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Museomics reveals evolutionary history of *Oreina* alpine leaf beetles (Coleoptera: Chrysomelidae)

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Abstract

The monophyletic status of the genus *Oreina* as well as its phylogenetic relation to the closely related genera *Chrysolina*, *Crosita* and *Cyrtonus* has been debated for several decades. To assess the status of the genus and understand its evolutionary history, we performed a museomics study on 148 museum specimens belonging to 25 of the 28 described *Oreina* species as well as 19 other chrysomelid species, mainly from the genus *Chrysolina*. Using innovative molecular methods relying on hyRAD hybridization capture, we succeeded in recovering 2235 shared nuclear loci. Phylogenomic analyses clearly demonstrated that *Oreina* species form a clade separated from *Chrysolina*. These analyses also revealed the position of *Chrysolina fastuosa* outside of the genus *Chrysolina*, supporting the following taxonomic status updates: *Fasta* **stat. rev.**, *Fasta fastuosa* **comb. nov.** Within the genus *Oreina*, we further propose the synonymization of *Oreina* (*Frigidorina*) **syn. n.** and *Oreina* (*Virgulatorina*) **syn. n.** with *Oreina* (*Chrysochloa*). Divergence time and ancestral range estimations suggested that *Oreina* originated approximately 53 Ma in the Alps. Ancestral host plant reconstruction revealed key shifts during *Oreina* diversification. Overall, our study reinforces the importance of museum collections for molecular analysis and the effectiveness of hybridization capture approaches for conducting phylogenomic studies and finely investigating controversial taxonomic debates.

KEYWORDS
alpine biogeography, beetle evolution, historical DNA, host plant evolution, phylogenomics

INTRODUCTION

The chrysomelid genus *Oreina* Chevrolat consists of 28 Palaearctic species (Kippenberg, 2010) distributed across the mountain, alpine and subnival zones (Kippenberg, 1994). Mountain ranges are characterized by strong topological breaks, which favour isolation of populations and putatively trigger diversification of biota (Cun & Wang, 2010; Schwery et al., 2015). In *Oreina*, such processes are considered to have been driven by a large number of dispersal and vicariance events (Triponez et al., 2013), which have produced the diversity found today, consisting of 26 species that occur in European mountain ranges and two in the Altai Mountains. *Oreina* species, classified into seven subgenera (Kippenberg, 2010), also include numerous subspecies and forms or morphs, having not been confirmed as valid species on genetic grounds so far (Kippenberg, 1994, 2010). *Oreina* beetles feed on Asteraceae and Apiaceae, from which they gather not only nutrients but also chemical elements that they might remobilize in their own defence chemistry against predators (Pasteels et al., 1995). Species feeding on Apiaceae or Asteraceae: Cardueae produce cardenolides de novo, whereas species feeding on

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Asteraceae sequester pyrrolizidine alkaloid N-oxides (PAs) from their host plants (Dobler et al., 1996; Ehmke et al., 1991; Pasteels et al., 1995). *Oreina* beetles showcase a wide variety of bright metallic colours based on a Bragg-mirror surface structure (Barry et al., 2020), except species of the subgenus *Oreina* (*Protorina*) Weise, which are dull-coloured (Kippenberg, 2008). The distinct colour polymorphism between and within certain species is remarkable (Figure 1). It has been explained by numerous selection pressures that vary in time, direction, and strength, and which apply not only to *Oreina* (Borer et al., 2010; van Noort, 2013) but also to closely related genera from the subtribe Chrysolinina Chen, such as *Chrysolina* Motschulsky, *Cyrtonus* Latreille and *Crosita* Motschulsky (Mikhailov, 2008; van Noort, 2013). The genera *Cyrtonus* and *Crosita* consist of 41 and 10 species, respectively, distributed in the Palaearctic region (Kippenberg & Mikhailov, in preparation). Their phylogenetic position with respect to other Chrysolinina genera remains uncertain as very few studies have addressed this question.

The genus *Chrysolina* is closely related to *Oreina* (Petitpierre, 2021). It is highly diverse and one of the most species-rich genera of chrysomelid beetles, with nearly 500 species divided into 70 subgenera (Bieńkowski, 2019). The main distribution of *Chrysolina species* is in the Palaearctic region (Bieńkowski, 2019). Like *Oreina*, they feed on Apiaceae and Asteraceae but also on other plant families, such as Scrophulariaceae, Lamiaceae, Plantaginaceae, Hypericaceae and Ranunculaceae (Bourdonné & Doguet, 1991; Jolivet & Petitpierre, 1976). For more than half a century, the respective systematic position and taxonomy of *Chrysolina* and *Oreina* have been strongly debated. Based on morphological characteristics, some authors proposed to place the genus *Oreina* as a subgenus of *Chrysolina* (Bechyné, 1958; Bourdonné & Doguet, 1991; Daccordi, 1994; Garin et al., 1999; Jurado-Rivera & Petitpierre, 2015; Petitpierre, 2021). Other authors instead argued for separating the two genera (Bieńkowski, 2019; Dobler et al., 1996; Hsiao & Pasteels, 1999; Kippenberg, 2010; Kippenberg & Döberl, 1999; Kühnelt, 1984). This debate is still ongoing despite additional and newer methods being applied to tackle it, such as host plant affiliation (Jolivet & Petitpierre, 1976), karyotype analysis (Bourdonné & Doguet, 1991; Petitpierre, 1978), allozyme studies (Dobler et al., 1996), and DNA fingerprinting and sequencing (Garin et al., 1999; Hsiao & Pasteels, 1999).

The field of phylogenomics is being reinvigorated by the development of innovative molecular methods allowing to integrate previously inaccessible samples such as specimens from natural history collections, a development now recognized as a new research field: museomics (Wolinsky, 2010). Natural history collections across the globe house over a billion biological specimens including rare or

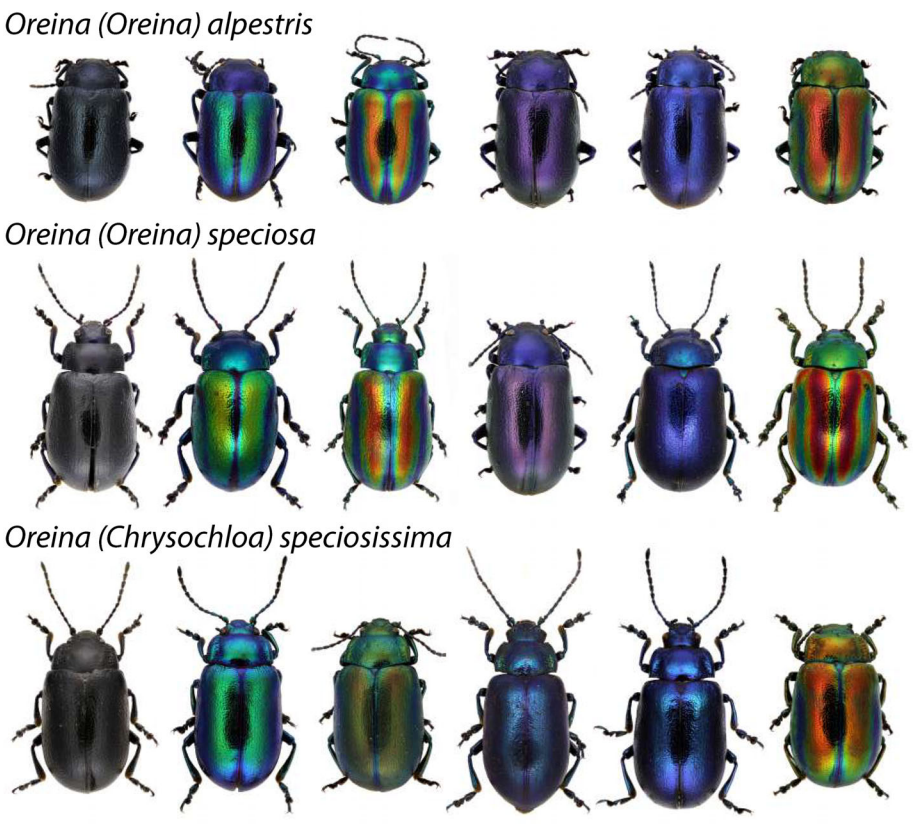

FIGURE 1 Example of colour pattern convergence between *Oreina* species: *Oreina alpestris* with different colour morphs (from left to right): ssp. *bannatica*, ssp. *polymorpha*, ssp. *marsicana*, ssp. *bannatica*, ssp. *variabilis* and ssp. *meridiana*. *Oreina speciosa* with different colour morphs: ssp. *pretiosa* auct, ssp. *pretiosa* auct, ssp. *pretiosa* auct, ab. *blühweissi*, ssp. *pretiosa* auct, ssp. *pretiosa* auct ('auct' indicates correct nomenclature is under discussion). *Oreina speciosissima* with different colour morphs: ssp. *convergens*, ssp. *speciosissima*, ssp. *troglodytes*, ssp. *troglodytes*, ssp. *speciosissima*, ssp. *fuscoaenea*. Picture credits: Matthias Borer.

extinct species, or species known from only very few specimens (Deng et al., 2019; Jin et al., 2020). The DNA in museum specimens, referred to as historical DNA (hDNA), is usually present in small quantities, and exhibits variable levels of degradation and fragmentation as a result of post-mortem damage triggered by sampling and storage conditions, and by the time that has passed since their collection (Billerman & Walsh, 2019). Consequently, it is challenging to analyse hDNA with methods such as classical genomic complexity reduction methods (e.g., RADseq), which are otherwise widely used for population genomics or phylogenomics approaches (Linck et al., 2017). Recent developments in DNA extraction, library preparation, enrichment and sequencing methods have enabled the emergence of new approaches for generating genomic data from these historical samples (reviewed in Card et al., 2021; Raxworthy & Smith, 2021). Because of its specificity and ability to recover small amounts of DNA, hybridization capture has revealed a powerful alternative. Among the several described approaches is HyRAD (Suchan et al., 2016), a method that relies on 'bench-top' probe design, as opposed to synthesized probes, based on a ddRADseq experiment (Peterson et al., 2012), produced from fresh samples characterized by high-quality DNA. Nuclear homologous fragments of hDNA are then hybridized to these probes, allowing only the targeted loci to be captured and eliminating all unwanted fragments such as contaminants and other technical sequences. The HyRAD method has proven particularly effective in recovering genetic information from collection samples, including from extinct species or populations, and providing key data for phylogenomic reconstructions (Toussaint et al., 2021).

In this study, we applied the HyRAD method to 148 museum specimens from 25 of the 28 known *Oreina* species and to one species for each of 13 *Chrysolina* subgenera as well as *Crosita* and *Cyrtonus* representatives. Our aims are to (1) infer a robust phylogeny of the genus *Oreina* and its closely related genera *Chrysolina*, *Crosita* and *Cyrtonus*; (2) clarify the division of genera and subgenera; (3) estimate divergence times between major clades; (4) investigate whether biotic (e.g., host plant relationship) or abiotic (biogeography) factors may have driven the diversification of *Oreina*.

MATERIALS AND METHODS

Taxon sampling

To investigate the evolution of species within *Oreina* and closely related genera, we selected 184 museum specimens from the Natural History Museum of Basel and private collection of H. Kippenberg. For 148 samples, sufficient genetic information has been recovered to allow their inclusion in the study (Table S1). These 148 voucher specimens (100 *Oreina*, 41 *Chrysolina*, 3 *Crosita*, 2 *Cyrtonus* and 2 *Timarcha* Latreille as the outgroup) represent a total of 44 species. Among the 28 described species of *Oreina*, only three species are missing in our sampling: *Oreina* (*Protorina*) *sibylla* Binaghi from the Apennines, initially integrated in the sampling but excluded from the final dataset because of poor genomic coverage and inconsistent phylogenetic positions; *Oreina* (*Allorina*) *canavesei* Bontems from the Graian Alps; and *Oreina* (*Chrysochloa*) *redikortzevi* Jacobson from the Altai Mountains, because no samples were available at the time of the laboratory work.

DNA extraction and HyRAD protocol

To design the probe set, DNA was extracted from eight fresh *Oreina* specimens (indicated in Table S1) using a QIAamp DNA extraction kit (QIAGEN) including a digestion step at 56°C with proteinase K. DNA was quantified using a Qubit assay (Thermo Fisher Scientific) and quality assessed with a Fragment Analyser (Advanced Analytical Technologies). We then applied double-digestion RAD-sequencing (ddRAD; Peterson et al., 2012) with the restriction enzymes PstI and MseI. DNA adapters including unique index specific to each specimen, PCR primer for amplification and sequencing, and T7-promoter sequence necessary for final transcription of probes into RNA were ligated. Libraries were size-selected using PippinPrep (Sage Science) on a 2% agarose cassette (SageScience) in range mode 200–350 bp. An aliquot of the eight individual libraries were sequenced on eight lanes of an Illumina MiSeq Nano sequencer with 300 bp paired-end reads. These eight libraries were transcribed into RNA using HiScribe T7 High Yield RNA Synthesis Kit (New England Biolabs) and equimolarly pooled to produce the final probe sets.

Historical DNA was extracted from one median leg of each specimen using a protocol detailed in Toussaint et al. (2021). Purified DNA, eluted in 25 µL, was quantified using a Qubit assay (Thermo Fisher Scientific) and quality assessed with a Fragment Analyser (Advanced Analytical Technologies). Shotgun libraries were prepared using protocol for degraded DNA (Tin et al., 2014). Hybridization capture and enrichment were performed as described in Toussaint et al. (2021). Samples were identified using dual-indexing tagging and pooled in equimolar quantities based on their respective concentrations. Sequencing was performed on an Illumina Hiseq2500 sequencer with 100 bp paired-end reads (Lausanne Genomic Technologies Facility, Switzerland).

PhyloHyRAD and locus reconstruction

The phyloHyRAD pipeline (Gauthier et al., 2020; Toussaint et al., 2021) was used to analyse the data. First, the construction of a loci reference catalogue was performed from ddRAD probe libraries. Paired-end reads were demultiplexed and cleaned using AdapterRemovalv2 (Schubert et al., 2016) and Cutadapt (Martin, 2011) to remove adaptors, bases with a quality score lower than 20 and reads smaller than 30 bp. Read quality was first checked using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Locus construction was performed using ipyrad (Eaton & Overcast, 2020) with a minimum depth of six and a clustering threshold of 0.80 (following testing with values 0.70, 0.80, and 0.90). Loci shared by at least two probes were retained in a reference catalogue, which was evaluated for contamination using the metagenomic sequence

classifier Centrifuge (Kim et al., 2016) and BLAST on non-redundant (nr) database and the reference genome of the potato beetle *Leptinotarsa decemlineata* Say also a member of the family Chrysomelidae (BioProject PRJNA171749).

The demultiplexed and cleaned paired-end reads from each historical sample were processed using AdapterRemovalv2 (Schubert et al., 2016) and Cutadapt v1.18 (Martin, 2011) with the same parameters as for the probe reads. In addition, terminal poly-Cs were removed using a custom Perl script (DropBpFastq_polyC.pl). Read quality was checked using FastQC. Cleaned reads from each historical sample were individually mapped onto the locus catalogue generated above using BWA-MEM v0.7.17 (Li, 2013). Indels were realigned using the GATK IndelRealigner (McKenna et al., 2010) and PCR duplicates were removed using MarkDuplicates from the Picard toolkit (http://broadinstitute.github.io/picard). Nucleotide mis-incorporation patterns were investigated using MapDamage2.0 (Jónsson et al., 2013), and base quality scores were rescaled according to their probability of representing a post-mortem DNA deamination event, to reduce the impact of DNA decay on downstream analyses. Individual consensus sequences were generated for each locus using the following scripts from samtools suite: mpileup, bcftools and vcfutils.pl (Li et al., 2009). Consensus sequences were cleaned using seqtk (https://github.com/lh3/seqtk) to retain bases with a phred quality >30. Cleaned consensus sequences were combined using a custom script, loci shared by at least two thirds of the samples were kept and aligned with MAFFT v7.407 using the --auto option to automatically select an appropriate strategy for the alignment (Katoh & Standley, 2013). Alignments were checked and manually cleaned in Geneious R11 (Biomatters) to remove short or problematic sequences. Two datasets were generated, one including all samples (dataset A: 100 *Oreina*, 41 *Chrysolina*, 3 *Crosita*, 2 *Cyrtonus* and 2 *Timarcha* as the outgroup) and a second one keeping only one sample per species with the lowest percentage of missing data (dataset B: 25 *Oreina*, 16 *Chrysolina*, 1 *Crosita*, 1 *Cyrtonus* and 1 *Timarcha* as the outgroup).

Phylogenomic inference

Phylogenetic inference was performed in IQ-TREE v2.0.5 (Minh et al., 2020) using the edge-linked partition model (Chernomor et al., 2016). First, the best partitioning schemes were estimated using PartitionFinder v2.1.1 (Lanfear et al., 2017) with the rcluster algorithm under the Akaike information criterion corrected (AICc), with a rcluster-max of 1000 and a rcluster-percent of 20. The resulting partitioning schemes were then used in IQ-TREE to select corresponding models of nucleotide substitution using ModelFinder (Kalyaanamoorthy et al., 2017) and the AICc across all available models in IQ-TREE. To avoid local optima, we performed 100 independent tree searches for each dataset in IQ-TREE. To estimate branch support, we calculated 1000 ultrafast bootstraps along with 1000 SH-aLRT tests in IQ-TREE (Guindon et al., 2010; Hoang et al., 2018). We used the hill-climbing nearest-neighbour interchange topology search strategy to avoid severe model violations leading to biased ultrafast bootstrap estimations (Hoang et al., 2018). The best tree for each analysis was selected based on the comparison of maximum likelihood scores.

Divergence time estimation

To generate a dataset that would be tractable for Bayesian inference of divergence times using relaxed clocks, we first selected loci with a minimum length of 200 bp and that were also recovered from the outgroup *Timarcha*. We then estimated phylogenetic trees for each selected locus using IQ-TREE and a model of nucleotide substitution selected using ModelFinder. All resulting gene trees were rooted to ensure subsequent calculations (see below). These phylogenetic trees and the best scoring tree from the species trees were used to conduct a 'gene shopping approach' as developed in SortaDate (Smith et al., 2018). Loci were filtered using the following three tree-based criteria: (1) clock-likeness measured as root-to-tip variance and deviation of terminals in a given topology from the mean rate; (2) total tree length; and (3) least topological conflict with the best scoring species tree measured through bipartition calculation of similarity. Loci were ultimately sorted first by topological concordance (criterion 3), clock likeliness (criterion 1) and finally by tree length (criterion 2) in order to create an unbiased dataset consisting of the most informative loci. We selected the 200 best scoring loci based on this filtering and concatenated them to produce the final alignment used in the divergence time estimation (dataset C).

Divergence time estimation analyses were performed in BEAST v1.10.4 (Suchard et al., 2018). Dataset C was partitioned a priori by locus for a total of 200 initial partitions, and the best partitioning scheme and models of substitution were determined with PartitionFinder2 (Lanfear et al., 2017) using the rclusterf algorithm with parameters rclustermax = 1000, rcluster-percent = 20 and min-subset size = 2000, and the Bayesian information criterion algorithm to select between competing models. Because this algorithm relies on only three models (GTR, GTR + G and GTR + I + G), we re-estimated a posteriori the best models in PartitionFinder2 using all those included in BEAST. We implemented clock partitioning by conducting analyses with one clock for each partition. We assigned a Bayesian log-normal relaxed clock model to the different clock partitions. We also tested different tree models by using a Yule (pure birth) or a birth–death model. To calibrate the relaxed clocks implemented in BEAST, we used a secondary calibration derived from the dated phylogeny of Tenebrionidae constructed by Kergoat et al. (2014), which among various outgroups includes *Timarcha* and *Chrysolina* species. We used the crown ages of *Timarcha* + *Chrysolina* (127 Ma, 95% CI = 107–147 Ma) to constrain the corresponding node in our topology with log-normal priors spanning the 95% credibility intervals of the estimates from Kergoat et al. (2014). The analyses consisted of 200 million generations with parameter and tree sampling every 5000 generations. We estimated marginal likelihood estimates (MLE) for each analysis using path-sampling and stepping-stone sampling (Baele et al., 2012), with 100 path steps, and chains running for 1 million

generations with a log-likelihood sampling every 1000 cycles. The Maximum Clade Credibility tree of each analysis with median divergence age estimates was generated in TreeAnnotator 1.10.4 (Suchard et al., 2018) after removing the first 25 million generations as burn-in.

Ancestral range estimation

We inferred the biogeographical history of *Oreina* using the R package BioGeoBEARS v1.1.1 (Matzke, 2013). We conducted the analyses under six models: the dispersal extinction cladogenesis (DEC) model (Ree & Smith, 2008), and the ML adaptation of the dispersal-vicariance (DIVA) model (Ronquist, 1997) and the Bayesian inference of historical biogeography for discrete areas (BayArea) model (Landis et al., 2013). With each previous model, founder event speciation was tested as an additional parameter (j). We used the best BEAST tree with outgroups pruned. Due to the complex geological history of the European mountain ranges, we opted for unconstrained inferences, without time-stratification, dispersal multiplier matrices, distance matrices, areas allowed matrices, and area-of-areas matrices. We designed a biogeographic regionalization in nine areas based on geological evidence (Faure & Ferrière, 2022) corresponding to C: Cantabric range; P: Pyrenees; M: Massif Central; A: Alps and Jura; I: Italian Apennines; N: Northern Europe comprising the Vosges, the Black Forest, the Bavarian and Bohemian Forest, the Ore Mountains and the Sudetes; K: Carpathian Mountains, R: Rhodope Mountains; S: Siberia (Table S2). The best model was selected based on log-likelihood and AIC scores.

Host plant reconstruction and diversification

Ancestral state reconstruction was carried out with the 'make.simmap' implemented in the R package phytools 0.6–60 (Revell, 2012). This Bayesian method uses stochastic character mapping to estimate the ancestral states and posterior probabilities for tips on a phylogeny. We used the best ML tree as input into simmap, along with a matrix of the 'host plant families' states distributed in proportion between 0 and 1 based on literature (Dobler et al., 1996; Hsiao & Pasteels, 1999; Pasteels et al., 1995; Pasteels & Rowel-Rahier, 1991) and our own observations to allow for multistates (Table S2). Three models for character evolution were tested and run for 1000 simulations: 'ER' (equal rates for all permitted transitions), 'SYM' (symmetric backward and forward rates for all permitted transitions), 'ARD' (all rates different for permitted transitions). The most likely model was selected after comparing likelihood and AIC.

RESULTS

Efficiency of HyRAD method for museomics

The DNA extraction performed on the historical samples resulted in variable concentrations of DNA (Table S1). Captures and enrichments performed using HyRAD allowed the sequencing of a large dataset composed of more than 329 million Illumina reads distributed among 184 samples with a mean number of reads by a sample of 1,791,154 (sd = 1,719,835). Application of the phyloHyRAD pipeline, which comprises the construction of a reference catalogue and the mapping of historical data, successfully retrieved enough phylogenomic data for 148 specimens out of 184 samples. The mapping step resulted in 32.2% (sd = 6.5%) of the mapped reads before cleaning and 22.1% (sd = 4.6%) after cleaning on average. From these mapping files, the reconstruction of the loci of interest allowed recovery of an average of 1684 (sd = 607) loci per sample. The heterogeneity of recovery among samples observed in these statistics does not seem to be related to the age of the specimens nor to specific treatments that the specimens may have undergone during storage such as the use of 'Scheerpeltz' solution (composition: 55% H_2O, 30% EtOH, 10% acetic acid and 5% ethyl acetate) (Table S1). This solution is known to harm DNA, but the specimens concerned present a number of loci similar to the other specimens, opening the possibility of including these museum specimens in genetic projects.

In addition, although the probes were composed of only *Oreina* species samples, they captured homologous loci across all samples, including those belonging to *Chrysolina*, *Crosita* and *Cyrtonus* but also to the outgroup species *Timarcha goettingensis* Linnaeus. As observed by Toussaint et al. (2021) in a previous study, to a certain extent the phylogenetic distance between the probes and the sample does not appear to impact capture efficiency. From the raw dataset, we kept the samples with the largest number of loci in order to maximize the information overlap and improve the phylogenetic resolution (Table S1). We also sought to keep the best coverage of the species. Thus, our final data matrix eventually encompasses 148 samples out of the 184 samples. This final dataset includes 2235 loci, a total length of 330,310 bp and a missing data rate of 32.9% (Table 1).

TABLE 1 Alignment statistics for each dataset, including the number of taxa, the alignment length, the percentage of missing data, the numbers and percentages of variable sites and of parsimony informative sites, and the GC content.

Dataset	No. of taxa	No. of loci	Align. length	Missing data	Variable sites	Parsimony informative sites	GC content
A	148	2235	330,310	32.9%	84,913 (25.7%)	41,762 (12.6%)	0.409
B	44	2235	330,310	35.5%	52,752 (16.0%)	22,800 (6.9%)	0.409
C	44	200	35,953	20.0%	5690 (15.8%)	2728 (7.6%)	0.416

Molecular phylogenetic inferences

Phylogenetic inference was performed on two datasets: dataset A comprising 148 specimens from 44 species (Figure 2) and dataset B composed of only one specimen per species (Figure 3). These inferences provide strong support for the monophyly of *Oreina* (dataset A: SH-aLRT = 100 and UFBoot = 100; dataset B: SH-aLRT = 100 and UFBoot = 100) and confirm the systematic position of *Oreina* outside of *Chrysolina*. The species *Chrysolina fastuosa* is recovered as sister to *Oreina* with strong branch support (dataset A: SH-

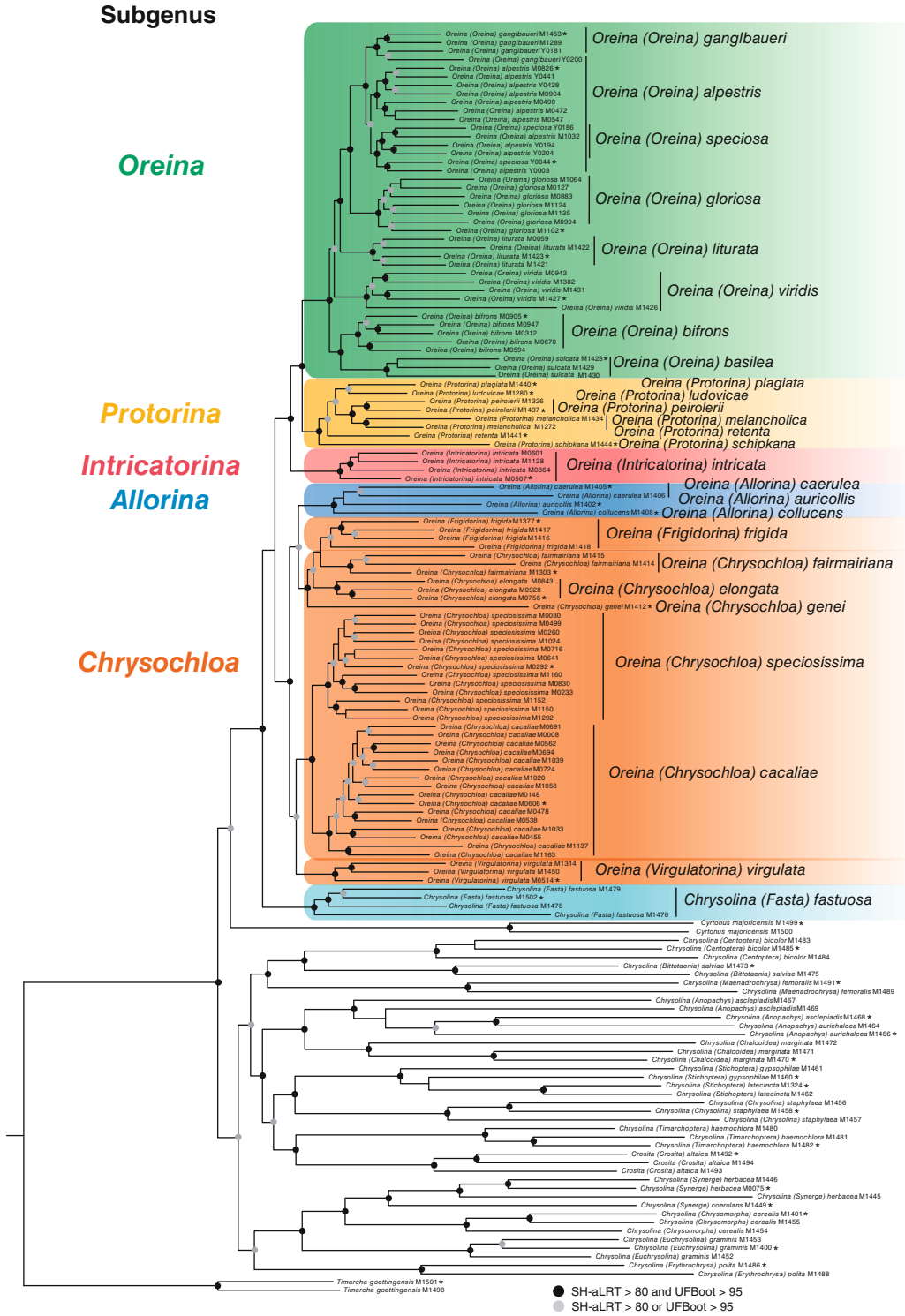

FIGURE 2 Best scoring maximum likelihood tree based on dataset A including 2235 loci and 148 samples. Branch support expressed in SH-aLRT and ultrafast bootstrap (UFBoot) is given as indicated in the caption. Samples used in the species phylogeny (Figure 3) are indicated with an asterisk.

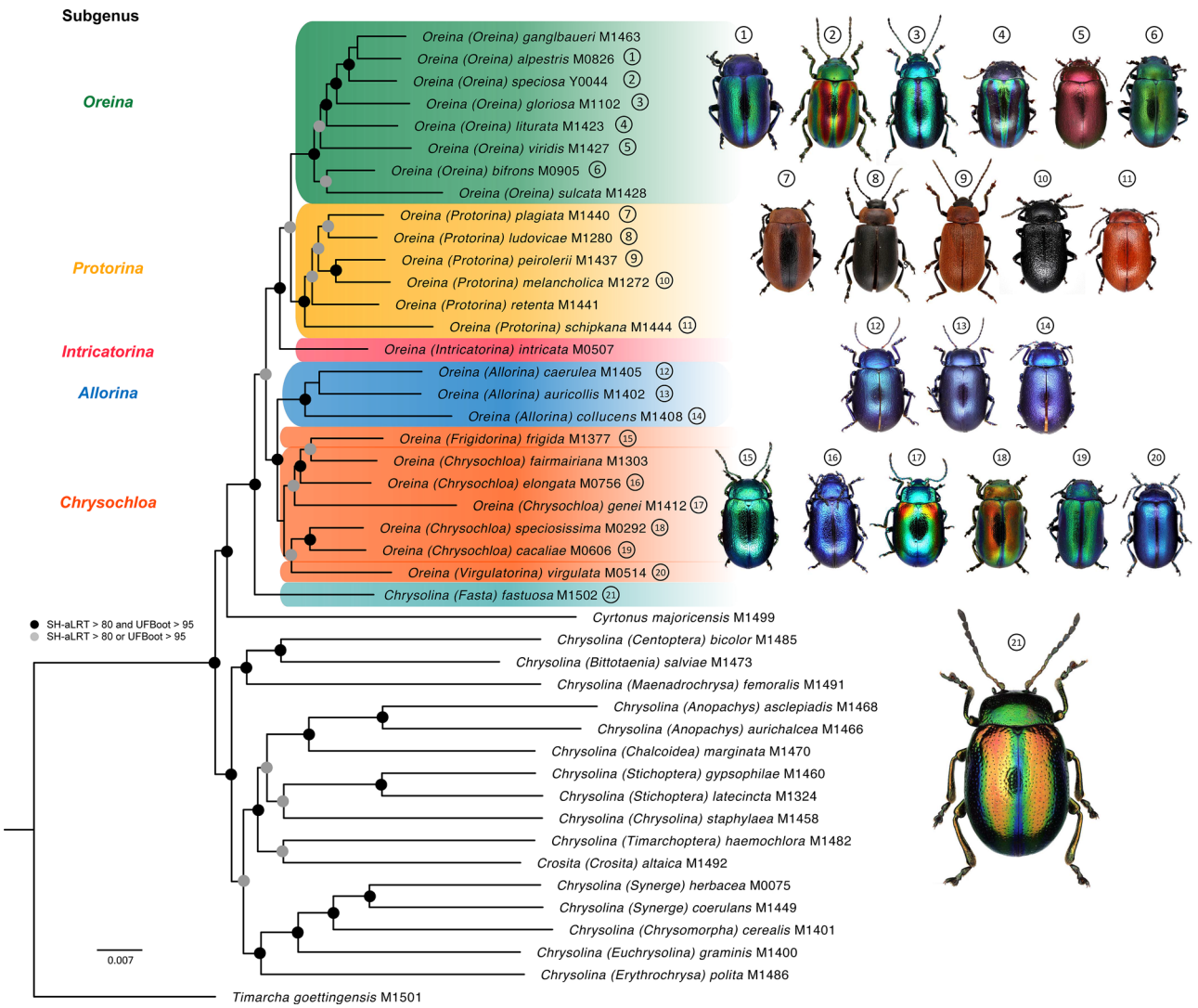

FIGURE 3 Best scoring maximum likelihood tree inferring species relationships based on dataset B (2,235 loci, 44 species). Branch support expressed in SH-aLRT and ultrafast bootstrap (UFBoot) is given as indicated in the caption. Picture credits: Matthias Borer. Photographs, with adapted scales, of collection specimens for most *Oreina* species and *Chrysolina* (*Fasta*) *fastuosa* have been included in front of each subgenus.

aLRT = 100 and UFBoot = 100; dataset B: SH-aLRT = 100 and UFBoot = 100) while *Cyrtonus majoricensis* Breit is recovered as sister to *Oreina* and *Chrysolina fastuosa* with strong branch support (dataset A: SH-aLRT = 94.4 and UFBoot = 94; dataset B: SH-aLRT = 99.2 and UFBoot = 99). In contrast, *Crosita altaica* Gebler, the only *Crosita* species included in our study, is recovered within *Chrysolina* (dataset A: SH-aLRT = 100 and UFBoot = 100; dataset B: SH-aLRT = 100 and UFBoot = 93), close to *Chrysolina* (*Timarchoptera*) *haemochlora* Gebler (Figures 2, 3).

Within the genus *Oreina*, we retrieved most of the previously described subgenera as monophyletic. *Oreina* (*Oreina*) is monophyletic with strong branch support (dataset A: SH-aLRT = 100 and UFBoot = 95; dataset B: SH-aLRT = 99.3 and UFBoot = 94) and includes eight species. A clade is formed by the species *Oreina* (*Oreina*) *ganglbaueri* Jakob, *Oreina* (*Oreina*) *alpestris* Schummel and *Oreina* (*Oreina*) *speciosa* Linnaeus. In the larger phylogeny (Figure 2), *O. ganglbaueri* is separated with strong branch support (SH-aLRT = 100 and UFBoot = 100). However, the individuals of the other two species, that is, *O. alpestris* and *O. speciosa*, are mixed and do not form separate clades. *Oreina* (*Protorina*) is monophyletic and includes the six analysed species (dataset A: SH-aLRT = 100 and UFBoot = 95; dataset B: SH-aLRT = 99.3 and UFBoot = 94). *Oreina* (*Intricatorina*), a monospecific subgenus, is monophyletic and placed as sister to the two previously mentioned subgenera (dataset A: SH-aLRT = 100 and UFBoot = 100; dataset B: SH-aLRT = 100 and UFBoot = 100). *Oreina* (*Allorina*) is monophyletic and strongly supported in the species phylogeny (dataset B: SH-aLRT = 100 and UFBoot = 100) but shows a more complex position in the phylogeny including all samples, being nested in the *Oreina* (*Chrysochloa*) clade with weak branch support (dataset A: SH-aLRT = 67.8 and UFBoot = 63).

The monospecific *Oreina* (*Frigidorina*) and *Oreina* (*Virgulatorina*) are placed within the subgenus *Chrysochloa*, which comprises five

studied species. The comparative examination of the global and species phylogenies reveals that *Oreina* (*Frigidorina*) *frigida* Weise is sister to *Oreina* (*Chrysochloa*) *fairmairiana* Gozis with low and moderate branch supports (dataset A: SH-aLRT = 42.7 and UFBoot = 38; dataset B: SH-aLRT = 81.7 and UFBoot = 87) and forms a clade together with *Oreina* (*Chrysochloa*) *elongate* Suffrian, and *Oreina* (*Chrysochloa*) *genei* Suffrian. In a second clade, *Oreina* (*Virgulatorina*) *virgulata* Germar is associated to *Oreina* (*Chrysochloa*) *speciosissima* Scopoli and *Oreina* (*Chrysochloa*) *cacaliae* Schrank with moderate branch support (dataset A: SH-aLRT = 86.7 and UFBoot = 90; dataset B: SH-aLRT = 99.7 and UFBoot = 78). The previous clades form a larger clade enclosing *O.* (*Chrysochloa*) plus *O.* (*Frigidorina*) *frigida* and *O.* (*Chrysochloa*) plus *O.* (*Virgulatorina*) *virgulata* with strong support in the species phylogeny (dataset B: SH-aLRT = 100 and UFBoot = 100).

Divergence time estimation and historical biogeography

The BEAST dating analysis based on a Yule model and a unique uncorrelated log-normal relaxed clock had a slightly better marginal likelihood (log marginal likelihood using Path Sampling MLPS = −127972.89; log marginal likelihood using Stepping-stone Sampling MLSS = −127972.31) than the birth–death model (MLPS = -127974.97; MLSS = −127974.15). However, in the two models, *Oreina* clade divergence time estimates are very similar with an origin of the crown *Oreina* at 46.6 Ma (95% HPD: 36.0–57.9 Ma) for the preferred Yule model and at 46.0 Ma (95% HPD: 35.6–56.8 Ma) for the birth–death model.

The ancestral range reconstruction analysis performed with BioGeoBEARS integrated the current known distributions and no other constraints. The six tested models presented very similar reconstructions despite having different likelihoods (ranging from −120.186 to −114.733 detailed in Table S3). The BAYAREALIKE+J model including dispersal, extinction, sympatry and founder processes had a slightly better likelihood than the other models (LnL = −114.733), so this is the reconstruction we present in Figure 3, but the other models propose close scenarios. The convergence of the results obtained with the different models highlights the robustness of the inferences made and the results obtained. These models revealed that the ancestor of *Oreina* likely originated in the Alps. The analysis did not identify large patterns of ancestral range change impacting particular clades. It would appear that colonization events occurred later at the species level. There were several cases of wide dispersal across Europe, as is the case for *O. alpestris*, *Oreina viridis* Duftschmid, *Oreina caerulea* Olivier, *O. speciosissima* and *O. virgulata*. Other colonization events were more localized, for example, towards the Iberian Peninsula for *O. ganglbaueri*, *Oreina ludovicae* Mulsant and *O. fairmairiana*. The clade composed of *Oreina bifrons* Fabricius and *Oreina sulcata* Gebler seemed to have undergone a progressive north-eastern dispersal, first for *O. bifrons* in north-eastern Europe and a long-distance dispersal for *O. sulcata*, which is found in Siberia (not shown on the map in Figure 4).

Host plant relationships

Of the three tested models for the ancestral host plant reconstruction (i.e., ER, SYM and ARD), the SYM model received better support (log-likelihood = −42.786; AIC = 91.572) than the two other models ER (log-likelihood = −50.468; AIC = 102.936) and ARD (log-likelihood = −42.577; AIC = 97.154). This reconstruction identified Asteraceae as the ancestral host plant family (Figure 5). The species *C.* (*Fasta*) *fastuosa* which is sister to *Oreina* is associated with different species of the family Lamiaceae showing that the switch to Asteraceae was made only by *Oreina*. In the rest of the phylogeny, between the different *Chrysolina* species, it seems that the ancestral host plants belonged to the Lamiaceae family, but this result should be interpreted with caution because of the low sampling for this genus. Within the genus *Oreina*, two other host switches are associated with two subgenera. First, *Oreina* (*Oreina*) is associated with a switch to plant species from the Apiaceae family. It should be noted that the same switch of host plant occurred in *O. fairmairiana*, a species phylogenetically surrounded by taxa feeding on Asteraceae (Figure 5). Secondly, *Oreina* (*Allorina*) is associated with a specialization in a tribe of the Asteraceae family, namely the Cardueae (Figure 5). Outside of this subgenus, other species such as *O. virgulata* are found on various species of Asteraceae including species of Cardueae. *Oreina schipkana*, the only species without a known host plant, is likely to feed on Asteraceae as revealed by the character reconstruction that allows to infer the current state of each species according to the reconstruction along the phylogeny (unless a recent switch such as in *O. fairmairiana* occurred).

DISCUSSION

New methods provided by museomics (Card et al., 2021; Raxworthy & Smith, 2021) such as the HyRAD approach (Gauthier et al., 2020; Suchan et al., 2016; Toussaint et al., 2021) used in this study allowed an extensive sampling of our focus genus *Oreina* with a total of 100 samples and 25 of the 28 known *Oreina* species. The HyRAD method thus allowed the recovery and sequencing of a large number of nuclear loci from collection samples whose DNA is often highly degraded, especially samples treated with 'Scheerpeltz' solution. Moreover, the evolutionary scale of this study, namely the focus on four closely related genera, seems to be a particularly appropriate scale to express the full potential of the HyRAD method. In contrast to other approaches widely used in phylogenomics such as Ultra Conserved Elements (UCEs, Faircloth et al., 2012) or Universal Single Copy Orthologs (USCOs, Dietz et al., 2021) that seek to synthesize loci in silico and capture relatively well-conserved regions or genes, the HyRAD approach is based on ddRAD loci randomly sampled across the genome. These regions are assumed to be more variable and more informative at a recent evolutionary scale (Lee et al., 2018). The robust phylogenies obtained from these loci have allowed important taxonomic revisions but also the study of evolutionary mechanisms involved in the emergence and diversification of

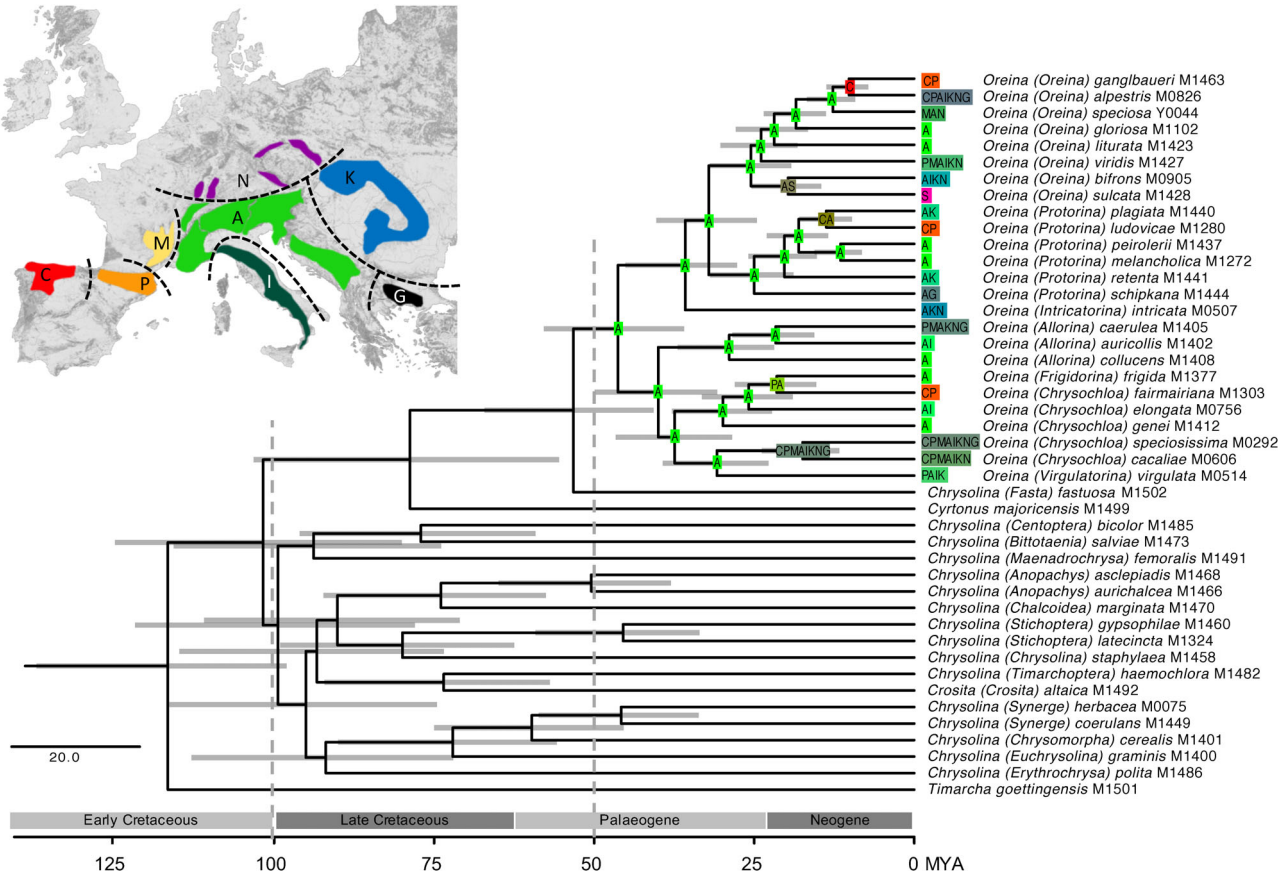

FIGURE 4 Bayesian divergence time estimates. Maximum clade credibility tree obtained from BEAST analysis. Node estimates are median ages, with 95%credibility intervals represented by a grey horizontal bar for each node. The most likely ancestral range for each *Oreina* node is given based on the results of the BAYAREALIKE+J model as estimated in BioGeoBEARS. C: Cantabric range; P: Pyrenees; M: Massif Central; A: Alps and Jura; I: Italian Apennines; N: Northern Europe comprising the Vosges, the Black Forest, the Bavarian and Bohemian Forest, the Ore Mountains and the Sudetes; K: Carpathian Mountains, G: Rhodope Mountains; S: Siberia. *Oreina sulcata*, distributed in Siberia, is not represented on the map.

alpine leaf beetles such as the role of biogeography and host plant relationships.

Monophyly and subgenera of *Oreina*

Our results clearly solve the systematic position of *Oreina* outside of *Chrysolina* as a monophyletic group, as advocated by certain authors (i.e., Bieńkowski, 2019; Dobler et al., 1996; Hsiao & Pasteels, 1999; Kippenberg, 2010; Kippenberg & Döberl, 1999; Kühnelt, 1984) but also questioned by others (Daccordi, 1994; Gomez-Zurita et al., 1999; Jurado-Rivera & Petitpierre, 2015).

Subgenus *Oreina* (*Oreina*)

Within *Oreina* (*Oreina*), the two sister species *O.* (*Oreina*) *speciosa* and *O.* (*Oreina*) *alpestris* are mixed and do not form separated clades, suggesting gene flow between the two species (Figure 2). This species complex has already been documented and seems to result from a complex biogeographic history (Triponez et al., 2011). Nevertheless, the males of these species can clearly be distinguished by their genitalia. The general shape is very similar, but the aedeagus tip in *O. speciosa* is much larger and more pronounced than in *O. alpestris*. An analogous pattern exists in the well-studied ground beetle subgenus *Carabus* (*Ohomopterus*) Reitter, specifically in the *Carabus iwawakianus* Nakane and *Carabus insulicola* Chaudoir group among the taxa *C. iwawakianus* and *Carabus maiyasanus* Bates (Fujisawa et al., 2019), where extensive gene flow could be detected. However, interspecific differences in genitalia morphology have been maintained. The *O. alpestris* and *O. speciosa* groups seem to be another example for such a pattern within the order of Coleoptera.

Subgenus *Oreina* (*Chrysochloa*)

The subgenus *Oreina* (*Chrysochloa*) shows different internal relationships in the large phylogeny (Figure 2) compared with the species phylogeny (Figure 3). These differences could be related to the longer

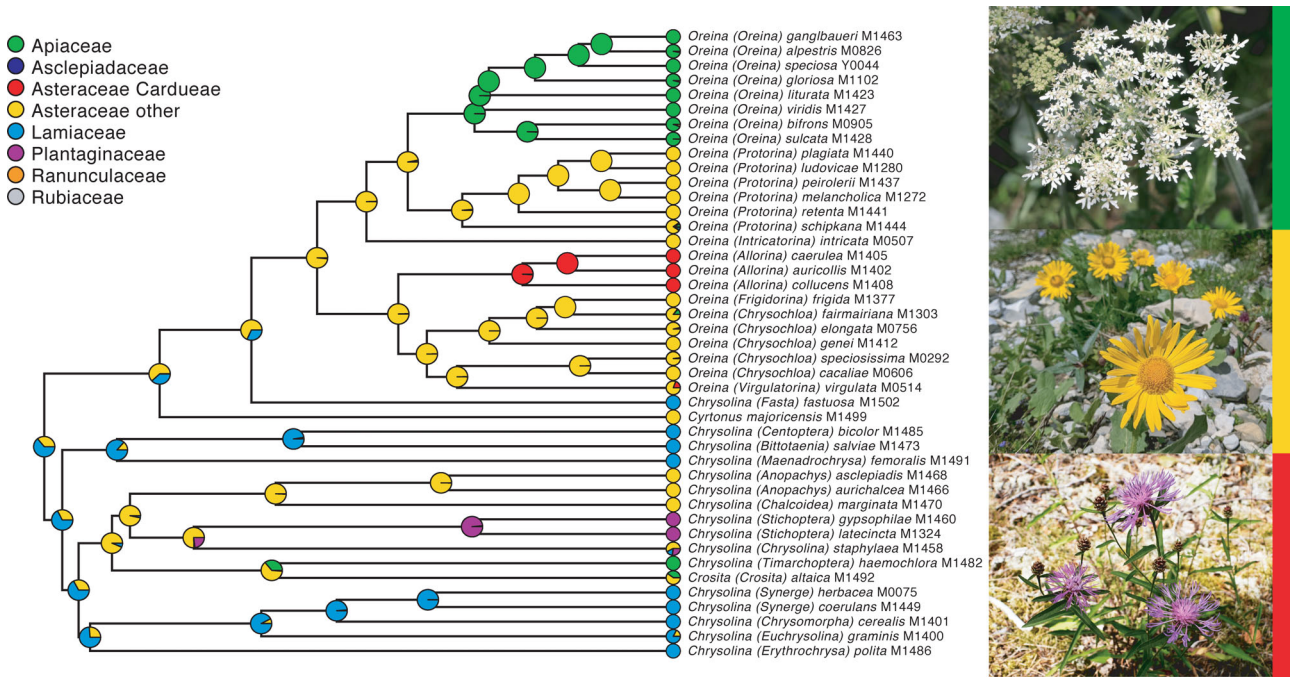

FIGURE 5 Ancestral host plant preference and diversification dynamics of *Oreina* and some closely related species. For the three main host plant families a picture of a representative species is indicated (top to bottom): *Heracleum phondylium* (source iNaturalist steve_mcwilliam), *Doronicum grandiflorum* (source iNaturalist phiro) and *Centaurea jacea* (source iNaturalist vesaoikonen).

branches observed in the samples with more missing data, leading to a long-branch attraction mechanism. The results expand the subgenus *Chrysochloa* by two species (*O. frigida* and *O. virgulata*) and confirm the taxonomy of a third species (*O. fairmairiana*). This result is congruent with the phylogeny obtained by Hsiao and Pasteels (1999), based on the analysis of molecular data across three mitochondrial regions. Moreover, Weise (1906, 1916) arranged these three species among the subgenus *Chrysochloa*. This, however, was a classification in two catalogues without any morphological discussion. Kühnelt (1984) introduced two monospecific subgenera, *Frigidorina* (for *O. frigida*) and *Virgulatorina* (for *O. virgulata*), justified by apparent morphological features, which seemed relevant to characterize these subgenera. The features are in the case of *O.* (*Frigidorina*): antennomeres 5 and 6 short, only slightly longer than broad, aedeagus short with two small denticles, and in the case of *O.* (*Virgulatorina*): last maxillar palpomere as thick as the penultimate, pronotum without lateral bulge, aedeagus short and broad. In contrast, *O. fairmairiana* has always been included in the *O.* (*Chrysochloa*) subgenus. However, it has distinct morphological characteristics and is the only species of this subgenus feeding on Apiaceae and Asteraceae. Therefore, its taxonomy was questionable. The phylogenetic result, however, does not agree with the morphologically founded classification of the subgenera *Frigidorina* and *Virgulatorina* supported by Kühnelt (1984) and Kippenberg and Döberl (1999) and the hesitation regarding *O. fairmairiana* appears unjustified. Consequently, we (except Kippenberg) propose to synonymize the two subgenera with *Oreina* (*Chrysochloa*), resulting in *Oreina* (*Virgulatorina*) **syn. nov.** and *Oreina* (*Frigidorina*) **syn. nov.** Species of the subgenus *Oreina* (*Chrysochloa*) as newly defined share the following morphological characteristics: The epipleura are narrow and do not reach the end of the third abdominal sternite. The upper side of the body has a metallic sheen. Further, the last maxillar palpomere is not particularly broad and not axe-shaped.

Systematic position of *Chrysolina fastuosa*, *Cyrtonus* and *Crosita*

The systematic classification of the species *Chrysolina fastuosa* (Scopoli, 1763) was questioned several times. Warchalowski (1994) proposed to replace the genus name *Dlochrysa*, partly also used as a subgenus, and introduced the subgenus *Fastuolina*. He assigned *Coccinella fastuosa* Scopoli, 1763 as the type species to the new monospecific subgenus *Fastuolina*. Warchalwoski justified this change by stating that *Dlochrysa* Motschulsky, 1860 and *Oreina* Chevrolat, 1837 are based on the same type species (details see Bontems, 1978), and therefore *Dlochrysa* Motschulsky, 1860 is an objective synonym of *Oreina* Chevrolat, 1837 and cannot be used as a subgeneric name for *Chrysolina*. Since Warchalowski did not provide any diagnosis for *Fastuolina*, the name was deemed unavailable by Petitpierre and Alonso-Zarazaga (Petitpierre, 2019). They proposed the new subgeneric name *Fasta* Petitpierre and Alonso-Zarazaga (Petitpierre, 2019) for the monospecific taxon *Chrysolina* (*Fasta*) *fastuosa* with the type species *Coccinela fastuosa* (Scopoli, 1763).

Morphologically, *C. fastuosa* differs from the other *Chrysolina* species mainly by the last tarsomere with two ventral teeth at the apical end and eyes with a very short ocular groove giving the eyes an oval

and not kidney-like shape. Our analysis confirms the special status of this species: *Chrysolina fastuosa* is sister to *Oreina*. This placement is justified by the morphological differences between genera *Chrysolina* and *Oreina*. Therefore, we elevate *Fasta* Petitpierre and Alonso-Zarazaga, 2019 **stat. rev.** from subgenus to genus, resulting in a revised status for *Fasta fastuosa* (Scopoli, 1763) **comb. nov.**

Cyrtonus majoricensis Breit is placed as sister to the clade comprising *Fasta* and *Oreina*, and thus we consider the genus *Cyrtonus* confirmed as a taxonomic unit, although more evidence is needed to reconsider the systematics of *Cyrtonus* by including other species from the genus. The position of *Crosita altaica* (Gebler, 1823), the only *Crosita* species included in our study, within the clade of *Chrysolina*, reflects the unsatisfactory taxonomic situation of the genus *Crosita*. In 1950, Bechyné transferred the species of the subgenus *Bittotaenia* Motschulsky from the genus *Chrysolina* to the genus *Crosita*, but Bieñkowski (2001) repositioned the subgenus *Bittotaenia* in the genus *Chrysolina*. This embedding of *Crosita* in the *Chrysolina* clade suggests that the morphological criteria of *Crosita* (i.e., hind wings absent, third tarsomere of posterior tarsi with a clear emargination at the apex and on the ventral side with a glabrous longitudinal stripe) are not sufficient for creating a clearly defined genus and that genus *Chrysolina* currently is a conglomerate of morphologically distinct taxa for which the phylogeny has not yet been fully clarified. However, a focused study on *Crosita* including all *Chrysolina* subgenera and additional *Crosita* species would be needed to clearly assess the status of this genus and to consider reliable taxonomic changes.

Oreina in time and space

The emergence of the genus *Oreina* is estimated with a stem-group age of 53.6 Ma (95% HPD: 40.7–67.1) and a crown-group age of 46.6 Ma (95% HPD: 36.0–57.9). This estimated age for the clade is consistent with the few known *Oreina* fossils, namely *Oreina amphyctionis* Heer, *Oreina hellenis* Heer and *Oreina protogeniae* Heer from the Sarmatian deposits in Germany dated to 11.6–12.7 Ma (Heer, 1847). Our results show that lineage diversification in *Oreina* has thus started during the second orogenic phase of the Alps at the end of the Late Cretaceous and the Eocene (Froitzheim et al., 1996).

Ancestral range estimations based on the definition of nine ranges revealed an origin of *Oreina* in the Alps. However, the current distribution of the species implies numerous relatively recent recolonizations. It is likely that species with a wide distribution have experienced multiple dispersals in and out of mountain massifs without a strong spatial structuring, as exemplified in *O. speciosa* (Triponez et al., 2011). Divergence events could imply peripatric or even sympatric speciation events, likely driven by abiotic or biotic differentiation not related to biogeographical scenarios. Still, the geographic distributions suggest several cases of vicariance particularly towards the Iberian Peninsula. This is the case for the clade composed of *O. ganglbaueri*, *O. alpestris* and *O. speciosa*. While *O. liturata* and *O. gloriosa* are only found in the Alps, *O. speciosa* is also present in the Massif Central. *O. alpestris* has a very wide distribution including both the Massif Central and the Pyrenean and Cantabric ranges. Finally, *O. ganglbaueri* is only found in the Iberian Peninsula, that is, the Pyrenees and the Cantabria. This distribution suggests a vicariance event from the Alps to south-western Europe in the clade comprising *O. alpestris*, *O. liturata* and *O. ganglbaueri* followed by the speciation of *O. ganglbaueri*, which is now a clearly separate species (Figure 2). Relatively similar patterns are found in the phylogeny, such as for *O. ludovicae* or *O. fairmariana* distributed in the Pyrenean and Cantabric ranges. Interestingly the latter case is also associated with a shift in host plant family (Asteraceae vs. Apiaceae). Divergence times between the three above-mentioned species pairs also suggest speciation through vicariance events between the Pyrenean and Cantabric ranges versus outside these two massifs: *O. plagiata* /*O. ludovicae* = 13.9 Ma (95% HPD: 9.7–17.9), *O. frigida* /*O. fairmairiana* = 21.6 Ma (95% HPD: 15.3–28.0), *O. ganglbaueri* + *O. alpestris* /*O. speciosa* = 12.8 Ma (95% HPD: 9.2–16.7). Such divergence times during the middle Miocene reveal that speciation occurred in a much warmer climate (Mid Miocene Climate Optimum, Methner et al., 2020) characterized by the existence of the Rhine rift, which separated the already-existing Pyrenean and Cantabric ranges from the Alps and further European massifs (Ziegler & Dèzes, 2005).

Host plant relationships and diversification

Results indicate an important role of the association with the host plant in the origin of *Oreina*. Indeed, the emergence of *Oreina* is associated with a host switch to Asteraceae. Due to the small number of *Chrysolina* taxa analysed, we cannot elaborate on the evolution of host plant use and diversification. A second host switch seems to have been important, from Asteraceae to Apiaceae at the origin of the subgenus *Oreina* (*Oreina*). Jolivet and Petitpierre (1976) first dealt with a synthesis concerning the host plants and the types of trophic selection of some *Chrysolina*, *Oreina* and *Crosita* species. By gathering the available data, they established a first preliminary grouping. Fifteen years later, Bourdonné and Doguet (1991) presented a phylogenetic hypothesis including host plants for the Palaearctic *Chrysolina* sensu *lato*. In that paper, they present a split of the studied *Oreina* subgenera, with *Oreina* feeding on Apiaceae and the other subgenera feeding on Asteraceae. This result has been later confirmed by molecular studies (Dobler et al., 1996; Hsiao & Pasteels, 1999) and corresponds to the result of our analysis (Figure 5). The same switch of host plant also occurred in *O. fairmairiana*, in the middle of a clade of species feeding on Asteraceae. *O. fairmairiana* and *O. sulcata* are the only species known to feed on host plants of both families, Asteraceae and Apiacae. These two species have never been included in previous phylogenetic studies, but this host plant switch could correspond to a convergence in the ability to feed on Apiaceae. The results also show a specialization of species from the subgenus *Allorina* on a tribe of the family Asteraceae, Cardueae. Overall, our results show a strong phylogenetic signal, with some opportunistic shifts in host plants, known to

occur in leaf beetles with chemical defences (Pasteels & Rowel-Rahier, 1991) and likely induced by ecological factors.

AUTHOR CONTRIBUTIONS

Jérémy Gauthier: Writing – original draft; methodology; visualization; formal analysis; investigation; software. **Matthias Borer:** Conceptualization; writing – original draft; validation; resources; funding acquisition. **Emmanuel F.A. Toussaint:** Methodology; validation; formal analysis; writing – review and editing. **Julia Bilat:** Methodology; formal analysis. **Horst Kippenberg:** Resources; validation. **Nadir Alvarez:** Conceptualization; funding acquisition; writing – review and editing; validation; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article (final alignments and trees) are available within a Github repository https://github.com/JeremyLGauthier/Oreina_phylogeny. The PHyRAD pipeline is available in the Github repository https://github.com/JeremyLGauthier/PHyRAD. Raw reads are available on the NCBI BioProject PRJNA926865.

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REFERENCES

Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A. & Alekseyenko, A.V. (2012) Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, 29, 2157–2167.

Barry, M.A., Berthier, V., Wilts, B.D., Cambourieux, M.C., Bennet, P., Pollès, R. et al. (2020) Evolutionary algorithms converge towards evolved biological photonic structures. *Scientific Reports*, 10, 12024.

Bechyné, J. (1958) Über die taxonomische Valenz der Namen von *Oreina* s.str. (Col. Phytophaga). *Bulletin de la Société Entomologique Suisse*, 31(1), 79–95.

Bieñkowski, A.O. (2001) A study on the genus *Chrysolina* Motschulsky, 1860, with a checklist of all the described subgenera, species, subspecies, and synonyms (coleoptera: Chrysomelidae: Chrysomelinae). *Genus*, 12, 105–235.

Bieñkowski, A.O. (2019) *Chrysolina* of the world – 2019 (Coleoptera: Chrysomelidae). Taxonomic review. Livny. Mukhametov G.V. Publ. 2019. 919 pp.

Billerman, S.M. & Walsh, J. (2019) Historical DNA as a tool to address key questions in avian biology and evolution: a review of methods, challenges, applications, and future directions. *Molecular Ecology Resources*, 19, 1115–1130.

Bontems, C. (1978) Nomenclature du genre *Oreina* Chevrolat (Col., Chrysomelidae, Chrysomelinae). *Nouvelle Revue d'Entomologie*, VIII(1), 69–80.

Borer, M., van Noort, T., Rahier, M. & Naisbit, R.E. (2010) Positive frequency-dependent selection on warning color in alpine leaf beetles. *Evolution; International Journal of Organic Evolution*, 64, 3629–3633.

Bourdonné, J.-C. & Doguet, C. (1991) Données sur la biosystématique des Chrysolina l.s. (Coleoptera: Chrysomelidae: Chrysomelinae). *Annales de la Société Entomologique de France (N.S.)*, 27(1), 29–64.

Card, D.C., Shapiro, B., Giribet, G., Moritz, C. & Edwards, S.V. (2021) Museum genomics. *Annual Review of Genetics*, 55, 633–659.

Chernomor, O., von Haeseler, A. & Minh, B.Q. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, 65, 997–1008.

Cun, Y.-Z. & Wang, X.-Q. (2010) Plant recolonization in the Himalaya from the southeastern Qinghai-Tibetan plateau: geographical isolation contributed to high population differentiation. *Molecular Phylogenetics and Evolution*, 56, 972–982.

Daccordi, M. (1994) Notes for phylogenetic study of Chrysomelinae, with descriptions of new taxa and a list of all the known genera (Coleoptera: Chrysomelidae, Chrysomelinae). Proc. of the 3rd Intern. symposium on the Chrysomelidae, Beijing, 1992, Leiden: 60–84.

Deng, J., Guo, Y., Cheng, Z., Lu, C. & Huang, X. (2019) The prevalence of single-specimen/locality species in insect taxonomy: an empirical analysis. *Diversity*, 11, 106.

Dietz, L., Eberle, J., Mayer, C., Kukowka, S., Bohacz, C., Baur, H. et al. (2021) Standardized nuclear markers improve and homogenize species delimitation in Metazoa. *Methods in Ecology and Evolution*, 14(2), 543–555.

Dobler, S., Mardulyn, P., Pasteels, J.M. & Rowell-Rahier, M. (1996) Host-plant switches and the evolution of chemical defense and life history In the leaf beetle genus Oreina. *Evolution; International Journal of Organic Evolution*, 50, 2373–2386.

Eaton, D.A.R. & Overcast, I. (2020) Ipyrad: interactive assembly and analysis of RADseq datasets. *Bioinformatics*, 36, 2592–2594.

Ehmke, A., Rowell-Rahier, M., Pasteels, J.M. & Hartmann, T. (1991) Sequestration of ingested [14C] senecionineN-oxide in the exocrine defensive secretions of chrysomelid beetles. *Journal of Chemical Ecology*, 17, 2367–2379.

Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T. & Glenn, T.C. (2012) Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, 61, 717–726.

Faure, M. & Ferrière, J. (2022) Reconstructing the Variscan terranes in the alpine basement: facts and arguments for an Alpidic Orocline. *Geosciences*, 12, 65.

Froitzheim, N., Schmid, S. & Frey, M. (1996) Mesozoic paleogeography and the timing of eclogite facies metamorphism in the Alps: a working hypothesis. *Eclogae Geologicae Helveticae*, 89, 81–110.

Fujisawa, T., Sasabe, M., Nagata, N., Takami, Y. & Sota, T. (2019) Genetic basis of species-specific genitalia reveals role in species diversification. *Science Advances*, 5, eaav9939.

Garin, C.F., Juan, C. & Petitpierre, P. (1999) Mitochondrial DNA phylogeny and the evolution of host-plant use in palearctic *Chrysolina* (coleoptera, Chrysomelidae) leaf beetles. *Journal of Molecular Evolution*, 48, 435–444.

Gauthier, J., Pajlokiv, M., Neuenschwander, S., Kaila, L., Schmid, S., Orlando, L. et al. (2020) Museomics identifies genetic erosion in two butterfly species across the 20th century in Finland. *Molecular Ecology Resources*, 20(5), 1191–1205.

Gomez-Zurita, J., Garin, C.F., Juan, C. & Petitpierre, E. (1999) Mitochondrial 16S rDNA sequences and their use as phylogenetic markers in leaf-beetles with special reference to the subfamily Chrysomelinae. In: Cox, M.L. (Ed.) *Advances in Chrysomelidae biology*. The Netherlands: Backhuys Publ. Oegstgeest, pp. 25–38.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59, 307–321.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2018) UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522.

Hsiao, T.H. & Pasteels, J.M. (1999) Evolution of host-plant affiliation and chemical defense in *Chrysolina – Oreina* leaf beetles as revealed by mtDNA phylogenies. In: Cox, M.L. (Ed.) *Advances in Chrysomelidae biology*, *1*. Leiden: Backhuys Publ, pp. 321–342.

Jin, M., Zwick, A., Ślipiński, A., Keyzer, R. & Pang, H. (2020) Museomics reveals extensive cryptic diversity of Australian prionine longhorn beetles with implications for their classification and conservation. *Systematic Entomology*, 45, 745–770.

Jolivet, P. & Petitpierre, E. (1976) Les plantes-hôtes connues des *Chrysolina* (Col. Chrysomelidae) essai sur les types de sélection trophique. *Annales de la Société Entomologique de France (NS)*, 12, 1, 123–149.

Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F. & Orlando, L. (2013) mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics*, 29, 1682–1684.

Jurado-Rivera, J.A. & Petitpierre, E. (2015) New contributions to the molecular systematics and the evolution of host-plant associations in the genus Chrysolina (coleoptera, Chrysomelidae, Chrysomelinae). *ZooKeys*, 547, 165–192.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.

Kergoat, G.J., Bouchard, P., Clamens, A.-L., Abbate, J.L., Jourdan, H., Jabbour-Zahab, R. et al. (2014) Cretaceous environmental changes led to high extinction rates in a hyperdiverse beetle family. *BMC Evolutionary Biology*, 14, 220.

Kim, D., Song, L., Breitwieser, F.P. & Salzberg, S.L. (2016) Centrifuge: rapid and sensitive classification of metagenomic sequences. *Genome Research*, 26, 1721–1729.

Kippenberg, H. (1994) 88. Familie: Chrysomelidae. In: Lohse, G.A. & Lucht, W.H. (Eds.) *Die Kiifer Mitteleuropas. Band 14. Supplementband mit Katalogteil*. Krefeld: Goecke & Evers, pp. 65–83.

Kippenberg, H. (2008) Revision der Untergattung Protorina Weise der Gattung *Oreina* Chevrolat (coleoptera: Chrysomelidae: Chrysomelinae). *Koleopterologische Rundschau*, 78, 367–418.

Kippenberg, H. (2010) Subfamily Chrysomelinae Latreille, 1802. In: Löbl, I. & Smetana, A. (Eds.) *Catalogue of Palaearctic coleoptera*, Vol. 6. Stenstrup, Denmark: Chrysomeloidea. Apollo Books, pp. 390, 924–443 ISBN 978-87-88757-84-2.

Kippenberg, H. & Döberl, M. (1999) 88. Familie: Chrysomelidae W.H. Lucht, B. Klausnitzer (Eds.), Die Käfer Mitteleuropas 15, 4. Supplementband, Goecke & Evers, Krefeld pp. 313–324.

Kühnelt, W. (1984) Monographie der Blattkafergattung Chrysochloa (coleoptera, Chrysomelidae). *Sitzungsberichten Österr. Akad. Wissenschaften. Mathem.-naturw*, 1984, 171–287.

Landis, M.J., Matzke, N.J., Moore, B.R. & Huelsenbeck, J.P. (2013) Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology*, 62(6), 789–804.

Lanfear, R., Frandsen, P.B., Wright, A.M., 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, 772–773.

Lee, K.M., Kivelä, S.M., Ivanov, V., Hausmann, A., Kaila, L., Wahlberg, N. et al. (2018) Information dropout patterns in restriction site associated DNA Phylogenomics and a comparison with multilocus sanger data in a species-rich moth genus. *Systematic Biology*, 67, 925–939.

Li, H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv [q-bio.GN]*.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N. et al. (2009) The sequence alignment/map format and SAMtools. *Bioinformatics*, 25, 2078–2079.

Linck, E.B., Hanna, Z.R., Sellas, A. & Dumbacher, J.P. (2017) Evaluating hybridization capture with RAD probes as a tool for museum genomics with historical bird specimens. *Ecology and Evolution*, 7, 4755–4767.

Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, 17, 10–12.

Matzke, N.J. (2013) BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R scripts. R Package, v. 0.2.1.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A. et al. (2010) The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, 20, 1297–1303.

Methner, K., Campani, M., Fiebig, J., Löffler, N., Kempf, O. & Mulch, A. (2020) Middle Miocene long-term continental temperature change in and out of pace with marine climate records. *Scientific Reports*, 10, 7989.

Mikhailov, Y.E. (2008) Body colouration in the leaf beetle genera *Oreina* Chevr. And *Crosita* Motsch. And trends in its variation. In: Jolivet, P., Santiago-Blay, J. & Schmitt, M. (Eds.) *Research on Chrysomelidae*, Vol. 1. Brill: Leiden, pp. 129–148.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. et al. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530–1534.

Pasteels, J.M., Dobler, S., Rowell-Rahier, M., Ehmke, A. & Hartmann, T. (1995) Distribution of autogenous and host-derived chemical defenses in *Oreina* leaf beetles (coleoptera: Chrysomelidae). *Journal of Chemical Ecology*, 21, 1163–1179.

Pasteels, J.M. & Rowel-Rahier, M. (1991) Proximate and ultimate causes for host plant influence on chemical defense of leaf beetles (coleoptera: Chrysomelidae). *Entomologia Generalis*, 15(4), 227–235.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S. & Hoekstra, H.E. (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, 7, e37135.

Petitpierre, E. (1978) Chromosome numbers and sexdetermining systems in fourteen species of. *Chrysomelinae (Coleoptera, Chrysomelidae) Caryologia*, 31(1978), 219–223.

Petitpierre, E. (2019) Fauna Iberica Coleoptera. *Chrysomelidae II*, 46, 406–408.

Petitpierre, E. (2021) The genetic and cytogenetic relationships among subgenera of *Chrysolina* Motschulsky, 1860 and *Oreina* Chevrolat, 1837 (coleoptera: Chrysomelidae: Chrysomelinae). *International Journal of Zoology and Animal Biology*, 4(3), 1–7.

Raxworthy, C.J. & Smith, B.T. (2021) Mining museums for historical DNA: advances and challenges in museomics. *Trends in Ecology & Evolution*, 36, 1049–1060.
Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, 57, pg. 4-14.
Revell, L.J. (2012) Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution / British Ecological Society*, 3, 217–223.
Ronquist, F. (1997) Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, 46, 195–203.
Schubert, M., Lindgreen, S. & Orlando, L. (2016) AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Research Notes*, 9, 88.
Schwery, O., Onstein, R.E., Bouchenak-Khelladi, Y., Xing, Y., Carter, R.J. & Linder, H.P. (2015) As old as the mountains: the radiations of the Ericaceae. *The New Phytologist*, 207, 355–367.
Smith, S.A., Brown, J.W. & Walker, J.F. (2018) So many genes, so little time: a practical approach to divergence-time estimation in the genomic era. *PLoS One*, 13, e0197433.
Suchan, T., Pitteloud, C., Gerasimova, N.S., Kostikova, A., Schmid, S., Arrigo, N. et al. (2016) Hybridization capture using RAD probes (hyRAD), a new tool for performing genomic analyses on collection specimens. *PLoS One*, 11, e0151651.
Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J. & Rambaut, A. (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, 4, vey016.
Tin, M.M.-Y., Economo, E.P. & Mikheyev, A.S. (2014) Sequencing degraded DNA from non-destructively sampled museum specimens for RAD-tagging and low-coverage shotgun phylogenetics. *PLoS One*, 9, e96793.
Toussaint, E.F.A., Gauthier, J., Bilat, J., Gillett, C.P.D.T., Gough, H.M., Lundkvist, H. et al. (2021) HyRAD-X exome capture museomics unravels giant ground beetle evolution. *Genome Biology and Evolution*, 13(7), evab112.
Triponez, Y., Arrigo, N., Pellissier, L., Schatz, B. & Alvarez, N. (2013) Morphological, ecological and genetic aspects associated with endemism in the Fly orchid group. *Molecular Ecology*, 22, 1431–1446.
Triponez, Y., Buerki, S., Borer, M., Naisbit, R.E., Rahier, M. & Alvarez, N. (2011) Discordances between phylogenetic and morphological patterns in alpine leaf beetles attest to an intricate biogeographic history of lineages in postglacial Europe. *Molecular Ecology*, 20, 2442–2463.
van Noort, T. (2013) Colour polymorphism in the leaf beetle genus '*Oreina*'. Thèse de Doctorat: Université de Neuchâtel, RERO-ID R007495350.
Warchalowski, A. (1994) Eine nomenklatorische Anderung innerhalb der Gattung *Chrysolina* Motschulsky. *Genus*, 2(3), 281.
Weise, J. (1906) Catalogus Coleopterorum Europae, Caucasi et Armeniae Rossicae. Editio secunda. Ed. Edmund Reitter.
Weise, J. (1916) in Junk, Coleopterorum Catalogus, Berlin, 24, pars 68 Chrysomelidae, 12, Chrysomelinae: 98–109.
Wolinsky, H. (2010) History in a single hair. *EMBO Reports*, 11, 427–430.
Ziegler, P.A. & Dèzes, P. (2005) Evolution of the lithosphere in the area of the Rhine rift system. *Geologische Rundschau: Zeitschrift Fur Allgemeine Geologie*, 94, 594–614.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Ancestral range estimations performed with BioGeoBEARS and the six tested model. Models and parameters are indicated above each tree. Node pie charts represent the likelihoods of ancestral states.

Table S1. Sample statistics: sample origin, conservation, DNA concentration, number of reads and loci, and percentage of missing data. Samples included in the dataset A are in black, dataset B in bold, probes in red, and excluded samples are in grey.

Table S2. For each species, the table includes known host plants and host plant family probability used for the host plant reconstruction, as well as known distributions and matrix used for the biogeography reconstruction.

Table S3. Parameter estimates of different models and comparison in BioGeoBEARS.

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