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Jumping to new hosts: the diversification of flea beetles (Coleoptera: Chrysomelidae: Alticini) in the context of their host plant associations

Harald Letsch[1,*], **Franziska Beran**[2]

[1]Division of Tropical Ecology and Animal Biodiversity, Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, Vienna, Austria,[2]Population Ecology Group, Institute of Ecology and Evolution, Friedrich Schiller University Jena, Jena, Germany *Corresponding author, mail: harald.letsch@univie.ac.at

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Flea beetles (Alticini) represent one of the most diverse groups within the family Chrysomelidae and are associated with more than 100 different plant families. Conspicuously, only 10 genera account for about a quarter of flea beetle diversity, whereas about 380 genera each comprise less than 10 species, indicating different rates of diversification within the Alticini. Here, we reconstructed the phylogenetic relationships of 608 species in 101 Alticini genera using mitogenomes and cytochrome oxidase I, and applied several frameworks of clade-specific diversification rate analyses. Increased diversification rates were consistently detected in the cosmopolitan genera *Altica* Goffroy, *Longitarsus* Berthold, and *Phyllotreta* Chevrolat, and in neotropical taxa of the subtribe Oedionychina. In addition, we tested whether the evolution of specialized interactions with plants of the order Brassicales influenced the diversification of *Phyllotreta* and *Psylliodes* Berthold flea beetles. Specialization on Brassicales was only associated with increased diversification rates in *Phyllotreta* but not in *Psylliodes*. Our results indicate that host associations *per se* do not explain different diversification rates and lay the groundwork for investigating the evolutionary drivers of rapid radiations in Alticini.

Key words: differential diversification, plant defense against herbivore, insect–host association, mitogenome

Introduction

Flea beetles (Alticini) form by far the largest tribe within leaf beetles (Chrysomelidae), comprising ca. 8,000 described species in over 534 genera worldwide (Nadein 2013, Nadein and Bezděk 2014). More recent studies suggest that there are about 10,000 species in 601 genera (Douglas et al. 2023, Konstantinov et al. 2023). Within the subfamily Galerucinae, Alticini are traditionally defined by a meta-femoral spring in the hindlegs that allows them to jump and effectively escape from predators (Furth 1988, Schmitt 2004, Nadein and Betz 2016, Ruan et al. 2020). However, the monophyly of leaf beetles with a jumping organ is challenged by other morphological characters, such as the shape of the spermatheca and the venation of the wings, that contradict the phylogenetic signal of the meta-femoral spring (Furth and Suzuki 1992, 1994). The placement of several genera that possess a jumping organ into Galerucini by recent phylogenetic analyses, corroborates the hypothesis that the ability to jump has evolved several times independently in Galerucinae (Ge et al. 2011, 2012, Nie et al. 2018, Douglas et al. 2023). Here we focus on Alticini sensu stricto, i.e., not including genera placed within Galerucini.

The current distribution of Alticini includes all continents except Antarctica, with their center of diversity in Central and South America (Scherer 1988). Worldwide, they are associated with more than 100 different plant families. Most Alticini genera and species live mono- or oligophagously on a restricted group of plant species, belonging to the same genus or family, usually characterized by a common secondary chemistry (Jolivet 1988). However, within Alticini, species richness of subgroups is extremely biased, with a quarter of flea beetle diversity being accounted for by only 10 species-rich genera. These include the cosmopolitan genera *Longitarsus*, *Chaetocnema* Stephens, *Altica*, *Phyllotreta*, and *Psylliodes*, as well as the mainly Neotropical genera *Asphaera* Duponchel and Chevrolat

and *Alagoasa* Bechyné. In contrast, about 380 genera comprise less than 10 known species (Nadein 2013).

Differential diversification in groups of herbivores is frequently explained by the insects' host plant association, as adaptation to a specific host or a specific plant defense mechanism might establish a whole new spectrum of ecological opportunities, which may lead to accelerated speciation rates (e.g., Ehrlich and Raven 1964, Winkler and Mitter 2008, McKenna et al. 2009). However, the role of host plants in the diversification of Alticini has not yet been analyzed, although physiological adaptions to host plant defenses have been intensively studied in several alticine genera (e.g. Dobler et al. 2000, Dobler 2001, Beran et al. 2014, 2018). For example, 2 species-rich genera, *Phyllotreta* (about 300 species) and *Psylliodes* (about 200 species) are mainly associated with cruciferous plants of the order Brassicales (Jolivet 1988, Gikonyo et al. 2019), which are protected against herbivores by characteristic phytochemical defense compounds, the mustard oil glucosides (glucosinolates). Upon herbivory, glucosinolates are hydrolyzed by β-thioglucosidase enzymes (myrosinases) to unstable aglucones that spontaneously rearrange to deterrent isothiocyanates well known for their toxicity to herbivorous insects (Jeschke et al. 2016, Blažević et al. 2020). *Psylliodes* and *Phyllotreta* have evolved several different strategies to cope with the "mustard oil bomb" of their cruciferous host plants. These include the detoxification of isothiocyanates via the mercapturic acid pathway, the desulfation of glucosinolates to prevent their activation by myrosinases, and the sequestration of glucosinolates (Beran et al. 2014, 2018, Ahn et al. 2019). Species of the genus *Phyllotreta* further deploy sequestered glucosinolates for their own defense using intrinsic myrosinase activity (Beran et al. 2014, Sporer et al. 2021).

A host shift to Brassicales has led to significant diversification in the butterfly subfamily Pierinae and the weevil subfamily Ceutorhynchinae, although an increased speciation rate following the host shift was only detected in Pierinae (Wheat et al. 2007, Edger et al. 2015, Letsch et al. 2018). However, within Alticini, the evolutionary history and consequences of specialization on Brassicales have not yet been investigated. Previous attempts to elucidate the phylogeny of Alticini resulted in differing hypotheses on the relationships of *Psylliodes* and *Phyllotreta* (Ge et al. 2012, Gomez-Rodriguez et al. 2015, Nie et al. 2018, 2020, Zhang et al. 2022, Douglas et al. 2023). More recent studies recovered both genera within the same clade but never as sister groups, which makes a common origin of feeding on Brassicales unlikely. To date, none of these studies could infer diversification dynamics in Alticini due to limited taxon sampling.

In the present study, we investigate the diversification dynamics among Alticini, with a specific focus on the crucifer-feeding genera. In particular, we address the following questions: (Q1) Do diversification rates in particular groups or genera of Alticini differ from the mean diversification rate across all Alticini? Unequal diversification rates could indicate that different ecological adaptations or interactions have shaped the diversification dynamics in Alticini. (Q2) Do diversification rates differ between *Psylliodes* and *Phyllotreta*? Different diversification rates between these genera would suggest that different adaptation strategies rather than the general association with crucifers would have fueled diversification in both genera.

To obtain a robust hypothesis on the phylogenetic relationships of Alticini, as well as a sufficient taxon sampling to infer diversification pattern, we applied a 2-step approach. Initial tree reconstruction analyses on 113 mitogenomes provided the phylogenetic backbone of Alticini. The resulting mitogenomic tree subsequently served as a constraint to reconstruct a "diversity tree" including 608 Alticini species, represented by complete cytochrome-c-oxidase I (COI) sequences. This tree is intended to represent the diversity in the included subtribes and genera. We then estimated the divergence times for the "diversity tree" and applied several clade-specific evolutionary models to investigate the diversification dynamics within Alticini and the potential impact of adaptation to Brassicales on the diversification of *Psylliodes* and *Phyllotreta*.

Materials and Methods

Mitogenome Data Set

The mitogenomes of 100 Alticini species in 44 genera and 4 Galerucini outgroup species were downloaded from the NCBI Genbank database (Supplementary Table S1a). Gene and RNA annotations of all mitogenomes were individually generated using the MITOS2 server of the University Leipzig (Bernt et al. 2013, Donath et al. 2019) and manually refined in Geneious Prime 2022.0.2. For 9 species of *Phyllotreta* and *Psylliodes*, the mitogenomes were assembled de novo from transcriptome data (Supplementary Table S1b). For this purpose, the raw reads in fastq format were corrected, trimmed, and assembled to the template mitogenome of *Psylliodes chlorophana* (NC_053362) in Geneious. The newly generated consensus sequences were annotated by MITOS2 and manually refined.

The protein coding genes of all 113 taxa were translated into amino acid sequences, which were subsequently aligned with Mafft v7.496 (Katoh and Standley 2013). The amino acid alignments were used as templates to align the corresponding nucleotide sequences using PAL2NAL (Suyama et al. 2006). The ribosomal small and large RNA sequences were aligned with the L-INS-i algorithm in Mafft (Katoh and Standley 2014). In order to extract only unambiguously aligned portions and to eliminate potentially randomly aligned regions of both the rRNA and protein coding gene alignments, we used Gblocks (Talavera and Castresana 2007) with the following settings: sequence type either codon (protein coding genes) or DNA (rRNA), a minimum of 5 positions for a block, allowed gap positions in half of the sequences and otherwise defaults. Masked protein coding gene and rRNA alignments were then concatenated with AMAS v0.96 (Borowiec 2016).

Tree reconstruction analyses For maximum likelihood (ML) tree reconstruction, we used the software IQ-TREE v1.7.18 (Nguyen et al. 2015, Chernomor et al. 2016, Kalyaanamoorthy et al. 2017). For the nucleotide data set, we a priori generated a partition scheme with all protein coding genes separated into codon positions and the rRNA genes separated into single partitions, resulting in 41 partitions in total. The best-fitting partition scheme and nucleotide substitution model for each partition was estimated with the ModelFinder algorithm in IQ-TREE. Subsequently, we conducted 10 independent ML analyses with an increased number of stop iterations (-stop 200). Branch support values were estimated with 1,000 ultrafast (UF) bootstrap replicates (Minh et al. 2013), an increased maximum number of iterations to stop (-nm 5000), and the "bnni" option (Hoang et al. 2018) to reduce the risk of branch support overestimation. For the amino acid data set, the partitioning and model schemes, as well as tree search were applied in a similar manner, employing the genes as single partitions.

We additionally conducted Bayesian Inference (BI) tree reconstructions, using the software PhyloBayes (MPI version 1.8c; Lartillot et al. 2013). PhyloBayes implements the CAT mixture models that were developed to account for across site heterogeneities in sequence evolution and to reduce potential effects of compositional and mutational bias (Lartillot et al. 2007). Previous studies have shown that these models are well suited to suppress potential long-branch

attraction artifacts in tree reconstruction (e.g. Struck et al. 2011, Boussau et al. 2014). We applied BI analyses in PhyloBayes using the CAT-GTR model, which allows rate variation across sites under the GTR model. Constant positions were included, as their exclusion might affect likelihood calculations (Whelan and Halanych 2017). We ran 2 independent chains with a minimum of 25,000 cycles with 1 tree sampled for each generation. We then evaluated the convergence of model parameters and tree space with the PhyloBayes tools *tracecomp* and *bpcomp* using a 20% burn-in. Convergence of topologies was ensured by calculating the maximum difference (maxdiff) in the bipartition frequencies of the 2 chains.

Cytochrome-c-Oxidase 1 (COI) Data Set

To reconstruct the "diversity tree", we downloaded an additional 499 sequences from the NCBI Genbank and the Barcode of Life Database (BOLD) respectively (Supplementary Table S2). Taxa were selected so that each available species was presented by 1 individual COI sequence. If sequences assigned to the same species show more than 3% sequence divergence, both sequences were retained to present different species. This resulted in a final alignment of 612 species in total, including all species from the mitogenome data set.

Tree reconstruction analyses Tree reconstruction analyses using the 612 taxa COI data set was performed in IQ-TREE, using either the best tree from the ML tree search on the mitogenomic nucleotide data set, or the MCC tree from the BI analyses of the mitogenomic amino acid data set as topological constraints. The COI data set was applied on nucleotide level and partitioned according to the codon positions. 50 independent ML analyses were conducted using tree search and node support parameters similar to the mitogenome ML tree reconstruction analyses. Subsequently, we ran additional 50 independent ML runs, using model parameters of the best run in the previous analyses.

Divergence time analyses Divergence time estimation analyses were conducted by Bayesian Inference (BI) in the software BEAST v2.6.7 (Bouckaert et al. 2019). The best tree as inferred in the 612 taxa COI data set tree reconstructions was used as fixed input tree topology. The root age was calibrated by †*Crepidocnema yantarica* (Moseyko et al. 2010) from the Oise amber of France (55.8–48.6 Ma), representing the oldest reliable appearance of flea beetles in the fossil record. As the position of the genus †*Crepidocnema* within Alticini is not defined, we applied it as stem fossil, thus calibrating the split Galerucini + Alticini (i.e., Galerucinae = root). The age of Galerucinae has been estimated between 95–55 Ma in previous analyses (Gómez-Zurita et al. 2007, Wang et al. 2014), suggesting that the clade might be older than †*Crepidocnema*. We therefore applied the fossil as a normal prior distribution with its youngest age used as lower bound and calibrated so that 95% of the distribution lay between the fossil age and a maximum of 95 Ma. Within Alticini, the genus *Crepidodera* was calibrated by †*Crepidodera svetlanae* (Bukejs 2014) from Baltic amber (37.2–33.9 Ma). As this fossils' position within the genus is not defined, we also applied †*Crepidodera svetlanae* as a stem fossil. Here we used a lognormal prior distribution with the fossils' youngest age as lower bound and calibrated so that 95% of the distribution lay between the fossil age and a maximum of 50 Ma.

Prior to the divergence time estimations, we tested the fit of 2 clock models, the uncorrelated lognormal relaxed clock (UCLN; Drummond et al. 2006), and the random local clock (RLC; Drummond and Suchard 2010). Both clock models were combined with either a Yule pure birth tree model and a Birth-Death tree model. The log marginal likelihood of all 4 candidate models was estimated by path sampling (PS) as implemented in BEAST 2.6.7 (Baele et al. 2012). PS analyses were run for 100 steps of each 100,000 generations, and the best-fitting tree model was selected according to BF based on the interpretation of Kass and Raftery (1995). The best-fit substitution model was implemented by bModelTest (Bayesian model test package for BEAST 2.6.7). Using the best-fitting model setup, we conducted 2 BEAST runs for 175 million generations each (sampling every 5,000 generations). The quantity of generations discarded as burn-in, the convergence and mixing of parameters and the effective sample sizes (ESS), were assessed by the Tracer software v1.7.1 (Rambaut et al. 2018). Post-burn-in samples were used to construct a maximum clade credibility (MCC) tree with median node heights in TreeAnnotator v2.6.7 (Bouckaert et al. 2019).

Diversification rate analyses To test for differential diversification among alticine groups, we applied several frameworks of clade-specific diversification rate analyses. We first estimated speciation and extinction rate dynamics over time and across branches in the MCC tree with the BAMM v2.5.0 software (Rabosky 2014) and the BAMMtools v2.1.9 package (Rabosky et al. 2014) for R (R Development Core Team 2019). Prior distributions on speciation (lambda) and extinction (mu) rates were calculated with BAMMtools (lambdaInitPrior = 2.03, lambdaShiftPrior = 0.02, muInitPrior = 2.03) and the incomplete taxon sampling for Alticini was taken into account using a global sampling fraction (ρ = 608/7,917). The BAMM analyses employed a reversible jump MCMC (rjMCMC) run of 8 chains and 10,000,000 generations sampled every 10,000 steps and a burn-in of 25%. Subsequently, we assessed ESS of the log-likelihood and the number of shift events, as well as the visualizations of rates and shifts with BAMMtools. Mean diversification rates along the branches of the tree were depicted as a phylorate plot, which represents the phylogeny with its branches colored to reflect diversification rates. The best overall shift configuration was shown by the maximum shift credibility (MSC) configuration, which maximizes the marginal probability of rate shifts along individual branches.

However, BAMM has been criticized due to potentially biased modeling of the probability of rate shifts in extinct lineages, which can lead to incorrect likelihood estimations (Moore et al., 2016, Laudanno et al., 2020). We therefore used an additional method to infer clade-specific diversification rates in the software RevBayes v1.1.1 (Höhna et al. 2016). The implemented lineage-specific birth–death shift model (LSBDS) principally relies on algorithms originally developed for state-dependent speciation and extinction (SSE) branching processes (Maddison et al. 2007, FitzJohn et al. 2009). It estimates branch-specific diversification rates independently from a focal trait and thus allowed us to infer diversification dynamics throughout the tree without a priori assigning the taxa to specific traits. We applied the LSBDS model according to the developers' suggestions (see https://revbayes.github.io/tutorials/divrate/branch_specific.html), using a discretized lognormal prior with 6 rate categories for estimation of diversification rate regimes. Missing species were accounted for by a global sampling fraction (ρ = 608/7,917) and 2 chains with 100,000 generations were run. Net diversification rate changes were finally visualized using the R package *RevGadgets* v1.0.0 (Tribble et al. 2022), by coloring the branches of the MCC tree.

To further test for specific diversification rates of crucifer-feeding and noncrucifer-feeding alticine groups, we conducted additional clade-specific diversification rate analyses with the software BayesRate v1.6.5 (Silvestro et al. 2011). This approach

estimates the fit of different models in which the rates vary between predefined clades. It employs a thermodynamic integration approach to calculate marginal likelihoods of different diversification models, and uses Bayes Factors (BF) to evaluate the best model, as well as clade-specific taxon sampling proportions to account for missing taxa. Based on the previous RevBayes analyses we subdivided our data set in 6 clades and tested 3 different rate regimes in BayesRate: (i) all clades have a single common diversification rate; (ii) *Altica*, *Longitarsus*, *Phyllotreta*, *Psylliodes* (crucifer-feeding species), Oedionychina, and the remaining species evolve under different diversification rates; and (iii) *Phyllotreta*, *Longitarsus*, and Oedionychina evolve at one rate, *Altica* evolves at a one rate, and *Psylliodes* and the remaining species evolve at one rate. The parameters for speciation and extinction (pure-birth vs. birth-death model for each clade) were estimated separately for all rate regimes and BF were calculated and compared according to Kass and Raftery (1995) to indicate the best-fitting model combination and rate regime. All MCMC analyses were run for 1 million generations, with sampling every 100 generations and discarding the first 100,000 generations as burn-in. Subsequently, Tracer was used to assess ESS and parameter convergence. Diagrams showing the 95% credibility intervals for individual (post-burn-in) net diversification rates were produced with the R-package diversitree v0.9.9 (FitzJohn 2012).

Explicit tests for the impact of crucifer-feeding on the diversification dynamics of Alticini were carried out in R using the package *hisse* v1.9.5 (Beaulieu and O'Meara 2016). The HiSSE (Hidden State Speciation and Extinction; Beaulieu and O'Meara 2016) approach is an extension of the popular BiSSE model (Binary State Speciation and Extinction; Maddison et al. 2007), which calculates the impact of an observed single character on diversification rates, as well as transitions between the characters' states. However, the BiSSE model is prone to Type 1 errors, meaning it is biased towards the detection of character-dependent diversification, although the observed character has evolved independently from diversification (Rabosky and Goldberg 2015). In contrast, HiSSE calculates potential correlations between the observed (focal) trait (here: state 0, noncrucifer-feeding; state 1, crucifer-feeding) by considering the presence of unobserved (hidden) traits (state 0A, 1A, hidden trait absent; state 0B, 1B, hidden trait present). In our case, a correlation between crucifer-feeding and high diversification rates in the presence of a hidden trait would suggest that other traits in combination with the host associations would trigger higher diversification rates. HiSSE also provides different null models that assume character-independent diversification without constraining diversification rates to be homogenous across the tree. These character-independent models (CID-2 and CID-4) have the same number of distinct parameters that can vary across the tree as their BiSSE and HiSSE counterparts, but model diversification unlinked to the observed character (Beaulieu and O'Meara 2016). In total, we fitted 15 different HiSSE models that estimated speciation, extinction, and transition rates between states 0 (noncrucifer-feeding) and 1 (crucifer-feeding) in 2 rate classes A and B (see SupplementaryTable S2). We included 4 BiSSE-like models without hidden states, 4 constrained models with hidden states either associated with crucifer-feeding (0A, 1A, 1B), or noncrucifer-feeding (0A, 1A, 0B), and 1 full HiSSE model with 2 hidden states and thus 4 different rate classes 0A, 1A, 0B, 1B. We also tested 8 character-independent model sets CID-2 and CID-4. We accounted for incomplete taxon sampling by providing a uniform sampling fraction for both crucifer-feeding and noncrucifer-feeding clades.

Results

Phylogenetic Relationships as Inferred by Mitogenomes

The backbone of the Alticini tree could only partly be resolved by the ML and BI analyses of the mitogenome data sets, which is reflected by the variable relationships with low node support among distinct groups in both ML and BI analyses (Fig. 1, Supplementary Figs. S1–4). All phylogenetic analyses consistently recovered highly supported sister group relationships of *Longitarsus*, *Aphthona* Chevrolat + *Bikasha* Maulik ("*Longitarsus* group"), *Altica*, *Macrohaltica* Bechyné and *Syphrea* Baly ("*Altica* group"), and *Dibolia* Latreille, *Argopistes* Motschulsky + *Apteropeda* Chevrolat ("Dibolia group"). We could furthermore consistently recover a clade that includes the genus *Blepharida* Chevrolat and its relatives, as well as *Nisotra* Baly and its relatives ("*Blepharida* group"), which appeared as sister group to most other alticine groups. Additionally, all analyses recovered the mainly neotropical subtribe Oedionychina, including the genera *Asphaera*, *Omphoita* Chevrolat, and *Philopona* Weise, as well as *Hemipyxis* Dejean and *Hyphasis* Harold. ML and BI analyses of the mitogenomic data sets were mostly consistent in recovering a highly supported clade comprising *Phyllotreta* and *Psylliodes* together with *Crepidodera* Chevrolat + *Xuthea* Baly, *Epitrix*, and *Chaetocnema* Stephens (Fig. 1, Supplementary Figs. S1–4). According to Ge et al. (2012), we refer to this clade as the "*Chaetocnema* group". However, within this group, the relationships differed between ML and BI analyses. While the former shows *Psylliodes* as sister to *Crepidodera* + *Xuthea* and *Phyllotreta* as sister to *Epitrix*, the latter shows *Phyllotreta* as sister to *Chaetocnema* and *Psylliodes* as sister to a clade consisting of *Epitrix* and *Phyllotreta* + *Chaetocnema*. The only exception were the ML analyses of the amino acid data set, which recovered *Phyllotreta* outside the "*Chaetocnema* group", as sister to *Phygasia* Baly. However, the ML analyses of the amino acid data set using the CAT-PSMF pipeline also showed a monophyletic "*Chaetocnema* group" including *Phyllotreta*. Within *Psylliodes*, all crucifer-feeding species formed a monophyletic group (Fig. 1).

Divergence Time Estimations

The best "diversity tree" was inferred with the constraint from the ML tree reconstruction of the mitogenome nucleotide data set (Supplementary Fig. S5), which was then applied as a fixed topology for the divergence time estimations. Bayes factor analyses suggested that the model with a pure birth tree model and a RLC fits the data best (Supplementary Table S4). The analyses based on this model (Fig. 2, Supplementary Fig. S5) recovered the emergence of Alticini at 62.7 Ma (median age; 95% height posterior density [HPD] = 76.8–49.0 Ma) and the onset of diversification of extant Alticini at 57.9 Ma (95% HPD = 70.9–46.5 Ma) in the Paleocene. Our estimations further recovered the origin of major Alticini groups in the Eocene. The "*Chaetocnema* group" diversified in the early Eocene at 51.0 Ma (95% HPD = 63.0–41.6 Ma). Within this group, the genera *Psylliodes* and *Phyllotreta* diversified at 45.9 Ma (95% HPD = 56.5–36.4 Ma) and 31.1 Ma (95% HPD = 39.6–24.1 Ma), respectively and the genera *Chaetocnema* and *Epitrix* diversified both in the middle Eocene at 40.9 Ma (95% HPD = 50.4–32.3 Ma) and 41.7 Ma (95% HPD = 53.1–32.7 Ma), respectively. In the "*Longitarsus* group", the early split between *Longitarsus* and *Aphthona* + *Bikasha* appeared in the middle Eocene at 44.7 Ma (95% HPD = 55.3–35.3 Ma). Both Oedionychina and the "*Altica* group" originated in the early Eocene at 38.0 Ma (95% HPD = 47.1–29.3 Ma) and 36.0 Ma (95% HPD = 45.3–27.8 Ma), respectively. Within the latter, the

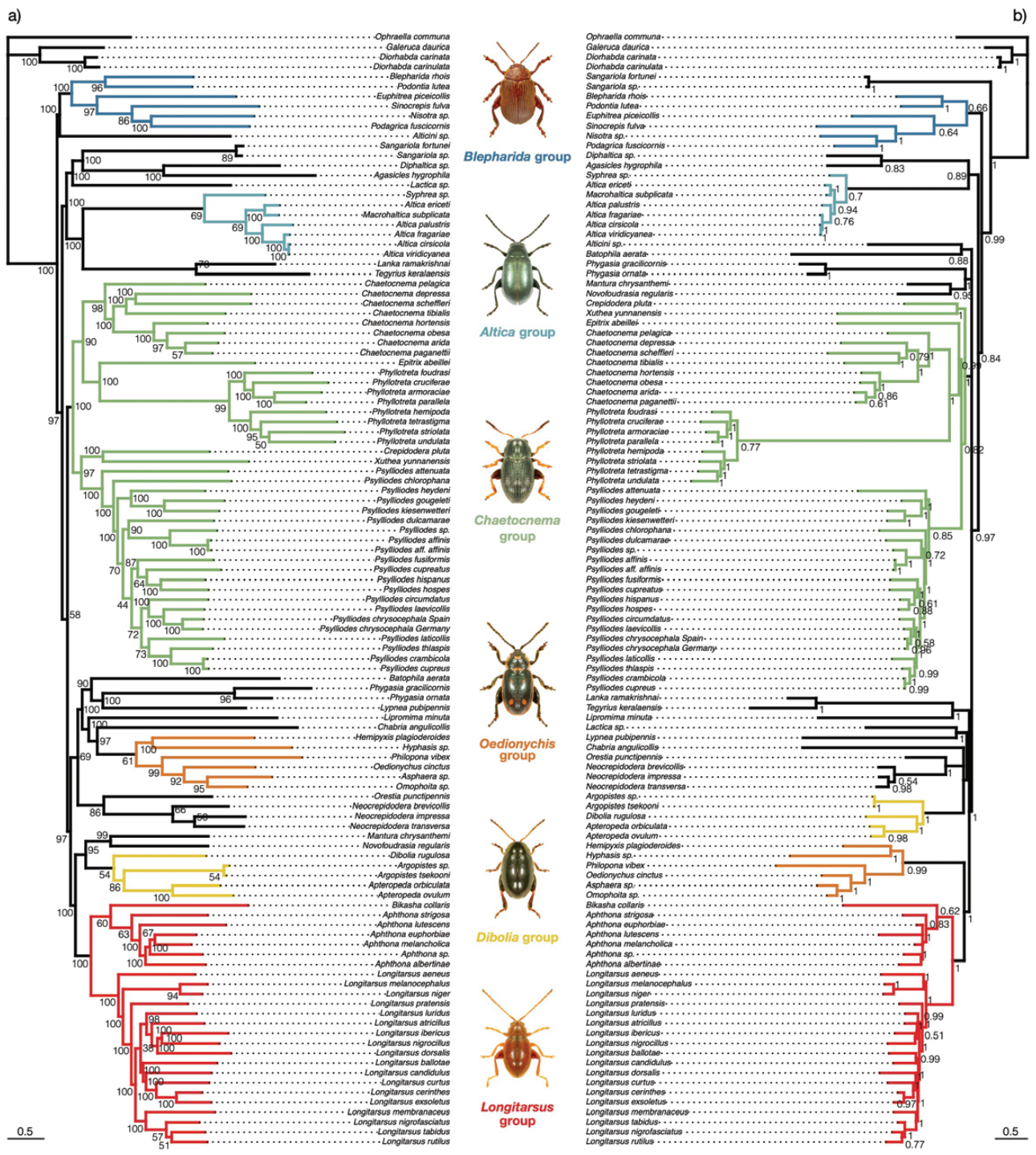

Fig. 1. Tree reconstruction results of the 113 taxa mitogenome data set. a) Maximum likelihood tree inferred from nucleotide data set using IQ-TREE. Node numbers show bootstrap support values. b) Bayesian tree inferred from the amino acid taxa data using PhyloBayes under the site-heterogeneous CAT-GTR model. Node numbers show the posterior probability values. Alticini representatives (from top to bottom): *Blepharida sacra, Altica bicarinata, Chaetocnema angustula, Oedionychis cincta, Dibolia alpestris,* and *Longitarsus gruevi*. Picture copyright: Lech Borowiec, Wroclaw, Poland, used with permission.

diversification of the genus *Altica* started in the early Miocene at 19.7 Ma (95% HPD = 24.7–15.5 Ma).

Diversification Rate Analyses

Both the BAMM and the LSBDS model analyses in RevBayes indicated increased net diversification in several groups of Alticini, as shown by the heat maps plotted on the MCC tree in Fig. 2a. Besides high diversification rates in some smaller clades near the tips, highest diversification was recovered in the genus *Altica*. Higher diversification rates were further predicted in Oedionychina and the genera *Longitarsus* and *Phyllotreta*, whereas no increased diversification was predicted for *Psylliodes*, except for a small clade of 5 crucifer-feeding *Psylliodes* species. The BayesRate analyses principally corroborated these results, as the best model identified by BF comparisons had different speciation and

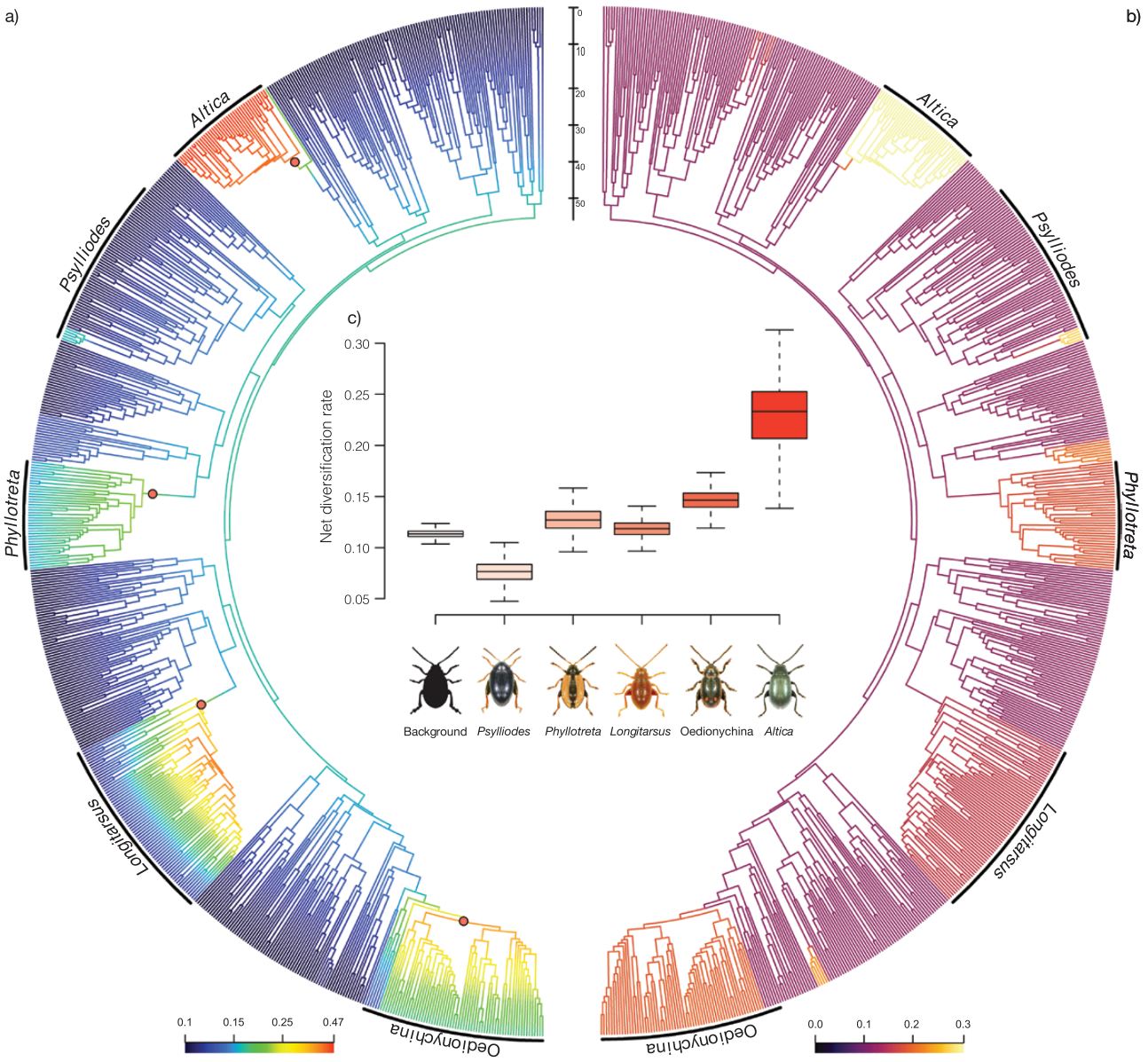

Fig. 2. Results of diversification rate analyses. a) Results of the branch-specific diversification rate analyses as inferred in BAMM, based on a uniform sampling strategy. Colors indicate relative speciation rates along each branch on the chronogram (increasing from blue to red). The red circles on the branches indicate regime shifts in the maximum shift credibility (MSC) configuration. b) Results of the branch-specific diversification rates as indicated by the LSBDS model in RevBayes. Colors indicate relative speciation rates along each branch on the chronogram (increasing from violet to yellow). c) Net diversification rates as indicated by the best BayesRate model. Alticini representatives (right to left): *Psylliodes chalcomera*, *Phyllotreta armoraciae*, *Oedionychis cincta*, *Longitarsus gruevi*, and *Altica bicarinata*. Picture copyright: Lech Borowiec, Wroclaw, Poland, used with permission.

extinction rates for all focal clades and the background (Table 1). This model indicated highest diversification in *Altica* (d = 0.23) and higher rates in Oedionychina (d = 0.15), as well as in the genera *Longitarsus* (d = 0.12) and *Phyllotreta* (d = 0.13). In contrast, diversification in crucifer-feeding *Psylliodes* species (d = 0.08) was recovered even lower than in the remaining Alticini (d = 0.11). The state-dependent analyses in HiSSE showed a CID-2 model fitting best to the data (Supplementary Table S5). Among the 15 models we compared in total, this model alone had a high AICw value (w = 0.99), thus indicating that crucifer-feeding in principle had no impact on diversification dynamics in Alticini.

Discussion

Diversification rate shifts in Alticini occurred at different times in several unrelated clades that are associated with different groups of host plants. Besides crucifer-feeding *Phyllotreta*, we could detect increased net diversification rates in *Longitarsus*, Oedionychina and most prominently in *Altica* (Q1). In contrast, we could not detect increased diversification rates in *Psylliodes*. This indicates that adaptation to Brassicaceae per se is not a driver of diversification in crucifer-feeding Alticini (Q2). The timing and pattern of diversification we recovered in our analyses rather implies different causes for the increased diversification in the respective groups. It is nevertheless conspicuous that the increase of diversification rates found in the 3 groups *Phyllotreta* (31.1 Ma; HPD = 39.6–24.1 Ma), Oedionychina (29.3 Ma; HPD = 36.9–22.8 Ma), and *Longitarsus* (29.5 Ma; HPD = 36.9–23.4 Ma), all occurred shortly after the transition from the Eocene to the Oligocene at 33.9 Ma. This time was characterized by a significant global cooling, including the development of permanent continental ice sheets in Antarctica (Zachos et al. 2001). While this

Table 1. Results of Bayes factor tests in the BayesRate analyses

#	Model	Marginal lnL	BF
1	|BG + Ps + Ph + Oe + Lo + Al|	−2505.15	30.14
2	|BG + Ph|Ps + Oe + Lo|Al|	−2441.43	20.08
3	|BG|Ps|Ph|Oe|Lo|Al|	−2304.89	0

Marginal likelihood values (lnL) and relative Bayes Factors (BF) are presented for each individual model scheme. Group abbreviations: *Psylliodes* (Ps), *Phyllotreta* (Ph), Oedionychina (Oe), *Longitarsus* (Lo) *Altica* (Al), Background, i.e., remaining taxa (BG).

cooling was probably responsible for the overall global decrease in floral richness and the extinction of humid-adapted clades in higher latitudes (Wolfe 1992, Morley 2003), it also induced radiations in more deciduous/dry-adapted floras in these regions (Jacobs et al. 1999, Bouchenak-Khelladi et al. 2014).

In Brassicaceae, the Eocene–Oligocene transition also marks the beginning radiation of the cool-adapted clades (Couvreur et al. 2009, Edger et al. 2015, Huang et al. 2015). Their radiation in Europe, the Mediterranean, and the Saharo-Arabian regions (Cardinal-McTeague et al. 2016) coincided with the emergence and radiation of the crucifer-adapted species of the genus *Psylliodes*, as well as the diversification of the genus *Phyllotreta*. Beside these flea beetle genera, also the weevil genus *Ceutorhynchus* Germar and butterflies of the subfamily Pierinae adapted to Brassicaceae in the Early Oligocene. Similar to *Phyllotreta*, Pierinae butterflies also experienced increased diversification rates after their colonization of Brassicaceae (Edger et al. 2015, Letsch et al. 2018). This pattern corroborates the hypothesis that climatic shifts favoring insect host plants indirectly induce diversification in clades adapted to these plant groups (Peña and Wahlberg 2008, Winkler et al. 2009, Letsch et al. 2018).

Within Oedionychina, the inferred diversification rate shift is also associated with the colonization of Central and South America in this group at 31.6 Ma (HPD = 39.9–24.9 Ma). It has previously been proposed that the endemic Alticini fauna of the Neotropics diversified after the breakup of the Gondwanan supercontinent in the Late Cretaceous with the separation of South America from Africa (Scherer 1988). However, our analyses show a much younger colonization of the Neotropical regions. Its timing and the early relationships in Oedionychina, with the Asian taxa *Hemipyxis*, *Hyphasis*, and *Philopona* as sister to the New World taxa, indicate that Central and South America was colonized via Asia and North America. The observed higher diversification rates in Neotropical taxa might be the result of new ecological opportunities after entering this continent. Alternatively, this pattern could also be primarily due to a lower extinction rate in these areas, compared to the Palearctic, where the Eocene–Oligocene boundary marks the decline of previously tropical warm and humid-adapted floras.

The availability of new resources due to climate changes in favor of insect host plants raises the question if specific adaptation modes to hosts are responsible for differential diversification pattern among Alticini. In our analyses, *Phyllotreta* and *Psylliodes* were not recovered as sister groups, and crucifer-feeding appeared as a derived character state in *Psylliodes* (Fig. 1). These patterns indicate that associations with Brassicales have evolved independently in both genera. This is also reflected by the different strategies in both genera to cope with the crucifers' defense system. *Psylliodes* metabolize isothiocyanates mainly via the mercapturic acid pathway, which probably plays only a minor role as a detoxification strategy in *Phyllotreta* (Beran et al. 2018, Sporer et al. 2021). A lineage-specific diversification of sulfatases is associated with the evolution of glucosinolate sulfatase activity in *Psylliodes*, but not in *Phyllotreta* (Ahn et al. 2019).

The ability to sequester glucosinolates has been demonstrated in both *Phyllotreta* and *Psylliodes*, but only *Phyllotreta* can benefit from sequestered glucosinolates using their own myrosinase enzymes (Beran et al. 2014, 2018, Sporer et al. 2020). Together, the observed differences in adaptations to glucosinolates support our finding that *Phyllotreta* and *Psylliodes* have adapted independently from each other to crucifers. Since we could only detect increased diversification rates for *Phyllotreta*, the mode of adaptation to Brassicaceae, i.e., the ability to exploit plant defense compounds for protection against natural enemies, might be associated with differential diversification among crucifer-feeding flea beetles. Indeed, there is a growing body of evidence that selection pressure from higher trophic levels has been an important driving force in coevolutionary interactions between herbivorous insects and their host plants (Beran and Petschenka 2022). Nevertheless, due to the successful diversification of Brassicaceae itself in the Late Eocene and Miocene in the Northern hemisphere (Cardinal-McTeague et al. 2016), the general adaptation of *Psylliodes* to Brassicaceae may have facilitated speciation events over time that led to their recent species richness, without a distinct pattern of rapid diversification. However, within *Psylliodes*, a small cluster of crucifer-feeding species also displayed slightly higher speciation rates. In addition to morphological differences, these species are known to differ in host use, habitat preference, and overall geographic distribution (Cox 2007, Rheinheimer and Hassler 2018), which could have been drivers of reproductive isolation. However, it is also notable that 3 of these species (P. *crambicola*, *P. luridipennis*, and *P. marcida*) are associated with coastal habitats.

The accumulation of secondary plant compounds for defense is also well known for the genus *Longitarsus*, but is centered around completely different metabolite classes and plant taxa. Host plants used by many species of this genus frequently contain either pyrrolizidine alkaloids (Boraginaceae and the tribes Eupatorieae and Senecioneae of the Asteraceae), or iridoid glycosides (Lamiaceae, Scrophulariaceae, and Plantaginaceae), which are generally deterrent and/or toxic for nonadapted herbivores (Bernays and Chapman 1977, Bowers and Puttick 1988, van Dam et al. 1995, Pentzold et al. 2014). In *Longitarsus*, sequestration is common in species adapted to plants containing pyrrolizidine alkaloids or iridoid glycosides (Dobler et al. 2000, 2011, Dobler 2001, Narberhaus et al. 2003, Pankoke and Dobler 2015). Other species in the same beetle genus only tolerate these defense compounds, but do not sequester them (Dobler 2001). In addition, certain species with host plants that do not contain pyrrolizidine alkaloids, or that only occasionally feed on hosts with pyrrolizidine alkaloids, are still able to sequester them (Dobler 2001, Narberhaus et al. 2003). The origin of adaptations to pyrrolizidine alkaloids and iridoid glycosides is so far equivocal. Previous attempts to elucidate the deep phylogenetic relationships within *Longitarsus* provided inconsistent results (Dobler 2001, Salvi et al. 2019) and although the mitogenome data of our study shows well supported clades, the COI data was not sufficient to provide robust hypotheses of deeper relationships within *Longitarsus*.

Adaptation to iridoid glycosides might also play a role in the anti-predator defense mechanisms of representatives of Oedionychina. Species of this subtribe often display a contrasting coloration pattern with a combination of dark and light spots or stripes (Konstantinov et al. 2022), which has been interpreted as warning colors to deter potential predators (Begossi and Benson 1988). Besides body coloration, unpalatability of several species of *Alagoasa*, *Omphoita* and *Aspheara* has been documented (Begossi and Benson 1988).

Species of these genera, as well as the genera *Capraita* Bechyné, *Walterianella* Bechyné, and *Kuschelina* Bechyné are specialized on members of the plant families that contain iridoid glycosides, such as Lamiaceae, Verbenaceae, Bigoniaceae, and Buddlejaceae. Data on sequestration of these compounds by oedionychine beetles is rare, but has been documented in *Walterianella bucki* Bechyné feeding on *Buddleja* Bartling (Duckett and Casari 2002).

The highest diversification rate was found in the genus *Altica*, which turned out to be younger than most other genera, emerging only at about 17 Ma in the Early Miocene. Nevertheless, *Altica* underwent a rapid radiation, and now presents one of the most speciose extant flea beetle genera. *Altica* species feed primarily on members of the Onagraceae and Lythraceae (Myrtales), as well as the Vitaceae and Haloragaceae, but a number of other plant families may be colonized as well (Jolivet and Hawkeswood 1995, Rheinheimer and Hassler 2018). Studies with 3 sympatric *Altica* species that have diverged very recently but are associated with distantly related host plants suggest that host plant switching led to reproductive isolation (Xue et al. 2011). Recently, candidate gene families involved in in the detection of chemical cues and in common detoxification pathways have been annotated in a draft genome assembly of *Altica viridicyanea* (Xue et al. 2021). While some gene families or subfamilies appear to be specifically expanded in *A. viridicyanea* compared to those in chrysomelid species belonging to other genera, comparisons with the genomes of the closely related species *A. cirsicola* and *A. fragariae* may provide deeper insights into the molecular basis of host switching and rapid adaptation in this genus. Moreover, infection with *Wolbachia*, a reproductive parasite that can induce cytoplasmic incompatibility, is thought to have influenced the evolution of *Altica* flea beetles (Jäckel et al. 2013). This genus thus represents an interesting model to study evolutionary drivers of rapid speciation in the Alticini.

Diet Breadth in Alticini

Diet breadth, the number of plant species used by an insect as hosts, has also been proposed as a possible driver of insect diversification. For example, alternating phases of host expansion and specialization may lead to an increase in net speciation rate, a scenario known as the oscillation hypothesis (Janz and Nylin 2008, Janz 2011). Although the number of host plant families may vary considerably between Alticini genera, the diet breadth of species within these genera may still be similar. For example, in both *Phyllotreta* and *Psylliodes*, most species feed on several species of the same plant family, whereas a few species have either more restricted or broader host use (Gikonyo et al. 2019). This pattern of diet breadth appears to be frequent also in other genera of Alticini. For example, in *Chaetocnema*, most species feed oligophagously on either Polygonaceae, Junaceae, and Cyperaceae, whereas other representatives of this genus are associated with all 3 families. In *Alagoasa*, many species are mainly associated with either Verbenaceae, Lamiaceae, Bignoniaceae, and Acanthaceae, but a few species, such as *Alagoasa decemguttata*, feed on several of these families (Begossi and Benson 1988). In *Longitarsus*, almost all species live oligophagously on 1 plant family (mainly Scrophulariaceae, Lamiaceae Plantaginaceae, Convolvulaceae, and Asteraceae (Salvi et al. 2019). In contrast, the pollen feeding species of the genus *Systena*, feed on a wide range of different host plant families (Clark et al. 2004). In our present study, we did not explicitly test for a correlation of diet breadth and diversification in Alticini, but the patterns described above suggest that differences in diet breadth do not play an important role in the diversification in Alticini.

Phylogenetic Implications for Alticini

Mitogenome data have been widely used in insect phylogenetics, and to date most molecular studies on Galerucinae rely on them (Ge et al. 2012, Gomez-Rodriguez et al. 2015, Nie et al. 2018, Hlaka et al. 2022, Zhang et al. 2022). However, the application of mitogenomes in insect phylogenetics is not always straightforward. Accelerated substitution rates and compositional heterogeneity are a common issue (e.g., Talavera and Castresana 2007, Timmermans et al. 2016). This is reflected by the relatively long branch exhibited by *Phyllotreta* in all our tree reconstructions, as well as in previous studies (Ge et al. 2012, Gomez-Rodriguez et al. 2015, Nie et al. 2018, Hlaka et al. 2022, Zhang et al. 2022), indicating elevated evolutionary rates in this taxon. Differences in evolutionary rates across targeted groups can be problematic in phylogenetic tree reconstructions, leading to "long-branch attraction" (LBA), i.e., a preferential clustering of long branches (long-branch attraction effect; Felsenstein 1978), irrespective of their true phylogenetic position. While primarily suspected as an inherent problem for parsimony methods (Schulmeister and Wheeler 2004), also probabilistic methods such as ML and BI can be affected, due to model violations (Brinkmann et al. 2005, Lartillot et al. 2007). However, the development of site-heterogeneous models of evolution, i.e., the CAT-GTR model (Lartillot and Philippe 2004) has been shown to alleviate problems caused by LBA (Lartillot et al. 2007). In our analyses, the position of *Phyllotreta* appeared equivocal and differed among data sets and reconstruction methods applied. ML analyses of the amino acid data set recovered *Phyllotreta* as sister group to *Phygasia*. We suspect this to be a consequence of potential long-branch attraction due to violations of the site-homogeneous models applied in this approach, as application of site-heterogeneous models consistently inferred *Phyllotreta* as part of the "*Chaetocnema* group" irrespective of the applied reconstruction method.

Although the phylogenetic position of *Phyllotreta* and other Alticini genera should be assessed with potentially less LBA-prone molecular data in the future, the position of *Phyllotreta* within the "*Chaetocnema* group" currently appears to be the most reliable hypothesis. In general, our analyses were largely consistent with several previous studies on Alticini phylogeny. The specific groups we refer to (see Fig. 1) have been defined by Ge et al. (2012) and are frequently recovered in phylogenetic analyses of Chrysomelidae and their subgroups (Ge et al. 2012, Gomez-Rodriguez et al. 2015, Nie et al. 2020, Hlaka et al. 2022, Zhang et al. 2022). However, the relationships among these groups are generally only weakly supported, depending on the specific taxon sampling, the data sets used (nucleotides vs. amino acids), and the tree reconstruction methods applied (Zhang et al. 2022). This indicates that mitogenomes have a restricted phylogenetic signal to elucidate the deeper relationships within Alticini. However, the most recent study on the phylogeny of Galerucini and Alticini based on anchored hybrid enrichment (AHE) data, could also not robustly resolve the deeper relationships among most subgroups of Alticini and did not consistently place *Phyllotreta* within the "*Chaetocnema* group" (Douglas et al. 2023).

The divergence time of Alticini in the Paleocene is consistent with previous studies that focused on that group (Gómez-Zurita et al. 2007), although it should be noted that studies targeting the divergence times of higher groups, such as Cerambycidae, recovered older ages for Galerucinae (Wang et al. 2014, Nie et al. 2021). Nevertheless, in these studies, Alticini were mainly represented by only a few taxa, making an age estimate of this group less robust.

Conclusions

Our analyses show that several groups of Alticini experienced periods of rapid radiations, most notably in the genus *Altica*, but also in the genus *Longitarsus*, the subtribe Oedionychina and in the crucifer-feeding genus *Phyllotreta*. We could not detect an increased diversification rate in the crucifer-feeding genus *Psylliodes*, which contradicts the hypothesis that adaptation to crucifers is associated with rapid radiations. The diversification pattern revealed in our study did not support an overall correlation of host plant association with beetle diversification, but at least in *Phyllotreta* and *Longitarsus*, the employment of sequestered plant secondary compounds as anti-predator defense mechanism could have been an important driving force in the evolution of these groups. While sequestration of glucosinolates in *Phyllotreta* and of pyrrolizidine alkaloids or iridoid glycosides in *Longitarsus* is well documented, evidence for a similar significance of this mechanism in species of the Oedionychina is still pending. Chemical defense mechanisms (including aposematism) frequently occur in Chrysomelidae, but evidence for sequestration of secondary plant compounds for defense is much rarer, given the enormous species richness of this beetle family and its intimate associations with their host plants. This could well indicate that successful adaptation to host plants, providing an additional anti-predator mechanism through sequestration of plant defense compounds, ultimately leads to increased diversification in these groups of Alticini.

To date, our taxon sampling is the largest for Alticini, yet it still represents probably less than 10 percent of the described alticine species. In addition, mitogenome data may not be optimal for inferring deeper phylogenetic relationships within this group. Nevertheless, our study highlights the potential of Alticini as a model group to understand the macroevolutionary mechanisms that have shaped the close interactions between herbivorous insects and their host plants. Future studies should focus on additional genomic data to consolidate the phylogenetic relationships. We hope that our findings will encourage studies on the potential causes of rapid radiations in *Altica* and Oedionychina. Especially the latter represent one of the largest chrysomelid radiations in the Americas.

Supplementary Material

Supplementary material is available at *Insect Systematics and Diversity* online.

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Specimen Collection Statement

The authors attest that all legal and regulatory requirements, including export and import collection permits, have been followed for the collection of specimens from source populations at any international, national, regional, or other geographic level for all relevant field specimens collected as part of this study.

Author Contributions

Harald Letsch (Conceptualization [Lead], Formal analysis [Lead], Investigation [Lead], Methodology [Lead], Resources [Supporting], Validation [Lead], Visualization [Lead], Writing – original draft [Lead], Writing – review & editing [Equal]), and Franziska Beran (Conceptualization [Supporting], Formal analysis [Supporting], Funding acquisition [Equal], Investigation [Supporting], Methodology [Supporting], Resources [Lead], Validation [Supporting], Visualization [Supporting], Writing – original draft [Supporting], Writing – review & editing [Equal])

Data Availability

Data from this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.hmgqnk9nk (Letsch, 2023).

References

Ahn SJ, Betzin F, Gikonyo MW, Yang ZL, Köllner TG, Beran F. Identification and evolution of glucosinolate sulfatases in a specialist flea beetle. Sci Rep. **2019**:9(1):15725. https://doi.org/10.1038/s41598-019-51749-x

Baele G, Li WLS, Drummond AJ, Suchard MA, Lemey P. Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. Mol Biol Evol. **2012**:30(2):239–243. https://doi.org/10.1093/molbev/mss243

Beaulieu JM, O'Meara BC. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. Syst Biol. **2016**:65(4):583–601. https://doi.org/10.1093/sysbio/syw022

Begossi A, Benson WW. Host plants and defense mechanisms in Oedionychina (Alticinae). In: Jolivet P, Petitpierre E, Hsiao TH, editors. Biology of Chrysomelidae, Series Entomologica, Dordrecht (The Netherlands): Springer Netherlands; **1988**. p. 57–71.

Beran F, Pauchet Y, Kunert G, Reichelt M, Wielsch N, Vogel H, Reinecke A, Svatoš A, Mewis I, Schmid D, *et al*. *Phyllotreta striolata* flea beetles use host plant defense compounds to create their own glucosinolate-myrosinase system. Proc Natl Acad Sci USA. **2014**:111(20):7349–7354. https://doi.org/10.1073/pnas.1321781111

Beran F, Petschenka G. Sequestration of plant defense compounds by insects: from mechanisms to insect–plant coevolution. Annu Rev Entomol. **2022**:67:163–180. https://doi.org/10.1146/annurev-ento-062821-062319

Beran F, Sporer T, Paetz C, Ahn S-J, Betzin F, Kunert G, Shekhov A, Vassão DG, Bartram S, Lorenz S, *et al*. One pathway is not enough: the cabbage stem flea beetle Psylliodes Chrysocephala uses multiple strategies to overcome the Glucosinolate-Myrosinase defense in its host plants. Front Plant Sci. **2018**:9:1754. https://doi.org/10.3389/fpls.2018.01754

Bernays EA, Chapman RF. Deterrent chemicals as a basis of oligophagy in *Locusta migratoria* (L.). Ecol Entomol. **1977**:2(1):1–18. https://doi.org/10.1111/j.1365-2311.1977.tb00861.x

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. **2013**:69(2):313–319. https://doi.org/10.1016/j.ympev.2012.08.023

Blažević I, Montaut S, Burčul F, Olsen CE, Burow M, Rollin P, Agerbirk N. Glucosinolate structural diversity, identification, chemical synthesis and metabolism in plants. Phytochemistry. **2020**:169:112100. https://doi.org/10.1016/j.phytochem.2019.112100

Borowiec ML. AMAS: a fast tool for alignment manipulation and computing of summary statistics. PeerJ. **2016**:4:e1660. https://doi.org/10.7717/peerj.1660

Bouchenak-Khelladi Y, Slingsby JA, Verboom GA, Bond WJ. Diversification of C4 grasses (Poaceae) does not coincide with their ecological dominance. Am J Bot. **2014**:101(2):300–307. https://doi.org/10.3732/ajb.1300439

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N, *et al*. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. PLoS Comput Biol. **2019**:15(4):e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Boussau B, Walton Z, Delgado JA, Collantes F, Beani L, Stewart IJ, Cameron SA, Whitfield JB, Johnston JS, Holland PWH, *et al*. Strepsiptera, phylogenomics and the long branch attraction problem. PLoS One. **2014**:9(10):e107709. https://doi.org/10.1371/journal.pone.0107709

Bowers MD, Puttick GM. Response of generalist and specialist insects to qualitative allelochemical variation. J Chem Ecol. **1988**:14:319–334. https://doi.org/10.1007/BF01022549

Brinkmann H, Giezen M. van der, Zhou Y, Poncelin de RG, Philippe H. An empirical assessment of long-branch attraction artefacts in deep eukaryotic phylogenomics. Syst Biol. **2005**:54:743–757. https://doi.org/10.1080/10635150500234609

Bukejs A. A new species of the genus Crepidodera Chevrolat (Coleoptera: Chrysomelidae) from Baltic amber. Zootaxa. **2014**:3815(2):286. https://doi.org/10.11646/zootaxa.3815.2.8

Cardinal-McTeague WM, Sytsma KJ, Hall JC. Biogeography and diversification of Brassicales: a 103 million year tale. Mol Phylogenet Evol. **2016**:99:204–224. https://doi.org/10.1016/j.ympev.2016.02.021

Chernomor O, von Haeseler A, Minh BQ. Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol. **2016**:65(6):997–1008. https://doi.org/10.1093/sysbio/syw037

Clark SM, LeDoux DG, Seeno TN, Riley EG, Gilbert AJ, Sullivan JM. Host plants of leaf beetle species occurring in the United States and Canada. Special Publication No. 2. Sacramento (CA): The Coleopterists Society; **2004**. p. 476.

Couvreur TL, Franzke A, Al-Shehbaz IA, Bakker FT, Koch MA, Mummenhoff K. Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). Mol Biol Evol. **2009**:27:55–71. https://doi.org/10.1093/molbev/msp202

Cox ML. Atlas of the seed and leaf beetles of Britain and Ireland (Coleoptera, Bruchidae, Chrysomelidae, Megalopodidae and Orsodacnidae). Newbury, England: Pisces Publications; **2007**.

Dobler S. Evolutionary aspects of defense by recycled plant compounds in herbivorous insects. Basic Appl Ecol. **2001**:2(1):15–26. https://doi.org/10.1078/1439-1791-00032

Dobler S, Haberer W, Witte L, Hartmann T. Selective sequestration of pyrrolizidine alkaloids from diverse host plants by *Longitarsus* flea beetles. J Chem Ecol. **2000**:26:1281–1298. https://doi.org/10.1023/A:1005444313447

Dobler S, Petschenka G, Pankoke H. Coping with toxic plant compounds–the insect's perspective on iridoid glycosides and cardenolides. Phytochemistry. **2011**:72(13):1593–1604. https://doi.org/10.1016/j.phytochem.2011.04.015

Donath A, Jühling F, Al-Arab M, Bernhart SH, Reinhardt F, Stadler PF, Middendorf M, Bernt M. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. Nucleic Acids Res. **2019**:47(20):10543–10552. https://doi.org/10.1093/nar/gkz833

Douglas HB, Konstantinov AS, Brunke AJ, Moseyko AG, Chapados JT, Eyres J, Richter R, Savard K, Sears E, Prathapan KD, *et al*. Phylogeny of the flea beetles (Galerucinae: Alticini) and the position of Aulacothorax elucidated through anchored phylogenomics (Coleoptera: Chrysomelidae: Alticini). Syst Entomol. **2023**:48(3):361–386. https://doi.org/10.1111/syen.12582

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. Relaxed Phylogenetics and dating with confidence. PLoS Biol. **2006**:4(5):e88. https://doi.org/10.1371/journal.pbio.0040088

Drummond AJ, Suchard MA. Bayesian random local clocks, or one rate to rule them all. BMC Biol. **2010**:8:114. https://doi.org/10.1186/1741-7007-8-114

Duckett CN, Casari SA. First descriptions of larval stages of *Walterianella bucki* Bechyné (Coleoptera: Chrysomelidae: Alticini) and notes on life history. Coleopt Bull. **2002**:56(2):170–181. https://doi.org/10.1649/0010-065x(2002)056[0170:fdolso]2.0.co;2

Edger PP, Heidel-Fischer HM, Bekaert M, Rota J, Glöckner G, Platts AE, Heckel DG, Der JP, Wafula EK, Tang M, *et al*. The butterfly plant arms-race escalated by gene and genome duplications. Proc Natl Acad Sci USA. **2015**:112(27):8362–8366. https://doi.org/10.1073/pnas.1503926112

Ehrlich PR, Raven PH. Butterflies and plants: a study in coevolution. Evolution. **1964**:18(4):586–608. https://doi.org/10.2307/2406212

Felsenstein J. Cases in which parsimony or compatibility methods will be positively misleading. Syst Zool. **1978**:27(4):401–410. https://doi.org/10.1093/sysbio/27.4.401

FitzJohn RG. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol Evol. **2012**:3(6):1084–1092. https://doi.org/10.1111/j.2041-210x.2012.00234.x

FitzJohn RG, Maddison WP, Otto SP. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Syst Biol. **2009**:58(6):595–611. https://doi.org/10.1093/sysbio/syp067

Furth DG. The jumping apparatus of flea beetles (Alticinae) — the metafemoral spring. In: Jolivet P, Petitpierre E, Hsiao TH, editors. Biology of Chrysomelidae, Series Entomologica. Dordrecht (The Netherlands): Springer Netherlands; **1988**. p. 285–297.

Furth DG, Suzuki K. The independent evolution of the metafemoral spring in Coleoptera. Syst Entomol. **1992**:17(4):341–349. https://doi.org/10.1111/j.1365-3113.1992.tb00555.x

Furth DG, Suzuki K. Character correlation studies of problematic genera of Alticinae in relation to Galerucinae (Coleoptera: Chrysomelidae). In: Proceedings of the third international symposium on the Chrysomelidae. Beijing (China): Citeseer; **1994**. p. 116–135.

Ge D, Chesters D, Gómez-Zurita J, Zhang L, Yang X, Vogler AP. Anti-predator defence drives parallel morphological evolution in flea beetles. Proc R Soc B Biol Sci. **2011**:278(1715):2133–2141. https://doi.org/10.1098/rspb.2010.1500

Ge DY, Gomez-Zurita J, Chesters D, Yang XK, Vogler AP. Suprageneric systematics of flea beetles (Chrysomelidae: Alticinae) inferred from multilocus sequence data. Mol Phylogenet Evol. **2012**:62:793–805. https://doi.org/10.1016/j.ympev.2011.11.028

Gikonyo MW, Biondi M, Beran F. Adaptation of flea beetles to Brassicaceae: host plant associations and geographic distribution of Psylliodes Latreille and Phyllotreta Chevrolat (Coleoptera, Chrysomelidae. Zookeys. **2019**:856:51–73. https://doi.org/10.3897/zookeys.856.33724

Gomez-Rodriguez C, Crampton-Platt A, Timmermans MJTN, Baselga A, Vogler AP. Validating the power of mitochondrial metagenomics for community ecology and phylogenetics of complex assemblages. Methods Ecol Evol. **2015**:6:883–894. https://doi.org/10.1111/2041-210X.12376

Gómez-Zurita J, Hunt T, Kopliku F, Vogler AP. Recalibrated tree of leaf beetles (Chrysomelidae) indicates independent diversification of angiosperms and their insect herbivores. PLoS One. **2007**:2(4):e360. https://doi.org/10.1371/journal.pone.0000360

Hlaka V, Biondi M, Allsopp E, van Asch B. *Argopistes sexvittatus* and *Argopistes capensis* (Chrysomelidae: Alticini): Mitogenomics and phylogeny of two flea beetles affecting olive trees. Genes. **2022**:13(12):2195. https://doi.org/10.3390/genes13122195

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: Improving the ultrafast bootstrap approximation. Mol Biol Evol. **2018**:35(2):518–522. https://doi.org/10.1093/molbev/msx281

Höhna S, Landis MJ, Heath TA, Boussau B, Lartillot N, Moore BR, Huelsenbeck JP, Ronquist F. RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. Syst Biol. **2016**:65(4):726–736. https://doi.org/10.1093/sysbio/syw021

Huang C-H, Sun R, Hu Y, Zeng L, Zhang N, Cai L, Zhang Q, Koch MA, Al-Shehbaz I, Edger PP, *et al*. Resolution of Brassicaceae phylogeny using nuclear genes uncovers nested radiations and supports convergent morphological evolution. Mol Biol Evol. **2015**:33(2):394–412. https://doi.org/10.1093/molbev/msv226

Jäckel R, Mora D, Dobler S. Evidence for selective sweeps by Wolbachia infections: phylogeny of Altica leaf beetles and their reproductive parasites. Mol Ecol. **2013**:22(16):4241–4255. https://doi.org/10.1111/mec.12389

Jacobs BF, Kingston JD, Jacobs LL. The origin of grass-dominated ecosystems. Ann Mo Bot Gard. **1999**:86(2):590–643. https://doi.org/10.2307/2666186

Janz N. Ehrlich and Raven revisited: Mechanisms underlying codiversification of plants and enemies. Annu Rev Ecol Evol Syst. **2011**:42(1):71–89. https://doi.org/10.1146/annurev-ecolsys-102710-145024

Janz N, Nylin S. The oscillation hypothesis of host-plant range and speciation. In: Tilmon KJ, editor. Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. Berkeley and Los Angeles (CA): Uiversity of California Press; **2008**. p. 203–215.

Jeschke V, Gershenzon J, Vassão DG. A mode of action of glucosinolate-derived isothiocyanates: detoxification depletes glutathione and cysteine levels with ramifications on protein metabolism in *Spodoptera littoralis*. Insect Biochem Mol Biol. **2016**:71:37–48. https://doi.org/10.1016/j.ibmb.2016.02.002

Jolivet P. Food habits and food selection of Chrysomelidae. Bionomic and evolutionary perspectives. In: Jolivet P, Petitpierre E, Hsiao TH, editors. Biology of Chrysomelidae, Series Entomologica. Dordrecht (The Netherlands): Springer Netherlands; **1988**. p. 1–24.

Jolivet P, Hawkeswood TJ. Host-plants of Chrysomelidae of the world. Leiden (The Netherlands): Blackhuys Publishers; **1995**.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. **2017**:14(6):587–589. https://doi.org/10.1038/nmeth.4285

Kass RE, Raftery AE. Bayes factors. J Am Stat Assoc. **1995**:90(430):773–795. https://doi.org/10.1080/01621459.1995.10476572

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. **2013**:30(4):772–780. https://doi.org/10.1093/molbev/mst010

Katoh K, Standley DM. MAFFT: iterative refinement and additional methods. In: Multiple sequence alignment methods. Totowa: Springer; **2014**. p. 131–146.

Konstantinov AS, Baselga A, Anderson RS, Carlton C, Gusarov VI, Ivie MA, Owens BE, Sokolov IM, Tishechkin AK. Moss-inhabiting beetles of the West Indies (Insecta: Coleoptera). J Insect Biodivers. **2023**:38(2):48–72. https://doi.org/10.12976/jib/2023.38.2.3

Konstantinov AS, Roie MV, Furth D, Clark SM, Riley EG. Flea beetles of the West Indies: subtribe Oedionychina Chapuis 1875, key to genera, new combinations, synonymy, checklist, and description of new genera and species (Coleoptera, Chrysomelidae, Galerucinae, Alticini). J Insect Biodivers. **2022**:33(1):1–56. https://doi.org/10.12976/jib/2022.33.1.1

Lartillot N, Brinkmann H, Philippe H. Suppression of long-branch attraction artefacts in the animal phylogeny using a site-heterogeneous model. BMC Evol Biol. **2007**:7(Suppl 1):S4. https://doi.org/10.1186/1471-2148-7-S1-S4

Lartillot N, Philippe H. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. Mol Biol Evol. **2004**:21(6):1095–1109. https://doi.org/10.1093/molbev/msh112

Lartillot N, Rodrigue N, Stubbs D, Richer J. PhyloBayes MPI: phylogenetic reconstruction with infinite mixtures of profiles in a parallel environment. Syst Biol. **2013**:62(4):611–615. https://doi.org/10.1093/sysbio/syt022

Laudanno G, Haegeman B, Rabosky DL, Etienne RS, Beaulieu J. Detecting lineage-specific shifts in diversification: a proper likelihood approach. Syst Biol. **2020**:70(2):389–407. https://doi.org/10.1093/sysbio/syaa048

Letsch H, Gottsberger B, Metzl C, Astrin JJ, Friedman ALL, McKenna DD, Fiedler K. Climate and host plant associations shaped the evolution of ceutorhynch weevils throughout the Cenozoic. Evolution. **2018**:72:1815–1828. https://doi.org/10.1111/evo.13520

Maddison WP, Midford PE, Otto SP. Estimating a binary character's effect on speciation and extinction. Syst Biol. **2007**:56(5):701–710. https://doi.org/10.1080/10635150701607033

McKenna DD, Sequeira AS, Marvaldi AE, Farrell BD. Temporal lags and overlap in the diversification of weevils and flowering plants. Proc Natl Acad Sci USA. **2009**:106(17):7083–7088. https://doi.org/10.1073/pnas.0810618106

Minh BQ, Nguyen MAT, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol. **2013**:30(5):1188–1195. https://doi.org/10.1093/molbev/mst024

Moore BR, Höhna S, May MR, Rannala B, Huelsenbeck JP. Critically evaluating the theory and performance of Bayesian analysis of macroevolutionary mixtures. Proc Natl Acad Sci USA. **2016**:113(34):9569–9574. https://doi.org/10.1073/pnas.1518659113

Morley RJ. Interplate dispersal paths for megathermal angiosperms. Perspect Plant Ecol Evol Syst. **2003**:6(1-2):5–20. https://doi.org/10.1078/1433-8319-00039

Moseyko AG, Kirejtshuk AG, Nel A. New genera and new species of leaf beetles (Coleoptera: Polyphaga: Chrysomelidae) from Lowermost Eocene French amber. Ann Soc Entomol Fr (NS). **2010**:46(1–2):116–123. https://doi.org/10.1080/00379271.2010.10697645

Nadein KS. Catalogue of Alticini genera of the World (Coleoptera: Chrysomelidae); **2013** [accessed 2022 July 12]?. http://www.zin.ru/Animalia/Coleoptera/eng/alticinw.htm

Nadein K, Betz O. Jumping mechanisms and performance in beetles. I. Flea beetles (Coleoptera: Chrysomelidae: Alticini). J Exp Biol. **2016**:219(13):2015–2027. https://doi.org/10.1242/jeb.140533

Nadein KS, Bezděk J. 2.7.8 Galerucinae Latreille, 1802. In: Leschen RAB, Beutel R, editors. Handbook of zoology. Coleoptera, beetles. Volume 3 morphology and systematics. Vol. 3. Berlin (Germany), Boston (MA): De Gruyter; **2014**. p. 251–259.

Narberhaus I, Theuring C, Hartmann T, Dobler S. Uptake and metabolism of pyrrolizidine alkaloids in *Longitarsus* flea beetles (Coleoptera: Chrysomelidae) adapted and non-adapted to alkaloid-containing host plants. J Comp Physiol B. **2003**:173(6):483–491. https://doi.org/10.1007/s00360-003-0356-6

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. **2015**:32(1):268–274. https://doi.org/10.1093/molbev/msu300

Nie R, Vogler AP, Yang X-K, Lin M. Higher-level phylogeny of longhorn beetles (Coleoptera: Chrysomeloidea) inferred from mitochondrial genomes. Syst Entomol. **2021**:46:56–70. https://doi.org/10.1111/syen.12447

Nie RE, Andujar C, Gomez-Rodriguez C, Bai M, Xue HJ, Tang M, Yang CT, *et al*. The phylogeny of leaf beetles (Chrysomelidae) inferred from mitochondrial genomes. Syst Entomol. **2020**:45:188–204. https://doi.org/10.1111/syen.12387

Nie RE, Breeschoten T, Timmermans MJTN, Nadein K, Xue HJ, Bai M, Huang Y, Yang X-K, Vogler AP. The phylogeny of Galerucinae (Coleoptera: Chrysomelidae) and the performance of mitochondrial genomes in phylogenetic inference compared to nuclear rRNA genes. Cladistics. **2018**:34(2):113–130. https://doi.org/10.1111/cla.12196

Pankoke H, Dobler S. Low rates of iridoid glycoside hydrolysis in two Longitarsus leaf beetles with different feeding specialization confer tolerance to iridoid glycoside containing host plants. Physiol Entomol. **2015**:40(1):18–29. https://doi.org/10.1111/phen.12085

Peña C, Wahlberg N. Prehistorical climate change increased diversification of a group of butterflies. Biol Lett. **2008**:4(3):274–278. https://doi.org/10.1098/rsbl.2008.0062

Pentzold S, Zagrobelny M, Rook F, Bak S. How insects overcome two-component plant chemical defence: plant β-glucosidases as the main target for herbivore adaptation. Biol Rev. **2014**:89(3):531–551. https://doi.org/10.1111/brv.12066

R Development Core Team. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; **2019**.

Rabosky DL. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS One. **2014**:9(2):e89543. https://doi.org/10.1371/journal.pone.0089543

Rabosky DL, Goldberg EE. Model inadequacy and mistaken inferences of trait-dependent speciation. Syst Biol. **2015**:64(2):340–355. https://doi.org/10.1093/sysbio/syu131

Rabosky DL, Grundler M, Anderson C, Shi JJ, Brown JW, Huang H, Larson JG, *et al*. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods Ecol Evol. **2014**:5:701–707.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in bayesian phylogenetics using Tracer 1.7. Syst Biol. **2018**:67:901–904.

Rheinheimer J, Hassler M. Die Blattkäfer Baden-Württembergs. Karlsruhe, Germany: Kleinsteuber Verlag; **2018**.

Ruan Y, Konstantinov AS, Shi G, Tao Y, Li Y, Johnson AJ, Luo X, Zhang X, Zhang M, Wu J, *et al*. The jumping mechanism of flea beetles (Coleoptera, Chrysomelidae, Alticini), its application to bionics and preliminary design for a robotic jumping leg. ZooKeys. **2020**:915:87–105. https://doi.org/10.3897/zookeys.915.38348

Salvi D, D'Alessandro P, Biondi M. Host plant associations in Western Palaearctic Longitarsus flea beetles (Chrysomelidae, Galerucinae, Alticini): a preliminary phylogenetic assessment. ZooKeys. **2019**:856:101–114. https://doi.org/10.3897/zookeys.856.32430

Scherer G. The origins of the Alticinae. In: Jolivet P, Petitpierre E, Hsiao TH, editors. Biology of Chrysomelidae, Series Entomologica. Dordrecht (The Netherlands): Springer Netherlands; **1988**. p. 115–130.

Schmitt M. Jumping flea beetles: structure and performance (Insecta, Chrysomelidae, Alticinae). Leiden (NL): Brill; **2004**.

Schulmeister S, Wheeler WC. Comparative and phylogenetic analysis of developmental sequences. Evol Dev. **2004**:6(1):50–57. https://doi.org/10.1111/j.1525-142x.2004.04005.x

Silvestro D, Schnitzler J, Zizka G. A Bayesian framework to estimate diversification rates and their variation through time and space. BMC Evol Biol. **2011**:11:311. https://doi.org/10.1186/1471-2148-11-311

Sporer T, Körnig J, Beran F. Ontogenetic differences in the chemical defence of flea beetles influence their predation risk. Funct Ecol. **2020**;34(7):1370–1379. https://doi.org/10.1111/1365-2435.13548

Sporer T, Körnig J, Wielsch N, Gebauer-Jung S, Reichelt M, Hupfer Y, Beran F. Hijacking the mustard-oil bomb: how a Glucosinolate-sequestering flea beetle copes with plant Myrosinases. Front Plant Sci. **2021**:12:645030. https://doi.org/10.3389/fpls.2021.645030

Struck TH, Paul C, Hill N, Hartmann S, Hösel C, Kube M, Lieb B, Meyer A, Tiedemann R, Purschke G, *et al*. Phylogenomic analyses unravel annelid evolution. Nature. **2011**:471(7336):95–98. https://doi.org/10.1038/nature09864

Suyama M, Torrents D, Bork P. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. Nucleic Acids Res. **2006**:34(Web Server issue):W609–W612. https://doi.org/10.1093/nar/gkl315

Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. **2007**:56(4):564–577. https://doi.org/10.1080/10635150701472164

Timmermans MJTN, Barton C, Haran J, Ahrens D, Culverwell CL, Ollikainen A, Dodsworth S, Foster PG, Bocak L, Vogler AP. Family-level sampling of mitochondrial genomes in Coleoptera: compositional heterogeneity and Phylogenetics. Genome Biol Evol. **2016**:8(1):161–175. https://doi.org/10.1093/gbe/evv241

Tribble CM, Freyman WA, Landis MJ, Lim JY, Barido-Sottani J, Kopperud BT, Höhna S, *et al*. RevGadgets: an R package for visualizing Bayesian phylogenetic analyses from RevBayes. Methods Ecol Evol. **2022**:13:314–323. https://doi.org/10.1111/2041-210X.13750

van Dam NM, Vuister LWM, Bergshoeff C, de Vos H, van Der Meijden E. The "Raison D'être" of pyrrolizidine alkaloids in *Cynoglossum officinale*: deterrent effects against generalist herbivores. J Chem Ecol. **1995**:21(5):507–523. https://doi.org/10.1007/bf02033698

Wang B, Ma J, McKenna DD, Yan EV, Zhang H, Jarzembowski EA. The earliest known longhorn beetle (Cerambycidae: Prioninae) and implications for the early evolution of Chrysomeloidea. J Syst Paleontol. **2014**:12(5):565–574. https://doi.org/10.1080/14772019.2013.806602

Wheat CW, Vogel H, Wittstock U, Braby MF, Underwood D, Mitchell-Olds T. The genetic basis of a plant–insect coevolutionary key innovation. Proc Natl Acad Sci USA. **2007**:104(51):20427–20431. https://doi.org/10.1073/pnas.0706229104

Whelan NV, Halanych KM. Who let the CAT out of the bag? Accurately dealing with substitutional heterogeneity in Phylogenomic analyses. Syst Biol. **2017**:66(2):232–255. https://doi.org/10.1093/sysbio/syw084

Winkler IS, Mitter C. The phylogenetic dimension of insect-plant interactions: a review of recent evidence. In: Tilmon K, editor. Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. Berkley (CA): University of California Press; **2008**.

Winkler IS, Mitter C, Scheffer SJ. Repeated climate-linked host shifts have promoted diversification in a temperate clade of leaf-mining flies. Proc Natl Acad Sci USA. **2009**:106:18103–18108. https://doi.org/10.1073/pnas.0904852106

Wolfe JA. Climatic, floristic, and vegetational changes near the Eocene/Oligocene boundary in North America. In: Prothero DR, Berggren WA, editors. Eocene-Oligocene biotic and climatic evolution. Princeton (NJ): Princeton University Press; **1992**. p. 421–436.

Xue H-J, Li W-Z, Nie R-E, Yang X-K. Recent speciation in three closely related sympatric specialists: inferences using multi-locus sequence, post-mating isolation and endosymbiont data. (R. DeSalle, ed.). PLoS One. **2011**:6(11):e27834. https://doi.org/10.1371/journal.pone.0027834

Xue H-J, Niu Y-W, Segraves KA, Nie R-E, Hao Y-J, Zhang L-L, Cheng X-C, Zhang X-W, Li W-Z, Chen R-S, *et al*. The draft genome of the specialist flea beetle *Altica viridicyanea* (Coleoptera: Chrysomelidae). BMC Genomics. **2021**:22(1):243. https://doi.org/10.1186/s12864-021-07558-6

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science. **2001**:292(5517):686–693. https://doi.org/10.1126/science.1059412

Zhang H, Song N, Yin X. Higher-level phylogeny of Chrysomelidae based on expanded sampling of mitogenomes. PLoS One. **2022**:17(1):e0258587. https://doi.org/10.1371/journal.pone.0258587