4.48, respectively), copulatory bulb with LA longer than L (Fig. 24), vulva VA not sclerotized; S attachment not projected backwards (Fig. 17). It can be easily distinguished from sympatric *D. nesiotes* by its larger size, a copulatory bulb with F absent (Fig. 24), vulva VA without frontal or lateral sclerotization and distal tips of S arms projected forward (Fig. 17). The three species can be diagnosed by fixed nucleotide differences in the DNA barcode for *cox1* as follows: position 242 (A/G/T), 263 (G/A/T), 338 (G/A/T), 347 (T/A/G), 368 (T/G/A), 434 (G/A/T), 506 (A/T/G), 533 (G/A/T) and 596 (G/A/T) in *D. simbeque* sp. nov., *D. alegranzaensis* and *D. nesiotes*, respectively (reference alignment deposited in TreeBASE, available at http://purl.org/phylo/treebase/phylows/study/TB2:S10950).

MALE (*holotype*): Figs 4, 7, 24–28. Carapace (Fig. 4) 6.12 mm long; maximum width 4.84 mm; minimum width 3.47 mm. Red orange, frontally darker, becoming lighter towards back; slightly foveate at borders, slightly wrinkled with small black grains mainly at the front. Frontal border roughly round, from 1/2 to 3/5 carapace length; anterior lateral borders convergent. AME diameter 0.26 mm; PLE 0.26 mm; PME 0.221 mm; AME separated from each other by approximately one diameter or more; PME approximately 1/2 PME diameter from PLE. Sternum reddish orange, frontally darker, becoming lighter towards back; very slightly wrinkled, mainly between legs and frontal border.

Chelicerae 3.57 mm long, approximately 2/5 of carapace length in dorsal view; fang medium-sized, 2.29 mm; basal segment dorsal, ventral sides completely covered with granulations. Chelicera inner groove medium-size, about 2/5 cheliceral length; armed with three teeth and lamina at base; B>D>M; D trapezoid, located roughly at centre of groove; B close to basal lamina; M at middle of B and D. Legs dark orange-coloured. Lengths of male described above; fe1 5.508 mm (all measurements in mm), pa1 3.93, ti1 5.61, me1 4.95, ta1 0.87, total 20.86; fe2 5, pa2 3.37, ti2 4.69, me2 4.39, ta2 0.82, total 18.26; fe3 3.82, pa3 2.29, ti3 2.86, me3 3.62, ta3 0.82, total 13.41; fe4 4.84, pa4 2.75, ti4 3.82, me4 4.6, ta4 1.02, total 17.03; fe Pdp 3.6, pa Pdp 1.73, ti Pdp 1.73, ta Pdp 1.58, total 8.62; relative length:

Table 7. Intraspecific spination variability of *Dysdera simbeque* sp. nov.

	Proximal	Medial-proximal	Medial-distal	Distal
Tibia 3 dorsal	1.1–2.1	0	0	0–1.0.0–1
Tibia 3 ventral	1.0–1.0–1	0	0	0.0.1
Tibia 4 dorsal	1.0–2.1	0	0	1.0.1
Tibia 4 ventral	1.0.1	0	0	0.2.0
		Number of rows		**Number of spines**
Femur 3 dorsal		0		0
Femur 4 dorsal		2		1–2/4–9

Table 8. *Dysdera simbeque* sp. nov. Leg measurements variability. Males (N = 4)/Females (N = 4).

	I	II	III	IV
Fe	5.1–5.5/4.5–5.0	4.5–5.0/4.1–4.3	3.3–4.1/3.2–3.4	4.4–4.8/4.2–4.5
Pa	3.5–3.9/3.2–3.5	3.1–3.5/2.8–3.0	2.1–2.3/1.9–2.0	2.6–2.7/2.4–2.7
Ti	5.0–5.6/4.0–4.3	4.1–4.7/3.5–4.0	2.5–2.8/2.3–2.5	3.6–3.8/3.5–3.7
Mt	4.3–4.9/3.6–4.0	4.0–4.4/3.2–3.6	3.2–3.6/2.9–3.1	4.3–4.6/3.9–4.2
Ta	0.8–1.0/0.87	0.8–0.9/0.8–0.7	0.8–0.8/0.7–0.8	0.87–1.02/0.87
TOTAL	19.1–20.8/16–17.6	16.8–18.2/14.4–15	11.9–13.4/11.3–11.8	16.1–17.0/15–16

Q3

1>2>4>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.2–1.1; distal 1.0.1; tb3v spines arranged in one band; proximal 1.0.1; with two terminal spines. Fe4d spines in two rows; forward 1–2; backward 9–10; tb4d spines arranged in two bands; proximal 1.2.1; distal 1.0.1; tb4v spines arranged in two bands; proximal 1.0.1; distal 1.0.1; with two terminal spines. Distal part of metatarsus III and IV densely covered with short hair. Claws with 8 teeth or less; only slightly longer than claw width.

Abdomen 8.38 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

Male copulatory bulb T as long as DD (Fig. 7); DD not bent, same T axis in lateral view; internal distal border markedly expanded. ES wider, more sclerotized than IS; IS continuous to tip. DD tip (Figs 24–27) straight in lateral view. C present, short; distal end on DD internal tip; poorly developed; located close to DD distal tip; proximal border sharply decreasing; distal border truncated, upper tip not projected, rounded, external side smooth. L well-developed; external border sclerotized, laterally markedly folded, distally projected; distal border divergent, continuous. LA present, sheet-like; longer than, distally not fused to L. F absent. AL present, well-developed; proximal border in posterior view smooth, not fused with DH. P (Fig. 28) fused to T; perpendicular to T in lateral view; lateral length from 1/2 to 2/3 of T width; ridge present, perpendicular to T; distinctly expanded, right-angled, upper margin smooth; not distally projected; back margin not folded.

FEMALE (*paratype* CRBA-4272): Figs 17–18. All characters as in male except: Carapace 6.27 mm long; maximum width 4.9 mm; minimum width 3.26 mm. AME diameter 0.29 mm; PLE 0.25 mm; PME 0.19 mm.

Chelicerae 3.21 mm long; fang medium-sized, 2.29 mm; M close to B. Lengths of female described above: fe1 5 mm (all measurements in mm), pa1 3.47, ti1 4.28, me1 4.03, ta1 0.87, total 17.65; fe2 4.33, pa2 3.06, ti2 3.77, me2 3.52, ta2 0.82, total 15.5; fe3 3.37, pa3 2.04, ti3 2.55, me3 3.06, ta3 0.82, total 11.83; fe4 4.44, pa4 2.7, ti4 3.72, me4 4.23, ta4 0.87, total 15.96; fe Pdp 3.26, pa Pdp 1.53, ti Pdp 1.27, ta Pdp 1.58, total 7.65; relative length 1>4>2>3. Spination: leg1, leg2 spineless; tb3d spines arranged in two bands; proximal 1.2.1; distal 1.0.0; tb3v spines arranged in one band; proximal 1.0.1; with two terminal spines. Fe4d spines in two rows; forward 2; backward 9; tb4d spines arranged in two bands; proximal 1.0.1; distal 1.0.1; tb4v spines arranged in two bands; proximal 1.0.1; distal 1.0.1; with two terminal spines.

Abdomen 8.13 mm long; cream-coloured; cylindrical. Abdominal dorsal hairs 0.12 mm long; thick, roughly straight, compressed, lanceolate; uniformly, thickly distributed.

Vulva (Figs 17–18) DA not distinguishable from VA, rectangular; DA twice as wide as long; DF wide in dorsal view. MF well-developed, completely sclerotized. VA frontal region completely sclerotized; posterior region sclerotized in most anterior area. S attachment not projected under VA; arms as long as DA, straight; tips dorsally projected; neck as wide as arms.

VARIATION: Male cephalothorax ranges in length from 5.9–6.8 mm, females from 5.8–6.2 mm (N = 4). The colour of the carapace varies between red orange to brownish orange in some specimens. Some have a greater number of short black hairs on the sternum and ventral part of the chelicerae. The size of the S arms and the development of the lateral sclerotization of the VA differ among specimens. Spination and leg measurement variability are listed in Tables 7 and 8, respectively.

REMARKS: Specimens were collected by hand from under stones, as well as in MSS (mesovoid shallow substratum) traps.

Phylogenetic analyses

The combined data matrix included 48 taxa representing 15 species and a total of 3749 characters (*cox1*: 1179 bp; *16S+L1:* 566 pb and 40 gap absence/presence characters; *nad1*: 343 pb; *28S*: 765 pb and 17 gap a/p chars; *H3*: 328 pb, *ITS-2*: 465 pb and 46 gap a/p chars).

Parsimony analyses of the combined data matrix yielded two trees of 4071 steps (CI: 47, RI: 74). All clades received jackknife supports above 70%, except for the position of *D. sanborondon* as sister species to *D. alegranzaensis* + *D. simbeque* sp. nov. (Fig. 29). The AIC criterion implemented in jMODELTEST selected the following models of nucleotide substitution for each gene fragment: TIM3+I+G for *cox1*; TIM2+I+G for *16S+L1*; TrN+I+G for *nad1*;

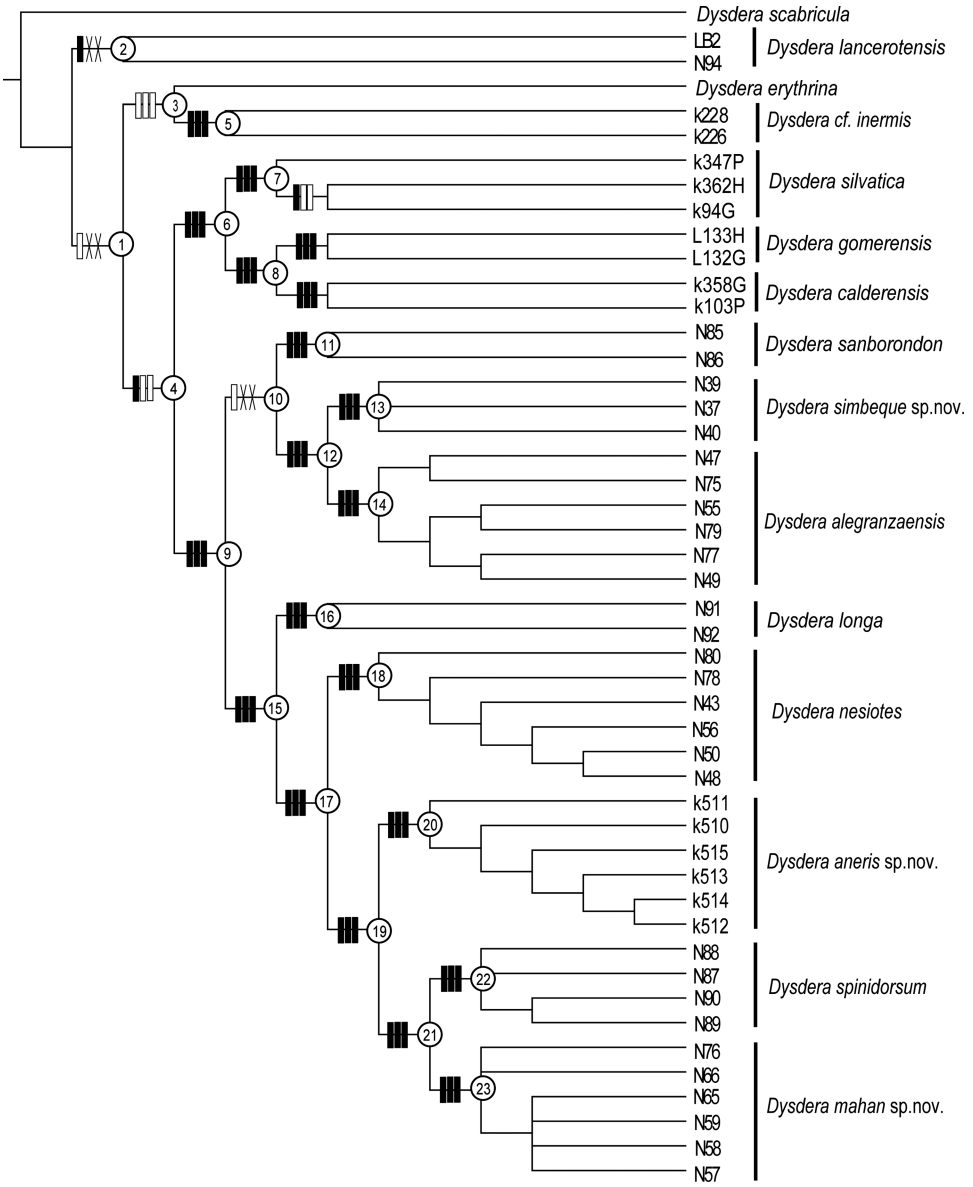


Fig 29. Strict consensus of two trees of 4071 steps (CI = 47, RI = 74) resulting from uniformly weighted parsimony analysis of the complete data set. Bars on branches denote support as follows: anterior bar refers to parsimony jackknife support, middle bar to maximum likelihood bootstrap support and posterior bar to posterior probability. Black bar: parsimony jackknife or ML bootstrap >70%, posterior probability >0.95%; white bar: parsimony jackknife or ML bootstrap <70%, posterior probability <0.95%; asterisk (*): this particular clade was not recovered in the analyses.

TIM2+G for *28S*; TrNef+I+G for *H3* and HKY+G for *ITS-2*. The Bayesian inference analyses were run during 4 million generations and the first 10% were discarded as burn-in. Maximum likelihood analyses yielded one tree of logL −2713.866745. All analyses agreed in supporting the monophyly of the eastern Canarian endemics, with the exclusion of *D. lancerotensis*, and in recovering the same internal topology. The only source of conflict across the different analyses was the position of *D. sanborondon*, which was a sister to *D. simbeque* sp. nov. + *D. alegranzaensis* in the parsimony analyses and sister to the remaining taxa in the model-based analyses, although both alternative positions were poorly supported (Fig. 29). PBS values (Table 9, see supplementary material which is available on the Supplementary tab of the article's Informaworld page at http://www.informaworld.com/mpp/uploads/tsab. . .) indicated low levels of character conflict across partitions (the largest negative value for the mitochondrial data set was −4 found in clade 4, and the largest nuclear −2, in clade 8).

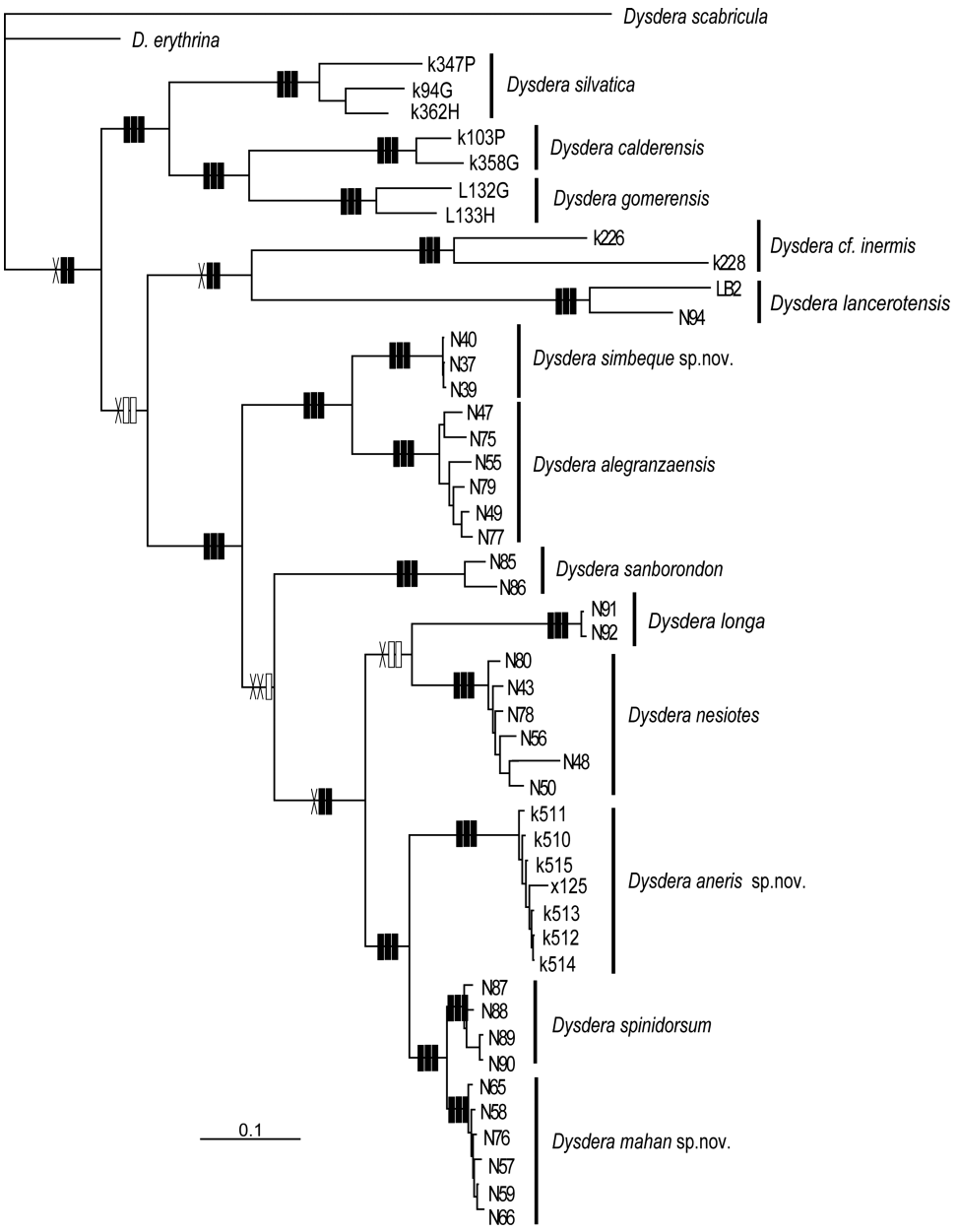


Fig 30. Bayesian majority rule consensus tree of the combined mitochondrial genes. Bars on branches denote support as follows: anterior bar refers to parsimony jackknife support, middle bar to maximum likelihood bootstrap support and posterior bar to posterior probability. Black bar: parsimony jackknife or ML bootstrap >70%, posterior probability >0.95%; white bar: parsimony jackknife or ML bootstrap <70%, posterior probability <0.95%; asterisk (*): this particular clade was not recovered in the analyses.

The ILD test revealed the existence of significant incongruence between the mitochondrial and nuclear datasets ($P = 0.001$). Visual inspection of the trees obtained from the independent analyses of the mitochondrial and nuclear genes revealed that the main source of conflict is the position of the species *D. longa*, which forms a monophyletic group with *D. nesiotes*, *D. aneris* sp. nov., *D. spinidorsum* and *D. mahan* sp. nov. based on the mitochondrial data (Fig. 30), and it is sister to *D. simbeque* sp. nov. + *D. alegranzaensis* according to the nuclear genes (Fig. 31). Another relevant topological difference between the two partial analyses is the position of *D. aneris* sp. nov., which is sister to *D. spinidorsum* + *D. mahan* sp. nov. according to mitochondrial data, but is a sister to *D. nesiotes* based on the nuclear genes. In addition, the nuclear genes did not support monophyly of the genotypes of *D. mahan* sp. nov., *D. spinidorsum*, *D. aneris* sp. nov. and *D. nesiotes*, and some of these species did, in fact, share nuclear genotypes.

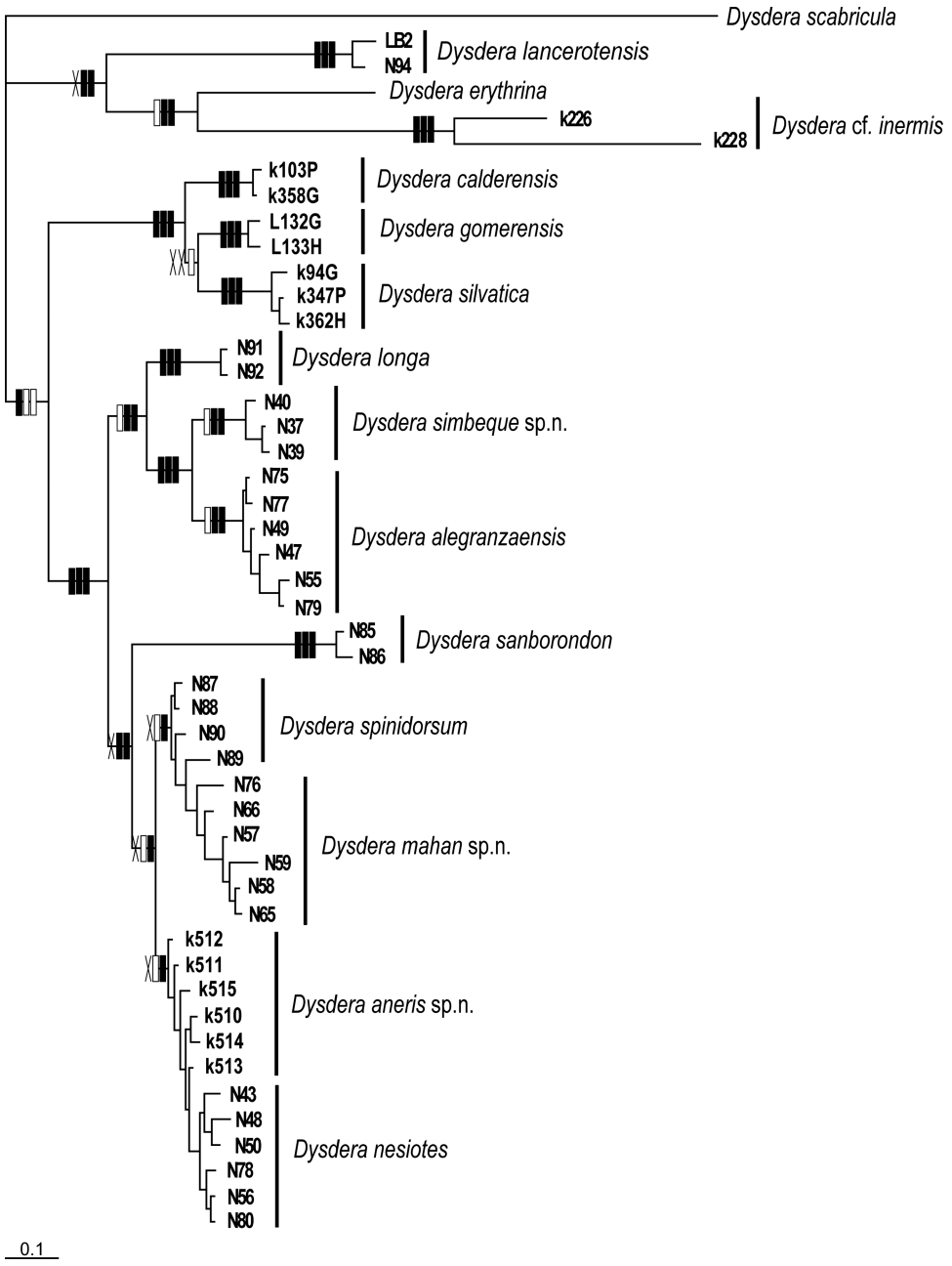


Fig 31. Bayesian majority rule consensus tree of the combined nuclear genes. Bars on branches denote support as follows: anterior bar refers to parsimony jackknife support, middle bar to maximum likelihood bootstrap support and posterior bar to posterior probability. Black bar: parsimony jackknife or ML bootstrap > 70%, posterior probability > 0.95%; white bar: parsimony jackknife or ML bootstrap < 70%, posterior probability < 0.95%; asterisk (*): this particular clade was not recovered in the analyses. **Q4**

Support for the conflicting topologies involving the last species, however, was very low in all analyses, and the ILD test applied only to these species failed to detect significant incongruence between the two partitions ($P = 0.1918$).

Comparisons of uncorrected genetic distances (*P*-values) between eastern Canarian species indicated that *D. spinidorsum* and *D. mahan* sp. nov. exhibited the lowest levels of genetic divergence for all gene fragments. Uncorrected pairwise distances within and between species revealed higher divergence levels for the mtDNA than for the nuclear *ITS-2* gene (Table 10, see supplementary material which is available on the Supplementary tab of the article's Informaworld page at http://www.informaworld.com/mpp/uploads/tsab. . .).

Divergence times

Preliminary cross-validation analyses selected the Langley-Fitch as the best clock method for analysing the mitochondrial data set. The clade age estimates and corresponding

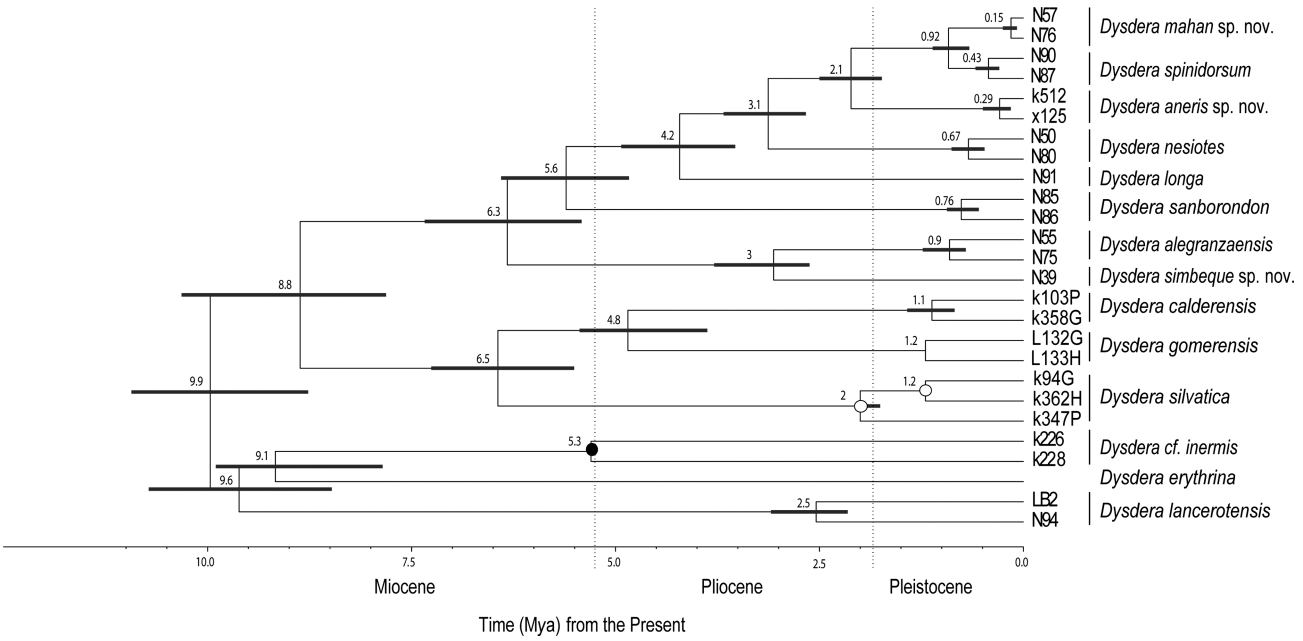


Fig 32. Chronogram obtained by the Langley–Fitch clock method based on the preferred Bayesian topology obtained by the simultaneous analyses of the *cox1*, *16S*, *nad1*, *28S* and *H3* partitions. Numbers on nodes are estimated lineage age and bars are confidence intervals based on bootstrap resampling of branch lengths. Open circles and filled circles correspond to maximum and fixed calibration points, respectively (see text for details).

confidence intervals are summarized in Fig. 32. The average rate of substitution estimated was 0.04916 per site per million years, corresponding to a pairwise genetic divergence of 9.8%, which is five times faster than the rates estimated by Macías-Hernández *et al.* (2008) using a single calibration point (1.75% pairwise divergence), and four times faster than universal rates reported for arthropod mitochondrial genes (DeSalle *et al.*, 1987; Brower, 1994), though it is similar to the rates estimated for *Dysdera lancerotensis* (10.2%) (Bidegaray-Batista *et al.*, 2007). In spite of the higher substitution rates, the estimated divergence time in the present study largely overlapped with the confidence intervals of the estimates made by Macías-Hernández *et al.* (2008).

Analysis of morphological variation

Few variables exhibited evidence of sexual dimorphism across the species examined (Fe1, Esc and Fang in *D. spinidorsum*; Fe1 in *D. alegranzaensis*; Fe1 and Q2 in *D. aneris* sp. nov.; and Fe1 and Mt4 in *D. longa*). We interpreted significant sex differences among the variables as random sampling variability due to the lack of a general pattern of sexual dimorphism, although some caution should be exercised regarding the Fe1 (significantly different between sexes in four species). Further analyses were conducted considering individuals of both sexes of each species. The Kruskal–Wallis test revealed significant interspecific differences in all morphological variables. The Wilcoxon matched pairs test detected significant differences in body size (P1) between all species compared except for the species pairs: *D. alegranzaensis – D. nesiotes* ($Z = 0.459$; $P = 0.65$), *D. alegranzaensis – D. aneris* sp. nov. ($Z = 1.172$; $P = 0.24$) and *D. mahan* sp. nov. – *D. spinidorsum* ($Z = 0.178$; $P = 0.859$). The first PCA axis accounted for 31.41% of the variance and was associated with the chelicera and fang lengths. The second axis accounted for 58.52% of the total variance and was associated with appendage length (Fe1, Mt4; Fig. 33). In the PCA plot, *D. mahan* sp. nov. clearly stands apart from all the other species. The Bray–Curtis similarity cluster defines four main groups: small species (*D. sanborondon*), medium-sized species (*D. alegranzaensis*, *D. nesiotes* and *D. aneris* sp. nov.), which is a large species (*D. longa*, *D. spinidorsum* and *D. simbeque* sp. nov.) and *D. mahan* sp. nov., a large species with long appendages (Fig. 34).

Discussion

Morphology represented the single major source of evidence to delimit species boundaries until the mid-20th century (Coyne, 1994). However, relying solely on morphological characters tends to oversimplify and underestimate diversity (Bond *et al.*, 2001; Bickford *et al.*, 2007). Continuous or barely partitioned diversity (i.e. polymorphism), low variability (cryptic species), sex- or life stage-restricted

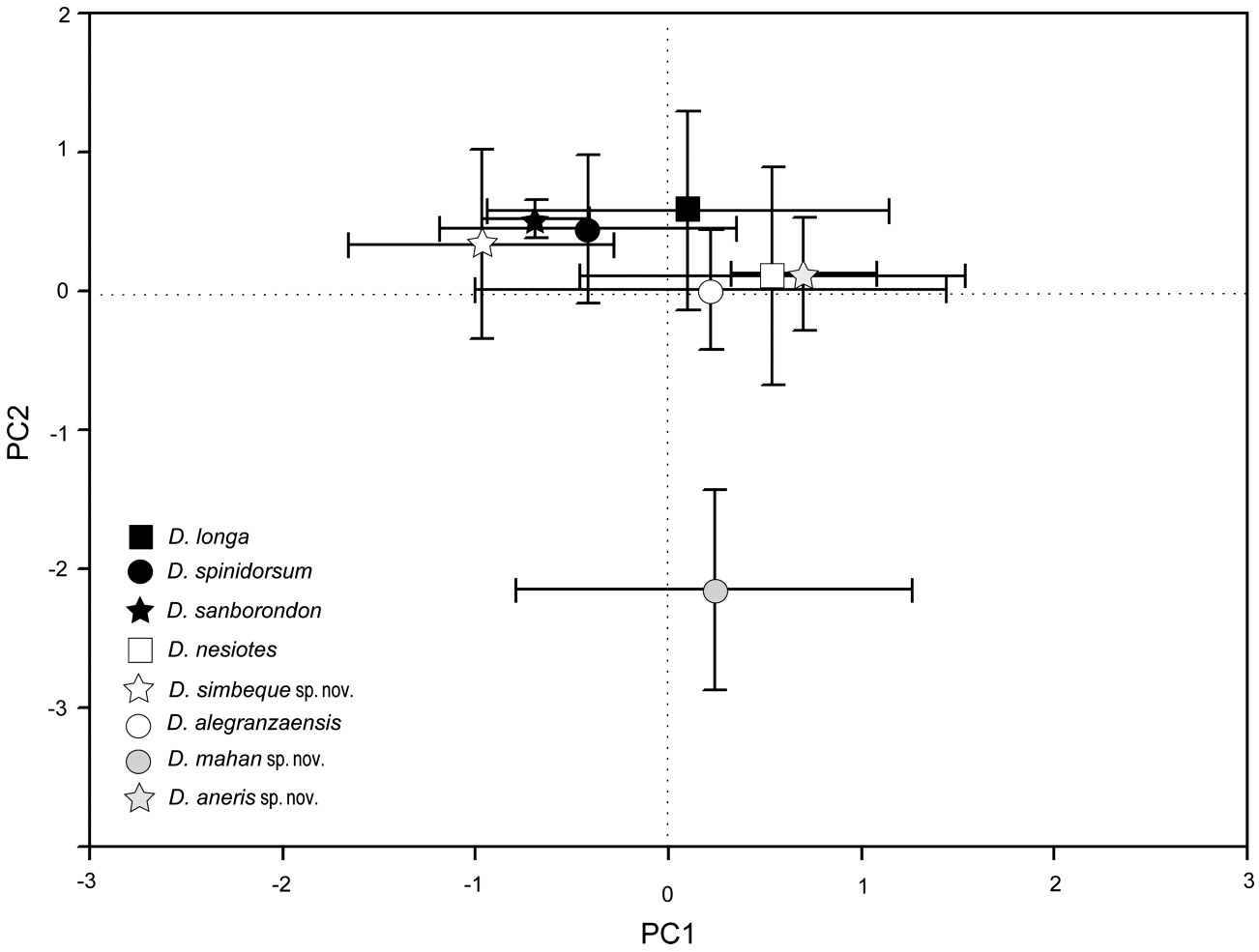


Fig 33. Principal components analysis. Plot of the first two discriminant axes, with the 95% of score variance on each axis.

diagnosis, or simple lack of expertise may hinder the use of morphology for species delimitation.

Species may arise through a plethora of different processes and circumstances, making species recognition and delimitation a daunting task based on any single data set. In recent years, there is an emerging consensus that the multifaceted nature of species can only be understood by reconciling evidence provided by multiple independent disciplines. The term 'integrative taxonomy' has been coined to refer to such a combined approach (Dayrat, 2005; Padial *et al.*, 2010; Schlick-Steiner *et al.*, 2010).

A former taxonomic revision of the endemic eastern Canarian *Dysdera* highlighted an unusual variability in the vulval morphology of the species *D. nesiotes* (see figs. 59–62 in Arnedo *et al.*, 2000), which was interpreted as intraspecific polymorphism. In the context of a molecular phylogeny of the group, Macías-Hernández *et al.* (2008) pointed out that *D. nesiotes* might actually include three independent lineages that were overlooked in the former revisionary work. The morphological study of a larger sample of specimens in combination with molecular data, geographic distribution and ecological preference data has now allowed us to reinterpret the morphological polymorphism observed in these species and to delimit and formally describe three new taxa.

Genetic divergence is not necessarily related to morphological differentiation (e.g. Orr & Smith, 1998). Significant genetic divergences may underlie morphologically homogeneous species, as seen in cryptic species complexes (Bond *et al.*, 2001; Hedin & Wood, 2002; Sinclair *et al.*, 2004; Boyer *et al.*, 2007). However, in adaptive radiations, remarkable phenotypic differentiation may occur in the absence of clear genetic discontinuities, as exemplified by the African cichlid fishes (Nagl *et al.*, 1998). Of the three new species described here, *D. mahan* sp. nov. exhibits the greatest morphological differences compared with its sister taxa (see Figs 33, 34). However, these two species exhibit the lowest number of pairwise genetic divergences of any sister species pair (3.6% in mtDNA), and both share common alleles in *ITS-2* and *H3*. The low degree of genetic differentiation suggests a relatively recent time of divergence, further confirmed by the molecular clock dating (0.9 Mya, 0.6–1.6 Mya), representing the most recent

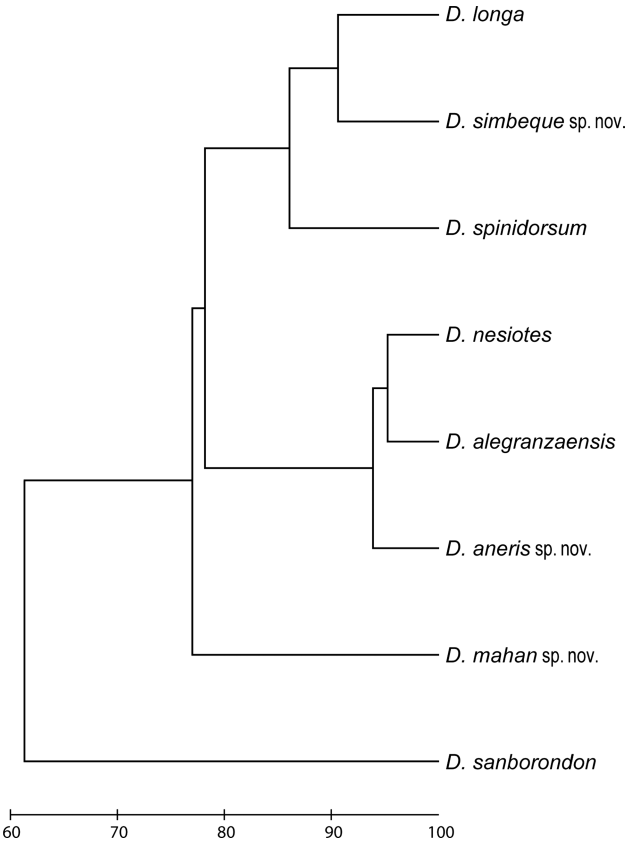


Fig 34. Dendrogram showing species similarity scores derived from somatic measurements estimated across a hierarchical agglomerative cluster using the Bray–Curtis distance.

speciation event in the group. Newly formed species achieve genealogical exclusivity in mitochondrial DNA long before they become distinct in nuclear markers, due to higher substitution rates and a smaller effective population size (Moore, 1995). Therefore, the retention of unsorted ancestral polymorphism seems the most plausible explanation for the lack of genealogical exclusivity in the nuclear genes. The habitat occupied by *D. mahan* sp. nov., the intertidal zone in pebble beaches, is unique among the Canarian *Dysdera* and represents the only true case of an ecological shift in this large species radiation. The decoupling of genetic divergence and morphological differentiation observed in this case could be the result of a natural selection acting on phenotypic traits that are adaptive for this unusual environment (Dieckmann, 2004). In spiders, genitalia are the most important source of diagnostic characters, which implies that genitalia have diverged rapidly relative to other structures (Eberhard, 1985). However, the absence of diagnostic characters in the male bulb of *D. mahan* sp. nov. compared with *D. nesiotes* and *D. aneris* sp. nov. provides evidence for the rapid evolution of somatic morphology in the former species and, hence, for the involvement of natural selection.

Dysdera aneris sp. nov. lies at the other end of a putative gradient of negatively correlated genetic and morphological differentiation. This species is well characterized from a genetic standpoint, shows high mtDNA pairwise divergences compared with closely related taxa (7.3–10%), and all its mtDNA and nuclear alleles are exclusive (except *28S*). Nevertheless, this species had been previously overlooked and misidentified as *Dysdera nesiotes*, which is not even its sister taxa according to the mitochondrial data. In fact, molecular markers provided crucial evidence to diagnose the new species based on vulval characters that were previously misinterpreted as intraspecific polymorphism. Significant molecular divergences in the absence of morphological changes are common in spider groups with poor dispersal capabilities, which therefore, have geographically isolated populations that are subject to similar environmental conditions (Bond *et al.*, 2001; Hedin, 2001). An infrequent, long-range dispersal event allowed *D. aneris* sp. nov. to diverge in isolation on an island with a similar environment and selective forces to the ancestral area, which preserved the original phenotype. Molecular dating puts an upper limit on the colonization of the Salvage Islands by *Dysdera* of approximately 2.1 Mya (1.5–2.5), similar to what has been found in the wall lizard *Teira dugesii* (Brehm *et al.*, 2003). This data corroborates a previous suggestion regarding the late Pliocene origin of the present-day biota of the Salvage Islands, following subaerial volcanism after island submergence (Geldmacher *et al.*, 2001).

The third new species described in this paper, *D. simbeque* sp. nov. co-exists on the same island with its sister species, although they do not overlap in their distribution range. These two species significantly differ from each other in body size and exhibit slight genitalia differences. Size segregation in these species is difficult to explain because the two species share the same ecological regime and are both syntopic with a third endemic, *D. nesiotes*. The significantly larger size of *D. simbeque* sp. nov. compared with *D. nesiotes* is in accordance with the expectations of trait segregation in sympatric species to avoid resource competition. The range size of *D. nesiotes*, on the other hand, fully overlaps with that of *D. alegranzaensis*. A comparative phylogeographic study of the two species is currently underway to investigate the patterns of vicariance and secondary sympatry (Macías-Hernández *et al*., in prep) that may explain the coexistence of close relatives with virtually identical morphology.

The use of DNA sequences as a complement to traditional morphology-based taxonomy represents an invaluable resource for phylogenetics (Tautz *et al.*, 2003). In addition, the advisable use of multiple loci for species delimitation improves the chances of obtaining more reliable trees (Maddison, 1997), and the patterns of congruence among multiple unlinked loci offer insights into relevant evolutionary processes, such as hybridization and introgression

(Funk & Omland, 2003). Our results illustrate that the simultaneous analysis of mitochondrial and nuclear genes improves the resolution of the tree topology and increases clade support. The ILD test, however, revealed significant incongruence between mtDNA and nDNA genes. The main areas of disagreement are the position of *D. longa* and *D. aneris* sp. nov., although an ILD test run on the clade including the species *D. aneris* sp. nov., *D. nesiotes*, *D. spinidorsum* and *D. mahan* sp. nov. was not significant. Instances of incongruence between mtDNA and nDNA genes are not uncommon among arthropods and other organisms (Funk & Omland, 2003). Several causes have been put forward to explain the apparent incongruence between mitochondrial and nuclear markers, including incomplete lineage sorting of ancestral polymorphisms and introgressive hybridization (Maddison, 1997), homoplasy in the data (Baker & DeSalle, 1997), and differences in analytical and methodological procedures (Brower, 1996). Although shared ancestral polymorphism and hybridization are difficult to detect in the first stages of the speciation process (Sota & Vogler, 2001), hybridization is more a likely cause of incongruence if populations co-occurred in sympatry (Rokas *et al.*, 2003). However, all the species investigated in this study with present-day overlapping distributions were found to be genealogically exclusive in both mitochondrial and nuclear markers. *Dysdera nesiotes*, on the other hand, is paraphyletic to *D. aneris* sp. nov. based on nuclear markers, but the two species are separated by more than 165 km of open sea, which rules out the involvement of hybridization. The patterns of non-monophyly in nuclear markers suggest that incomplete lineage sorting may be a better explanation of the incongruence between genome partitions. The independent networks of each nuclear gene exhibited incongruent patterns of reticulation and allele sharing among the species *D. nesiotes*, *D. aneris* sp. nov., *D. spinidorsum* and *D. mahan* sp. nov. The sister-group relationship of *D. aneris* sp. nov. and *D. nesiotes*, which was supported by nuclear genes, could, hence, be the result of the retention in *D. nesiotes* and *D. aneris* sp. nov. of nuclear alleles already present in the common ancestor shared with closer relatives. A similar argument could be invoked to explain the incongruent position of *D. longa* in the trees recovered from each genome partition. Incomplete lineage sorting has been proposed in a variety of organisms as the main factor causing incongruence between species and gene trees and among unlinked loci (Funk & Omland, 2003). The faster rate of fixation of ancestral mtDNA polymorphisms compared with nuclear genes suggests that mitochondrial gene trees are more likely to reflect true species relationships than a nuclear-encoded gene (Moore, 1995, 1997). However, in the presence of gene flow between diverging populations, mtDNA may be homogenized between the populations more readily than nuclear DNA, and thus, mtDNA may appear to be paraphyletic when nuclear genes may be monophyletic (Ballard & Rand, 2005).

In the present study, the combined analyses used resolve instances of incongruence between genomic partitions mostly in favour of the mitochondrial partition. This may simply reflect the larger amount of variable characters in the mtDNA partition, as suggested by the higher pairwise divergences observed (Table 10, see supplementary material which is available on the Supplementary tab of the article's Informaworld page at http://www.informaworld.com/mpp/uploads/tsab. . .).

Nevertheless, the number of nodes in the combined tree with negative PBS values is low, and it is similar in both genomic partitions (2 and 3 for mitochondrial and nuclear genes, respectively (Table 9, see supplementary material which is available on the Supplementary tab of the article's Informaworld page at http://www.informaworld.com/mpp/uploads/tsab. . .).

In fact, only the *H3* dataset shows negatively partitioned Bremer support values at combined topology node 15 (Fig. 32), which is the major area of disagreement between partitions, while the other nuclear partitions are either positive or zero at this node. The nuclear genes may not actually be as incongruent with the mitochondrial genes as suggested by the ILD. It has been shown that the ILD test is prone to reporting significant conflict between character partitions when they differ only in the amount of noise (Quicke *et al.*, 2007), there are few characters, or the substitution rate is not homogeneous (Darlu & Lecointre, 2002). However, the high support for the conflicting clade between the genome partitions suggests otherwise (Mason-Gamer & Kellogg, 1996).

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