PENSOFT
SENCKENBERG world of biodiversity
Leibniz Association

Phylogeographic analyses of western Palearctic *Scaurus* (Coleoptera: Tenebrionidae) reveal undetected taxonomic substructure along the pre-Saharian Atlantic Coast of western Africa

Carmen Duque-Amado[1], Mario García-París[1], Alberto Sánchez-Vialas[1]

1 *Museo Nacional de Ciencias Naturales (MNCN-CSIC), c/ José Gutiérrez Abascal 2, 28006, Madrid, Spain*

https://zoobank.org/4CAF4C53-004E-4EE0-B502-7242A1BA2660

Corresponding author: Carmen Duque-Amado (carmen.duque@mncn.csic.es)

Received 19 July 2024
Accepted 16 October 2024
Published 27 December 2024

Academic Editors Anna Hundsdörfer, Michael Schmitt

Citation: Duque-Amado C, García-París M, Sánchez-Vialas A (2024) Phylogeographic analyses of western Palearctic *Scaurus* (Coleoptera: Tenebrionidae) reveal undetected taxonomic substructure along the pre-Saharian Atlantic Coast of western Africa. Arthropod Systematics & Phylogeny 82: 707–717. https://doi.org/10.3897/asp.82.e132546

Abstract

Phylogeography stands as a key tool to explore evolutionary patterns and processes, playing a crucial role in delimiting evolutionary units. Identifying these units is essential for providing robust taxonomic decisions. In this study, we present a comprehensive phylogeographical framework of *Scaurus uncinus* (Forster, 1771) and *Scaurus gigas* Waltl, 1835 across the Iberian Peninsula and North-western Africa, where they are widely co-distributed, coexisting in several localities on both sides of Strait of Gibraltar. Our results show that the Strait of Gibraltar did not act as a geographical barrier for these species, revealing shared mitochondrial haplotypes and nuclear alleles between populations on both sides. However, the Souss Valley in Morocco appears to have historically served as a significant geographical barrier within the *S. gigas* lineage, leading to the divergence of two morphologically distinct sublineages, one to the north (*S. gigas*) and the other to the south (*S. ferreri* stat. nov.). In addition, we point out a case of cytonuclear discordance between *S. uncinus* and *S. gigas* in the southwest corner of the Iberian Peninsula, suggesting the occurrence of an ancient event of genetic introgression between the two species.

Keywords

Allele networks, cyto-nuclear discordances gene flow, genetic isolation, haplotype networks, introgression, mitochondrial DNA

1. Introduction

Understanding the patterns and processes underlying the origin and distribution of lineages, both at intra- and supra- specific levels, is fundamental for testing taxonomic hypotheses (Szymura and Barton 1986; Babik et al. 2005; Dufresnes and Martínez-Solano 2020; Sánchez-Vialas et al. 2020, 2021; Kamiński et al. 2021, Sainz-Escudero et al. 2022). In this context, phylogenetic and phylogeographic analyses have become key tools for delimiting evolutionary units, contribute to resolving long-standing taxonomic problems (Mas-Peinado et al. 2021; Sainz-Escudero et al. 2021; Cuesta-Segura et al. 2023).

Complex evolutionary dynamics, encompassing hybridization among morphologically similar species or lineages have usually driven to intense taxonomic debates (Dufresnes et al. 2020; Doniol-Valcroze et al. 2021). Gene

Copyright *Carmen Duque-Amado* et al.: This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

flow across well-defined species can determine different outcomes along the speciation continuum, including reinforcement (strengthening preexisting reproductive isolating mechanisms between lineages) (Servedio and Noor 2003; Pfenning 2016), melting pots (Dinis et al. 2019), lineage fusion (Taylor et al. 2006), instant speciation (Ramsey and Schemske 1998; Gonçalves and Brito 2020), and introgression (Zieliński et al. 2013; Racimo et al. 2015). Within this context, the understanding of these processes is required to perform solid taxonomic revisions.

The extend of hybrids zones and the fate of the parental lineages depends on the strength of the barriers to gene flow (Dufresnes et al. 2020). In this sense, restricted and localized introgression is consistent with strong selection against hybrids, which also is of paramount importance to determine the taxonomic status of the evolving lineages (Dufresnes et al. 2020). The hallmark of gene flow between closely related species can be noticed as cyto-nuclear discordances (Wielstra and Arntzen 2020). This phenomenon is well-documented in regions of climatic instability, where range shifts promote secondary contact and lineages admixture (Hewitt 1999, 2004). This is the case of the western Palearctic, where cyclic climatic oscillations during the Pleistocene had a significant influence on the genetic diversity and distribution of the local biota (Hewitt 2004; Gómez and Lunt 2006). The integration of phylogeographic hypotheses and congruence analysis enables more accurate taxonomic investigations of morphologically difficult to interpret groups.

Scaurus Fabricius, 1775 (Coleoptera: Tenebrionidae) is a group of thermophilic darkling beetles comprised by 25 species widespread through the Mediterranean basin, with extensions into the Atlantic and Saharan regions (Escalera 1914; Labrique 2009, 2020; Iwan and Löbl 2020, Nabozhenko et al. 2020). Some species of this genus exhibit partially overlapping distributions, making them an excellent system for comparative phylogeographic studies. This applies to *Scaurus uncinus* (Forster, 1771) and *S. gigas* Waltl, 1835, two species with partially sympatric distributions across both sides of the Gibraltar Strait (Cartagena et al. 2002; Cartagena and Galante 2005; Español 1957, 1960, 1963; Labrique 1995a, 1995b). The former species extends over the western Mediterranean basin and the Atlantic coast of Morocco, whereas the latter, *S. gigas*, is restricted to the Atlantic coastal plains from the Oued Draa in Morocco to the southwestern corner of the Iberian Peninsula (Español 1960; Labrique 2004). Although no seasonal differences have been identified between these species, habitat segregation may be occurring; *S. gigas* inhabits sandy coastal areas and does not venture into inland regions, whereas *S. uncinus* occupies a broader range of open habitats and is rarer in coastal plains (Español 1960; López-Pérez 2010). Idiosyncratic patterns of distribution across the Gibraltar Strait have been reported in ecologically similar species of Tenebrionoidea, such as in *Pimelia* Fabricius, 1775, in which for some species the Strait of Gibraltar act as a geographical barrier but clearly not for others (Mas-Peinado et al. 2021). However, whether this barrier accounts for phylogeographical structuration in *Scaurus* is unknown.

The genus *Scaurus* is characterized by a conserved general morphology, which resulted in a complex taxonomic history for several species, including *S. uncinus* and *S. gigas* (see Labrique 2004). Whereas *S. uncinus* presents a relatively uniform morphology across most of its distribution, the southern Moroccan populations of *S. gigas* are diagnosable from other conspecifics (Español 1943, 1960). These populations were initially classified as subspecies, named *Scaurus uncinus ferreri* Español, 1943, based on specimens from Sidi Ifni, but were later transferred as a synonym of *S. gigas* (Labrique 2004). Currently, *S. gigas* encompasses two additional synonyms from northern Morocco: *Scaurus hespericus* (Solier, 1838) (Type locality: Tanger, Mahon and Gibraltar) and *Scaurus maroccanus* (Fairmaire, 1875) (Type locality: Mogador). Despite their partly overlapping geographic ranges on either sides of the Gibraltar Strait, no comparative phylogeographic studies have been conducted between the species pair *S. uncinus* and *S. gigas*, and consequently, their evolutionary history remains enigmatic. Given these shortfalls, a revision of the diversity and distribution of *S. uncinus* and *S. gigas* across most of their range is needed.

In this work, we perform comparative phylogeographic analyses by combining mitochondrial and nuclear gene fragments of *S. uncinus* and *S. gigas* sampled over an extensive area that covers most of their respective distribution ranges. We aim to explore (1) the intraspecific patterns of mitochondrial and nuclear genetic variation in both species, (2) the existence of shared phylogeographic patterns and barriers to gene flow, (3) the strength of the reproductive barriers between these pairs of species, and (4) the nature of the morphological differentiation among the populations of *S. gigas*.

2. Materials and methods

2.1. DNA extraction, sequencing, and sequence alignment

Thirty-seven specimens of *S. gigas* (N=14), *S. ferreri* (N=3), and *S. uncinus* (N=20) from the Iberian Peninsula and Morocco were preserved in 96° ethanol at 6°C for molecular DNA analyses. All specimens were injected with 96° ethanol before preservation. The studied specimens have been deposited in the entomological collection of the Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain.

Genomic DNA was extracted from leg tissue using the Qiagen DNeasy commercial kit. PCR was used to amplify fragments of the cytochrome B (Cyt *b*) (30 specimens) and the nuclear internal transcribed spacer (ITS2) (33 specimens) genes. Although attempts were made to amplify genetic material from all 37 specimens, complete success was not achieved across all specimens. The Cyt *b* was amplified using the set of primers CB-J-10933/CB-N-11367 (Kergoat et al. 2014) and CB-3/CB-4 (Barra-

clough et al. 1999), whereas the ITS2 was amplified using the set of primers CAS28sB1d and CAS5p8sFt (Ji et al. 2003). PCRs were performed in 25 µl, including 23 µl of the PCR products (15.6 µl of H_2O, 2.5 µl of a reaction buffer with $MgCl_2$ [2 mM], 1 µl of dNTP [10 mM], 2.5 µl of $MgCl_2$ [50 mM], 0.5 µl of each primer [10 mM], and 0.2 µl of Taq polymerase [5U/mL]) and 2 µl of DNA. PCR conditions for Cyt *b* with the set of primers CB-J-10933/CB-N-11367 were as follows: 3 min at 94°C, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min, with a final single extra extension step at 72°C for 10 min; and with the set of primers CB-3 and CB-4: 3 min at 94°C, followed by 5 cycles of denaturation at 92°C for 1 min, annealing at 38°C for 1 min and extension at 72°C for 1 min, followed by 40 cycles of denaturation at 92°C for 1 min, annealing at 48°C for 1 min and extension at 72°C for 1 min, with a final single extra extension step at 72°C for 10 min. For ITS2 PCR conditions were: 5 min at 96°C, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min, with a final single extra extension step at 72°C for 10 min. PCR products were checked in a 1.5% agarose gel and the products of expected length were purified and directly sequenced at Macrogen Inc. (Macrogen Europe, Madrid, Spain).

2.2. Haplotype and allelic networks

Sequences were collapsed into haplotypes using ALTER (Glez-Peña et al. 2010). Haplotype (Cyt *b*) and allele (ITS2) networks were built using a minimum spanning network (Bandelt et al. 1999) and Farris's maximum-parsimony (MP plotted in Pop Art (Leigh and Bryant 2015).

3. Results

The studied populations of *Scaurus gigas* from the Iberian Peninsula and North Africa are represented by 12 Cyt *b* haplotypes, three are only found in the Iberian Peninsula, eight exclusively in Morocco, and a single one is shared at both sides of the Strait of Gibraltar (Fig. 1; Table 1). The studied populations from Aglou and Deghaimis (Morocco) present two haplotypes separated between each

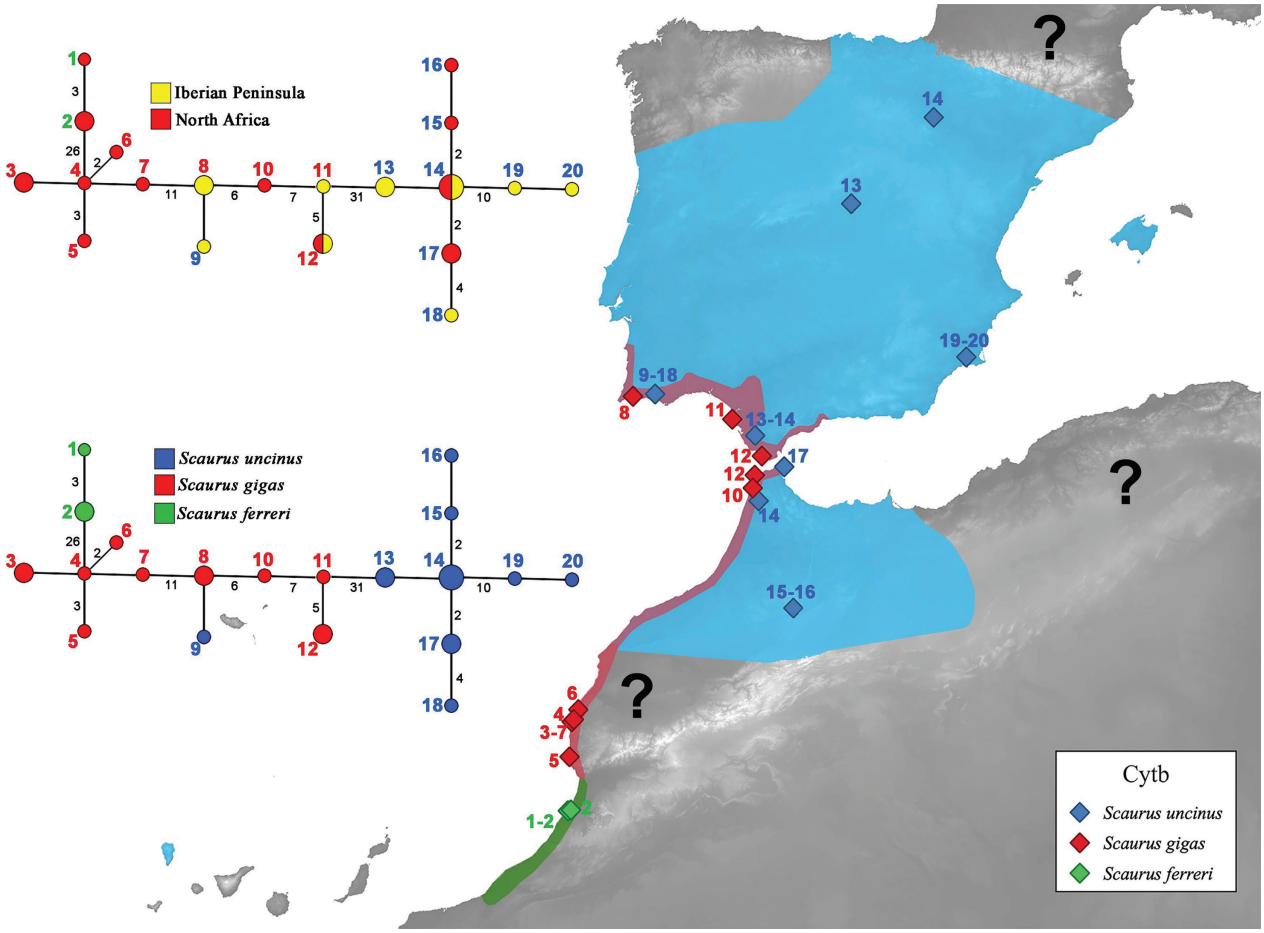

Figure 1. Haplotype network based on Cyt *b* haplotypes for *Scaurus gigas* (Waltl, 1835) (red), *Scaurus uncinus* (Forster, 1771) (blue), and *Scaurus ferreri* Español, 1960 (green). Inferred intermediate haplotypes are counted with black numbers. Colors correspond to the geographic origin of the specimens. The size of the circles indicates the relative frequency of sequences belonging to a particular allele. The presence of *S. uncinus* outside the blue shaded area requires confirmation because it is easy to be confused with other species of similar morphology and the records are old and imprecise.

Table 1. Species identities, collecting localities, voucher numbers and GenBank accession numbers for the samples analyzed in this study.

Code	Species	Locality	Coordinates	Haplotype (Cyt *b*)	Cyt *b* Genbank code	Allele (ITS2)	ITS2 Genbank code
CDAS0047	*Scaurus gigas*	Morocco: Essaouira: Ouassane – Sidi Kaouki	31º22′48″N, 9º47′37″W	4	P0438088	1	PQ435559
CDAS0048	*Scaurus gigas*	Morocco: Essaouira: Ouassane – Sidi Kaouki	31º22′48″N, 9º47′37″W	6	P0438089	1	PQ435560
CDAS0050	*Scaurus gigas*	Portugal: Faro: Lagos: Meia Praia	37º06′59″N, 8º39′08″W	8	P0438082	3	PQ435566
CDAS0051	*Scaurus gigas*	Portugal: Faro: Lagos: Meia Praia	37º06′59 “N, 8º39′08″W	8	P0438083	3	PQ435567
CDAS0112	*Scaurus gigas*	Spain: Cádiz: Chipiona	36°44′12″N, 6°25′45″W	11	P0438085	—	—
CDAS0114	*Scaurus gigas*	Spain: Cádiz: Playa de Bolonia	36°5′17″N, 5°46′21″W	12	P0438086	3	P0435568
CDAS0115	*Scaurus gigas*	Morocco: Tanger: Cap Espartel	35º45′5″N, 5º55′56″W	12	P0438087	—	—
CDAS0117	*Scaurus gigas*	Morocco: Tnine Chtouka (Tnine-des-Ghtouka)	33°18′5″N, 8°9′18″W	—	—	3	PQ435569
CDAS0118	*Scaurus gigas*	Morocco: between Diet and Larache	35º31′34″N, 5º58′42″W	10	P0438084	—	—
CDAS0119	*Scaurus gigas*	Morocco: Road Essaouira-Moulay Bouzerktoun	31º35′46″N, 9º39′48″W	3	P0438090	1	P0435561
CDAS0120	*Scaurus gigas*	Morocco: 4 km S El Ghazoua	31º25′09″N, 9º45′10″W	3	P0438091	1	PO435562
CDAS0121	*Scaurus gigas*	Morocco: 4 km S El Ghazoua	31º25′09″N, 9º45′10″W	7	P0438092	1	PQ435563
CDAS0124	*Scaurus gigas*	Morocco: 3 km SW Afra (Imsouane)	30º45′52″N, 9º49′16″W	5	P0438093	2	PQ435564
CDAS0125	*Scaurus gigas*	Morocco: 3 km SW Afra (Imsouane)	30º45′52″N, 9º49′16″W	—	—	2	P0435565
CDAS0129	*Scaurus ferreri*	Morocco: Aglou Beach	29°49′05″N, 9°49′31″W	1	P0438094	4	PQ435570
CDAS0130	*Scaurus ferreri*	Morocco: Aglou Beach	29°49′05″N, 9°49′31″W	2	P0438095	4	PQ435571
CDAS0133	*Scaurus ferreri*	Morocco: Deghaimis	29º49′59″N, 9º45′55″W	2	PQ438096	4	P0435572
CDAS0001	*Scaurus uncinus*	Spain: Cadiz: Medina Sidonia	36º26′59″N, 5º55′8″W	13	P0438097	5	P0435573
CDAS0002	*Scaurus uncinus*	Spain: Cadiz: Medina Sidonia	36º26′59″N, 5º55′8″W	14	P0438104	5	P0435574
CDAS0006	*Scaurus uncinus*	Spain: Madrid: Sanchinarro, Valde-fuentes	40°30′07″N, 3°38′33″W	13	P0438099	5	PO435575
CDAS0007	*Scaurus uncinus*	Spain: Madrid: Sanchinarro, Valde-fuentes	40°30′07″N, 3°38′33″W	13	P0438098	6	P0435581
CDAS0008	*Scaurus uncinus*	Spain: Murcia: Fuente Álamo de Murcia	37º43′12″N, 1º08′02″W	19	P0438109	5	P0435576
CDAS0009	*Scaurus uncinus*	Spain: Murcia: Fuente Álamo de Murcia	37º43′12″N, 1º08′02″W	20	PQ438110	8	P0435584
CDAS0013	*Scaurus uncinus*	Spain: Navarra: Ablitas	41º57′43″N, 1º36′12″W	—	—	5	P0435577
CDAS0014	*Scaurus uncinus*	Spain: Navarra: Ablitas	41º57′43″N, 1º36′12″W	14	P0438105	9	P0435585
CDAS0017	*Scaurus uncinus*	Morocco: E of Ifrane	33°28′38″N, 5°01′39″W	—	—	10	P0435586
CDAS0018	*Scaurus uncinus*	Morocco: E of Ifrane	33°28′38″N, 5°01′39″W	—	—	10	P0435587
CDAS0024	*Scaurus uncinus*	Morocco: Tanger	35º17′58″N, 5º51′02″W	14	P0438101	7	P0435582
CDAS0025	*Scaurus uncinus*	Morocco: Tanger	35º17′58″N, 5º51′02″W	14	P0438100	7	PQ435583
CDAS0027	*Scaurus uncinus*	Portugal: Faro: Loulé: Lombada	37º09′59″N, 8º09′30″W	9	P0438081	5	P0435578
CDAS0028	*Scaurus uncinus*	Portugal: Faro: Loulé: Lombada	37º09′59″N, 8º09′30″W	18	P0438106	5	PQ435579

Code	Species	Locality	Coordinates	Haplotype (Cyt *b*)	Cyt *b* Genbank code	Allele (ITS2)	ITS2 Genbank code
CDAS0029	*Scaurus uncinus*	Morocco: 15 km S-SW Timahdite	33°24′20″N, 5°07′17″W	15	P0438107	—	—
CDAS0030	*Scaurus uncinus*	Morocco: 15 km S-SW Timahdite	33°24′20″N, 5°07′17″W	16	P0438108	10	PQ435588
CDAS0109	*Scaurus uncinus*	Spain: Ceuta: Cala Desnarigado, Monte Hacho	35°53′45″N, 5°16′54″W	17	P0438102	5	P0435580
CDAS0110	*Scaurus uncinus*	Spain: Ceuta: Cala Desnarigado, Monte Hacho	35°53′45″N, 5°16′54″W	17	P0438103	10	PQ435589
CDAS0092	*Scaurus uncinus*	Morocco: El Hajeb	33°42′10″N, 5°21′30″W	—	—	11	PQ435591
CDAS0093	*Scaurus uncinus*	Morocco: El Hajeb	33°42′10″N, 5°21′30″W	—	—	10	P0435590

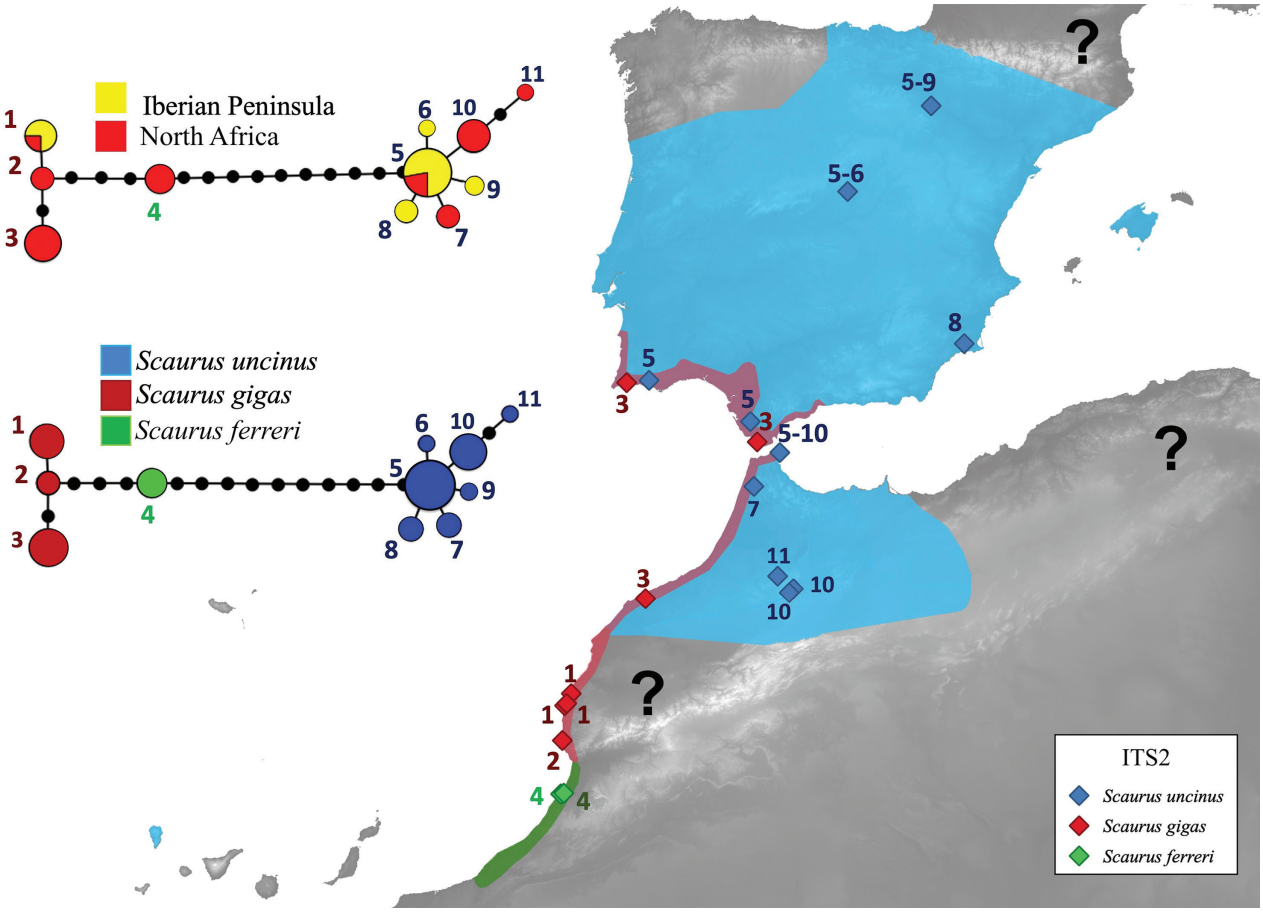

Figure 2. Allelic network based on partial sequences of the nuclear second Internal Transcribed Spacer (ITS2) for *Scaurus gigas* (Waltl, 1835) (red), *Scaurus uncinus* (Forster, 1771) (blue), and *Scaurus ferreri* Español, 1960 (green). Inferred intermediate alleles are represented by small black circles. Colors correspond to the geographic origin of the specimens. The size of the circles indicates the relative frequency of sequences belonging to a particular allele.

other by 4-point mutations, but they are notably distant (26-point mutations) from all other haplotypes.

The studied populations of *Scaurus uncinus* presented eight Cyt *b* haplotypes. This species shows lower haplotype diversity than *S. gigas*. Four haplotypes are only found in the Iberian Peninsula, three only in Morocco, and one is shared across the Strait of Gibraltar. The studied population from Murcia (Spain) is separated by 10 mutations from the closest haplotype (Fig. 1).

Allele networks based on ITS2 for the studied populations of *S. gigas* (15 specimens) and *S. uncinus* (20 specimens) were represented by four and seven alleles, respectively (Fig. 2). The studied populations of *S. gigas* present one allele shared between North African and Iberian populations. All the other alleles are only present in Africa. The studied populations from Aglou and Deghaimis (Morocco) present a single allele separated by four-point mutations from the rest of the Iberian and African haplotypes (Fig. 2).

Scaurus uncinus, shows a higher allelic diversity than *S. gigas*, with seven ITS2 alleles observed. The most common allele is present both in the Iberian Peninsula,

where it is widespread (at Faro, Cádiz, and Madrid), and in North Africa (at Ceuta). Also, three alleles are observed in North Africa and another three different alleles in the Iberian Peninsula. A single specimen morphologically identified as *S. uncinus* (CDAS0027, See Table 1) has mitochondrial DNA of *S. gigas* being represented by the Cyt *b* haplotype number 9 (Fig. 1; Table 1) and with nuclear DNA in concordance with its morphology being characterized by the ITS2 allele number 5 (Fig. 2; Table 1).

4. Discussion

The Strait of Gibraltar, located between northwestern Africa and southwestern Europe, has acted as a geographical barrier for dispersion in different groups of small vertebrates and arthropods (Busack et al. 1985; Mas-Peinado et al. 2021; Sánchez-Vialas et al. 2020), although in some other cases it had no effect at all (Gutiérrez-Rodríguez et al. 2017; Mas-Peinado et al. 2022). The effect of the Strait and its evolutionary consequences are highly variable even for different species within the same genus (Mas-Peinado et al. 2021, 2022). The phylogeographic pattern shown by *S. uncinus* and *S. gigas* agree with those species that have been able to disperse across this marine strait, such as other tenebrionids like species of the subgenera *Amblyptera* Solier, 1836 and *Amblypteraca* Mas-Peinado et al. 2018 of *Pimelia* (Mas-Peinado et al. 2022). The studied molecular markers of *S. uncinus* and *S. gigas* present shared haplotypes and alleles between the Iberian Peninsula and northern Morocco (Figs 1, 2).

The large degree of genetic structure found in the *S. gigas* lineage indicates a long history of geographic isolation and diversification in the African continent. This pattern is reversed in *S. uncinus*, which presents a pattern of subtle genetic structuring only among the European populations. The main nuclear allele of *S. uncinus* is widespread over the central and southwestern region of the Iberian Peninsula, but it is also present in northern Morocco. This allelic distribution could be related to a rapid range expansion into the African continent (Recuero and García-París 2011; Rodríguez-Flores et al. 2017; Cox et al. 2019). These data support the existence of recent intraspecific migration and gene flow through the Strait of Gibraltar for both species. However, the direction of the colonization seems to be different in each taxon. Hight levels of haplotypic diversity and internal recent speciation processes in the *S. gigas* lineage occur in the African continent suggesting that colonization of Europe took place from Africa; while in the *S. uncinus* lineage larger haplotypic diversity and geographic structuring occur within the European populations suggesting the opposite pattern of colonization, from Europe to Africa.

Scaurus gigas has its meridional limit at the surroundings of the Draa valley in Morocco (Español 1960). Populations of *S. gigas* from the Draa valley to Cabo Cantín (= Cabo Beddouza, 35 km North of Safi) were described by Español (1943) with subspecific status as *S. uncinus ferreri* (type locality: Sidi Ifni), morphologically characterized by presenting a poorly marked to absent dorsal carina in the anterior half of the elytra, which is well marked in the northern populations. Español (1943) ascription of these populations to *S. uncinus* was a result of an historical nomenclatural confusion stemming from early misidentifications. This confusion led to what is now recognized as *S. gigas* being incorrectly identified as *S. uncinus*, and *S. punctatus* being assigned to what is currently known as *S. uncinus* (Labrique 2004). During Labrique's (2004) reassessment of the taxonomy of these species, *S. uncinus ferreri* was included in the synonymy of *S. gigas* (Labrique 2004; Iwan and Löbl 2020).

However, our results reveal the presence of a distinct haplotypic and nuclear lineage, closely related to *S. gigas*, found at the western foothills of the Anti-Atlas Mountains, south of the Souss Valley, specifically at Aglou Beach and Deghaimis. This population exhibits a morphology consistent with the type series of *S. u. ferreri* housed at the Museu de Ciències Naturals of Barcelona, confirming its alignment with specimens from Aglou and Deghaimis. The Aglou Beach population is located approximately 57 km north of the type locality of *S. g. ferreri*, well within the geographic range outlined by Español (1943) for the southern subspecies (Fig. 4F). The phylogeographic structure of the clade *Scaurus gigas – S. ferreri* in Morocco is likely derived from a latitudinal vicariant process of differentiation thought the Atlantic coast of Morocco, marked and more diverse in the South, and followed by a more recent northward expansion to the Iberian Peninsula, where populations are genetically more homogeneous (Figs 1, 2). Within this framework, it is noteworthy that in the southern distribution range of *S. gigas – S. ferreri* clade, there are no obvious current geographic barriers to dispersal that could prevent gene flow between populations located at both sides of the Souss Valley. Some studies suggest orography as the key factor influencing the maintenance of fine patterns of allopatric lineage structuration (Habel et al. 2012). However, *S. gigas – S. ferreri* are coastal species that do not occur far from the coastline, what makes difficult to infer if orography (Anti-Atlas) is accounting for their differentiation. The coastal area of the Souss Valley primarily comprises fluvial terraces with aeolian dunes (Bhiry and Occhietti 2004), flanked by the High Atlas Mountains to the North by and the Anti-Atlas to the South. Over time, this region has undergone significant environmental changes since the Miocene, including uplifted mountains and shifting environmental conditions (Aït Hssaine 1994; Bhiry and Occhietti 2004). These dynamic geological and ecological factors may have potentially promoted speciation among the reported lineages of *Scaurus*. A plausible similar pattern is evidenced by geckos of the genus *Saurodactylus* Fitzinger, 1843 and lizards of the *Acanthodactylus pardalis* species group. In the case of *Saurodactylus*, two lineages arised allopatrically (now species), which contact in the Souss valley without signs of genetic admixing (Rosado et al. 2017), while for the *Acanthodactylus pardalis* species group, the Anti-Atlas establishes a

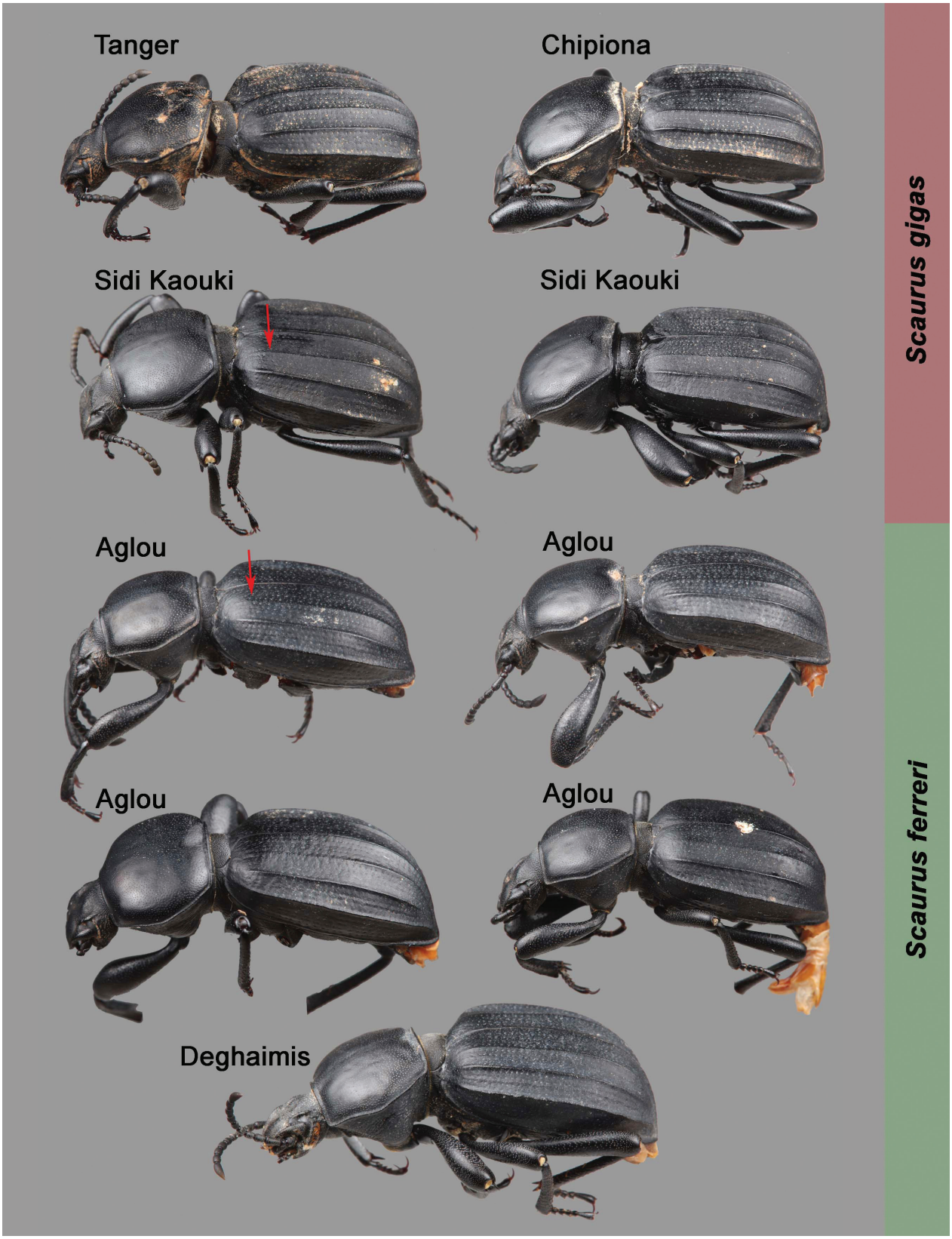

Figure 3. Morphological differences between *S. gigas* (Tanger, Chipiona and Sidi Kaouki) and *S. ferreri* (Aglou and Deghaimis) (see Table 1 for locality details). Note the poorly marked carina on the anterior elytral region in *S. ferreri*, which are relatively well-marked in *S. gigas* (indicated by a red arrow in one selected specimen for each species).

significant distribution gap between two sister species, one found in the Souss Valley, in the north of the Anti-Atlas, and the other to the South (Tamar et al. 2017). In our sampling we found strict concordance between nuclear and mtDNA markers depicting an isolated lineage south to the Souss Valley with no shared nuclear alleles between populations belonging to distinct mitochondrial populations in *S. gigas* – *S. ferreri*.

Figure 4. Live specimens and typical habitat of *S. gigas* (**a**–**c**) and *S. ferreri* (**d**–**f**). Specimens photographed are from (a) Meia Praia, Lagos (Portugal), (b) Chipiona, Cádiz (Spain), (d) Aglou Plage (Morocco) and (e) Deghaimis (Morocco). Landscape images represent (c) the northernmost range of *S. gigas* (near Sines, Portugal) and (f) one of the northern locations of *S. ferreri* at Aglou Plage (Morocco).

Although, our sampling across the Souss valley is very limited to test for the existence of complete reproductive isolation between the two lineages, the concordance between morphology (Figs 3, 4) and molecular markers made us considered them differentiated enough as to represent two independent evolutionary units. We, therefore, propose here the formal recognition of the southernmost populations of the *S. gigas* lineage, to be part of an independent species, and therefore revalidating the name *Scaurus ferreri* Español, 1943 stat. nov. for these populations (https://zoobank.org/C3150EA6-EE3D-447D-BB50-D22D80F4DF2C).

As expected by their morphological distinctiveness (Español 1943, 1960), genetic admixture between *S. gigas* and *S. uncinus* was almost absent across their contact zone. However, we observed a striking case of cyto-nuclear discordance in a single specimen morphologically identified as *S. uncinus* from Faro, southern Portugal. This specimen has a Cyt *b* haplotype of *S. gigas*, but its nuclear ITS2 allele as well as its morphology are within the variability of *S. uncinus*. This pattern of cyto-nuclear discordance observed in a single specimen, agrees with a possible hybrid origin for the specimen (Doniol-Vacroze et al. 2021). The low frequency of hybrids (n=1/30) across a wide potential area of sympatry (southwestern Atlantic coast of Europe and northwestern Atlantic coast of Africa), suggests that reproductive barriers between *S. gigas* and *S. uncinus* are strong, but not complete enough to prevent single episodes of hybridization (Dufresnes et al. 2020).

Transcontinental intraspecific patterns of colonization appear to be much common than previously expected between Africa and Europe for relatively large, flightless, xeric-adapted, species of terrestrial arthropods as darkling beetles (Condamine et al. 2013; Sánchez-Vialas et al. 2021; Mas-Peinado et al. 2018, 2022; Tinaut and Ruano 2021). Their low ability for dispersal is likely counteracted by large population densities and a strong survival ability in hostile environments. When darkling-beetle populations are located in coastal areas, as it is the case of the species of *Scaurus* studied here, they might be passively transported during exceptional climate disruptions (strong storms and tornados) or catastrophic volcanic episodes (ending in tsunamis) as it has been considered for other species as frogs (*Hyla meridionalis* Boettger, 1874) (Recuero et al. 2007) or tenebrionid beetles (*Archaeoglenes* Broun, 1893, *Amblypteraca* Mas-Peinado, Buckley, Ruiz & García-París, 2018) (Iwan et al. 2015; Mas-Peinado et al. 2022). While other representatives of Tenebrionoidea use complex strategies for dispersal, as blister beetles with larval phoresy (Sánchez-Vialas et al. 2021), darkling beetles seem to rely on passive dispersal favored by episodic events or even human accidental transport, as it seems likely the case for some populations of *S. uncinus*, resulting from heavy maritime transport from the Iberian coast to the Spanish city of Ceuta in northern Africa.

5. Declarations

Conflict of interest. The authors declare no conflict of interest.

Data availability statement. The molecular data newly generated for this study is available in GenBank. Accession numbers P0438082–P0438110 for Cyt *b* and PQ435559-PQ435590 for ITS2 sequences.

Funding. The authors declare that his study was funded by the project-grant PID2019-110243GB-100/AEI/10.13039/501100011033 (Ministerio de Ciencia e Innovación) to Mario García-Paris.

6. Acknowledgements

We thank E. Recuero Gil, J. Gutiérrez Rodríguez, J. L. Ruiz García, P. Mas-Peinado, N. Percino Daniel, N. Rosas Ramos, A. Zaldívar, G. García Martín, F. Alda, I. Martínez-Solano, J. Miller, M. Carrillo, L. Puerta Rodríguez, J. Morales-García, C. Settanni, P. Jurado-Angulo, L. Saínz Escudero, K. López-Estrada, J.E. Uribe Arboleda, and M. Calvo Revuelta for their help with fieldwork. We especially thank J. L. Ruiz for his advice and assistance at the Instituto de Estudios Ceutíes. We are also grateful to Pablo Vicent Castelló for his support during the lab work, as well as E. Karen López Estrada and Lucía Sainz Escudero for their help with the molecular analyses. We express our deep gratitude to Mercedes París, curator of Entomology at the Museo Nacional de Ciencias Naturales (Madrid), for her invaluable work and availability. Special thanks to Dr. Berta Caballero López, curator of the arthropod collection at the Museu de Ciències Naturals (Barcelona), and Sergi Gago Carrión for their speed and efficiency in providing us with study materials when time was of the essence. We also thank Javier Diéguez-Uribeondo, director of the 'Master en Biodiversidad en Áreas Tropicales y su Conservación, UIMP-CSIC,' for his unwavering support, dedication to teaching, and for playing the role of scientific matchmaker by introducing the authors of this article.

7. References

Aït Hssaine A (1994) Gèomorphologie et Quaternaire du piémont de Taroudant–Oulad Teima, vallée du Souss, Maroc. Ph.D thesis, Université de Montréal, 245 pp. https://www.elibrary.ru/item.asp?id=5622020

Babik W, Branicki W, Crnobrnja-Isailović J, Cogălniceanu D, Sas I, Olgun K, Poyarkov NA, García-París M, Arntzen JW (2005) Phylogeography of two European newt species—discordance between mtDNA and morphology. Molecular Ecology 14(8): 2475–2491. https://doi.org/10.1111/j.1365-294X.2005.02605.x

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barraclough TG, Hogan JE, Vogler AP (1999) Testing whether ecological factors promote cladogenesis in a group of tiger beetles (Coleoptera: Cicindelidae). Proceedings of the Royal Society B: Biological Sciences 266: 1061–1067. https://doi.org/10.1098/rspb.1999.0744

Bhiry N, Occhietti S (2004) Fluvial sedimentation in a semi-arid region: the fan and interfan system of the Middle Souss Valley, Morocco. Proceedings of the Geologists' Association 115(4): 313–324. https://doi.org/10.1016/S0016-7878(04)80011-7

Busack SD, Maxson LR, Wilson MA (1985) *Pelobates varaldii* (Anura: Pelobatidae): a morphologically conservative species. Copeia 1985(1): 107–112. https://doi.org/10.2307/1444797

Cartagena MC, Galante E (2005) Ecología de las especies de *Scaurus* en el Sudeste Ibérico (Coleoptera, Tenebrionidae). Sessió conjunta de Entomologia ICHN-SCL (13): 37–46. https://publicacions.iec.cat/repository/pdf/00000002/00000046.pdf

Cartagena MC, Viñolas A, Galante E (2002) Biodiversidad de tenebriónidos (Coleoptera: Tenebrionidae) en saladares ibéricos. Butlletí de la Institució Catalana d'Història Natural 70: 91–104. https://core.ac.uk/download/pdf/39023414.pdf

Cox K, McKeown N, Antonini G, Harvey D, Solano E, Van Breusegem A, Thomaes A (2019) Phylogeographic structure and ecological niche modelling reveal signals of isolation and postglacial colonization in the European stag beetle. PLoS One 14(4), e0215860. https://doi.org/10.1371/journal.pone.0215860

Condamine FL, Soldati L, Clamens AL, Rasplus JY, Kergoat GJ (2013) Diversification patterns and processes of wingless endemic insects in the Mediterranean Basin: historical biogeography of the genus *Blaps* (Coleoptera: Tenebrionidae). Journal of Biogeography 40(10): 1899–913. https://doi.org/10.1111/jbi.12144

Cuesta-Segura AD, Jurado-Angulo P, Jiménez-Ruiz Y, García-París M (2023) Taxonomy of the Iberian species of *Pseudochelidura* (Dermaptera: Forficulidae). European Journal of Taxonomy 860: 81–115. https://doi.org/10.5852/ejt.2023.860.2053

Dinis M, Merabet K, Martínez-Freiría F, Steinfartz S, Vences M, Burgon JD, Elmer KR, Donaire D, Hinckley A, Fahd S, Joger U, Fawzi A, Slimani T, Velo-Antón G (2019) Allopatric diversification and evolutionary melting pot in a North African Palearctic relict: The biogeographic history of *Salamandra algira*. Molecular Phylogenetics and Evolution (130): 81–91. https://doi.org/10.1016/j.ympev.2018.10.018

Doniol-Vacroze P, Ursenbacher S, Mebert K, Ghielmi S, Laddaga L, Sourrouille P, Karis M, Crochet P (2021) Conflicting relationships of *Vipera walser* inferred from nuclear genes sequences and mitochon-

drial DNA. Journal of Zoological Systematics and Evolutionary Research 59(8): 2307–2320. https://doi.org/10.1111/jzs.12543

Dowling TE, Secor CL (1997) The role of hybridization and introgression in the diversification of animals. Annual review of Ecology and Systematics 28(1): 593–619. https://doi.org/10.1146/annurev.ecolsys.28.1.593

Dufresnes C, Martínez-Solano I (2020) Hybrid zone genomics supports candidate species in Iberian *Alytes obstetricans*. Amphibia-Reptilia 41: 105–112. https://doi.org/10.1163/15685381-20191312

Dufresnes C, Pribille M, Alard B, Gonçalves H, Amat F, Crochet PA, Dubey S, Perrin N, Fumagalli L, Vences M, Martínez-Solano I (2020) Integrating hybrid zone analyses in species delimitation: lessons from two anuran radiations of the Western Mediterranean. Heredity 124: 423–438. https://doi.org/10.1038/s41437-020-0294-z

Español F (1943) Misión Científica E. Morales Agacino, Ch. Rungs y B. Zolotarevsky a Ifni y Sáhara Español. Tenebrionidae (CoI.). 1ª Parte. Eos 19: 119–148. http://hdl.handle.net/10261/148309

Español F (1957) Nuevos datos sobre los Tenebriónidos de Menorca. Bolletí de la Societat D'història Natural de Les Balears 4: 9–12.

Español F (1960) Los *Scaurus* de España (Col. Tenebrionidae). Eos 36(4): 141–155.

Español F (1963) Datos para el conocimiento de los tenebriónidos del Mediterráneo occidental. XXIII. Adiciones al catálogo de Tenebriónidos de Andalucía occidental. Eos 39(1–2): 187–209. https://digital.csic.es/bitstream/10261/140216/1/1954-30-1_2-012.pdf

Glez-Peña D, Gómez-Blanco D, Reboiro-Jato M, Fdez-Riverola F, Posada D (2010) ALTER: program-oriented conversion of DNA and protein alignments. Nucleic Acids Research 38: 14–18. https://doi.org/10.1093/nar/gkq321

Gómez A, Lunt DH (2007) Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In: Weiss, S. and Ferrand, N. (Eds.) Phylogeography of southern European refugia. Springer, Dordrecht, 155–188 pp. https://doi.org/10.1007/1-4020-4904-8_5

Gonçalves DV, Brito JC (2020) Second Sahelian amphibian endemism suggested by phylogeography of Groove crowned Bullfrog (*Hoplobatrachus occipitalis*) in western Sahel and hints of polyploid species formation. Journal of Zoological Systematics and Evolutionary Research 58 (1): 262–274. https://doi.org/10.1111/jzs.12321

Gutierrez-Rodriguez J, Barbosa AM, Martinez-Solano I (2017) Integrative inference of population history in the Ibero-Maghrebian endemic *Pleurodeles waltl* (Salamandridae). Molecular Phylogenetics and Evolution 112: 122–137. https://doi.org/10.1016/j.ympev.2017.04.022

Habel JC, Husemann M, Schmitt T, Zachos FE, Honnen AC, Petersen B, Parmakelis A, Stathi I (2012) Microallopatry caused strong diversification in *Buthus* scorpions (Scorpiones: Buthidae) in the Atlas Mountains (NW Africa). PLoS One 7(2): e29403.

Hewitt GM (1999) Post-glacial re-colonization of European biota. Biological journal of the Linnean Society 68(1–2): 87–112. https://doi.org/10.1111/j.1095-8312.1999.tb01160.x

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 359 (1442): 183–195. https://doi.org/10.1098/rstb.2003.1388

Iwan D, Kamiński MJ, Raś M (2015) Amphi-Indian Ocean Disjunction in the Trans-Pacific Genus *Archaeoglenes* Brown (Coleoptera: Tenebrionidae: Phrenapatinae): New Taxonomic and Distributional Data. The Coleopterists Bulletin 69(14): 152–166. https://doi.org/10.1649/0010-065X-69.mo4.152

Iwan D, Löbl I (2020) Catalogue of Palaearctic Coleoptera. Vol. 5. Tenebrionoidea. Revised and updated 2nd Edition. Brill, Leiden, 104–475 pp.

Ji YJ, Zhang DX, He LJ (2003) Evolutionary conservation and versatility of a new set of primers for amplifying the ribosomal internal transcribed spacer regions in insects and other invertebrates. Molecular Ecology Notes 3(4): 581–585. https://doi.org/10.1046/j.1471-8286.2003.00519.x

Kamiński MJ, Smith AD, Kanda K, Iwan D, Kergoat GJ (2021) Old origin for an European-African amphitropical disjunction pattern: New insights from a case study on wingless darkling beetles. Journal of Biogeography 49: 130–141. https://doi.org/10.1111/jbi.14288

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Briefings in bioinformatics 9(4): 286–298. https://doi.org/10.1093/bib/bbn013

Kergoat GJ, Soldati L, Clamens AL, Jourdan H, Jabbour-Zahab R, Genson G, Bouchard P, Condamine FL (2014) Higher level molecular phylogeny of darkling beetles (Coleoptera: Tenebrionidae). Systematic Entomology 39(3): 486–499. https://doi.org/10.1111/syen.12065

Labrique H (1995a) Étude des types de Scaurus F. décrits par Escalera (Coleoptera, Tenebrionidae). Bulletin de la Société Entomologique de France 100(5): 511–513. https://www.persee.fr/doc/bsef_0037-928x_1995_num_100_5_17199

Labrique H (1995b) Les *Scaurus* du groupe *tingitanus* Peyerimhoff (Coleoptera, Tenebrionidae). Bulletin de la Société entomologique de France 100: 119–126. https://www.persee.fr/doc/bsef_0037-928x_1995_num_100_2_17133

Labrique H (2004) Etude des *Scaurus* décrits par Forster, Fabricius et Waltl (Coleoptera, Tenebrionidae). Publications de la Société Linnéenne de Lyon 73(8): 333-340. https://www.persee.fr/doc/linly_0366-1326_2004_num_73_8_13538

López-Pérez JJ (2010) Corología de los Scaurini Billberg, 1820 (Col., Tenebrionidae, Tenebrioninae) provincia de Huelva, Sur-Oeste de la Península Ibérica. Boletín de la Asociación española de Entomología 34(1–2): 7–14. https://dialnet.unirioja.es/servlet/articulo?codigo=3927804

Larson EL, White TA, Ross CL, Harrison RG (2014) Gene flow and the maintenance of species boundaries. Molecular Ecology 23(7): 1668–1678. https://doi.org/10.1111/mec.12601

Leigh JW, Bryant D (2015) POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9): 1110–1116. https://doi.org/10.1111/2041-210X.12410

Mallet J (2005) Hybridization as an invasion of the genome. Trends in ecology & evolution 20(5): 229–237. https://doi.org/10.1016/j.tree.2005.02.010

Mas-Peinado P, Buckley D, Ruiz JL, García-París M (2018) Recurrent diversification patterns and taxonomic complexity in morphologically conservative ancient lineages of *Pimelia* (Coleoptera: Tenebrionidae). Systematic Entomology 43(3): 522–48. https://doi.org/10.1111/syen.12291

Mas-Peinado P, Ruiz JL, Merkl O, Buckley D, García-París D (2021) Taxonomy of the North Moroccan and Iberian species of the subgenus *Amblypteraca* (Coleoptera: Tenebrionidae: Pimeliinae: *Pimelia*). Zootaxa 49633: 457–482. https://doi.org/10.11646/zootaxa.4963.3.4

Mas-Peinado P, García-París M, Ruiz JL, Buckley D (2022) The Strait of Gibraltar is an ineffective palaeogeographic barrier for some flightless darkling beetles (Coleoptera: Tenebrionidae: Pimelia). Zoological Journal of the Linnean Society 195(4): 1147–1180. https://doi.org/10.1093/zoolinnean/zlab088

Nabozhenko MB, Labrique H, Korkutan A, Keskin A (2020) Contribution to the knowledge of the genus *Scaurus* Fabricius, 1775 (Coleoptera: Tenebrionidae: Scaurini) from Turkey. Кавказский энтомологический бюллетень 16(1): 141–151. https://doi.org/10.23885/181433262020161-141151

Pfennig KS (2016) Reinforcement as an initiator of population divergence and speciation. Current Zoology 62(2): 145–154. https://doi.org/10.1093/cz/zow033

Pyron RA, O'Connell KA, Lemmon EM, Lemmon AR, Beamer DA (2022) Candidate-species delimitation in *Desmognathus* salamanders reveals gene flow across lineage boundaries, confounding phylogenetic estimation and clarifying hybrid zones. Ecology and Evolution 12(2): e8574. https://doi.org/10.1002/ece3.8574

Racimo F, Sankararaman S, Nielsen R, Huerta-Sánchez E (2015) Evidence for archaic adaptive introgression in humans. Nature Reviews Genetics 16(6): 359–371. https://doi.org/10.1038/nrg3936

Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annual review of ecology and systematics 29: 467–501. https://doi.org/10.1146/annurev.ecolsys.29.1.467

Recuero E, Iraola A, Rubio X, Machordom A, García-París M (2007) Mitochondrial differentiation and biogeography of *Hyla meridionalis* (Anura: Hylidae): an unusual phylogeographical pattern. Journal of Biogeography 34(7): 1207–1219. https://doi.org/10.1111/j.1365-2699.2007.01688.x

Recuero E, García-París M (2011) Evolutionary history of *Lissotriton helveticus*: Multilocus assessment of ancestral vs. recent colonization of the Iberian Peninsula. Molecular Phylogenetics and Evolution 60: 172–180. https://doi.org/10.1016/j.ympev.2011.04.006

Rodríguez-Flores PC, Jiménez-Ruiz Y, Forró L, Vörös J, García-París M (2017) Non-congruent geographic patterns of genetic divergence across European species of *Branchinecta* (Anostraca: Branchinectidae). Hydrobiologia 801: 47–57. https://doi.org/10.1007/s10750-017-3266-4

Rosado D, Rato C, Salvi D, Harris DJ (2017) Evolutionary history of the Morocco lizard-fingered geckos of the *Saurodactylus brosse*ti complex. Evolutionary Biology 44(3): 386–400. https://doi.org/10.1007/s11692-017-9417-8

Sainz-Escudero L, Alonso M, Sánchez-Vialas A (2022) Diversity and distribution of Anostraca in temporary ponds in Western Africa with description of a new species of *Streptocephalus* Baird, 1852 (Pancrustacea: Branchiopoda: Streptocephalidae). Zootaxa 5213(4): 388–412. https://doi.org/10.11646/zootaxa.5213.4.4

Sánchez-Vialas A, García-París M, Ruiz JL, Recuero E (2020) Patterns of morphological diversification in giant *Berberomeloe* blister beetles (Coleoptera: Meloidae) reveal an unexpected taxonomic diversity concordant with mtDNA phylogenetic structure. Zoological Journal of the Linnean Society 189 (4): 1249–1312. https://doi.org/10.1093/zoolinnean/zlz164

Sánchez-Vialas A, Recuero E, Jiménez-Ruiz Y, Ruiz JL, Marí-Mena N, García-París M (2021) Phylogeny of Meloini blister beetles (Coleoptera, Meloidae) and patterns of island colonization in the Western Palaearctic. Zoologica Scripta 50(3): 358–375. https://doi.org/10.1111/zsc.12474

Stahlke AR, Bitume EV, Özsoy ZA, Bean DW, Veillet A, Clark MI, Clark EI, Moran P, Hufbauer RA, Hohenlohe PA (2022) Hybridization and range expansion in tamarisk beetles (*Diorhabda* spp.) introduced to North America for classical biological control. Evolutionary Applications 15: 60–77. https://doi.org/10.1111/eva.13325

Servedio MR, Noor MA (2003) The role of reinforcement in speciation: theory and data. Annual Review of Ecology, Evolution, and Systematics: 339–364. https://doi.org/10.1146/annurev.ecolsys.34.011802.132412

Szymura JM, Barton NH (1986) Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40(6): 1141–1159. https://doi.org/10.1111/j.1558-5646.1986.tb05740.x

Tamar K, Geniez P, Brito JC, Crochet PA (2017) Systematic revision of *Acanthodactylus busacki* (Squamata: Lacertidae) with a description of a new species from Morocco. Zootaxa 4276(3): 357–386. https://doi.org/10.11646/zootaxa.4276.3.3

Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow JL (2006) Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. Molecular Ecology 15(2): 343–355. https://doi.org/10.1111/j.1365-294X.2005.02794.x

Tinaut A, Ruano F (2021) Biogeography of Iberian ants (Hymenoptera: Formicidae). Diversity 13(2): 88. https://doi.org/10.3390/d13020088

Wielstra B, Arntzen JW (2020) Extensive cytonuclear discordance in a crested newt from the Balkan Peninsula glacial refugium. Biological Journal of the Linnean Society 130(3): 578–585. https://doi.org/10.1093/biolinnean/blaa062

Zieliński P, Nadachowska-Brzyska K, Wielstra B, Szkotak R, Covaciu-Marcov SD, Cogălniceanu D, Babik W (2013) No evidence for nuclear introgression despite complete mtDNA replacement in the Carpathian newt (*Lissotriton montandoni*). Molecular Ecology 22(7): 1884–1903. https://doi.org/10.1111/mec.12225