

Phylogenetic patterns in zopherine beetles are related to ecological niche width and dispersal limitation

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Abstract

Niche conservatism has been proposed as the mechanism driving speciation in temperate montane clades through range fragmentation during climatic oscillations. Thus, a negative relationship between speciation rates and niche width is expected. Here, we test this prediction using American zopherine beetles. Our phylogenetic analyses recovered two clades in addition to that of the genus *Zopherus*: the genera *Verodes* and *Phloeodes*, which originated most likely in the Eocene, and diversified during the Miocene and the Pliocene. The assessment of clade niche width in relation to clade diversity supported the proposition of narrow niches leading to a higher probability of range fragmentation during climatic oscillations, thus increasing speciation. Additionally, almost all current populations of *Phloeodes* and *Verodes* are located within regions that retained favourable climatic conditions across warm and cold Pleistocene periods, suggesting that dispersal limitation is a strong factor controlling clade distribution. In sum, our results suggest that (i) niche width is a major determinant of the probability of speciation in temperate montane clades, by controlling the probability of potential range fragmentation and (ii) dispersal limitation is also a major determinant of the speciation process, by increasing the fragmentation of realized ranges even when potential distributions are cyclically fused during climatic oscillations. When dispersal limitation is extreme, as in zopherine beetles, populations persist just in those areas that have retained suitable conditions during extremes of past climatic oscillations. Paradoxically, this relict condition confers zopherine beetles great resilience for facing future climate change.

Keywords: climate change, dispersal, niche conservatism, past climates, speciation, species distribution models

Received 29 April 2011; revision received 23 September 2011; accepted 2 October 2011

Introduction

Investigating the role of ecological niche on speciation processes and thus on phylogenetic patterns is a central research agenda in the interface of evolutionary, biogeographical and ecological studies. The degree to which an ecological niche is conserved across evolutionary time has been subject of an important debate, as available evidence supports the existence within clades of

both niche conservatism (Kozak & Wiens 2006) and niche divergence (Graham *et al.* 2004; Losos 2008). These contradictory findings seem to be related to the scale of the question being asked, as 'niches are presumably neither identical nor completely different between close relatives' (Wiens 2008), but also probably to the fact that the ecological niche of species could interact with demographical processes leading to population isolation and speciation in different ways. Indeed, it has been recently proposed that speciation processes could be driven by antagonistic processes in temperate and tropical regions (Kozak & Wiens 2007). The

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rationale for this assumption relies on Janzen's (1967) hypothesis that lower seasonal temperature variation in the tropics would lead to higher biotic stratification in tropical mountains and thus to tropical species having narrower niches than temperate ones. Linked to this idea, Kozak & Wiens (2007) hypothesized that niche conservatism should predominate in speciation processes in temperate zones but niche divergence should predominate in speciation processes in the tropics, because of the contrasting selection for narrower niches in the tropics and wider niches in temperate regions. Using plethodontid salamanders, they found support for this hypothesis, as allopatric sister species showed much greater temperature overlap in the temperate region than in the tropics.

Although this pattern has not yet been tested in other taxa, we assume a high relevance of niche conservatism for speciation processes in temperate zones as a working hypothesis and propose that a further test for this idea could be the analysis of the influence of niche width on phylogenetic patterns. Our approach is rooted in the concept of ecological niche above the species level (Hadly *et al.* 2009), which assumes that ecological niches are attributes of supraspecific clades, primarily controlled by intrinsic life history traits and conserved among species within clades. So if niche conservatism is a process driving speciation, as hypothesized by Kozak & Wiens (2006, 2007) for temperate clades, the width of a clade's ecological niche should determine the probability of speciation events through population isolation during climatic oscillations (Fig. 1). The rationale behind is that potential distribution of clades (i.e. the areas with suitable climatic conditions) moves upwards and downwards in mountains during climatic oscillations. Upwards shifts of potential distributions can result in the fragmentation of the suitable area and, therefore, the wider the niche of a clade, the lower probability of suffering fragmentations of its geographical range, eventually leading to speciation events. Therefore, a negative relationship between speciation rates and niche width is expected.

Here, we test this prediction using two sister clades of zopherid beetles (Coleoptera: Zopheridae). A recent comprehensive phylogenetic hypothesis for Coleoptera based on molecular data (Hunt *et al.* 2007) strongly supports the monophyly of Zopheridae (s. str.) with Monommatidae, but shows no evidence of close relationships of this clade with Colydiidae, challenging previous interpretations on the phylogenetic position and diagnosis of Zopheridae that was considered to include Colydiidae and Monommatidae (Slipinski & Lawrence 1999). While the debate will continue at deep phylogenetic nodes, the content and diagnosis of the zopherid tribe Zopherini (as recognized by Slipinski & Lawrence

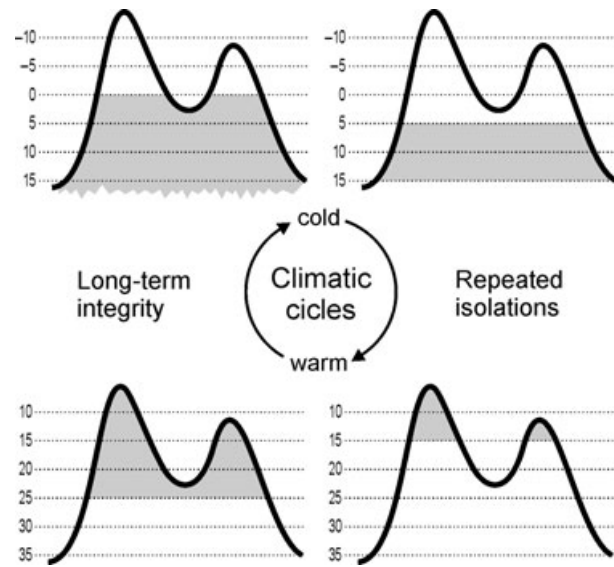


Fig. 1 Schematic representation of the hypothesis being tested in this study. Climatic oscillations between cold (upper row) and warm (lower row) periods make clade potential ranges move upwards and downwards in parallel to changing climate. A clade with a wide niche (left column) has a greater probability of retaining the integrity of its geographical range, whereas a clade with a narrow niche (right column) has a greater probability of suffering fragmentations of its geographical range, eventually leading to speciation events.

1999) have experienced relatively few modifications since Gebien's World catalogues of Tenebrionidae (Gebien 1910, 1937). However, the generic structure used by Foley & Ivie (2008) differs significantly from previous hypotheses by García-París *et al.* (2006). The taxonomic arrangement presented by Foley & Ivie (2008) follows a phylogenetic hypothesis based on morphological data. This phylogenetic hypothesis, although comprehensive in taxa analysed, lacks any statistical support and therefore may represent a misleading basis to support a robust supra specific taxonomy. In this work, we generate a robust molecular phylogenetic hypothesis for North American mainland lineages of Zopherini using both mitochondrial and nuclear sequence data for 16 species. With this robust phylogenetic hypothesis at hand, our aims are (i) to estimate the relative antiquity (Pangaeon vs. Mio-Pliocene) of the older splits among branches of the main continental lineages, based on sequence divergence; (ii) to assess whether clades with narrower ecological niches are those presenting higher diversification, as a further test of the relevance of niche conservatism in speciation processes of temperate montane clades; (iii) to estimate the migration ability of clades by comparing the location of current populations with their respective potential distribution of clades under present and past climatic

conditions; and (iv) to estimate the effect of future climatic change conditions by comparing present localities with future potential distributions while taking into account the estimated migration ability.

Material and methods

Sampling and sequencing

We obtained tissue samples from a total of 111 individuals corresponding to 16 different species and coming from 53 populations from Guatemala to Southwest USA. Additionally, three individuals of *Zopherus* were used as outgroups. Sampling effort was especially strong in Central Mexico where most diversity is concentrated (Table 1, Fig. 2a, b). Tissue was extracted from the femur of frozen specimens, and total genomic DNA was extracted using a phenol–chloroform protocol (Sambrook & Russell 2001), preceded by a digestion with proteinase K. Polymerase chain reaction (PCR) was used to amplify 630 bp of the mitochondrial cytochrome oxidase I gene (COI), using the primers LCO1490 (Folmer *et al.* 1994) and COI-H (Machordom *et al.* 2003). To improve the resolution of phylogenetic analyses, additional fragments were sequenced for 32 specimens representing all lineages found with the COI data set: 340 bp of the nuclear histone 3 (H3) gene was sequenced using the primers HexAF and HexAR (Ogden & Whiting 2003), as well as another mitochondrial fragment of 760 bp including a small piece of the ND1 gene, the tRNA Leucine and part of the 16S ribosomal RNA using the primers 16Sbr and 16Sar (Palumbi *et al.* 1991). PCRs were performed in a total volume of 25 μ L, including one unit of Taq polymerase (Biotools, 5 U/mL), 2.5 μ M of each primer, 0.4 mM of dNTPs, 1.5 mM of MgCl₂ and 67 mM of a reaction buffer (Tris–HCl, pH = 8.3, Biotools). PCRs consisted of 35 cycles with a denaturing temperature of 94 °C (30 s), annealing at 42 °C (LCO1490 + COI-H and MAR + 16Sar primer pairs) or 54 °C (HexAF + HexAR primer pair) (45 s) and extension at 72 °C (90 s). Double-strand templates were cleaned using sodium acetate and ethanol to precipitate the PCR products and then re-suspended in 22 μ L of ddH₂O. Sequencing reactions were performed for both strands and sequenced on an ABI PRISM 3730 DNA sequencer as explained in Martínez-Solano *et al.* (2006).

Sequence alignment and phylogenetic analyses

All sequences were compiled using Sequence Navigator™ version 1.0.1 (Applied Biosystems) and aligned manually. Data were analysed with the software jModelTest 0.1.1 (Posada 2008) to determine the substitution

model, under the Akaike Information Criterion (AIC), that best fit our data for subsequent analyses. To analyse the combined data set, we considered three partitions, one for each gene, and employed two different approaches. Maximum likelihood (ML, Felsenstein 1981) analyses were performed using the heuristic search algorithm implemented in GARLI v2.0 (Zwickl 2006) with model parameters estimated with jModelTest. We also used GARLI v2.0 to perform nonparametric bootstrapping (1000 pseudoreplicates) to assess the stability of internal branches. Bayesian phylogenetic analyses were conducted with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) and a partitioned data set. We initiated the analysis with random starting trees and run four Metropolis coupled Monte Carlo Markov chains (three heated, one cold) for 20×10^6 generations, sampling every 1000 generations. Analysis was run four times and checked for stationarity and convergence of the chains with Tracer v1.5 (Rambaut & Drummond 2007). All ESS values for each run were above a threshold of 200. We discarded 25% of the trees as burn-in. Posterior clade probabilities were used to assess nodal support. To analyse the COI data set, we used also ML (heuristic search and 1000 nonparametric bootstrap pseudoreplicates) and bayesian analyses (20×10^6 generations, sampling every 1000 generations and the first 5000 trees discarded as burn-in). Additionally, we applied a neighbour-joining (NJ) analysis under the minimum evolution objective function with uncorrected ('p') distances, and ties were broken systematically with the program PAUP*4.0b10 (Swofford 2002). This analysis was selected to get an insight on intraspecific diversification within terminal clades.

We used BEAST v1.6.1 (Drummond & Rambaut 2007) to estimate divergence times among clades. Times to the most recent common ancestors (TMRCA) were obtained using estimated substitution rates for tenebrionid beetles: 0.0168 ± 0.0018 for *cox1*, 0.0054 ± 0.0009 for 16S and 0.0017 ± 0.0003 for nuclear DNA (Papadopoulou *et al.* 2010). We performed several trials including analyses of each gene independently and of the combined data set, with and without outgroups. The analyses were run under an uncorrelated lognormal relaxed clock model and the Birth and Death process speciation tree prior with a length of chain of 50 millions sampled every 1000 generations. Each analysis was performed four times, and the results were compared in Tracer v1.5 and combined with logcombiner v1.6.1 with a 10% of burnin for each run.

Climatic niches

Climatic niches were estimated using climatic envelopes for each clade. As our aim was to assess the

Table 1 Localities, voucher information and GenBank Accession nos for the sequences and specimens used along the molecular study. All specimens were collected by the authors from 2001 to 2008

Species	Locality	Field no.	COI	16S	H3
<i>Verodes inaequalis</i>	México: México: Nevado de Toluca, 9.2 km desde Raíces	ZZ162	JN703505	JN703602	JN703634
<i>V. inaequalis</i>	México: México: Nevado de Toluca, 9.2 km desde Raíces	ZZ163	JN703506	—	—
<i>V. inaequalis</i>	México: México: 3.1 km al oeste del cruce Toluca-Carretera del Nevado	ZZ169	JN703507	—	—
<i>V. inaequalis</i>	México: México: 3.1 km al oeste del cruce Toluca-Carretera del Nevado	ZZ170	JN703508	—	—
<i>V. inaequalis</i>	México: Puebla: La Malinche	ZK075	JN703509	—	—
<i>V. inaequalis</i>	México: México: Río Frío	ZZ114	JN703510	—	—
<i>V. inaequalis</i>	México: México: Río Frío	ZZ115	JN703511	—	—
<i>V. inaequalis</i>	México: México: Paso de Cortés, Falda del Volcán Popocatepetl	ZZ104	JN703512	—	—
<i>V. inaequalis</i>	México: México: Cerro del Ajusco	ZZ124	JN703513	—	—
<i>V. inaequalis</i>	México: México: Cerro del Ajusco	ZZ125	JN703514	—	—
<i>V. inaequalis</i>	México: Hidalgo: El Chico, carretera del parque Km. 14	ZZ171	JN703515	—	—
<i>V. inaequalis</i>	México: Hidalgo: El Chico, carretera del parque Km. 14	ZZ172	JN703516	—	—
<i>V. inaequalis</i>	México: Hidalgo: 3 km al oeste de Mineral del Monte	ZZ121	JN703517	JN703603	JN703635
<i>V. inaequalis</i>	México: Veracruz: 4.8 km al sur de Atlahuilco	ZK083	JN703518	JN703604	JN703636
<i>V. inaequalis</i>	México: Veracruz: Puerto del Aire	ZK084	JN703519	—	—
<i>V. inaequalis</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZK089	JN703520	—	—
<i>V. inaequalis</i>	México: Veracruz: Huitzila	ZZ146	JN703521	—	—
<i>V. inaequalis</i>	México: Puebla: 3.8 km W Zoquitlán	ZK080	JN703522	JN703605	JN703637
<i>V. inaequalis</i>	México: Puebla: 3.8 km W Zoquitlán	ZK081	JN703523	—	—
<i>V. inaequalis</i>	México: Puebla: Lagunas de San Bernardino: Col. 24 Febrero	ZK094	JN703524	—	—
<i>V. inaequalis</i>	México: Veracruz: La Joya	ZZ154	JN703525	—	—
<i>V. inaequalis</i>	México: Jalisco: Nevado de Colima, 2900 m	ZZ107	JN703526	—	—
<i>V. inaequalis</i>	México: Jalisco: Nevado de Colima, 2900 m	ZZ108	JN703527	—	—
<i>V. inaequalis</i>	México: Veracruz: RMO El Ataque, 16 km S Huayacocotla	ZZ079	JN703528	JN703606	JN703638
<i>V. inaequalis</i>	México: Veracruz: RMO El Ataque, 16 km S Huayacocotla	ZZ080	JN703529	—	—
<i>Verodes carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZK110	JN703530	—	—
<i>V. carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZK111	JN703531	—	—
<i>V. carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZK112	JN703532	JN703607	JN703639
<i>V. carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZK087	JN703533	—	—
<i>V. carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZK086	JN703534	—	—
<i>V. carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZZ003	JN703535	—	—
<i>V. carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZZ004	JN703536	—	—
<i>V. carinatus</i>	México: Puebla: Nicolás Bravo: Huitzique	ZK113	JN703537	—	—
<i>V. carinatus</i>	México: Veracruz: Puerto del Aire: El Sumidero	ZK088	JN703538	—	—
<i>V. carinatus</i>	México: Puebla: Lagunas de San Bernardino: Colonia 24 Febrero	ZK117	JN703539	—	—
<i>V. carinatus</i>	México: Puebla: Nicolás Bravo: Huitzique	ZK092	JN703540	—	—
<i>V. carinatus</i>	México: Puebla: Lagunas de San Bernardino: Colonia 24 Febrero	ZK114	JN703541	—	—
<i>V. carinatus</i>	México: Puebla: Lagunas de San Bernardino: Colonia 24 Febrero	ZK115	JN703542	—	—
<i>V. carinatus</i>	México: Puebla: Lagunas de San Bernardino: Colonia 24 Febrero	ZK116	JN703543	—	—
<i>V. carinatus</i>	México: Puebla: Lagunas de San Bernardino: Colonia 24 Febrero	ZK095	JN703544	—	—
<i>V. carinatus</i>	México: Puebla: Lagunas de San Bernardino: Colonia 24 Febrero	ZK097	JN703545	—	—
<i>V. carinatus</i>	México: Oaxaca: Puerto Soledad (carretera Huautla-Teotitlán)	ZZ011	JN703546	—	—
<i>V. carinatus</i>	México: Puebla: 3.8 km al oeste de Zoquitlán	ZK082	JN703547	JN703608	JN703640
<i>Verodes</i> sp. nov. B	México: Veracruz: 5 km al noreste de La Joya	ZZ156	JN703548	JN703609	JN703641
<i>Verodes</i> sp. nov. B	México: Veracruz: 5 km al noreste de La Joya	ZZ158	JN703549	JN703610	JN703642
<i>Verodes interruptus</i>	México: Oaxaca: Sola de Vega: La Cumbre	ZK099	JN703550	JN703611	JN703643
<i>V. interruptus</i>	México: Oaxaca: Sola de Vega: La Cumbre	ZK108	JN703551	—	—
<i>V. interruptus</i>	México: Oaxaca: Sola de Vega: La Cumbre	ZK107	JN703552	—	—
<i>V. interruptus</i>	México: Oaxaca: R.M.O. El Tejocote	ZZ075	JN703553	—	—
<i>V. interruptus</i>	México: Oaxaca: R.M.O. El Tejocote	ZZ076	JN703554	—	JN703644
<i>V. interruptus</i>	México: Oaxaca: San Miguel Suchixtepec	ZK100	JN703555	JN703612	JN703645
<i>V. interruptus</i>	México: Oaxaca: 10 km al oeste de Santa María Lachixio	ZK102	JN703556	—	—
<i>V. interruptus</i>	México: Oaxaca: 10 km al oeste de Santa María Lachixio	ZK105	JN703557	JN703613	JN703646

Table 1 Continued

Species	Locality	Field no.	COI	16S	H3
<i>V. interruptus</i>	México: Oaxaca: 10 km al oeste de Santa María Lachixio	ZK106	JN703558	JN703614	JN703647
<i>V. interruptus</i>	México: Oaxaca: 25 km al oeste de Zaachila	ZZ053	JN703559	—	—
<i>Verodes insignis</i>	México: Oaxaca: Concepción Pápalo	ZK079	JN703560	JN703615	JN703648
<i>V. insignis</i>	México: Oaxaca: 24 km al norte de San Juan del Estado	ZZ017	JN703561	—	JN703649
<i>V. insignis</i>	México: Oaxaca: 24 km al norte de San Juan del Estado	ZZ018	JN703562	—	—
<i>V. insignis</i>	México: Oaxaca: Cerro Corral de Piedra, 9 km al oeste de La Cumbre	ZZ029	JN703563	—	—
<i>V. insignis</i>	México: Oaxaca: Cerro Corral de Piedra, 9 km al oeste de La Cumbre	ZZ030	JN703564	—	—
<i>Verodes sparsus</i>	Guatemala: Chimaltenango: Tecpán, 2906 m	Zg8	JN703565	JN703616	JN703650
<i>V. sparsus</i>	Guatemala: Chimaltenango: Tecpán, 2906 m	Zg9	JN703566	—	—
<i>V. sparsus</i>	Guatemala: Totonicapán: Rancho de Teja, 3139 m	Zg2	JN703567	—	—
<i>V. sparsus</i>	Guatemala: Totonicapán: Rancho de Teja, 3139 m	Zg7	JN703568	—	—
<i>V. sparsus</i>	Guatemala: Huehuetenango: Aldea Yoch, Santa Eulalia, 2617 m	Zg3	JN703569	—	—
<i>V. sparsus</i>	Guatemala: Huehuetenango: Yulhuitz, 2526 m	Zg4	JN703570	—	—
<i>V. sparsus</i>	Guatemala: Huehuetenango: San Mateo Ixtatán, 3034 m	Zg1	JN703571	JN703617	JN703651
<i>Verodes sp. nov. A</i>	México: Oaxaca: Loma del Viento—San Isidro Buenos Aires	ZZ013	JN703572	—	—
<i>Verodes sp. nov. A</i>	México: Oaxaca: Loma del Viento—San Isidro Buenos Aires	ZZ014	JN703573	—	—
<i>Verodes sp. nov. A</i>	México: Oaxaca: Puerto de Peña Verde—Santa María Pápalo	ZZ160	JN703574	JN703618	JN703652
<i>Verodes exsculptus</i>	México: Oaxaca: 24 km al norte de San Juan del Estado	ZZ026	JN703575	JN703619	JN703653
<i>V. exsculptus</i>	México: Oaxaca: Cerro Corral de Piedra, 9 km al oeste de La Cumbre	ZZ041	JN703576	—	—
<i>V. exsculptus</i>	México: Oaxaca: Cerro Corral de Piedra, 9 km al oeste de La Cumbre	ZZ042	JN703577	—	—
<i>V. exsculptus</i>	México: Veracruz: RMO El Ataque, 16 km al sur de Huayacocotla	ZZ083	JN703578	JN703620	JN703654
<i>Verodes anceps</i>	México: México: Paso de Cortés, Falda del Volcán Popocatepetl	ZZ096	JN703579	JN703621	JN703655
<i>V. anceps</i>	México: México: Paso de Cortés, Falda del Volcán Popocatepetl	ZZ097	JN703580	—	—
<i>V. anceps</i>	México: México: Falda del Volcán Popocatepetl, Amecameca	ZZ130	JN703581	JN703622	JN703656
<i>V. anceps</i>	México: México: Falda del Volcán Popocatepetl, Amecameca	ZZ131	JN703582	JN703623	—
<i>Verodes championi</i>	México: Guerrero: Puerto del Gallo	ZZ090	JN703583	—	—
<i>Verodes sp. nov. C</i>	México: Oaxaca: 25 km al oeste de Zaachila	ZZ072	JN703584	—	—
<i>Verodes sp. nov. C</i>	México: Oaxaca: 25 km al oeste de Zaachila	ZZ073	JN703585	—	—
<i>Verodes sp. nov. C</i>	México: Oaxaca: 10 km al oeste de Santa María Lachixio	ZK103	JN703586	—	JN703657
<i>Verodes sp. nov. C</i>	México: Oaxaca: 10 km al oeste de Santa María Lachixio	ZK104	JN703587	JN703624	JN703658
<i>Verodes guatemalensis</i>	Guatemala: Quiché: Nebaj, 1928 m	Zg5	JN703588	JN703625	JN703659
<i>Verodes aequalis</i>	México: Oaxaca: RMO Corral de Piedra, 14 km al oeste de La Cumbre	ZZ138	JN703589	JN703626	JN703660
<i>V. aequalis</i>	México: Oaxaca: RMO Corral de Piedra, 14 km al oeste de La Cumbre	ZZ139	JN703590	—	—
<i>V. aequalis</i>	México: Oaxaca: Concepción Pápalo	ZK077	JN703591	—	—
<i>V. aequalis</i>	México: Veracruz: 5 km al norte de Tlaquilpa	ZZ144	JN703592	JN703627	JN703661
<i>Phloeodes diabolicus</i>	USA: California: Riverside Co.: c. 6 mi NO Aguanga	AS08002	JN703593	JN703628	JN703662
<i>P. diabolicus</i>	USA: California: Los Angeles Co.: Fort Tejon Rd., Littlerock	AS08003	JN703594	—	—
<i>P. diabolicus</i>	USA: California: Santa Barbara Co.: Tepusquet Canyon Rd.	AS08004	JN703595	JN703629	JN703663
<i>Phloeodes plicatus</i>	USA: California: Santa Barbara Co.: Tepusquet Canyon Rd.	AS08005	JN703596	JN703630	JN703664
<i>Phloeodes ashei</i>	México: Hidalgo: cerca de Tepeoco, Sierra de Zacualtipán	ZK119	JN703597	JN703631	JN703665
<i>P. ashei</i>	México: Veracruz: Huayacocotla: Zilacatipán	ZZer	JN703598	JN703632	JN703666
<i>Zopherus</i>	México: Puebla: Nicolás Bravo: Huitzique	ZK090	JN703599	JN703633	JN703667
<i>Zopherus</i>	México: Puebla: Nicolás Bravo: Huitzique	ZK091	JN703600	—	—
<i>Zopherus</i>	México: Sonora: Tecoripa	ZZ173	JN703601	—	JN703668

COI, cytochrome oxidase I; H3, histone 3.

influence of climatic niche on speciation processes within clades, we considered that the suitable climatic conditions for any of the populations included in a given clade represent the climatic niche of that clade.

This concept assumes that ecological niches are primarily controlled by intrinsic life history traits and conserved among species within clades (Hadly *et al.* 2009). Thus, the clade climatic niche is conceptualized as the

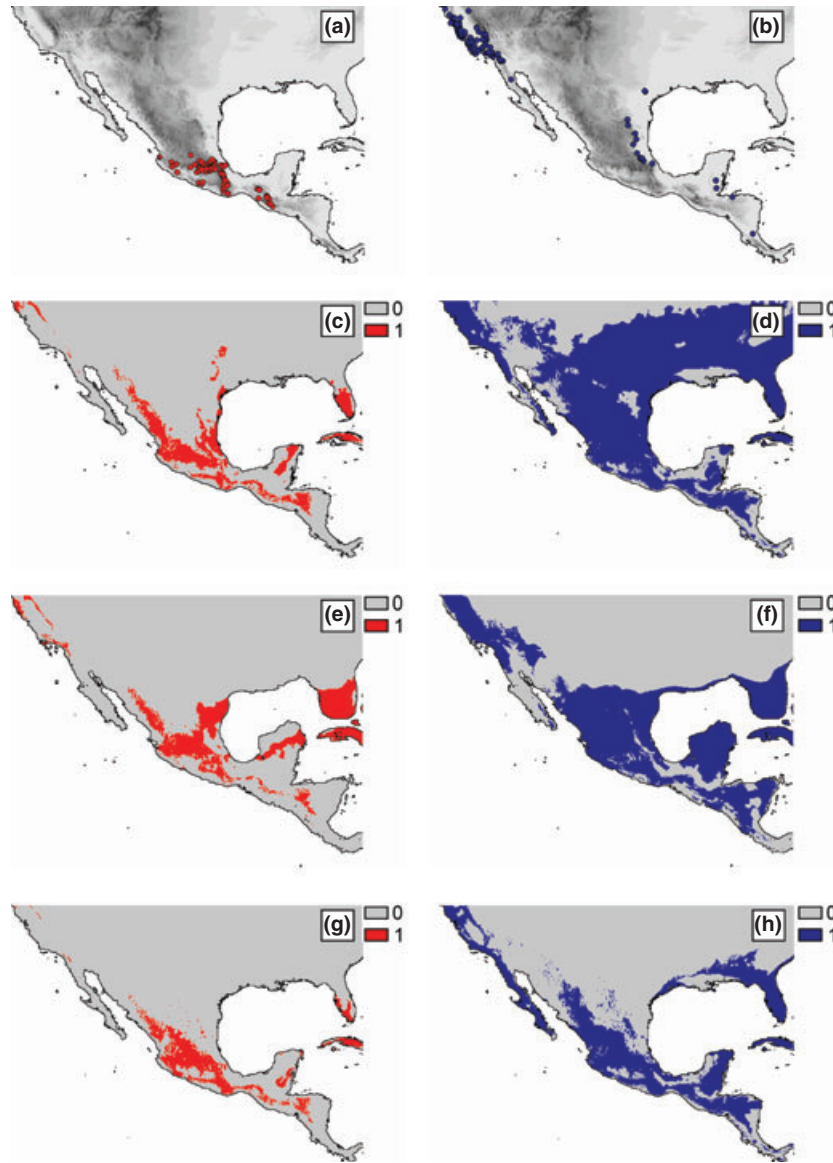


Fig. 2 Observed distributions of *Verodes* (a) and *Phloeodes* populations (b), and projected potential distributions for both clades (left column: *Verodes*; right column: *Phloeodes*) under current climatic conditions (c, d) and for last glacial maximum (e, f) and last interglacial maximum (g, h).

whole range of climatic conditions in which any of the populations belonging to the clade can develop. Therefore, we computed the maximum and minimum values of climatic variables within the observed distribution of a given clade, for the following variables: mean annual temperature (TANN), maximum temperature of the warmest month (TMAX), minimum temperature of the coldest month (TMIN), annual precipitation (PANN), precipitation of wettest quarter (PWET) and precipitation of driest quarter (PDRY) (variables bio1, bio6, bio7, bio12, bio16 and bio17 taken from Hijmans *et al.* 2005). Climatic data for 141 populations of *Phloeodes* (Fig. 2a) and for 143 populations of

Verodes (Fig. 2b) were obtained after georeferencing the collection localities of specimens included in this study, those deposited in collections (Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México; California Academy of Sciences; Essig Museum, University of California at Berkeley; Museo Nacional de Ciencias Naturales, CSIC), those extracted from an exhaustive bibliographic search (see Foley & Ivie 2008; and references therein), as well as data obtained along field sampling carried out by the authors at least once a year from 1996 to 2010 in Mexico (García-París *et al.* 2006; Baselga *et al.* 2009) and in 2005 to Guatemala, covering most of the mountain

systems of the region with and without previous records of zopherid beetles. The multidimensional space delimited by the maximum and minimum values of the six climatic variables was considered an estimate of the clade's climatic niche. To estimate the potential clade distribution, the niche was projected into space by considering as suitable all the 2.5 min grid cells with environmental conditions falling within the estimated niche. Therefore, potential range size is a measure of niche width. This algorithm is a strictly presence-only method, analogous to BIOCLIM (Busby 1991). It was selected because it is strictly a presence-only method, thus allowing us to approximate the potential distributions of clades rather than their realized distributions (Jiménez-Valverde *et al.* 2008), and because of its simplicity, which allows a direct link between maximum and minimum climatic values and projected distributions. Potential distributions of clades were spatially projected in the area between 180° and 79°E, 5° and 40°N for current climatic conditions (Hijmans *et al.* 2005), as well as for past conditions for the last glacial maximum (LGM) conditions (Braconnot *et al.* 2007) and last interglacial maximum (LIG) conditions (Otto-Bliesner *et al.* 2006) and future conditions for 2050 (CCM3 model, Govindasamy *et al.* 2003). LGM and LIG represent coldest and warmest climates during the last Pleistocene glacial cycle and date from about 21 000 and 120 000–140 000 kya, respectively. To assess the robustness of our results, potential distributions were also estimated using two different presence-only methods, BIOCLIM (Busby 1991) and DOMAIN (Carpenter *et al.* 1993). These models were performed in R (R Development Core Team 2009) using the R package *dismo* (Hijmans *et al.* 2010).

Results

The estimated substitution models were TrN + I + G for COI and HKY + I + G for 16S and H3. Sequences were deposited in GenBank, and Accession nos are given in Table 1. COI sequences yielded a total of 85 haplotypes among the ingroup species: 23 in *Verodes inaequalis*, 13 in *V. carinatus*, 2 in *Verodes* sp. nov. B, 10 in *V. interruptus*, 5 in *V. insignis*, 7 in *V. sparsus*, 3 in *Verodes* sp. nov. A, 4 in *V. exsculptus*, 2 in *V. anceps*, 1 in *V. championi*, 4 in *Verodes* sp. nov. C, 1 in *V. guatemalensis*, 4 in *V. aequalis*, 2 in *Phloeodes ashei*, 1 in *P. plicatus* and 3 in *P. plicatus*. Haplotypic diversity between species is not comparable through our data, because sampling is uneven among species.

The phylogenetic analysis of these haplotypes (Fig. 3) resulted in a poorly resolved tree at the internal nodes, but all the species were grouped in monophyletic clades with high statistical support. Only the *V. carinatus* and

V. interruptus clades presented low ML bootstrap values (54 and 60, respectively), but both were strongly supported by bayesian posterior probabilities. The addition of new characters to the phylogenetic analyses, corresponding to the 16S and H3 sequences, allowed a higher resolution of relationships among clades (Fig. 4), specially in the bayesian reconstruction. All ingroup haplotypes were split into two main clades each one corresponding to the genus *Phloeodes* and *Verodes*, respectively. Within the *Phloeodes* clade, the Central Mexican species *P. ashei* would be the sister lineage of a group including the Californian taxa. In the *Verodes* clade, the ML analysis does not resolve statistically the relationships among lineages. The bayesian analysis yielded a well-resolved topology with *V. inaequalis* being the sister lineage of the rest of species. The southernmost, Guatemalan species, *V. sparsus* and *V. guatemalensis*, share a clade with *V. aequalis* from central-eastern Mexico and do not appear to be sister species. In general, sister lineages in *Verodes* present parapatric distributions.

The estimations of TMRCA for the main clades provide different mean values but overlapping value ranges for each gene. The result of the combined analyses (Table 2) also lays on the ranges observed with each gene and provides an approximate temporal framework for the main clades: 30.4 (23.1–38.5) million years ago (Ma) for the ancestor of *Verodes*, 33.9 (25.6–44.1) Ma for *Phloeodes* and 53.7 (40.3–68.2) Ma to the most recent common ancestor of both clades.

Climatic niche was estimated to be much narrower for *Verodes* than for *Phloeodes*, especially regarding water requirements, and in lesser extent temperature requirements (Table 3): ranges for all variables (excepting TMAX) were narrower for *Verodes*, but the greatest difference was found for PANN, as no population of *Verodes* was observed in localities with annual precipitation lower than 555 mm, whereas populations of *Phloeodes* occur in arid environments with annual precipitation as low as 127 mm. The spatial projection of potential distributions of both clades under current climatic conditions stressed the relevance of niche width differences. The potential distribution of *Phloeodes* occupies 69% of the cells of 2.5 min comprised in the study area (Fig. 2c), whereas the potential distribution of *Verodes* is limited to 11% of the cells (Fig. 2d). The potential distribution of *Phloeodes* under LGM (Fig. 2e) and LIG (Fig. 2g) conditions was substantially reduced, occupying 35 and 26% of the cells, respectively, although in both cases, it was substantially more extended than the potential distribution of *Verodes*, which occupied 12 and 8% of the cells, respectively (Fig. 2f, h). Alternative species distribution models yielded similar projections for present, LGM and LIG conditions (Figs S1–S2, Supporting information).

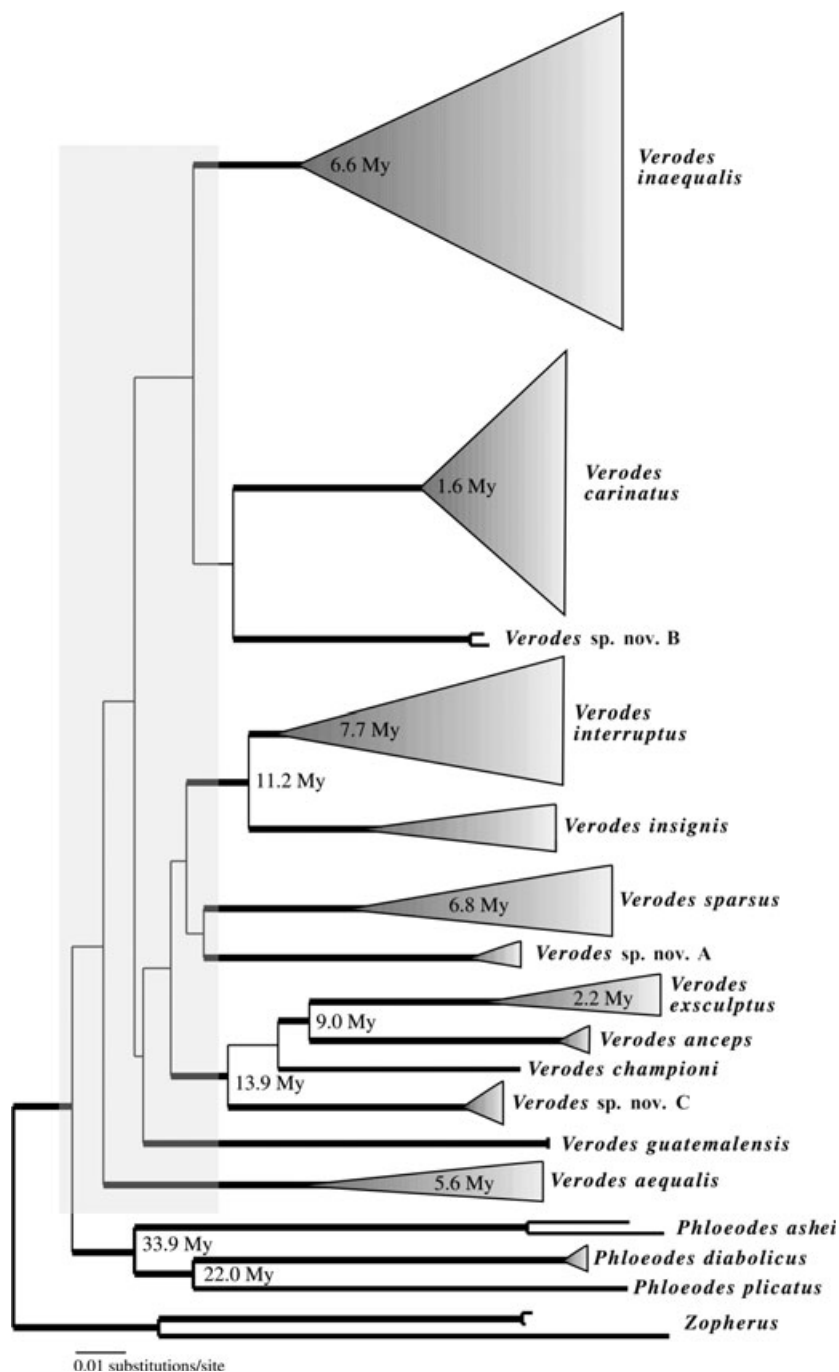


Fig. 3 Phylogenetic signal obtained from the complete COI data set of North American Zopherini beetles. Bayesian and NJ analyses show monophyly at the species level. Triangles indicate haplotype diversity within species; point of triangle indicates first branching event while triangle base is proportional to number of specimens. Shade at the base of the phylogenetic reconstruction indicates area of low branch support. Estimated times to the most recent common ancestors are indicated for selected nodes (see Table 2 for HPD intervals).

Almost all current populations of *Phloeodes* and *Verodes* are located within or very close to regions that retained favourable climatic conditions across all the analysed times (Fig. 5a, b): the mean distance between

current presences and the area of overlap between the three potential ranges for present, LGM and LIG was 5.1 km (SD = 24.7) for *Phloeodes* and 6.1 km (SD = 7.3) for *Verodes*. This indicates a very limited migration

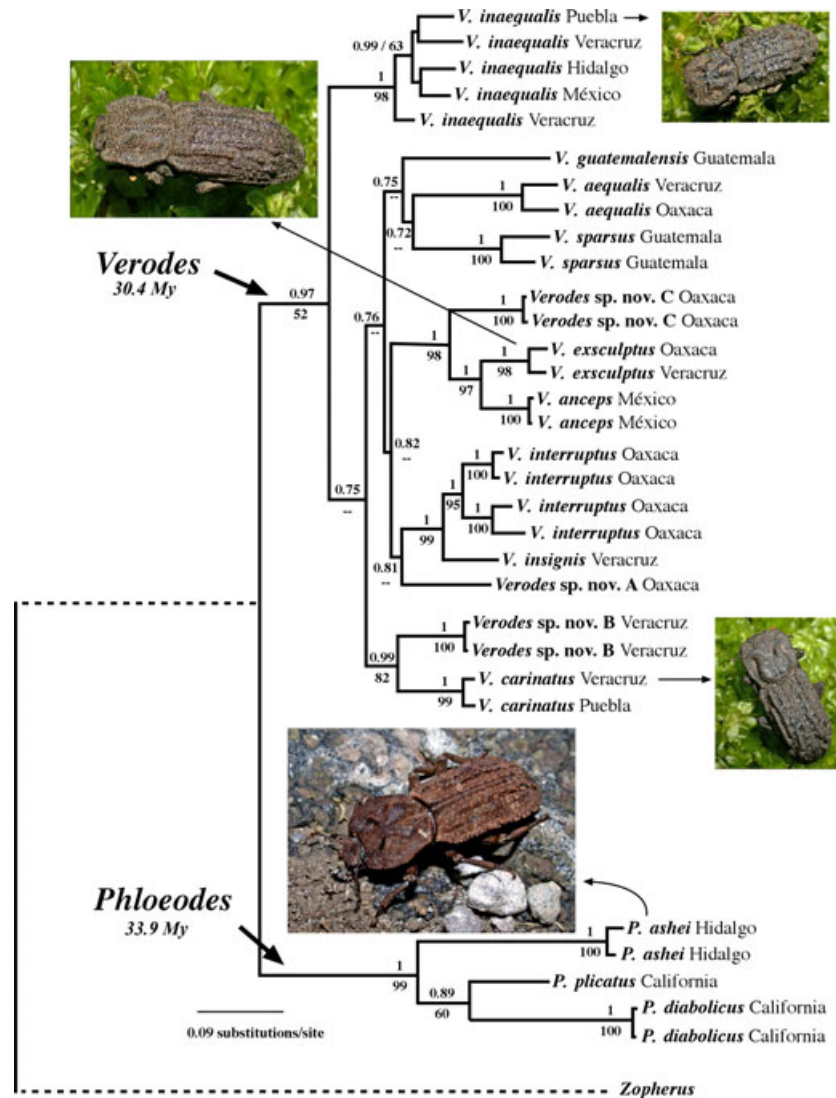


Fig. 4 Bayesian phylogenetic reconstruction of relationships among Zopherini beetles from North America. Analyses were based on sequence data from mitochondrial genes, part of COI, a small portion of ND1, tRNA Leucine, and part of the 16S ribosomal RNA, and nuclear histone 3 (H3) gene. Posterior probability values of relevant nodes are indicated above branches. Nonparametric maximum likelihood bootstrap values >60% are indicated below branches. Estimated times to the most recent common ancestors are indicated for *Verodes* and *Phloeodes* clades (see Table 2 for HPD intervals). Field images represent an overview of the morphological diversity within the group.

ability of zopherines, as almost no population (excepting two locations for *Phloeodes*, located about 180 km of the overlap area) appears in currently suitable areas that were unsuitable in the past.

The potential distributions of both clades for 2050 were estimated to be smaller than current ones. The area with climatically suitable conditions is estimated to be reduced 18% for *Phloeodes* (from current 69% of cells to 56% in 2050) and 36% for *Verodes* (from current 11% to 7% in 2050). However, all currently known populations of *Phloeodes* and *Verodes* will still fall within their respective potential distributions in 2050 (Fig. 5c, d).

Discussion

Our phylogenetic analyses clearly recovered two American zopherine clades in addition to the well-delimited genus *Zopherus*: the genera *Verodes* and *Phloeodes*. These two clades present similar amounts of genetic divergence across species (TMRCA ranges overlap for both genera—Table 2; and maximum genetic ‘p’ distances are similar among taxa of *Verodes* COI = 20.3%, and among *Phloeodes* COI = 20.2%), suggesting that speciation processes within the two clades occurred during the same time period. Our estimates

Table 2 Estimated time to the most recent common ancestors (TMRCA) for the main clades

Clade	Mean (Ma)	Lower HPD 95%	Upper HPD 95%
<i>Verodes</i>	30.43	23.12	38.54
<i>Phloeodes</i>	33.92	24.59	44.15
<i>Verodes inaequalis</i>	6.57	4.89	8.51
<i>Verodes interruptus</i>	7.67	5.43	10.15
<i>Verodes interruptus-insignis</i>	11.28	8.27	14.92
<i>Verodes aequalis</i>	5.95	3.95	8.37
<i>Verodes sparsus</i>	6.81	4.64	9.55
<i>Verodes</i> sp. nov. C	0.37	0.15	0.68
<i>Verodes exsculptus</i>	2.23	1.43	3.22
<i>Verodes anceps</i>	0.3	0.11	0.55
<i>Verodes anceps-exsculptus</i>	9.04	6.53	12.04
<i>V. anceps-exsculptus</i> -sp. nov. C	13.91	10.41	18.11
<i>Verodes</i> sp. nov. B	0.25	0.07	0.5
<i>Verodes carinatus</i>	1.62	0.97	2.42
<i>V. carinatus</i> -sp. nov. B	13.42	9.32	18.09
<i>Phloeodes ashei</i>	1.73	1.06	2.56
<i>Phloeodes diabolicus</i>	0.32	0.13	0.6
<i>Phloeodes diabolicus-plicatus</i>	21.98	15.23	29.66

Table 3 Maximum, minimum and range values for locations where populations of *Phloeodes* or *Verodes* are present

Variable	<i>Phloeodes</i>			<i>Verodes</i>		
	Max	Min	Range	Max	Min	Range
TANN (°C)	25.6	3.4	22.2	25.9	7.4	18.5
TMAX (°C)	37.1	19.4	17.7	35.2	16.7	18.5
TMIN (°C)	19.9	-7.9	27.8	16.6	-1.8	18.4
PANN (mm)	3246	127	3119	1690	555	1135
PWET (mm)	1241	65	1176	984	276	708
PDRY (mm)	298	1	297	175	15	160

PANN, annual precipitation; PDRY, precipitation of driest quarter; PWET, precipitation of wettest quarter; TANN, mean annual temperature; TMAX, maximum temperature of the warmest month; TMIN, minimum temperature of the coldest month.

place the most recent common ancestors of both groups most likely in the Eocene, with diversification occurring basically during the Miocene and the Pliocene. The assessment of clade niche width in relation to clade species richness supported the proposition of narrow niches leading to a higher probability of range fragmentation during climatic oscillations, thus increasing speciation. The projection of potential distributions further supported this hypothesis, by showing the spatial consequences of differences in niche width on clade potential distributions during climatic oscillations. Whereas *Phloeodes* retained the integrity of its potential distribution during all warm and cold periods because of its wide niche (Fig. 2d, f, h), *Verodes* suffered severe fragmentations of its potential distribution in arid or warm periods (Fig 2c, g), which may have led to a higher diversification of *Verodes* (16 species, as currently

shown, plus some additional undescribed species not included in the phylogenetic analyses) compared with *Phloeodes* (10 species).

Our phylogenetic hypothesis does not support Foley & Ivie's (2008) results based on morphological data. A well-supported monophyletic unit in our tree is composed by representatives of *Sesapsis* and *Phloeodes* (sensu Foley & Ivie 2008) and not between *Verodes* and *Phloeodes* as suggested by those authors. Genetic divergence across species of *Verodes* is of the same magnitude than across species of the *Sesapsis*—*Phloeodes* clade (as depicted in branch lengths in the NJ tree in Fig. 3), indicating an almost coetaneous speciation process within the two clades as also supported by our TMRCA estimates, based on a relaxed molecular clock that takes into account the possible heterogeneity of substitution rates among lineages. Foley & Ivie (2008)

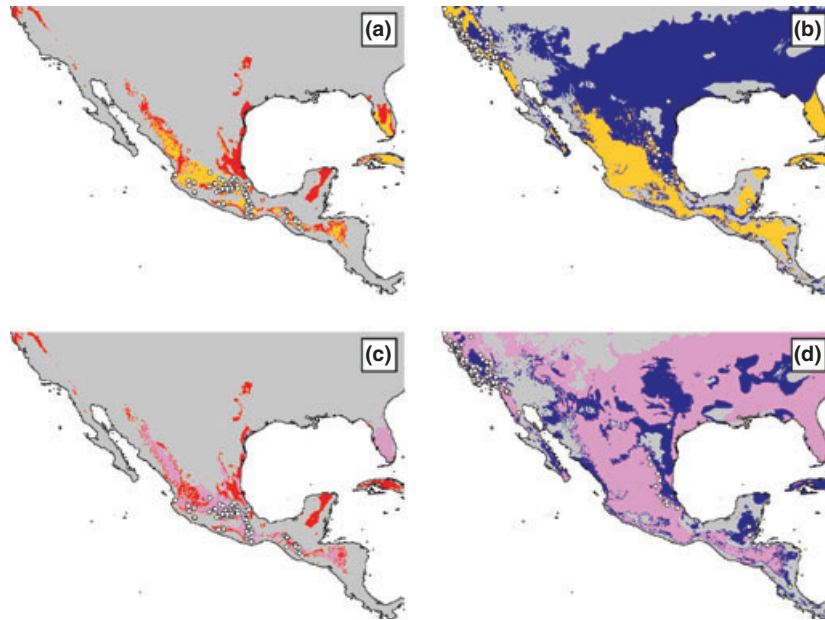


Fig. 5 Position of current populations (white points) of *Verodes* (left column) and *Phloeodes* (right column): (a–b) with respect to current potential distribution (red and blue, respectively) and area of overlap between current, last glacial maximum and last interglacial maximum potential distributions (yellow); (c–d) with respect to current potential distribution (red and blue, respectively) and potential distribution predicted for 2050 (violet).

proposed that separation between African and North American taxa occurred after the splitting between *Sesapsis* and *Phloeodes*. Our findings suggest that if Foley & Ivie's (2008) hypothesis is correct, then colonization from North America to Africa must have occurred very recently, during the local speciation process that took place in Mexican cloud forests, precluding thus any possibility of ancient vicariance between African and American faunas. Because no palaeogeographic support can be found for this scenario, we rather reject Foley & Ivie's (2008) phylogenetic hypothesis, based on both tree topology, and historical biogeography.

Given that *Phloeodes* and *Verodes* clades comprise different number of species, we investigated how clade's niche width could affect speciation processes. Phylogenetic niche conservatism in combination with climatic oscillations has been hypothesized to promote allopatric speciation in montane clades (Wiens 2004; Kozak & Wiens 2006). Further implications of the proposed mechanism have been addressed in relation to the study of diversity patterns (i.e. Hawkins *et al.* 2006; Wiens *et al.* 2007; Buckley *et al.* 2010). In contrast, further predictions of the original hypothesis in relation with speciation processes have not been addressed, to our knowledge. However, a clear implication of the original hypothesis (Wiens 2004) is that niche width of a clade is expected to determine the probability of speciation, as the narrower the niche, the higher the probability of suitable habitats being fragmented. We note

that niche width is also expected to determine extinction probability, as the narrower the clade's niche, the higher the probability of suitable habitats totally disappearing during climatic oscillations. Therefore, net diversification rates could attain very similar values for clades with contrasting niche widths through different combinations of speciation and extinction rates. For this reason, we argue that even moderate differences in absolute richness between clades of the same age could be reflecting strong effects of niche width on speciation probabilities. We found a clear difference between niche widths of two zopherine clades, *Verodes* and *Phloeodes*, in parallel with their respective species richness. This association between niche traits and clade diversities lacks statistical power, and more clades should be studied to assess the generality of our finding. However, the projection of potential distributions (based on clade niches) for different periods of contrasting climate (present, LGM, LIG) provides further support for our interpretation by showing a plausible mechanism of range fragmentation leading to gene flow reduction and, eventually, speciation.

It should be noted that dispersal limitation plays a significant role in combination with niche conservatism in boosting speciation processes. Climatic oscillations may produce cyclical fragmentations and fusions of clade ranges, and time between a given fragmentation and subsequent fusion could not be enough for effective speciation. In this scenario, if after fragmentation and

subsequent fusion individuals from different fragments are able to re-colonize the other fragments, gene flow would be recovered and speciation would never occur. However, if dispersal limitation precludes re-colonization, gene flow would be interrupted for larger periods than cyclic fragmentation–fusion events, eventually allowing speciation. Thus, even if potential distributions are fused again during some periods, the realized distributions may still remain fragmented because of dispersal limitation. In other words, niche conservatism could be the factor responsible of range fragmentation through the extinction of intermediate populations, but dispersal limitation would be a major determinant of speciation through the maintenance of realized distributions isolated even when potential distributions are fused. In our study, dispersal limitation appears to be extremely relevant, as current known populations of *Phloeodes* and *Verodes* are located within or very close to areas that retained favourable climatic conditions across all the analysed times (i.e. overlap between the three potential ranges for present, LGM and LIG; see Fig. 5). This finding suggests that re-colonization ability is extremely limited in *Phloeodes* and *Verodes*: current populations are those that survived during successive climatic oscillations and respective range fragmentations, and almost none is located in currently suitable places far from the temporal overlap area. Similar patterns are also documented for other animal groups inhabiting mesic forests from Central Mexico (Flores-Villela & Gerez 1994). In particular, plethodontid species of the genera, *Chiropterotriton*, *Bolitoglossa*, *Thorius* or *Pseudoeurycea*, are confined to highly restricted areas and scattered through the mountain systems despite their long specific evolutionary history (Hanken & Wake 2001; Parra-Olea 2003; Parra-Olea *et al.* 2004, 2005). These patterns suggest that limited dispersal precluding range expansions, in combination with climatic oscillations and niche conservatism, might be a crucial factor promoting speciation in small geographical areas isolated by elevation gradients (Wiens *et al.* 2007).

From a conservation perspective, the latter finding could be cause of concern considering the predicted changes in future climate because, as suggested for other organisms in the same geographical area (Sinervo *et al.* 2010), zopherine populations are unable to track suitable climatic conditions. Our results show that significant portions of potentially suitable areas would be reduced by 2050, but, interestingly, almost all currently known populations will still remain within this reduced potential ranges. Therefore, climate change will probably not be a direct major threat for these zopherine beetles. From an evolutionary perspective, what this overlap between present populations and past and future suitable conditions suggests is that *Phloeodes* and

Verodes populations are located in areas of extraordinary climatic resilience, allowing the maintenance of zopherine populations over long time periods in an evolutionary scale. This extraordinary long-term biological stability would probably be true for many other animals and plants, explaining the role of Mexican and Central American mountains as biodiversity hotspots (García-París *et al.* 2000; Parra-Olea 2002; Ornelas-García *et al.* 2008).

In conclusion, our study lends support to the hypothesis of niche conservatism being a relevant factor driving speciation in temperate montane clades (Wiens 2004). Moreover, our results suggest that when niche conservatism is the triggering mechanism of allopatric speciation, (i) niche width is a major determinant of the probability of speciation, by controlling the probability of range fragmentation; and (ii) dispersal limitation is also a major determinant of speciation processes, by increasing the fragmentation of realized distributions even when potential distributions are cyclically fused during climatic oscillations. When dispersal limitation is extreme, as in zopherine beetles analysed here, populations persist just in those areas that have retained suitable conditions during extremes of past climatic oscillations. Paradoxically, this relict condition confers zopherine beetles great resilience for facing future climate change.

Acknowledgements

We thank David B. Wake and James Hanken for their companionship and support during field trips in the mountains of Mexico, and to the late Marina Alcobendas for her invaluable assistance and collaboration in the laboratory. Research for this study was funded by grants CGL2009-10111 to A.B and CGL2010-15786 to M.G.-P. (Spanish Ministry of Science and Innovation), and grant UC-MEXUS 022043 to G.P.-O. Collecting permits were provided by SEMARNAT (México).

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Data accessibility

DNA sequences have been deposited in GenBank under Accession nos JN703505–JN703668. Details regarding individual samples are available in Table 1.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Projected potential distributions of *Verodes* and *Phloeodes* clades using BIOCLIM.

Fig. S2 Projected potential distributions of *Verodes* and *Phloeodes* clades using DOMAIN.

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