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Phylogeographic structure of two sympatric species of *Mediimorda* Méquignon, 1946 (Mordellidae, Coleoptera)

A. M. SANZ-LAPARRA [1,2]*, M. GARCÍA-PARÍS [2], & E. K. LÓPEZ-ESTRADA [2,3]

[1]*Department of Natural History, University Museum of Bergen, University of Bergen, Bergen, Norway,* [2]*Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain, and* [3]*Department of Biodiversity and Conservation, Real Jardín Botánico (RJB-CSIC), Madrid, Spain*

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Abstract
Sympatric distribution and syntopy are not common in closely related species since sporadic crosses may lead to the formation of hybrid zones disrupting the differentiation of sympatric incipient species. In Central Spain two morphologically similar species of *Mediimorda* Méquignon, 1946, *M. batteni* Plaza-Infante, 1985 and *M. bipunctata* (Germar, 1827) are strictly sympatric. *Mediimorda batteni* is endemic to the Iberian Peninsula while *M. bipunctata* is widely distributed in the Western Palaearctic Region. Intriguingly, sympatric distribution among closely related taxa that exhibits little morphological variation is not common. This makes us wonder if we are really facing two independent evolutionary units of *Mediimorda* as previously proposed or just two phenotypic morphs of a single taxon. To test for this hypothesis, we performed Bayesian and phylogeographic analyses based on mitochondrial (*cox1*) and nuclear (ITS2) data and estimated divergence time of clades. Additionally, a morphological revision and construction of potential distribution models were included to determine possible niche differences. Our results corroborate the existence of two lineages well differentiated, that probably diverged during the Pliocene. According to the morphology, each clade corresponds to the previously recognized *M. batteni* and *M. bipunctata*. The absence of hybridization and ecological segregation suggests that sympatric and syntopic distribution was accomplished long time after the speciation event that separated the two taxa took place. We propose that the divergence between clades was originated by allopatric speciation during the Late Pliocene subsequently followed by range shifts during the Pleistocene climatic oscillations, which resulted in the current syntopy of the two taxa.

Keywords: *Syntopy*, *tumbling flower beetles*, *molecular analyses*, *Western Palaearctic Region*

1. Introduction

Sympatric distribution and syntopy rarely occur between closely related species as the cohesion and stability of recently diverged species might be altered by hybridization (Mallet 2005). Sporadic crosses between syntopic populations may lead to the formation of hybrid zones disrupting the differentiation of sympatric incipient species (Woodruff 1973; Mallet 2005; Prado et al. 2016). Sympatry can also lead to competitive exclusion, as one of the two species might go extinct or be displaced when they share the same habitat and environmental conditions (e.g. Hardin 1960; Beaver & Baldwin 1975; Lopes et al. 2015).

Two species of tumbling flower beetles of the genus *Mediimorda* Méquignon, 1946 (Mordellidae), *M. batteni* Plaza-Infante, 1985 and *M. bipunctata* (Germar, 1827), are syntopic across a large area in Central Spain. *Mediimorda batteni* is endemic to the Iberian Peninsula while *M. bipunctata* is widely distributed in the Western Palaearctic Region (Plaza-Infante 1985; Leblanc 2002). The two species are not only syntopic in Central Spain but can also be found together from mid-June to mid-August on the same flowers of several Apiaceae species, such as *Daucus carota* L., *Thapsia villosa* L., *Eryngium campestre* L., *Foeniculum*

*Correspondence: A. M. Sanz-laParra, Department of Natural History, University Museum of Bergen, University of Bergen, P.O. Box 7800, Bergen NO5020, Norway. Email: annui.parra@uib.no; anuimalla@gmail.com

vulgare Mill. (Plaza-Infante 1985). To complicate matters *M. bipunctata* and *M. batteni* are very similar in external morphology, which makes them impossible to differentiate in the field, although they can be separated by characters in the male genitalia (Plaza-Infante 1985; Leblanc 2002, 2007).

Limited morphological differentiation, similar habitat requirements, strict local syntopy, and a close phylogenetic relationship make the study of *M. bipunctata* and *M. batteni* a particular interesting case to analyze some of the possible factors allowing local sympatry. Alternatively, *M. bipunctata* and *M. batteni* may still be at an incipient stage of speciation and might not have developed complete reproductive isolation, in which case the morphological divergence observed may reflect just intraspecific variability.

The present study first aimed to identify how many well-differentiated evolutionary units of *Mediimorda* are present in Central Spain, by analysing levels of congruence between nuclear (internal transcribed spacer 2 - ITS2) and mitochondrial (cytochrome *c* oxidase subunit 1 - *cox1*) markers, and with respect to the previously proposed morphological diagnostic traits. The results demonstrated that *M. batteni* and *M. bipunctata* constitute two monophyletic groups with enough evidence to be treated at the species level. For these analyses, a sample of 105 specimens of Iberian *Mediimorda* were used. Based on climatic data, areas of high suitability for each species were identified and the time of lineage diversification within each clade was also estimated. The phylogeographic patterns of the populations of both species in the Iberian Peninsula were analysed. It is proposed that the current sympatric distribution of these two taxa in the central areas of the Iberian Peninsula may have resulted from secondary contact driven by historical climatic oscillations.

2. Materials and methods

2.1. Taxon sampling

Specimens were collected in 27 different sites across the Iberian Peninsula. Information on the location of previously known populations was obtained by an exhaustive bibliographic search and from label data of the specimens in the collection at the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN-CSIC). Three to five specimens from each locality were used to perform molecular analyses to identify maker variability within populations (Table I). Field collections were carried out in most of the areas in Central and Southern Spain where *M. batteni* is present (Plaza-Infante 1985). Specimens were located visually in the field, captured by hand, and georeferenced. Once collected, some specimens were preserved in ethanol, the rest dry-mounted for morphological study. All specimens are deposited at the MNCN.

2.2. DNA extraction and sequencing

Total DNA was extracted from 108 specimens, of which 105 belonged to *Mediimorda* and three specimens to other genera of Mordellidae (Table I). The entire specimen was used for DNA extraction. Specimens were perforated on the right side of the thorax, so they could be recovered for morphological study after DNA extraction. Total genomic DNA was extracted using the Qiagen DNeasy extraction kit (Qiagen®), following the protocol described by the manufacturer. We generated partial sequences of two molecular markers with varying molecular evolutionary rates, the mitochondrial *cox1* and the ITS2 nuclear marker, to obtain phylogenetic resolution at different phylogenetic levels. For the *cox1*, we used the set of primers Jerry-C1-J-2183 and Pat-TL2 -N-3014 (Simon et al. 1994), while for the nuclear ITS2, we used the pair of primers CAS5p8sFc and CAS28sB1d (Ji et al. 2003).

Polymerase chain reactions (PCR) were performed in 25 µl, including 23 µl of the PCR mix (16.35 µl of H_2O; 2.5 µl of Nzytech reaction buffer with $MgCl_2$; 1 µl of dNTP; 1.75 µl of $MgCl_2$; 0.5 µl of each primer and 0.4 µl of Taq polymerase (Nzytech, 5 U/µl)) and 2 µl of specimen DNA for *cox1*; and 24 µl of the PCR mix (17.8 µl of H_2O; 2.5 µl of Nzytech reaction buffer with $MgCl_2$; 1 µl of dNTP; 1.5 µl of $MgCl_2$; 0.5 µl of each primer and 0.2 µl of Taq polymerase (Nzytech, 5 U/ µl)) and 1 µl of specimen DNA for ITS2. ITS2 PCR conditions were as follows: 5 min at 96°C for initial denaturation, 40 cycles of denaturation at 94°C (1 min), annealing at 55–60°C (1 min) and extension at 72°C (1 min) with a final extension at 72°C for 10 min. *Cox1* PCR conditions followed Gunter et al. (2013). Amplified products were checked in a 1.5% agarose gel and products with the expected length were purified and directly sequenced by Sanger at Macrogen Spain Inc. (Macrogen Europe, Amsterdam, The Netherlands). Chromatograms were assembled and edited using Geneious Prime 2021.0.3 (http://www.geneious.com/).

2.3. Phylogenetic analyses

Phylogenetic analyses were performed on separate data sets for each gene (*cox1* and ITS2) including our own generated sequences of *Mediimorda* and the closely related genera *Mordellistena*, *Variimorda* and *Stenalia* as outgroups. *Cox1* data set included 108 terminals and 810 pb while ITS2 data set contained

Table I. List of specimens included in this study, their species identity, collecting localities, Entomology collection number at the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN) and GenBank accession numbers.

Taxon	Locality	MNCN Entomology collection number	GenBank *cox1* accession number	GenBank ITS2 accession number
Mediimorda batteni	Spain: Ávila: 5 km Northwest of Valdelavía	325603, 325604, 325605	OQ863174, OQ863175, OQ863176	
Mediimorda batteni	Spain: Ávila: 6 km South of Las Navas del Marqués	331074, 325541, 325542, 325543, 325544, 325545	OQ863090, OQ863112, OQ863113, OQ863114, OQ863115, OQ863116	
Mediimorda batteni	Spain: Ávila: Rivera de Corneja	325537, 325538, 325539, 325540	OQ863108, OQ863109, OQ863110, OQ863111	
Mediimorda batteni	Spain: Ávila: San Bartolomé de Béjar	325606, 325607, 325608	OQ863177, OQ863178, OQ863179	
Mediimorda batteni	Spain: Burgos: 1 km South of Pardilla	325526, 325527, 325529, 325530	OQ863099, OQ863100, OQ863101, OQ863102	
Mediimorda batteni	Spain: Burgos: Villahizán	325521, 325522, 325523, 325524, 325525	OQ863094, OQ863095, OQ863096, OQ863097, OQ863098	
Mediimorda batteni	Spain: Cáceres: 4 km East of Hervás	325533, 325535	OQ863105, OQ863107	OQ914284, OQ914285
Mediimorda batteni	Spain: Cuenca: 1 km East of Paredes	325581, 325582, 325583	OQ863152, OQ863153, OQ863154	
Mediimorda batteni	Spain: Cuenca: 1 km Northeast of Alcalá de la Vega	325614, 325615, 325616, 325617	OQ863185, OQ863186, OQ863187, OQ863188	
Mediimorda batteni	Spain: Cuenca: 2 km Southwest of Monteagudo de las Salinas	331081, 325584, 325585, 325586, 325587, 325588	OQ863093, OQ863155, OQ863156, OQ863157, OQ863158, OQ863159	
Mediimorda batteni	Spain: Cuenca: 5 km East of Campillo de Altobuey	325612, 325613	OQ863183, OQ863184	
Mediimorda batteni	Spain: Cuenca: Puerto de Cabrejas	325556, 325557, 325558, 325559, 325560	OQ863127, OQ863128, OQ863129, OQ863130, OQ863131	
Mediimorda batteni	Spain: Granada: Pista Llanada de Sedella, Sierra Tejeda	325650, 325651, 331052, 331054	OQ863189, OQ863190, OQ863191, OQ863192	OQ914288
Mediimorda batteni	Spain: Guadalajara: 2.3 km Southeast of Uceda	325609, 325610	OQ863180, OQ863181	OQ914280, OQ914281
Mediimorda batteni	Spain: Guadalajara: 2.5 km East of Puebla de Valles	325547, 325548, 325549, 325550	OQ863118, OQ863119, OQ863120, OQ863121	OQ914282, OQ914283
Mediimorda batteni	Spain: Guadalajara: 4 km Northeast of Tamajón	325578, 325579, 325580	OQ863149, OQ863150, OQ863151	
Mediimorda batteni	Spain: Guadalajara: Hueva	325551, 325552, 325553, 325554, 325555	OQ863122, OQ863123, OQ863124, OQ863125, OQ863126	
Mediimorda batteni	Spain: León: Puente del Alba 1 km North of La Robla	325569, 325570, 325571	OQ863140, OQ863141, OQ863142	OQ914287
Mediimorda batteni	Spain: Madrid: 2 km South of La Cabrera	325592	OQ863163	
Mediimorda batteni	Spain: Madrid: El Pardo	331068, 331069, 325589	OQ863088, OQ863089, OQ863160	OQ914286
Mediimorda batteni	Spain: Navarra: 1 km South of Ayesa	325593, 325594, 325595, 325596	OQ863164, OQ863165, OQ863166, OQ863167	OQ914289
Mediimorda batteni	Spain: Palencia: 1 km Southeast of Palenzuela	325597, 325598, 325599	OQ863168, OQ863169, OQ863170	
Mediimorda batteni	Spain: Palencia: Villamediana	325600, 325601, 325602	OQ863171, OQ863172, OQ863173	
Mediimorda batteni	Spain: Salamanca: 1 km Southeast of La Hoya	331079, 325566, 325567, 325568	OQ863092, OQ863137, OQ863138, OQ863139	
Mediimorda batteni	Spain: Segovia: Alto del León	325575, 325576, 325577	OQ863146, OQ863147, OQ863148	
Mediimorda batteni	Spain: Valencia: 1 km Northeast of Villargordo del Cabriel	325561, 325562, 325563, 325564, 325565	OQ863132, OQ863133, OQ863134, OQ863135, OQ863136	OQ914290
Mediimorda batteni	Spain: Valladolid: 6 km South of Boecillo	325572, 325573, 325574	OQ863143, OQ863144, OQ863145	

(*Continued*)

Table I. (Continued).

Taxon	Locality	MNCN Entomology collection number	GenBank *cox1* accession number	GenBank ITS2 accession number
Mediimorda bipunctata	Spain: Cáceres: 4 km East of Hervás	325531, 325532, 325534	OQ863103, OQ863104, OQ863106	OQ914297, OQ914295, OQ914296
Mediimorda bipunctata	Spain: Guadalajara: 2.3 km Southeast of Uceda	331078, 325611	OQ863091, OQ863182	OQ914293, OQ914294
Mediimorda bipunctata	Spain: Guadalajara: 2.5 km East of Puebla de Valles	325546	OQ863117	
Mediimorda bipunctata	Spain: Madrid: 2 km South of La Cabrera	325590, 325591	OQ863161, OQ863162	OQ914291, OQ914292
Mordellistena	Spain: Zaragoza: 5 km Southeast of Sos del Rey Católico	325644	OQ863195	
Stenalia	Spain: Navarra: 2 km North of Rípodas	325635	OQ863194	
Variimorda	Spain: Zaragoza: 3 km North of Urriés	325634	OQ863193	OQ914298

19 terminals and 230 pb. Both matrices were aligned separately, *cox1* matrix was aligned manually while ITS2 was aligned using the multiple alignment in Geneious Prime 2021.0.3 software using gaps. Selection of the best model of substitution and best codon partition scheme was calculated using PartitionFinder2 (Lanfear et al. 2017). The corresponding evolutionary model for the *cox1* was GTR +G (1, 3), F81 + G (2). For the ITS2 gene, the evolutionary model used was JC. Bayesian inference method was implemented in the MrBayes v.3.2.6 program (Huelsenbeck & Ronquist 2001; Ronquist et al. 2012) and consisted of two simultaneous runs of 50 million generations each, sampling trees every 10,000 generations. Mixing and convergence among runs were evaluated by checking the average standard deviation of split frequencies and the Effective Sample Size (ESS) values and Potential Scale Reduction Factor (PSRF) for each parameter. A majority consensus tree was reconstructed after discarding the first 25,000 sampled trees as burn-in. All analyses were run in the public resource CIPRES Science Gateway v.3.3 (Miller et al. 2009).

Phylogeographic analyses were performed independently using *cox1* mitochondrial data and ITS2 nuclear data. DNA Sequence Polymorphism v.6.12.03 (Rozas et al. 2017) was used to collapse sequences to haplotypes or unique alleles. Allele networks were constructed through Population Analysis with Reticulate Trees (PopART) v.1.7 (Leigh & Bryant 2015) applying a TCS algorithm (Clement et al. 2002) to estimate relationships among alleles.

The divergence times for each splitting event was estimated in BEAST v.2.5.2 (Bouckaert et al. 2019) using the *cox1* dataset and applying a Bayesian uncorrelated lognormal relaxed clock model (Drummond et al. 2006). The model of the molecular substitution was estimated with the package bModelTest (Bouckaert & Drummond 2017). Mean and standard deviation of the substitution rate followed the rate estimated by Papadopoulou et al. (2010). Accordingly, the *ucld.mean* parameter for the *cox1* marker was assigned a lognormal distribution in real space, with initial value: Log(Mean): 0.0168 and Log(Stdev): 0.0018. The Yule model (Heled & Drummond 2012) was used as a tree prior. Analysis was run for 100 million generations, sampling every 1000 generation. To assess the convergence of the Markov Chain Monte Carlo (MCMC) run, the trace plots and effective sample sizes were inspected in Tracer v.1.7.2 (Rambaut et al. 2018). After discarding the burn-in (25%), the remaining trees were employed to build a maximum-clade credibility (MCC) tree with 95% high-posterior density (HPD) credibility intervals of ages using TreeAnnotator v.2.6.4 (Rambaut & Drummond 2021) included in the BEAST package. Trees were visualized with FigTree v.1.4.4 (Rambaut 2018).

2.4 *Morphological analyses*

Species identification was carried out following Plaza-Infante (1985) and Leblanc (2002, 2007). Specimens were also dissected to extract the male paramera of the genitalia, used as an identification character for species of *Mediimorda*.

Extended depth-of-focus images, of dry-mounted specimens were taken on a Leica DFC450 stereomicroscope, with magnification up to 120×, illuminated with diffuse light, using the LAS X software from Leica Microsystems.

2.5. *Map modelling*

To obtain the potential distribution of each species, species distribution models (SDMs) were calculated and the macroclimatic niche of the species were estimated using the Maximum Entropy Algorithm (Maxent) v.3.3.3k (Phillips et al. 2006; Phillips & Dudík 2008). Based on the sampled specimens, collection material (MNCN) and literature records (Ermisch 1969; Batten 1976; Plaza-Infante 1985; Ruzzier 2013), a total of 83 localities including all the known localities of *M. batteni* and many localities of *M. bipunctata*, were used. See Supplementary Table S1 for more details. A set of bioclimatic variables downloaded from WorldClim v 2.0 with a resolution of 30s (~1 km) were used (Fick & Robert 2017). To only consider the relevant biological variables into consideration a correlation and variance inflation factor analysis (vif) was performed using the *corrgram* (Wright 2018) and *HH* (Heiberger 2020) packages in R (v.4.0.1 www.r-project.orgi). After removing the variables with high correlation values (Pearson's correlation coefficient >0.8 and vif >10), the Mean Diurnal Range (Bio02), Isothermality (Bio03), Maximum Temperature of Warmest Month (Bio05) and Precipitation of Wettest Month (Bio13) were used to generate the model. To construct the Maxent model, default configuration with 10 replicates and a regularization multiplier (beta) of 2 were used, to produce a general response shape representing a more biologically realistic behaviour (Radosavljevic & Anderson 2014). To omit the less suitable records, the 10th percentile training presence threshold was set. The model was evaluated using the "area under the curve" (AUC) values obtained for the training data. With the Maxent model, the response curves of the climatic variables were also calculated, to show which ranges of the environmental conditions are more favourable for the distribution of the species. To better understand the species distribution, the resultant models were converted in a presence/absence map using the 10-percentile training presence threshold as a binary threshold.

3. Results

3.1 *Phylogenetic results and divergence time estimates*

Bayesian hypotheses derived from the mitochondrial *cox1* dataset recovered two well-supported groups (clades A and B) with posterior probability (PP) of 1 and 0.99 respectively. These clades can be morphologically assignable to *M. bipunctata* (clade A) and *M. batteni* (clade B) (Figure 1). The Bayesian phylogram derived from the nuclear marker ITS2 was completely congruent with the mitochondrial (Figure 2). Populations of *M. bipunctata* (clade A) are distributed in Central Spain while *M. batteni* (clade B) has a wider distribution encompassing the central and northern parts of the Iberian Peninsula, with a locality from southern Spain (Figures 1 and 2). Our divergence time estimation suggests that these two clades began to diverge during the Pliocene [3.42 Ma Mean; 95% highest posterior density (HPD) = 5.74–1.39 Ma] (Figure 3).

3.2 *Phylogeographic structure*

The specimens of *M. batteni* (clade B) analysed for mitochondrial *cox1* are represented by 47 haplotypes in the TCS network, depicting a multiple star pattern and high haplotype diversity with 38 different single individual haplotypes, characteristic for populations that have been stable in a geographic area for a comparatively long period (Figure 4(a)). Haplotypes of *M. batteni* are widespread across the Iberian Peninsula, including the Central Mountain System, the Iberian Mountain System, the Northern Iberian Plateau, the Pyrenees and Sierra Tejeda. One haplotype present in 20 individuals is found in specimens distributed throughout the region, evidencing a total absence of geographic structure within the species. Specimens of *M. bipunctata* (clade A) are represented by three different haplotypes occurring in populations in the Central Mountain System and constituting a cluster that is widely separated from the specimen representing *M. batteni* (clade B) (Figure 4(b)).

Phylogeographic analyses of the ITS2 nuclear data gave two different nuclear alleles; one isolated allele corresponding to *M. bipunctata* that is separated by 10 positions from the other allele representing *M. batteni* (Figure 5).

3.3 *Morphological analyses*

After reviewing various morphological characters likely useful to discriminate between *M. batteni* and *M. bipunctata*, we agree with Plaza-Infante

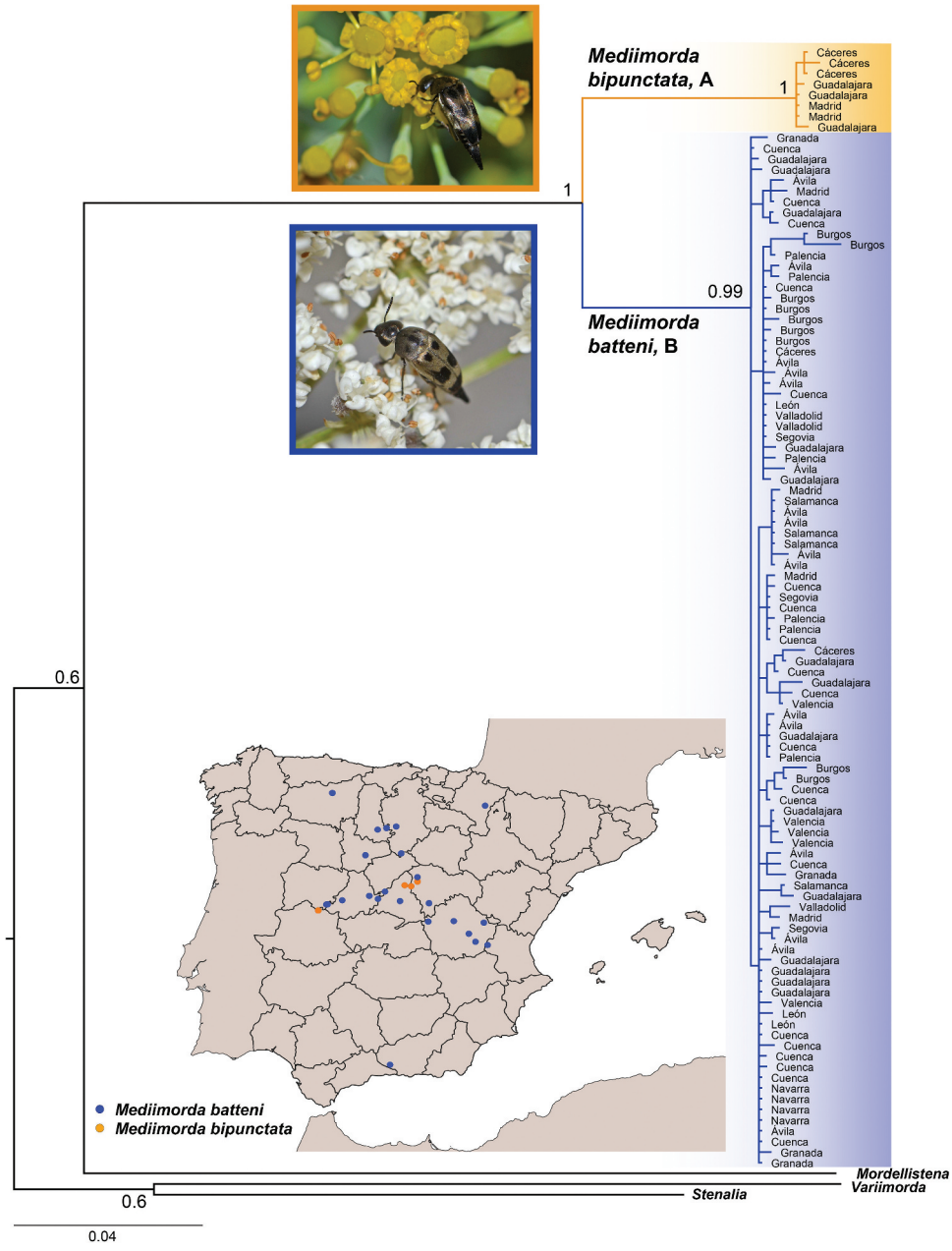

Figure 1. Bayesian phylogenetic tree based on *cox1* mitochondrial data. The colours represent the different mitochondrial lineages recovered in this study: *Mediimorda bipunctata* (Germar, 1827), clade A (Orange) and *Mediimorda batteni* Plaza-Infante, 1985, clade B (blue). Numbers near the main nodes indicate Posterior Probabilities values (PP). Localities of the specimens are represented in the Iberian Peninsula map, where all specimens of clade A share the same locality with clade B.

(1985) and Leblanc (2002, 2007) that characters in the male genital paramera are the most useful. Genital paramera are longer in *M. batteni* (>1 mm) than in *M. bipunctata* (<1 mm) and the shape of the paramera also differs between the two species. In *M. batteni* dorsal branch of the right paramere is straight and pointed apically (Figure 7 (b)), while it is curved and blunt in *M. bipunctata* (Figure 7(e)); the apex of the ventral branch is comparatively broad in *M. batteni*, while it is more curved and slender in *M. bipunctata* (Figure 7(b,e)). In *M. batteni* the apex of the left paramere is spatulated (Figure 7(c)), while it is pointed in *M. bipunctata* (Figure 7(f)). On the other hand, coloration is quite variable and cannot be used as a diagnostic character.

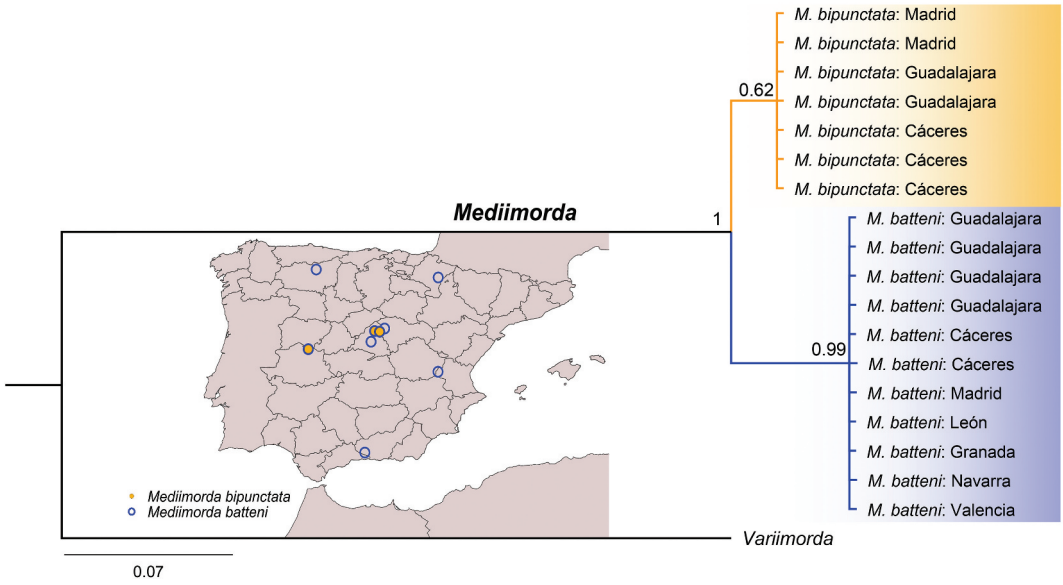

Figure 2. Bayesian phylogenetic tree based on ITS2 nuclear data. The colours represent the different species: *Mediimorda bipunctata* (Germar, 1827) (Orange) and *Mediimorda batteni* Plaza-Infante, 1985 (blue). Posterior probabilities (PP) are indicated for each of the main clades. Localities of the specimens are represented in the Iberian Peninsula map.

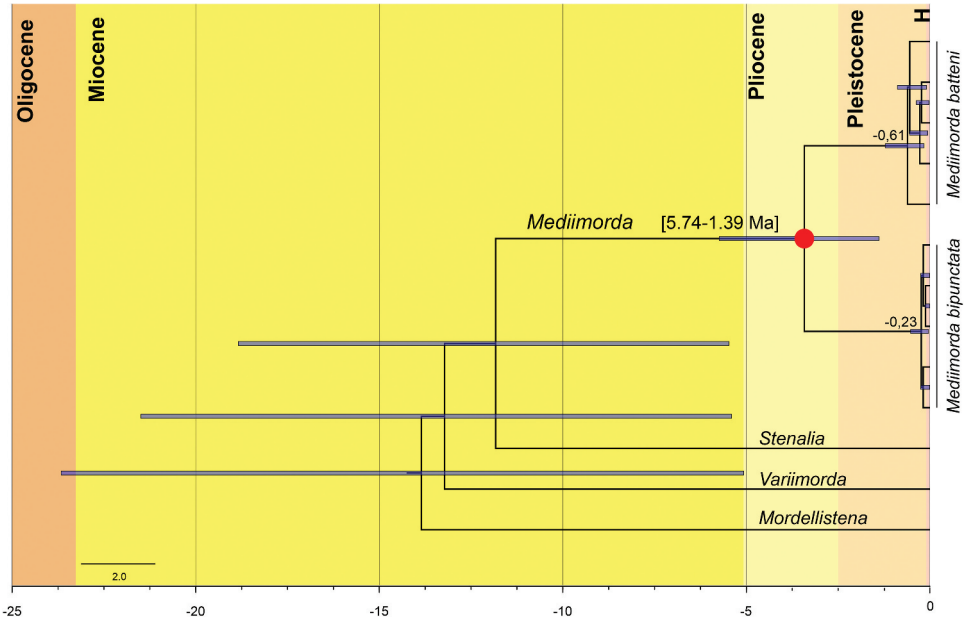

Figure 3. Chronogram showing lineage divergence times based on *cox1* mitochondrial data. The x-axis depicts time indicated in million years (Ma) and the corresponding geological periods. The 95% highest posterior density (HPD) intervals are represented by blue bars.

3.4 Climatic niche

The climatic niche models (Figures 6 and S1) depicting the potential distribution of the two species had high AUC test values for both *M. batteni* (AUC = 0.849) and *M. bipunctata* (AUC = 0.952). The models show that the two species have an almost overlapping potential distribution in the Iberian Peninsula, with highly suitable areas in the central and north-eastern parts of the peninsula (Figures 6, S1 and S3).

A Jackknife test showed that the four bioclimatic variables contributed unequally to the model. For *M. batteni* isothermality (Bio03) and precipitation of wettest month (Bio13) where the variables that accounted for the highest gain, while for

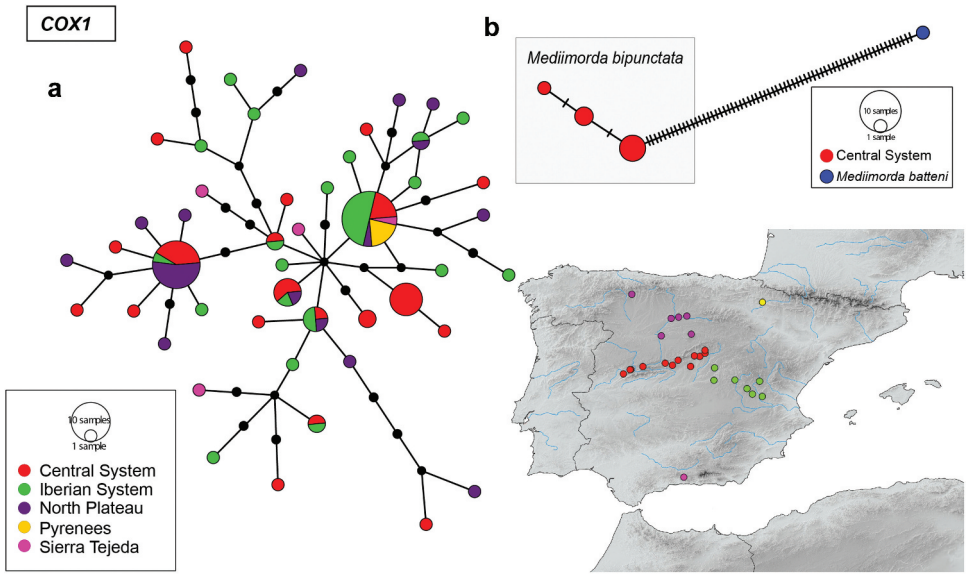

Figure 4. (a) TCS network of *Mediimorda batteni* Plaza-Infante, 1985 based on *cox1* mitochondrial marker. (b) TCS network of Iberian *Mediimorda bipunctata* (Germar, 1827) based on *cox1* mitochondrial marker, including one specimen of *M. batteni*. The size of the circles indicates the relative frequency of sequences belonging to a particular allele. Inferred intermediate haplotypes are represented by small black circles. Mutations are represented by hatch marks. Colours correspond to the geographic origin of the specimens as seen in the Iberian Peninsula map, showing the absence of geographic structure in the network.

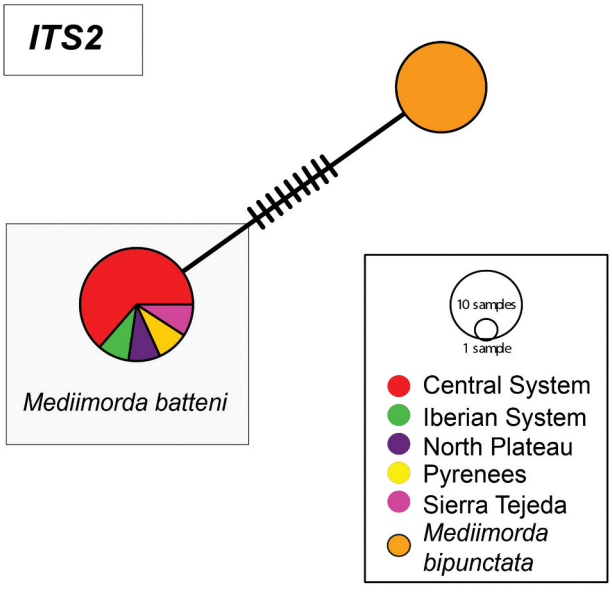

Figure 5. Allele network of *Mediimorda batteni* Plaza-Infante, 1985 and *Mediimorda bipunctata* (Germar, 1827) based on ITS2 nuclear marker. The size of the circles indicates the relative frequency of sequences belonging to a particular allele. Mutations are represented by hatch marks. Colours correspond to the geographic origin of the specimens and Orange haplotype correspond to a *M. bipunctata* specimen.

M. bipunctata it was the maximum temperature of warmest month (Bio05). The response curves of the bioclimatic variables show similar responses for climatic niches for both *M. batteni* and *M. bipunctata*, except that *M. batteni* shows a wider range of suitability for Bio03 and a more restricted range for Bio13 (Figure S2).

4. Discussion

4.1. Species differentiation and morphological change

Bayesian phylogenetic reconstruction analyses based on mitochondrial (*cox1)* (Figure 1) and nuclear (ITS2) (Figure 2) data support the existence of two well-differentiated lineages of *Mediimorda* in Central Spain. Mitochondrial and nuclear networks (Figures 4(b) and 5) depict two isolated clusters corresponding to *M. batteni* and *M. bipunctata*. Congruence between mitochondrial and nuclear DNA data suggests the absence of gene exchange between *M. batteni* and *M. bipunctata*, providing evidence for historical isolation between the two lineages that exists today, with no signals of hybridization (Crandall et al. 2000).

Our divergence time estimates suggest that *M. bipunctata* and *M. batteni* began to diverge around 3.42 Ma ago, during the Pliocene (Figure 3). Early and Middle Pliocene were characterised by warm climate, while the climate became highly variable during Late Pliocene (Haywood et al. 2000, 2009; Fedorov et al. 2013; Burke et al. 2018). In many organisms, speciation frequently took place during the glacial

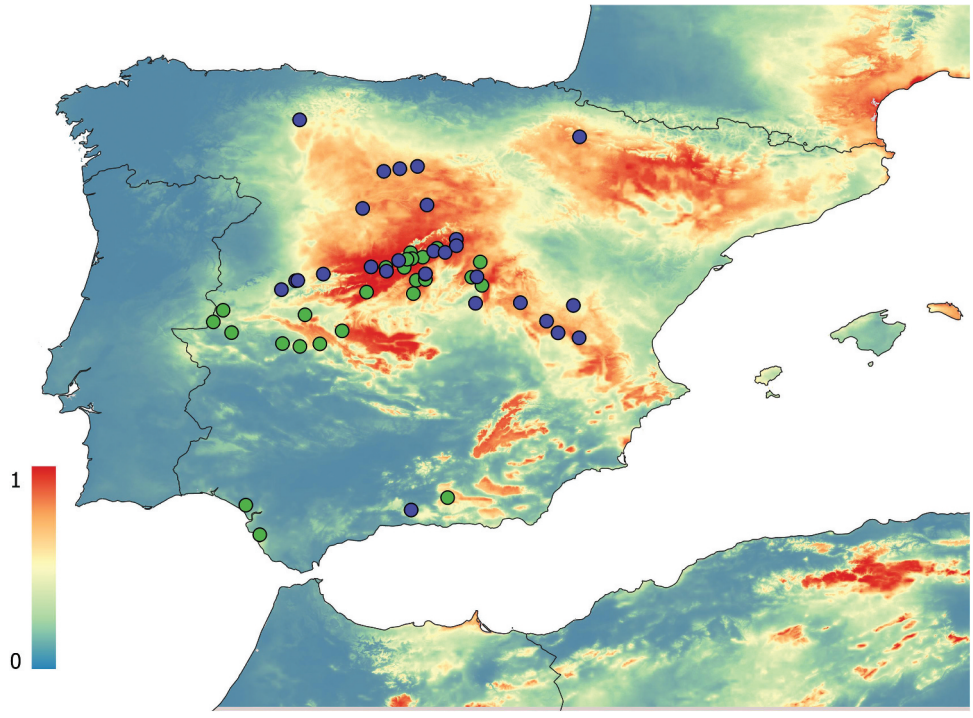

Figure 6. Potential geographic distribution of *Mediimorda batteni* Plaza-Infante, 1985. Red indicates areas of high suitability, and blue, areas of low suitability. Blue dots correspond to sampled specimens and green dots to previously published data (Supplementary Table S1).

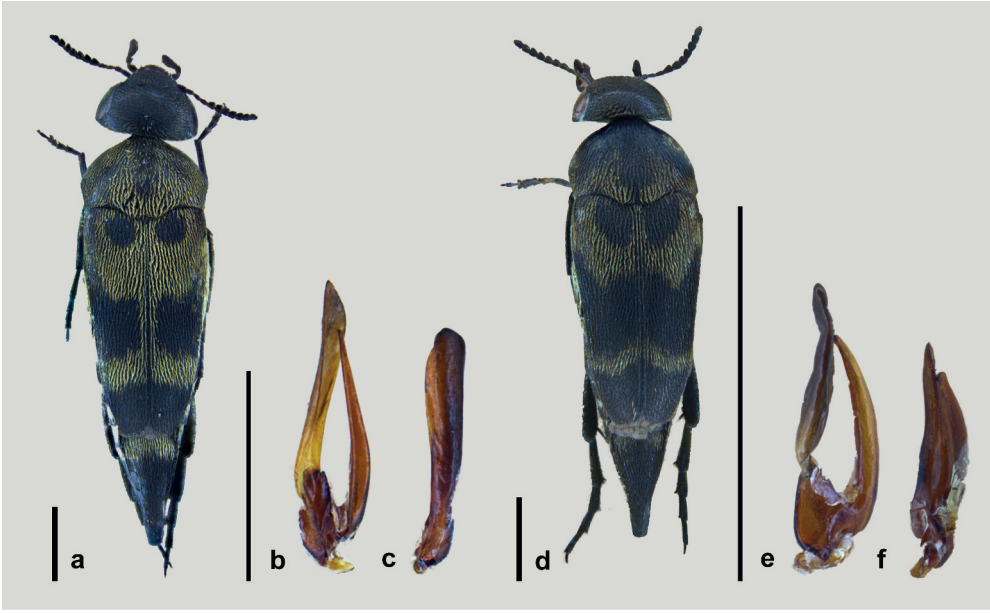

Figure 7. (a) *Mediimorda batteni* Plaza-Infante, 1985 habitus (MNCN_Ent 304450, holotype), (b) right paramere, (c) left paramere. (d) *Mediimorda bipunctata* (Germar, 1827) habitus (MNCN_Ent 305305), (e) right paramere, (f) left paramere. Scale bar 1 mm.

periods in Late Pliocene and Pleistocene (Veith et al. 2003; Carisio et al. 2004). In Southern Europe the dramatic climate changes often lead to isolation of populations in glacial refugia (Veith et al. 2003; Carisio et al. 2004; Hewitt 2011a; Moura et al. 2019). *Mediimorda* species commonly inhabit warm Mediterranean grasslands, and the major climatic changes during

Late Pliocene might have resulted in isolation and subsequent speciation.

Despite their relatively high genetic divergence, the two lineages of *Mediimorda* are very similar in external morphology (Figure 7(a,d)). Although slight differences between *M. batteni* and *M. bipunctata* can be seen in the colour pattern of the dorsal setation (Figure 7(a,d)), these differences can be the result of individual variation (see Leblanc 2007). Morphological similarity can be a consequence of ecological stability as the two species likely experienced similar environmental pressures throughout their evolutionary history (Zhao et al. 2019). Alternatively, the similarity can be a consequence of morphological stasis, i.e. slow evolution rates in morphological characters (Hedin 2001). General morphological homogeneity within the family Mordellidae is discussed by Liljeblad (1945); Jackman and Lu (2002); Serrahima (2011); Ruzzier (2013). This morphological homogeneity might be caused by morphological stasis derived from developmental or evolutionary constrains, acting very early during the origin of the family (see e.g. Smith 1983; Wake et al. 1983; Eldredge et al. 2005). The strong similarity in external morphology observed in *M. batteni* and *M. bipunctata* could thus also be a consequence of morphological stasis that not only act at the family level, but also at lower taxonomic levels.

However, *M. bipunctata* and *M. batteni* can be separated by the size and shape of the male genitalia (Figure 7) (Plaza-Infante 1985; Leblanc 2002, 2007). The distinctive development of insect genitalia can be caused by character displacement between closely related species with similar external morphology and behaviour (Shapiro & Porter 1989; Eberhard et al. 1998). The long isolation between *M. bipunctata* and *M. batteni* during Pliocene might have provided enough time for the male genitalia to differentiate enough to give complete reproductive isolation. External morphological traits have on the other hand not diverged noticeably due to the general trend of stasis in *Mediimorda*. However, this hypothesis cannot be tested without a phylogeny of the genus that establish if *M. batteni* and *M. bipunctata* are sister species or not.

4.2. *Sympatry and life history*

The phylogeographic analyses (Figures 1 and 2) together with the presence/absence map (Figure S3) demonstrate the partial sympatric and syntopic distribution of *M. batteni* and *M. bipunctata* in Central Spain. The presence/absence map shows that the predicted area of presence of *M. bipunctata* and *M. batteni* largely overlaps. In fact, all specimens of *M. bipunctata* were collected in localities where *M. batteni* also was present. Observations on the ecology of *M. batteni* agree with previous descriptions of its habitat; the species is usually found during hot summer days in semiarid grasslands and ruderal ecosystems where flowers of Apiaceae are abundant (Abdul-Rassoul 2010; Serrahima 2011; Ruzzier et al. 2017; Selnekovič & Ruzzier 2019).

Potential distribution models (Figures 6 and S1) and ecological response curves (Figure S2) show that *M. bipunctata* and *M. batteni* share a similar ecological niche and suggests that there is no ecological segregation between them. Considerable overlap was found in the climatic niches. Both species were constrained by a set of environmental conditions: Temperature of the Warmest Month (Bio05), Mean Diurnal Range (Bio02) and Precipitation of Wettest Month (Bio13). The optimal values of these climatic variables were found to be high maximum temperatures and moderate diurnal temperature fluctuations. However, the response curves for *M. batteni* and *M. bipunctata* varied for two of the climatic variables. Values of isothermality (Bio03) were lower in *M. batteni* than in *M. bipunctata*, which indicates that *M. batteni* prefers niches where the diurnal temperature oscillations relative to the annual oscillations are larger than in *M. bipunctata*. For precipitation of wettest month (Bio13), *M. batteni* shows a narrower range of suitability with preference for lower precipitation levels. The climatic variables Bio03 and Bio13 may thus explain the preference of *M. batteni* for the continental climate in Central Spain. This subtle difference in the ecology may be the key factors letting them coexist in sympatry until one excludes the other by competition according to Hardin's principle (Hardin 1960).

In conclusion, it is plausible that *M. bipunctata* and *M. batteni* diverged in two different geographic areas by allopatric speciation, in which geographical barriers initiated reproductive isolation and subsequent population divergence. Their current sympatric distribution may be the result of range changes occurring after the allopatric speciation, as range movements over time can produce areas of sympatry even though the distribution of sister species are not expected to overlap following the speciation event (Barraclough & Vogler 2000). *Mediimorda bipunctata* may have extended its geographic range westwards from Plio-Pleistocene refugium like the Balkan Peninsula (Hewitt 1999, 2011b), reaching the Iberian Peninsula during the Quaternary. The Iberian Peninsula might have served as a glacial

refugium for *M. batteni*, and *M. bipunctata* could have established contact with *M. batteni* populations in the Iberian Peninsula after the two species were fully reproductive isolated. This would explain the present sympatric distribution and the absence of hybridization between these two closely related species.

Although the geographic distribution of *M. batteni* seems to be restricted to the Iberian Peninsula by climatic conditions, the allele network reflects high intraspecific genetic diversity with no specific geographic structure, suggesting a high level of dispersal within its geographical range (Figure 4(a)). The lack of geographic population structure as seen in *M. batteni*, is not common, even among flying Coleoptera (Abellán et al. 2007; Drag et al. 2018; Brunetti et al. 2019). However, the absence of geographic structure suggests that *M. batteni* comprises a single panmictic population that is widely distributed in the Iberian Peninsula. Among the factors that may explain the absence of geographic structure, is a high dispersal rate combined with a large effective population size (see Marandel et al. 2018). Field records and previous literature on mordellids demonstrate that these beetles can be very abundant during spring and summer throughout most of their range. During the present study more than 200 specimens of *M. batteni* were observed on a single flowering *Eryngium campestre*. Therefore, *M. batteni* population size could be another reason to favour the existence of a panmictic structure over the entire species range (Marandel et al. 2018), but population and demographic analyses need to be performed to corroborate this hypothesis.

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Supplementary material

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ORCID

A. M. Sanz-laParra http://orcid.org/0000-0003-0580-6890
M. García-París http://orcid.org/0000-0002-9361-9405
E. K. López-Estrada http://orcid.org/0000-0001-5539-4826

References

Abdul-Rassoul MS. 2010. Tumbling flower beetles (Coleoptera, Mordellidae) of Iraq. Bulletin of the Iraq Natural History Museum 11(2):1–5.

Abellán P, Gómez-Zurita J, Millán A, Sánchez-Fernández D, Velasco J, Galián J, Ribera I. 2007. Conservation genetics in hypersaline inland waters: Mitochondrial diversity and phylogeography of an endangered Iberian beetle (Coleoptera: Hydraenidae). Conservation Genetics 8(1):79–88. DOI: 10.1007/S10592-006-9150-9.

Barraclough TG, Vogler AP. 2000. Detecting the geographical pattern of speciation from species-level phylogenies. The American Naturalist 155(4):419–434. DOI: 10.1086/303332.

Batten R. 1976. Mordellidae (Coleoptera) from the South of France and the Pyrenees. Entomologische Berichten 36 (11):164–171.

Beaver DL, Baldwin PH. 1975. Ecological overlap and the problem of competition and sympatry in the Western and Hammond's Flycatchers. The Condor 77(1):1–13. DOI: 10.2307/1366754.

Bouckaert RR, Drummond AJ. 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology 17(1):1–11. DOI: 10.1186/S12862-017-0890-6/FIGURES/6.

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, du Plessis L, Popinga A, Rambaut A, Rasmussen D, Siveroni I, Suchard MA, Wu C, Xie D, Zhang C, Stadler T, Drummond AJ. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Computational Biology 15(4):e1006650. DOI: 10.1371/JOURNAL.PCBI.1006650.

Brunetti M, Magoga G, Iannella M, Biondi M, Montagna M. 2019. Phylogeography and species distribution modelling of Cryptocephalusbarii (Coleoptera: Chrysomelidae): Is this

alpine endemic species close to extinction? ZooKeys 856:3–25. DOI: 10.3897/ZOOKEYS.856.32462.

Burke KD, Williams JW, Chandler MA, Haywood AM, Lunt DJ, Otto-Bliesner BL. 2018. Pliocene and Eocene provide best analogs for near-future climates. Proceedings of the National Academy of Sciences of the United States of America 115 (52):13288–13293. DOI: 10.1073/PNAS.1809600115/SUPPL_FILE/PNAS.1809600115.SM08.GIF.

Carisio L, Cervella P, Palestrini C, DelPero M, Rolando A. 2004. Biogeographical patterns of genetic differentiation in dung beetles of the genus *Trypocopris* (Coleoptera, Geotrupidae) Inferred from MtDNA and AFLP analyses. Journal of Biogeography 31(7):1149–1162. DOI: 10.1111/J.1365-2699.2004.01074.X.

Clement M, Snell Q, Walker P, Posada D, Crandall K. 2002. TCS: Estimating gene genealogies. Parallel and Distributed Processing Symposium 3:184. DOI: 10.1109/IPDPS.2002.1016585.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15(7):290–295. DOI: 10.1016/S0169-5347(00)01876-0.

Drag L, Hauck D, Rican O, Schmitt T, Shovkoon DF, Godunko RJ, Curletti G, Cizek L. 2018. Phylogeography of the endangered saproxylic beetle Rosalia longicorn, *Rosalia alpina* (Coleoptera, Cerambycidae), corresponds with its main host, the European beech (*Fagus sylvatica*, Fagaceae). Journal of Biogeography 45(12):2631–2644. DOI: 10.1111/JBI.13429.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed Phylogenetics and Dating with Confidence. PLOS Biology 4(5):e88. DOI: 10.1371/JOURNAL.PBIO.0040088.

Eberhard WG, Huber BA, Briceño RD, Salas I, Rodriguez V. 1998. One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. Evolution 52(2):415–431. DOI: 10.1111/J.1558-5646.1998.TB01642.X.

Eldredge N, Thompson JN, Brakefield PM, Gavrilets S, Jablonski D, Jackson JB, Lenski RE, Lieberman BS, McPeek MA, Miller W. 2005. The dynamics of evolutionary stasis. Paleobiology 31(S2):133–145. DOI: 10.1666/0094-8373(2005)031[0133:TDOES2.0.CO;2.

Ermisch K. 1969. Ergebnisse der Albanien-Expedition 1961 des Deutschen Entomologischen Institutes. 76. Beitrag. Coleoptera: Mordellidae. Beiträge zur Entomologie = Contributions to Entomology 19(7–8):845–859.

Fedorov AV, Brierley CM, Lawrence KT, Liu Z, Dekens PS, Ravelo AC. 2013. Patterns and mechanisms of early Pliocene warmth. Nature 496(7443):43–49. DOI: 10.1038/NATURE12003.

Fick SE, Robert JH. 2017. WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37(12):4302–4315. DOI: 10.1002/JOC.5086.

Gunter NL, Leavengood JM, Bartlett JS, Chapman EG, Cameron SL. 2013. A molecular phylogeny of the checkered beetles and a description of Epiclininae a new subfamily (Coleoptera: Cleroidea: Cleridae). Systematic Entomology 38(3):626–636. DOI: 10.1111/SYEN.12019.

Hardin G. 1960. The competitive exclusion principle. Science 131(3409):1292–1297. DOI: 10.1126/science.131.3409.1292.

Haywood AM, Dowsett HJ, Valdes PJ, Lunt DJ, Francis JE, Sellwood BW. 2009. Introduction. Pliocene climate, processes and problems. Philosophical transactions of the royal society A: Mathematical. Physical and Engineering Sciences 367(1886):3–17. DOI: 10.1098/RSTA.2008.0205.

Haywood AM, Sellwood BW, Valdes PJ. 2000. Regional Warming: Pliocene (3 Ma) Paleoclimate of Europe and the Mediterranean. Geology 28(12):1063. DOI: 10.1130/0091-7613(2000)28<1063:RWPMPO>2.0.CO;2.

Hedin MC. 2001. Molecular insights into species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae: Hypochilidae). Molecular Phylogenetics and Evolution 18(2):238–251. DOI: 10.1006/MPEV.2000.0882.

Heiberger RM. 2020. HH: Statistical analysis and data display: Heiberger and Holland. R Package Version 3.1-40. Available: https://CRAN.R-project.org/package=HH.

Heled J, Drummond AJ. 2012. Calibrated tree priors for relaxed phylogenetics and divergence time estimation. Systematic Biology 61(1):138–149. DOI: 10.1093/SYSBIO/SYR087.

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

Hewitt GM. 2011a. Quaternary phylogeography: The roots of hybrid zones. Genetica 139(5):617–638. DOI: 10.1007/S10709-011-9547-3.

Hewitt GM. 2011b. Mediterranean Peninsulas: The evolution of hotspots. In: Zachos FE, Habel JC, editors. Biodiversity hotspots distribution and protection of conservation priority areas. Berlin, Heidelberg: Springer. pp. 123–147. DOI: 10.1007/978-3-642-20992-5_7.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8):754–755. DOI: 10.1093/bioinformatics/17.8.754.

Jackman JA, Lu W. 2002. 101. Mordellidae. In: Arnett RH, Thomas MC, Skelley PE, Frank JH, editors. American Beetles. Polyphaga: Scarabaeoidea through Curculionoidea. Vol. II. Boca Raton: CRC Press. pp. 423–430.

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. DOI: 10.1046/J.1471-8286.2003.00519.X.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34 (3):772–773. DOI: 10.1093/MOLBEV/MSW260.

Leblanc P. 2002. Description de deux nouvelles espèces ouest-européennes du genre *Mediimorda* Méquignon, 1946 (Coleoptera Mordellidae). Publications de La Société Linnéenne de Lyon 71(8):317–324. DOI: 10.3406/LINLY.2002.13414.

Leblanc P. 2007. Révision des espèces ouest-paléarctiques du genre *Mediimorda* Méquignon, 1 946 et description de deux nouvelles espèces d'Afrique du Nord (Coleoptera Mordellidae). Publications de la Société Linnéenne de Lyon 76(9):235–250. DOI: 10.3406/LINLY.2007.13665.

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

Liljeblad E. 1945. Monograph of the family Mordellidae (Coleoptera) of North America, north of Mexico. Miscellaneous Publications, Museum of Zoology, University of Michigan Press, Ann Arbor, Michigan, No. 62

Lopes CM, De Barba M, Boyer F, Mercier C, da Silva Filho PJS, Heidtmann LM, Galiano D, Kubiak BB, Langone P, Garcias FM, Gielly L, Coissac E, de Freitas TRO, Taberlet P. 2015. DNA metabarcoding diet analysis for species with parapatric vs sympatric distribution: A case study on subterranean rodents. Heredity 114(5):525–536. DOI: 10.1038/hdy.2014.109.

Mallet J. 2005. Hybridization as an invasion of the genome. Trends in Ecology & Evolution 20(5):229–237. DOI: 10.1016/J.TREE.2005.02.010.

Marandel F, Lorance P, Andrello M, Charrier G, Le Cam S, Lehuta S, Trenkel VM. 2018. Insights from genetic and demographic connectivity for the management of rays and skates. Canadian Journal of Fisheries and Aquatic Sciences 75(8):1291–1302. DOI: 10.1139/cjfas-2017-0291.

Miller M, Zhuang M, Chen T, Pfeiffer W. 2009. The CIPRES Science Gateway V. 3.3. Available: https://www.phylo.org/index.php/. Accessed Jul 2021 4.

Moura CCM, Bastian HV, Bastian A, Wang E, Wang X, Wink M. 2019. Pliocene origin, ice ages and postglacial population expansion have influenced a panmictic phylogeography of the European Bee-Eater *Merops Apiaster*. Diversity 11 (1):12. DOI: 10.3390/D11010012.

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: The mid-Aegean trench calibration. Molecular Biology and Evolution 27 (7):1659–1672. DOI: 10.1093/MOLBEV/MSQ051.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190(3–4):231–259. DOI: 10.1016/J.ECOLMODEL.2005.03.026.

Phillips SJ, Dudík M. 2008. Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. Ecography 31(2):161–175. DOI: 10.1111/J.0906-7590.2008.5203.X.

Plaza-Infante E. 1985. Contribución al conocimiento del género *Mediimorda* Meq., 1946; *Mediimorda batteni* nov. sp. de la fauna española (Col., Mordellidae). Eos: Revista Española de Entomología 61:265–273.

Prado FD, Fernandez-Cebrián R, Hashimoto DT, Senhorini JA, Foresti F, Martínez P, Porto-Foresti F. 2016. Hybridization and genetic introgression patterns between two South American catfish along their sympatric distribution range. Hydrobiologia 788 (1):319–343. DOI: 10.1007/S10750-016-3010-5.

Radosavljevic A, Anderson RP. 2014. Making better Maxent models of species distributions: Complexity, overfitting and evaluation. Journal of Biogeography 41(4):629–643. DOI: 10.1111/JBI.12227.

Rambaut A. 2018. FigTree v.1.4.4. Available: http://tree.bio.ed.ac.uk/software/figtree/. Accessed Mar 2021 9.

Rambaut A, Drummond AJ. 2021. TreeAnnotator v.2. 6.4. Available: http://beast.community/.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5):901–904. DOI: 10.1093/SYSBIO/SYY032.

Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3):539–542. DOI: 10.1093/SYSBIO/SYS029.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez- Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34 (12):3299–3302. DOI: 10.1093/MOLBEV/MSX248.

Ruzzier E. 2013. Taxonomic and faunistic note on Italian Mordellidae (Coleoptera Tenebrionoidea) with redescription of *Falsopseudotomoxia argyropleura* (Franciscolo, 1942) n. comb. Bollettino della Società Entomologica Italiana 145 (3):103–115. DOI: 10.4081/BollettinoSEI.2013.103.

Ruzzier E, Ghahari G, Horák J. 2017. A checklist of the Iranian Mordellidae (Coleoptera: Tenebrionoidea). Zootaxa 4320 (1):146–158. DOI: 10.11646/zootaxa.4320.1.8.

Selnekovič D, Ruzzier E. 2019. New distributional records for sixteen Mordellidae species from the Western Palearctic (Insecta, Coleoptera, Mordellidae). ZooKeys 894:151–170. DOI: 10.3897/zookeys.894.39584.

Serrahima I. 2011. Catálogo provisional de los Mordellidae (Coleoptera) de Cataluña (España). Boletín de La Sociedad Entomológica Aragonesa (S.E.A) 48:375–381.

Shapiro AM, Porter AH. 1989. The lock-and-key hypothesis: Evolutionary and biosystematic interpretation of insect genitalia. Annual Review of Entomology 34(1):231–245. DOI: 10.1146/annurev.en.34.010189.001311.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6):651–701. DOI: 10.1093/AESA/87.6.651.

Smith JM. 1983. The genetics of stasis and punctuation. Annual Review of Genetics 17(1):11–25. DOI: 10.1146/annurev.ge.17.120183.000303.

Veith M, Kosuch J, Vences M. 2003. Climatic oscillations triggered post-Messinian speciation of Western Palearctic brown frogs (Amphibia, Ranidae). Molecular Phylogenetics and Evolution 26(2):310–327. DOI: 10.1016/S1055-7903(02)00324-X.

Wake DB, Roth G, Wake MH. 1983. On the problem of stasis in organismal evolution. Journal of Theoretical Biology 101 (2):211–224. DOI: 10.1016/0022-5193(83)90335-1.

Woodruff DS. 1973. Natural hybridization and hybrid zones. Systematic Biology 22(3):213–218. DOI: 10.1093/SYSBIO/22.3.213.

Wright K. 2018. Corrgram: Plot a correlogram. R Package Version 1.13. Available: https://CRAN.R-project.org/package=corrgram.

Zhao Q, Zhang H, Wei J. 2019. Climatic niche comparison across a cryptic species complex. PeerJ 2019(5):e7042. DOI: 10.7717/PEERJ.7042/SUPP-9.