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Cryptic diversity within the *Poecilochirus carabi* mite species complex phoretic on *Nicrophorus* burying beetles: Phylogeny, biogeography, and host specificity

Julia Canitz[1,2] | Derek S. Sikes[3] | Wayne Knee[4] | Julia Baumann[5] | Petra Haftaro[1] | Nadine Steinmetz[1] | Martin Nave[1] | Anne-Katrin Eggert[6] | Wenbe Hwang[7] | Volker Nehring[1]

[1]Institute for Biology I, University of Freiburg, Freiburg, Germany

[2]Senckenberg German Entomological Institute, Müncheberg, Germany

[3]University of Alaska Museum, University of Alaska Fairbanks, Fairbanks, Alaska, USA

[4]Canadian National Collection of Insects, Arachnids, and Nematodes, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

[5]Institute of Biology, University of Graz, Graz, Austria

[6]School of Biological Sciences, Illinois State University, Normal, Illinois, USA

[7]Department of Ecology and Environmental Resources, National University of Tainan, Tainan, Taiwan

Correspondence
Volker Nehring, Institute for Biology I, University of Freiburg, Hauptstraße 1, Freiburg, Germany.
Email: volker.nehring@biologie.uni-freiburg.de

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Abstract

Coevolution is often considered a major driver of speciation, but evidence for this claim is not always found because diversity might be cryptic. When morphological divergence is low, molecular data are needed to uncover diversity. This is often the case in mites, which are known for their extensive and often cryptic diversity. We studied mites of the genus *Poecilochirus* that are phoretic on burying beetles (Silphidae: *Nicrophorus*). *Poecilochirus* taxonomy is poorly understood. Most studies on this genus focus on the evolutionary ecology of *Poecilochirus carabi sensu lato*, a complex of at least two biological species. Based on molecular data of 230 specimens from 43 locations worldwide, we identified 24 genetic clusters that may represent species. We estimate that these mites began to diversify during the Paleogene, when the clade containing *P. subterraneus* branched off and the remaining mites diverged into two further clades. One clade resembles *P. monospinosus*. The other clade contains 17 genetic clusters resembling *P. carabi s.l.*. Among these are *P. carabi sensu stricto*, *P. necrophori*, and potentially many additional cryptic species. Our analyses suggest that these clades were formed in the Miocene by large-scale geographic separation; cospeciation of mites with the host beetles can be largely ruled out. Diversification also seems to have happened on a smaller scale, potentially due to adaptation to specific hosts or local abiotic conditions, causing some clusters to specialize on certain beetle species. Our results suggest that biodiversity in this genus was generated by multiple interacting forces shaping the tangled webs of life.

KEYWORDS
coevolution, *Poecilochirus austroasiaticus*, *Poecilochirus monospinosus*, *Poecilochirus subterraneus*, speciation

1 | INTRODUCTION

Coevolution between species can speed up evolution and can contribute to biodiversity through symbiont specialization (Hoberg et al., 1997; Paterson et al., 2010; Thompson, 2009; Yoder & Nuismer, 2010). Parasitidae mites are a prime example of a taxon in which symbiont specialization has led to impressive diversity (Magalhães et al., 2007; Perotti & Braig, 2009). These mites tend to live hidden lives and their morphological adaptations can be subtle, so that the diversity is often cryptic and underestimated (García-Varela et al., 2011).

There are 20 morphologically described species of the mite genus *Poecilochirus* G. & R. Canestrini, 1882 (Mesostigmata: Parasitidae). The deutonymphs can be distinguished morphologically (e.g., based on body size, patterning of the sternal shield, length of the opisthosomal l1 setae, shape of coxal setae; Baker & Schwarz, 1997; Hyatt, 1980; Perotti & Braig, 2009; Ramaraju & Madanlar, 1998; Wise et al., 1988). However, morphological differences between species are often small, thus the diversity of the genus remains unclear (Mašán, 1999).

The best-studied species is *Poecilochirus carabi*. This species' final juvenile stage (deutonymph) is phoretic on adult burying beetles (Silphidae: *Nicrophorus*) for dispersal (Schwarz & Koulianos, 1998). The mites attach between the beetles' legs and are carried to the beetles' brood chamber, where mites can feed on carrion, microorganisms, fly eggs and larvae, and sometimes beetle eggs and larvae (Brown & Wilson, 1992; De Gasperin & Kilner, 2015; Schwarz & Müller, 1992; Springett, 1968). The deutonymphs develop into adults and reproduce (Schwarz & Koulianos, 1998), and the mite offspring leave the brood chamber with the beetles.

There is no evidence that the mites affect host beetle fitness during the phoretic dispersal. However, some observations suggest that mites may affect the beetles' reproductive fitness. Depending on the environmental conditions, the mites can either directly reduce beetle brood weight and offspring number, for example, by predating on beetle eggs and larvae (De Gasperin & Kilner, 2015; Nehring et al., 2019; Schedwill et al., 2020), or have positive effects on beetle fitness by helping to fend off other competitors such as blowflies, nematodes, or other beetles (Springett, 1968; Sun et al., 2019; Wilson & Knollenberg, 1987). In any case, the two symbionts have probably coevolved.

The genus *Nicrophorus* consists of more than 60 species (Sikes et al., 2002, 2016). Burying beetles originated in the Cretaceous (99–127 Ma) in Eurasia, colonized the Western hemisphere, and have probably migrated back to Eurasia more than once (Hatch, 1927; Peck & Anderson, 1985; Sikes & Venables, 2013). Today, only *N. vespilloides* and *N. investigator* are distributed in both the eastern and western hemispheres (Sikes et al., 2008, 2016). Often, multiple *Nicorphorus* species occur in sympatry (e.g Brown & Wilson, 1992; Dekeirsschieter et al., 2011) but vary in their habitat preferences, diel activities, and reproductive seasons (Anderson, 1982; Burke et al., 2020; Esh & Oxbrough, 2021; Majka, 2011; Scott, 1998).

It has been shown that not all burying beetle species carry identical *P. carabi* mites. In central Europe, for example, two reproductively isolated populations of *P. carabi* occur sympatrically and have been named *Poecilochirus carabi sensu stricto* and *Poecilochirus necrophori* (Baker & Schwarz, 1997; Hyatt, 1980; Müller & Schwarz, 1990). The mites can switch between host individuals (Schwarz & Koulianos, 1998). They can recognize their main host by olfactory cues and prefer specific *Nicrophorus* species over others (Korn, 1982; Müller & Schwarz, 1990). When the preferred host species is not available, the mites will mount other host species, but their fitness may be reduced when they reproduce along with the less favorable host (Brown & Wilson, 1994; Nehring et al., 2017). Such host switches may counteract host specialization (Thompson, 2009).

Field and laboratory studies indicate that *P. necrophori* is a host specialist primarily found on *Nicrophorus vespillo*, while *P. carabi s.s.* is prevalent on at least three different *Nicrophorus* species (*N. vespilloides*, *N. investigator*, and *N. humator*), but rarely on *N. vespillo* (Schwarz, 1996). Furthermore, two reproductively isolated populations of *P. carabi* are specialized on two sympatric North American *Nicrophorus* species (*N. tomentosus* and *N. orbicollis*) and differ in morphology (Brown, 1989; Brown & Wilson, 1992), but their relationship to the European species is unknown. These populations are considered to belong to a cryptic species complex, termed *P. carabi sensu lato* (Baker & Schwarz, 1997; Mašán, 1999).

Several molecular analyses have been conducted for *Nicrophorus* (Sikes et al., 2008, 2016; Sikes & Venables, 2013), but are missing for *Poecilochirus* species. Here, we use molecular data to understand the evolutionary history of *Poecilochirus* mites that are phoretic on *Nicrophorus* beetles, and *P. carabi s.l.* in particular. We predicted that genetic diversity within the genus *Poecilochirus* is currently underestimated, and that host specificity is also evident in the mites' genetics. We sequenced two nuclear (ITS, LSU) and one mitochondrial DNA marker (COI) of *Poecilochirus* mites collected with their *Nicrophorus* hosts on four continents. We documented the genetic diversity of the mites, reconstructed the phylogenetic relationships, and estimated evolutionary divergence times. Our analyses contribute to a better understanding of speciation in global symbiotic systems, where geographic separation and host specialization interact to isolate populations.

2 | MATERIALS AND METHODS

We collected *Poecilochirus* mites from burying beetles from North and South America, Europe, and Asia, and used morphological, molecular, and behavioural data to delimit species. We also reconstructed phylogenetic relationships, and analysed the biogeography and host specificity of the main mite clusters.

2.1 | Sampling

We focused on mites from burying beetles that morphologically resemble *P. carabi* deutonymphs in their habitus (sternal and dorsal shields, body length c. 1 mm; 218 individuals). Samples originated from 43 different locations ranging from Alaska (USA) and Ecuador, through Europe, Central Asia, Japan and Melanesia (Figure S1). Mites were collected together with their host beetles, including 31 *Nicrophorus* species and one species of carabid beetle (*Pterostichus melanarius*). Specimens were sampled from the wild between 1998 and 2020, and were preserved in 96% ethanol or propylene glycol, or kept dry (Table S1). Several specimens of a German population (Mooswald, Freiburg) could be identified as *P. necrophori* or *P. carabi s.s.*; these mites were collected with *N. vespillo* and *N. vespilloides*, respectively, and preferred their main carrier over the other beetle species in three consecutive choice tests (for details see Nehring et al., 2017).

In addition, specimens identified as *P. subterraneus* deutonymphs (n = 12) and *Macrocheles* sp. female adults (n = 2) were added to the data set as the outgroup in phylogenetic analyses (Table S1). Mite vouchers are deposited in the Sikes Research Collection at the University of Alaska Fairbanks; the Canadian National Collection of Insects, Arachnids, and Nematodes; and the Acarological Collection at the University of Graz.

2.2 | Molecular methods

We extracted DNA from 232 mites. Two different methods were used for DNA extraction. We applied either the Phenol/Chloroform method where the whole individual was ground up in liquid nitrogen, or a nondestructive approach using the DNeasy Blood and Tissue Kit (Qiagen). For the nondestructive method, we incubated the entire specimen in 50 µl lysis buffer (ATL buffer) and 10 µl Proteinase K for approximately 24 h at 56°C. After 6–8 h, an additional 5 µl Proteinase K was added. After the lysis step, we removed the specimens and followed the default instructions of the DNeasy Kit protocol and eluted the DNA in deionized distilled water. In 30 thermocycler cycles, we amplified a fragment of the cytochrome oxidase I gene (COI), the internal transcribed spacer gene (ITS), and the gene encoding the large subunit of rRNA (LSU; primer pairs in Data S1). PCR products were purified and sent to Macrogen Europe Inc. for forward and/or reverse Sanger sequencing.

2.3 | Identification of genetic clusters

The chromatograms of COI, ITS and LSU sequences of 230 *Poecilochirus* samples were quality-checked. Heterozygous sites were detected manually by peak overlaps in the chromatogram and were translated into the IUPAC nucleotide code. Sequences were aligned separately for each gene using default settings of the Geneious Prime implementation MAFFT v.7.450 (Katoh & Standley, 2013). Obvious artificial sequence insertions were removed from the alignment. Alignments were concatenated to a supermatrix with "N"s symbolizing missing data/genes. The supermatrix was used for the phylogenetic reconstruction with IQtree multicore version 1.6.12 (Nguyen et al., 2015). We used IQtree's model finder (Kalyaanamoorthy et al., 2017) to select accurately best-fitting evolutionary models for each gene of the supermatrix. The models TPM2u+F+I+G4, TIM3+F+G4, and HKY+F+G4 were chosen for the COI, ITS, and LSU gene block, respectively. The phylogenetic approach with IQtree ran with 10,000 bootstrap replicates using the ultra-fast bootstrap approximation (Minh et al., 2013) and a parametric approximate likelihood-ratio test (SH-aLTR; Anisimova et al., 2011) for branch support. We set *P. subterraneus* as the outgroup. The phylogenetic tree was visualized with FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited using Inkscape version 1.0.1 (https://inkscape.org).

To obtain the most likely number of species, we applied the Poisson tree process (PTP) model implemented in the multirate (m) PTP tool version 0.2.4 (Kapli et al., 2017). The model is suitable for single gene alignments, therefore the mPTP analysis ran with the alignment and phylogeny received by COI genes only. The mPTP analysis was conducted with the following parameters: --mcmc 100,000,000; --mcmc_sample 10,000; --mcmc_burnin 500,000; --mcmc_runs 4; --mcmc_startrandom.

To support cluster delineation, mean uncorrected p-distances within and between clusters were calculated and were also based on COI sequences using MEGA version 10.1.7 (Kumar et al., 2018). Finally, we used the R packages maptools v1.0–2 and scatterpie v0.1.5 (R Core Team, 2020) to visualize the geographic distribution and relative abundance of phylogenetic clusters at each sample locality.

2.4 | Morphological identification

Mite specimens that were not destroyed during the DNA extraction (n = 95) were mounted and clarified in Heinze-PVA medium and stored in an oven at 50°C until total clarification. Morphological and morphometric analyses of mites were performed using differential interference contrast in a compound microscope (Reichert Diavar, Vienna). Identification of mites was based on the key by Hyatt (1980) and the description of *P. monospinosus* by Wise et al. (1988).

2.5 | Host specificity

Host specificity was calculated by the Shannon-Wiener Diversity index (H') and Evenness using LibreOffice version 6.2.8.2. We performed these calculations for three European and three North American clusters that contained enough samples and host species. In addition, we used a χ^2-test to investigate whether the frequency of host species occupied by a mite cluster deviates from the overall host species frequency in the same geographical area (R Stats

package v3.6.2). The host species frequency was derived from the number of mites that we sequenced from each beetle species; whenever possible, we had selected mites from different beetle individuals. Subsequently, the χ^2 value of each cluster ($\chi^2_{(cluster)}$) was set in relation to the theoretical χ^2 maximum of the respective cluster ($\chi^2_{(max)}$). A high quotient of $\chi^2_{(cluster)}/\chi^2_{(max)}$ suggests host specificity of the mite clusters for that area.

2.6 | Phylogenetic inference

We used the 38 samples for which all three genes were sequenced for phylogenetic analyses. These samples covered 16 of the previously identified clusters. We applied Maximum Likelihood (ML) and Bayesian Inference (BI) approaches and used four different methods for assigning branch support values - parametric approximate likelihood-ratio test (SH-aLRT), ultrafast bootstrapping (UFBoot), standard bootstrapping (SBS), and posterior probability (PP). Phylogenetic analyses were carried out with IQTree (ML; aLRT/UFBoot), RaxML version 8.2.4 (ML; SBS; Stamatakis, 2014), and MrBayes 3.2.7a (BI; PP; Ronquist et al., 2012). The input for all analyses is a concatenated alignment of the three genes generated with Geneious Prime 2020.1. All analyses were conducted with the data partitioned by gene and *P. subterraneus* specimens were used as the outgroup. Best-fitting substitution models were found using IQtree. Models were adjusted to the most similar substitution model RaxML and MrBayes can run with. For the IQtree analysis the same parameter settings were used as described above. For the RaxML analysis, we chose the rapid bootstrapping algorithm (-f a; -# 10,000; -T 20). For our Bayesian approach, we started MrBayes with default prior parameters and 1,000,000 generations. Afterwards, parameter values were checked for reliability with Tracer v. 1.7.1 (Rambaut et al., 2018). Trees were summarized with a burn-in of 10%. Phylogenies were plotted with FigTree and illustrated with InkScape.

2.7 | Divergence time analysis

For divergence time analysis, we combined the data of 25 *Poecilochirus* specimens with additional Mesostigmata taxa (including our own *Macrocheles* sequences). *Poecilochirus* samples were chosen by the availability and quality of COI and LSU sequence and covered 12 of the genetic clusters. The complete data set consists of 40 individuals, of which 26 represent the hyporder Parasitiae (one family), 13 the hyporder Dermanyssiae (10 families), and one the infraorder Uropodina (two families), which serves as the outgroup (Table S2). Certain taxa were represented by chimeric sequences, meaning that the COI and LSU sequences did not originate from the same individual but from the same genus or family (Table S2).

The analysis was conducted with Beast v. 2.6.3 (Bouckaert et al., 2019) which includes the Fossilized-Birth-Death Process model (Stadler, 2010; Stadler et al., 2018). Besides the assumption that every living lineage can experience speciation at rate λ or go extinct at rate μ, the Fossilized-Birth-Death Process model allows the treatment of known fossil calibration points as part of the tree prior at node times. We ran Beast2 under this model using fossil data available for five taxa (Table S3). Monophyly was fixed for samples of the families Parasitidae, Macrochelidae and Digamasellidae, as well as for the infraorder Gamasina, and the superfamilies Dermanyssoidae and Eviphidoidae. Our analysis is based on the COI and LSU genes of which each represents a separate partition. We set the substitution model to be unlinked and determined the GTR and TN93 as best-fitting models for the COI and LSU partition, respectively. The Clock and Tree model were set to be linked and the analysis ran with the Relaxed Clock Log Normal model. We set the five fossil calibration points to the clade nodes where the fossils are assumed to belong, and ran Beast2 with 1,000,000 generations. Stationarity was reached when all ESS values were above 200 and data were equally distributed in Tracer v. 1.7.1. The final divergence time phylogeny was assembled with TreeAnnotator v2.6.3 (included in Beast2 package). Results were plotted using FigTree and edited with InkScape.

2.8 | Biogeography and ancestral-area estimation

The divergence time reconstruction was the basis for a biogeographical analysis. We used the R package BioGeoBears (Matzke, 2014) which performs inferences of biogeographic histories on phylogenies. With BioGeoBears, different models of how biogeography may evolve on phylogenies can be tested on a given dated tree. Currently the package includes the dispersal-extinction-cladogenesis model (DEC), a likelihood version of the dispersal-vicariance model (DIVALIKE) and the Bayesian analysis of biogeography (BAYAREALIKE). Moreover, it provides an extended version for the models by the consideration of additional free parameters like 'j' ("jump dispersal") or 'x' (geographical distances) while modelling. The "jump dispersal" parameter simulates the founder-event speciation. It describes that at the time of cladogenesis one daughter lineage inherits the ancestral range, and the other lineage occupies a new area through a rare, long-distance colonization event, and founds an instantly genetically isolated population (Matzke, 2014; Zhang et al., 2017). Since the biogeography of *Poecilochirus* is our focus, we pruned the dated tree to a subset containing only *Poecilochirus* specimens (excl. *P. subterraneus*) for the BioGeoBears analysis. We divided the Northern Hemisphere into six areas: Western North America (W), Eastern North America (N), Europe (E), Northern Asia + Japan (A), Southern Asia (S), and South East Asia ranging to the Solomon Islands (I). In an initial analysis, we tested whether the existence of only the Bering Land Bridge or both the North Atlantic Land Bridge and the Bering Land Bridge might fit the data better. Three model types were tested in three different versions for each scenario (M0 = DEC, DIVALIKE, BAYAREALIKE; M1=DEC+J, DIVALIKE+J, BAYAREA+J, and M2 = DEC+J+X, DIVALIKE+J+X, BAYAREA+J+X), and the likelihood and Akaike Information Criterion with sample correction

(AICc) were compared between both scenarios. We continued with the scenario showing the lowest negative log likelihood value -lnL and lowest AICc values in most of the models and compared nested models using a likelihood-ratio test (same model type: M0 vs. M1 and M1 vs. M2). The AICc was used to compare among the model types.

A more likely scenario is obtained by running the biogeographical models under a time-stratified analysis. In such an analysis, BioGeoBears can take into account geographical changes and different difficulty levels for dispersal occurring over time. Our time-stratified analysis included three time slices. We tried to represent the geographic conditions of the Eocene/Oligocene, the Miocene, and present conditions. For this scenario we ran the models DEC/DEC+J and DIVALIKE/DIVALIKE+J.

3 | RESULTS

3.1 | Sequence data

We obtained 429 high-quality DNA sequences. Of these, 193 COI, 136 ITS, and 79 LSU sequences belonged to mites that resemble *P. carabi*, ten COI, six ITS, and three LSU sequences belonged to *P. subterraneus*, and one COI and one LSU sequence were generated from two *Macrocheles* specimens (Table S1). These sequence data have been submitted to GenBank under the accession numbers MW890765–MW890966 (COI), MW893012–MW893060 and MW893063–MW893153 (ITS), and MW893154–MW893193 and MW893196–MW893239 (LSU). The average length of the COI, ITS and LSU sequences were 655, 509 and 645 bp, respectively.

3.2 | Identification of genetic clusters

We identified 24 different genetic clusters by the IQtree approach that was based on a concatenated supermatrix of the COI, ITS, and LSU genes obtained from 230 *Poecilochirus* mites (Table 1, Figure S2). Of these, three clusters belong to the outgroup *P. subterraneus*. The largest cluster in the ingroup consisted of 89 samples (Europe-1), and seven clusters were represented by only one mite individual (singletons). Depending on the cluster, the number of different host species ranged from 1 to 8, and the number of sampling locations varied from 1 to 13 (Table 1). We named the clusters according to their main distribution areas. Except for Asia-2, all identified clusters with multiple individuals were supported by high branch support values (SH-aLRT >80%; UFBoot >95%) but several relationships among clusters were weakly supported (e.g., Asia-1 and Asia-2/Bali singleton). Especially at deeper phylogenetic splits, support values were low, indicating a more fragile tree topology (Figure S2).

The mPTP analysis covered 16 clusters and five singletons. Its results revealed that most of these clusters can be delineated as species on the molecular level of the COI gene. Four independent MCMC runs yielded the highest frequencies for species numbers between 19 and 21, with the highest likelihood score for a multi-coalescent rate (-lnL = 886.071) calculated for 20 species (including two *P. subterraneus* clusters, Figure S3).

The mean uncorrected p-distance of the COI gene within clusters was 0.78% ranging from 0.1% (Asia-1) to 1.9% (Asia-3). Among clusters, the overall mean p-distance was 15.48% with a range between 6.03% (Asia-1 vs. Bali singleton) and 21.06% (*P. subterraneus* [Germany] vs. USA-2). The mean p-distance between the known species *P. carabi s.s.* and *P. necrophori* (Europe-1 vs. Europe-2) was 10.21%, and that between *P. carabi*/*P. necrophori* and *P. monospinosus* (Europe1/Europe2 vs. USA-2/USA-3) was on average 19.46% (Table S4).

The mite clusters were each restricted to one of three major geographical regions (the European, Asian, and American continent). In North America, five different clusters occurred. While the North American cluster was distributed from Alaska/Canada over the Western to the Eastern USA, the USA-1 cluster was only found in the North-Eastern part of the USA (Illinois, Ohio, and Connecticut). The USA-2 and USA-3 clusters occurred only in Illinois and Ohio, and in Florida (South-Eastern USA), respectively. The Canada cluster appeared in Calgary/Alberta. In South America the Ecuador cluster was present (Figure 1, Table S1). The clusters Europe-1, Europe-2, and Europe-3 were distributed across Europe, although the Europe-2 cluster also contained a sample from Kazakhstan. Samples of the Eurasia cluster occurred in Latvia and Japan. All singletons and three additional genetic clusters (Asia-1, Asia-2 and Asia-3) were distributed across the Asian continent. On the Japanese Islands, we identified a distinct Japan cluster in addition to the Eurasia cluster (Figure 1, Table S1).

3.3 | Morphological identification

We morphologically identified 95 *Poecilochirus* specimens that resembled *P. carabi*, covering 19 different genetic clusters. Of these, 90 specimens from 16 genetic clusters match the *P. carabi* description of Hyatt (1980). The specimens from the Japan cluster differed slightly from Hyatt's description of *P. carabi* by a weakly sclerotized body and long podosomal and opisthosomal shields (Tables S1 and S5).

The single intact specimen of the USA-2 cluster (sample ID: oh-pus2) corresponded to *Poecilochirus monospinosus* Wise et al. (1988). All individuals of the USA-3 cluster resembled *P. monospinosus* as well, but differed in the setal pattern. The Sichuan singleton (CH-N.con) morphologically resembled *Poecilochirus austroasiaticus* Vitzhum 1930, but it was larger than reported by Hyatt (1980). These morphological results prompted our definition of *P. carabi s.l.*, which hereafter includes all genetic clusters except USA-2, USA-3, the Sichuan singleton, and the three *P. subterraneus* clusters.

TABLE 1 List of genetic clusters of *Poecilochirus*

	No. of mites	Host species (no. mites found on each host species)	Country of origin (no. of different sampling locations)
Clusters			
Asia−1	6	*N. nepalensis* (6)	Taiwan (1)
Asia−2	11	*N. concolor* (1), *N. melissae* (1), *N. nepalensis* (2), *N. schawalleri* (2), *N. sinensis* (2), *N. smefarka* (2), *N. vespilloides* (1)	China (1), Russia (1), Taiwan (1), India (1)
Asia−3	4	*N. melissae* (1), *N. nepalensis* (3)	India (1), China (1)
Canada	2	*N. hybridus* (2)	Canada (1)
Ecuador	2	*N. didymus* (2)	Ecuador (1)
Eurasia	6	*N. investigator* (2), *N. vespillo* (1), *N. vespilloides* (1)	Japan (1), Latvia (1)
Europe−1	89	*N. humator* (12), *N. interruptus* (6), *N. investigator* (6), *N. vespillo* (2), *N. vespilloides* (62), *Pterostichus melanarius* (1)	Germany (7), England (1), Austria (1), France (1), Scotland (1), The Netherlands (1), Poland (1)
Europe−2	27	*N. humator* (1), *N. interruptus* (6), *N. lunatus* (1), *N. vespillo* (16), *N. vespilloides* (3)	Germany (3), Poland (1), Kazakhstan (1), France (1), Latvia (1), The Netherlands (1), Austria (1)
Europe−3	10	*N. antennatus* (2), *N. germanicus* (2), *N. humator* (3), *N. interruptus* (2), *N. vespilloides* (1)	Czech Rep (1), France (1), Austria (1), England (1)
Japan	3	*N. quadripunctatus* (3)	Japan (1)
North America	31	*N. defodiens* (8), *N. hebes* (2), *N. investigator* (6), *N. nigrita* (2), *N. orbicollis* (4), *N. sayi* (3), *N. tomentosus* (4), *N. vespilloides* (2)	USA (6), Canada (2)
USA−1	13	*N. orbicollis* (7), *N. pustulatus* (1), *N. tomentosus* (5)	USA (3)
USA−2	6	*N. pustulatus* (6)	USA (2)
USA−3	5	*N. carolina* (5)	USA (1)
Singletons			
Philippines (PH-N.apo)	1	*N. apo*	Philippines
Bali (IND-N.ins)	1	*N. insularis*	Indonesia (Bali)
Solomon (SI-N.kie)	1	*N. kieictus*	Solomon Islands
Sulawesi (IDN-N.cha)	1	*N. charon*	Indonesia (Sulawesi)
Liaoning (CH-N.jap)	1	*N. japonicus*	China (Liaoning)
Russia (RUS-N.mor)	1	*N. morio*	Russia
Sichuan (CH-N.con)	1	*N. concolor*	China (Sichuan)
Outgroup *P. subterraneus*			
Psub-NA	5	*N. sayi* (5)	Canada (1); USA (1)
Psub-GER1	5	*N. humator* (5)	Germany (1)
Psub-GER2	2	*N. humator* (2)	Germany (1)

The number of mite individuals, the host species with the number of mite individuals sequenced from each host species, and the country of occurrence with the number of its different localities are listed for each cluster.

3.4 | Host specificity

We focused on six clusters that contained more than six mite specimens each and that were found in more than one location - three European and three North American clusters. Among the European clusters, Europe-1 and Europe-3 were each associated with five, and Europe-2 with four *Nicrophorus* species (Table 2; Figure 2).

Both Shannon-Wiener Diversity Index and Evenness were highest for the cluster Europe-1 and lowest for Europe-3 (Table 2). The clusters USA-2, USA-1 and North America were found on one, three and eight *Nicrophorus* species, respectively (Table 2; Figure 2). The χ^2 ratio ranged from 0.003 to 0.082 in Europe and from 0.014 to 0.256 in North America (Table 2). The higher the quotient, the more the samples from a cluster were concentrated on specific host species.

3.5 | Phylogenetic inference

The tree topologies inferred by the Maximum Likelihood and Bayesian analyses were consistent. The phylogeny comprised 37 mite individuals covering 16 genetic clusters. Two *P. subterraneus*

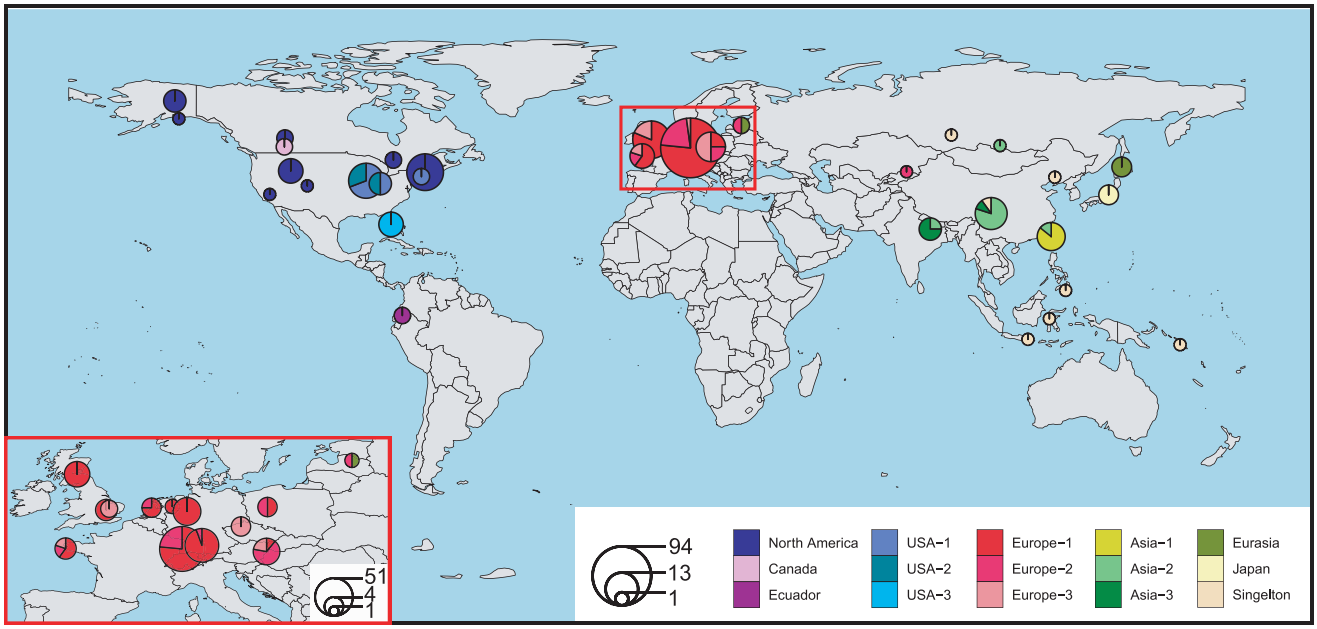

FIGURE 1 Distribution of the *Poecilochirus* clusters excluding *P. subterraneus*. Pie charts show the relative proportion of the different clusters at each location. Each cluster is represented by another colour (except singletons) and pie size reflects the sample size at each location. The European distribution is enlarged in the left bottom corner (red rectangle)

TABLE 2 Host specificity indices for three European (n = 124) and three North American (n = 50) clusters. The number of mite specimens found on each host, the Shannon–Wiener Diversity Index (H'), Evenness, χ^2 value and χ^2 ratio are listed for each cluster

	Host species[a]							H'	Evenness	$\chi^2_{(cluster)}$	$\chi^2_{(cluster)}/\chi^2_{(max)}$
Cluster	N. vs	N. vo	N. hum	N. int	N. inv	N. ant	N. ger				
Europe-1	62	2	12	6	6	0	0	0.93	0.479	19.1*	0.003
Europe-2	3	16	1	6	0	0	0	0.54	0.277	55.0*	0.035
Europe-3	1	0	3	2	0	2	2	0.15	0.076	50.3*	0.082
	N. def	N. heb	N. inv	N. nig	N. orb	N. pus	N. say	N. tom	N. vs			
North America	7	2	6	2	5	0	3	4	2	1.52	0.690	10.7	0.014
USA-1	0	0	0	0	7	1	0	5	0	0.58	0.266	13.9	0.045
USA-2 (*P. monospinosus*)	0	0	0	0	0	6	0	0	0	0.25	0.116	36.9*	0.256

[a]N. vs = N. vespilloides, N. vo = N. vespillo, N. hum = N. humator, N. int = N. interruptus, N. inv = N. investigator, N. ant = N. antennatus, N. ger = N. germanicus, N. def = N. defodiens, N. heb = N. hebes, N. nig = N. nigrita, N. orb = N. orbicollis, N. pus = N. pustulatus, N. say = N. sayi, N. tom = N. tomentosus.

*Significant: $\chi^2_{(cluster)}$ > critical value of 12.59 (df = 6; α = 0.05) for the European clusters; $\chi^2_{(cluster)}$ > critical value of 15.51 (df = 8; α = 0.05) for the North American clusters.

samples served as the outgroup (Figure 3). Monophyly of the previously defined genetic clusters was confirmed by the four different support values (SH-aLRT, UFBoot; SBS, and PP). The topology depicted a basal separation into two clades (PP = 1; aLRT/UFBoot/SBS = 100). One clade consisted of mites identified probably as *P. monospinosus* (USA-2 and USA-3), while the other included all clusters of *P. carabi s.l.*. Within this *P. carabi s.l.* clade, the most recent common ancestor of the USA-1, Canada, and Ecuador clusters split off first, but the close relationship between the Canada and Ecuador cluster showed lower branch support (aLRT = 35.4; UFBoot = 79; SBS = 61; PP = 0.79). Subsequently, the Asia-3 and then the Japan cluster branched off (PP = 1; aLRT/UFBoot/SBS>98). The remaining clusters diverged into two clades with a weak support (aLRT = 77.6; UFBoot = 81; SBS = 57; PP = 0.87). The close relationship between the North America/Eurasia and the Asian cluster had consistently high support through all but one branch value (aLRT = 93.8; UFBoot = 90; SBS = 67; PP = 0.99). In contrast, values within the clade including the European clusters and the Liaoning and Russia singletons (CH-N.jap/RUS-N.mor) were low and varied among analyses (Figure 3).

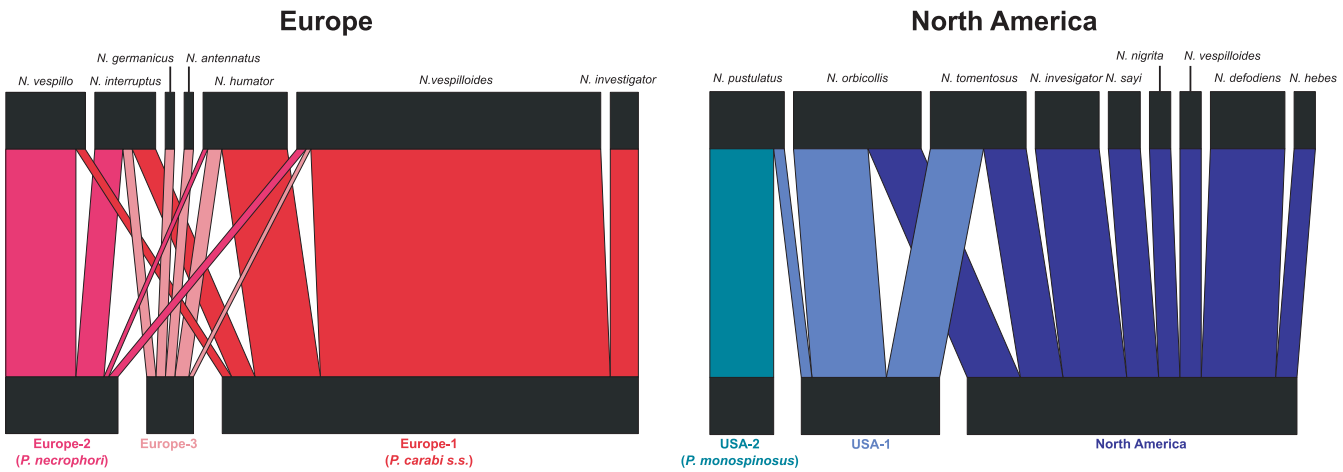

FIGURE 2 Association network between host species and the six genetic clusters tested for host specificity. The map illustrates the weighted association between mite clusters and *Nicrophorus* species for the clusters Europe-1, Europe-2 and Europe-3, as well as the clusters USA-1, USA-2 and North America. The thicker the bars, the more mite individuals are associated with the respective host species

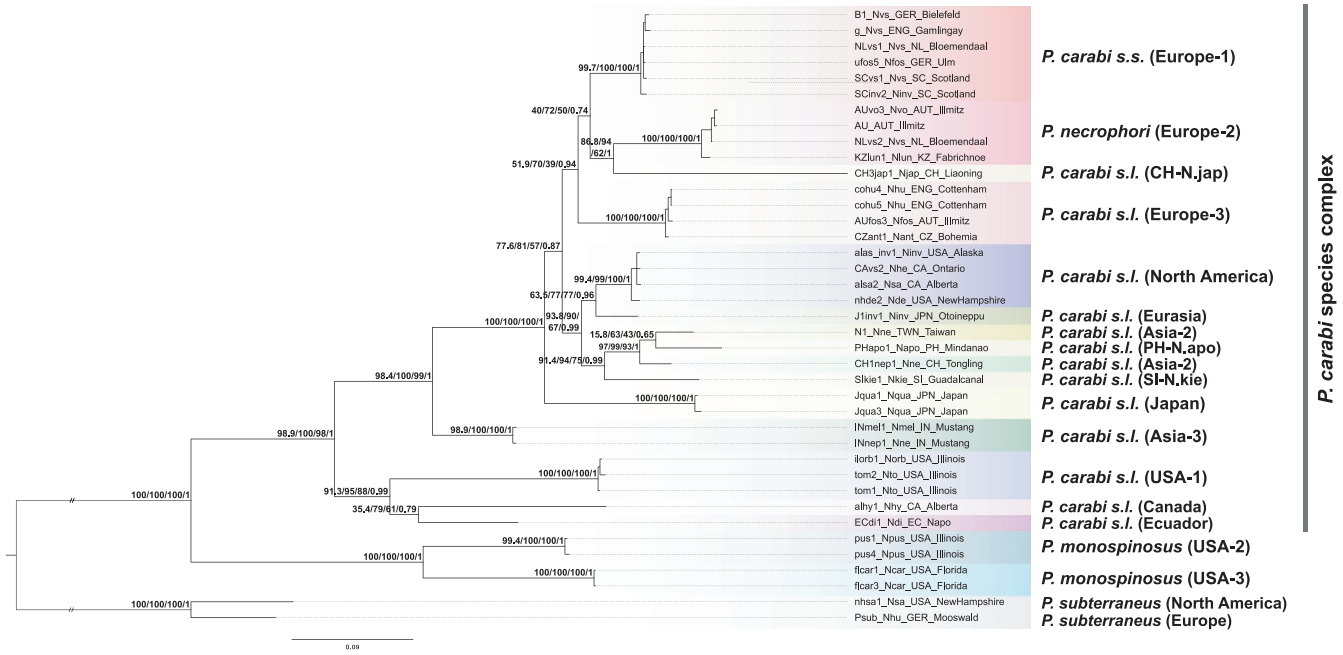

FIGURE 3 The phylogeny of *Poecilochirus carabi s.l.* as inferred by MrBayes. Branch labels represent the branch support values obtained by the likelihood ratio test/ultrafast bootstrapping/standard bootstrapping/posterior probability. Genetic clusters are indicated by colours. Basal branches are trimmed and the scale indicates the estimated substitutions per site. Species names are those of the best-fitting species description

3.6 | Divergence time analysis

The divergence time analysis included 40 specimens covering 10 genetic clusters of *P. carabi s.l.*, 2 clusters from each *P. monospinosus* and *P. subterraneus*, respectively, and 15 additional Mesostigmata taxa. The phylogenetic tree generated by Beast2 had high support values at all but two branches (split between Phytoseiidae and Podocinidae: PP = 0.79, and split among Asia-2 samples: PP = 0.81) (Figure S4). The relaxed-clock model suggested an origin of the Mesostigmata and the divergence into Parasitiae and Dermanyssiae in the Late Jurassic and Cretaceous, respectively (Figure S4). The first diversification within Parasitiae occurred in the early Eocene (~26–90 Ma). The segregation of *P. subterraneus* was suggested to occur in the mid Eocene around 44 Ma (95% CI: 23–74 Ma). The *P. monospinosus* clade branched off during the transition from the Eocene to the Oligocene at 34.7 Ma (95% CI: 18–59 Ma). Diversification of the *P. carabi s.l.* clade started in the Oligocene with the separation of USA-1 (29.5 Ma; 95% CI: 15–50 Ma). All remaining divergence events occurred during the late Oligocene/Miocene (~5–25 Ma; Figure S4).

3.7 | Biogeography and ancestral-area estimation

Our data best fit the assumption that mites dispersed via both the North Atlantic and Bering Land Bridge. This scenario received higher log-likelihood and lower AICc values in six out of nine models than the scenario considering only the Bering Land Bridge (Table S6). As the BAYAREALIKE model type yielded the lowest percentage of weighted AICc in both scenarios (<4%; Table S6), we excluded this model type from further analyses.

Within the scenario that considers both land bridges, *p*-values of the likelihood ratio tests were significant when comparing M0 and M1 (DEC: $p = .04$; DIVALIKE: $p = .04$), but were nonsignificant for the M1 and M2 comparison (DEC: $p = .06$; DIVALIKE: $p = .08$). Hence, the more complex M2 model was rejected for both model types. Regarding the weighted AICc values, DEC+J and DIVALIKE+J yielded the highest percentage with 39% and 45%, respectively (Table S7).

In the time-stratified scenario, the comparison of nested models resulted in an acceptance of the M1 model in all cases ($p_{(LRT)} < 0.05$). The AICc and weighted AICc values were lowest and highest, respectively, for the DIVALIKE+J model (Table 3). A dispersal rate of $d < 0.001$, an extinction rate of $e = 0.55$, and a relative per-event weight of founder-event speciation of $j = 1.28$ was estimated. The most likely ancestral distribution areas of the time-stratified DIVALIKE+J model are visualized in Figure 4.

The distribution of the last common ancestor of *P. carabi s.l.* and *P. monospinosus* was estimated to range from Eurasia to Eastern North America (EAN). Vicariance was inferred in the branching off of the common ancestor of the USA-2 and the USA-3 cluster (EAN → EA+N), and of the ancestor of the Japan cluster (EA → E+A). Six long-distance dispersals with founder-event speciation were suggested to explain the origin of both the USA-1 and the Asia-3 cluster (EA → EA+N; EA → EA+S), the divergence of the Europe-1/Europe-2 clusters (E → E+A) and the North America/Eurasia clusters (WN → WN+A), and two cladogenesis events within the Asian clade (I → I+S; S → S+I). However, the proportion of the most likely ancestral states deviated just slightly at several cladogenesis events (Figure S5).

4 | DISCUSSION

Our study identified 24 distinct genetic *Poecilochirus* clusters, some of which probably represent four different named species: *P. subterraneus*, *P. monospinosus*, *P. carabi s.s.*, and *P. necrophori*. The phylogenetic and species delimitation analyses indicate that many of the other genetic clusters are probably cryptic species which, to the best of our knowledge, have not yet been formally described. We cannot infer with certainty the geographical origin of *Poecilochirus* with our data set, but the mites appear to have migrated more than once between Asia, Europe and North America. We also found indication that some mite clusters are specialized on particular *Nicrophorus* species, which may have driven speciation, but this pattern appears to be largely concealed by the effects of multiple migrations between continents. It is difficult to separate these interwoven factors in the evolution of mite species, which obfuscates our understanding of the importance of coevolution with hosts and sympatric speciation in *Poecilochirus*. However, we can state with certainty that all speciation events we could infer happened more than five million years ago, with no indication of recent speciation events or ongoing segregation among extant populations.

4.1 | Cryptic diversity and host specificity of *Poecilochirus* mites

Cryptic species have been uncovered by molecular investigations across many mite groups (Beaurepaire et al., 2015; Knee, Beaulieu, Skevington, Kelso, Cognato, et al., 2012; Knee et al., 2012; Schäffer & Koblmüller, 2020). Based on our molecular analyses, we propose that *P. carabi s.l.* consists of at least 17 genetic clusters. Genetically, samples within each cluster are very similar (p-distance <2%), but clusters differ clearly and consistently from each other with a mean COI divergence of at least 6%. Given that the well-studied biological species *P. necrophori* and *P. carabi s.s.* (Baker & Schwarz, 1997; Müller & Schwarz, 1990; Nehring et al., 2017) are 10.2% divergent in their COI sequences, we suggest that most of the clusters we documented, at least those with enough samples (e.g., Europe-3 or North America), represent separate biological but not yet described species. The results of the different phylogenetic approaches, the species delimitation, and the divergence time analyses support this interpretation. To clarify the species status of the individual genetic clusters, more comprehensive analyses are needed - for example, additional behavioural and morphological tests, or a genomic approach; as well as the study of type specimens of previously named species in this complex.

TABLE 3 Statistics of the BioGeoBears analysis testing four different models in the time-stratified scenario with log likelihood, likelihood ratio test (LRT; p(LRT)), sample corrected AIC (AICc) and weighted AICc values

Model	-lnL	LRT	$p_{(LRT)}$	AICc	# param	# tips	Weighted AICc
DEC	−35.65	4.128	0.04	75.47	2	13	26%
DEC+J	−33.59			75.84	3	13	21%
DIVALIKE	−36.5	7.191	0.001	77.17	2	13	11%
DIVALIKE+J	−32.91			74.48	3	13	42%

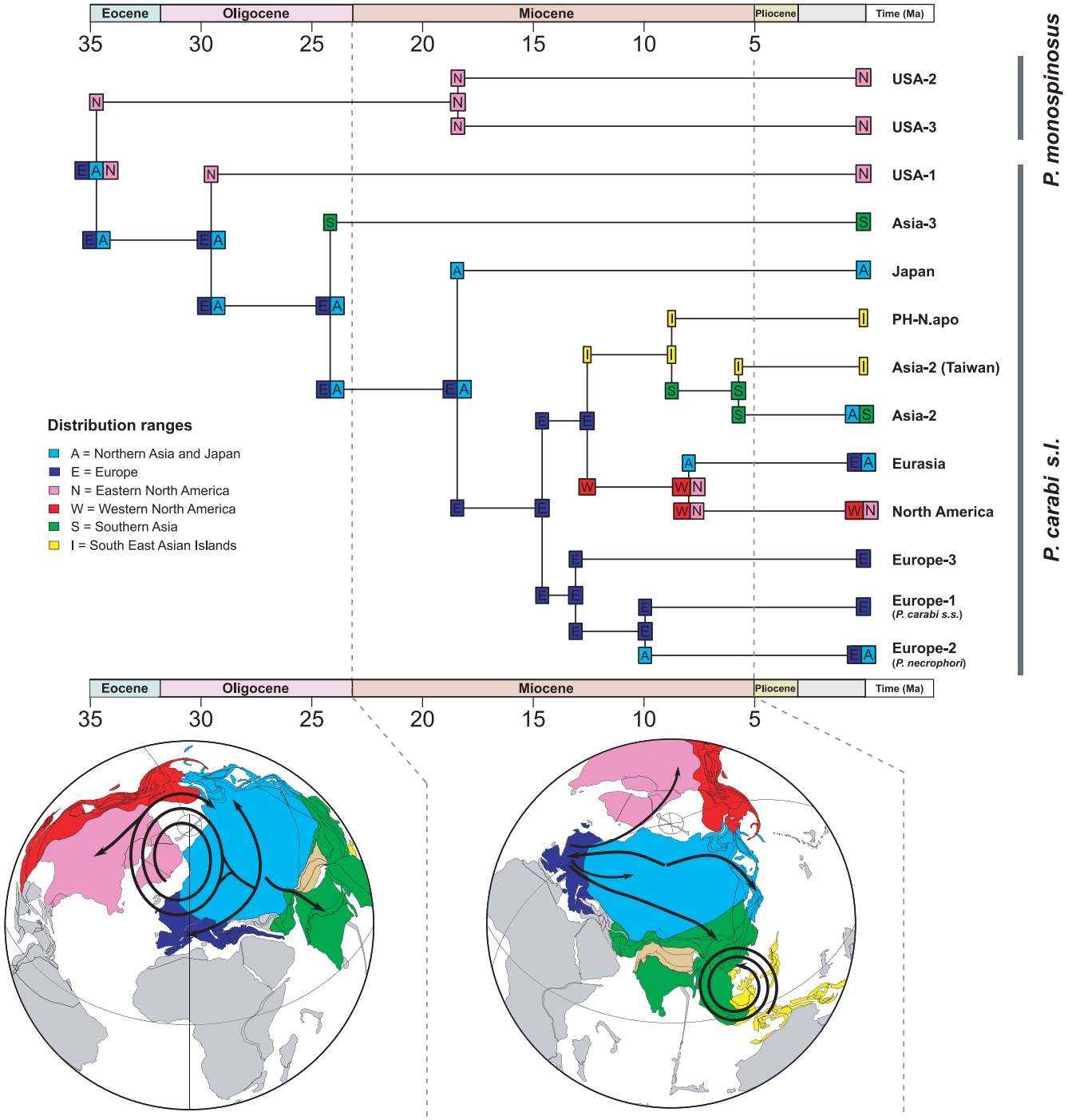

FIGURE 4 Ancestral state estimation of the DIVALIKE+J model inferred by the time-stratified scenario. Plate tectonic maps are illustrated for 25 Ma and for 12 Ma by the ODSN Plate Tectonic Reconstruction Service (https://www.odsn.de/odsn/services/paleomap/paleomap.html). Black arrows on the maps show dispersals with and without founder-event speciation

4.2 | *Poecilochirus subterraneus*

Poecilochirus subterraneus served as the outgroup in our study. The species has previously been observed in Europe (mostly on different *Nicrophorus* species; Hyatt, 1980; Korn, 1982), North America (*N. investigator*, *N. nigrita*; Grossman & Smith, 2008; Sikes, 1996), and Asia (*N. quadripunctatus*, Satou et al., 2000). Here, we sequenced American and European mites resembling the *P. subterraneus* description and found that while mites from both continents clustered together, they segregated into three distinct clusters - one from North America and two from Europe. While species delimitation may be unreliable because of limited sampling among the *P. subterraneus* clusters, our data indicate that *P. subterraneus* might be more diverse than previously thought.

4.3 | The clades *P. monospinosus* (USA-2 & USA-3) and *P. cf. austroasiaticus* (Sichuan singleton)

The mites that morphologically resemble the description of *P. monospinosus* fall into two separate genetic clusters that we found on two different host species. USA-3 ($n = 5$) have been sampled from *N. carolina* in Florida only. We did not have any samples available from other beetle species in Florida, thus we cannot speculate about any potential specialization on *N. carolina*.

We found USA-2 mites only associated with *N. pustulatus*. This host association persisted across two locations. In both, *N. pustulatus* occurs sympatrically with other host species (*N. orbicollis*, *N. tomentosus*). In our data set, only one mite from another genetic cluster was found on *N. pustulatus*. USA-2 was also the cluster with the second lowest Shannon-Wiener index and evenness, and by far the highest χ^2 ratio, a measure that takes into account the sampling data quality of the cluster in question. USA-2 thus appears to be a strict monospecific host specialist on *N. pustulatus*. *Nicrophorus pustulatus* has a unique ecology; it has been reported to reproduce on snake eggs and in bird nests on dead nestlings, and it occurs predominantly in the forest canopy, while other *Nicrophorus* species are typically found near the ground (DeMarco & Martin, 2020; Smith et al., 2007; Wettlaufer et al., 2018). *Nicrophorus pustulatus* thus occupies a distinct ecological niche that may isolate the mites, and possibly select for adaptations specific to this niche. Other families of phoretic mites associated with *N. pustulatus* showed no apparent host specificity for this beetle species (Knee, 2017; Knee et al., 2012), indicating that this beetle's unique niche has not caused mite divergence in every case. Previously, *P. monospinosus* had only been described from poultry manure, preying on fly eggs and larvae – it has not been documented on beetle hosts (Wise et al., 1988). This raises the question whether mites of the original description are an aberrant lineage not associated with beetles or whether *P. monospinosus* is more general in its host usage and occurs with and without beetles.

The mite individual from *N. concolor* found in Central China (Sichuan Province) is particularly interesting because morphologically it resembles *P. austroasiaticus* more than *P carabi s.s.* or any other described species. A discovery of this species in Central China and the association with *N. concolor* is an unexpected observation, as so far *P. austroasiaticus* has only been recorded in Siberia/Northwestern China and Europe on animal corpses, or in association with silphid beetles including *N. investigator* (Hyatt, 1980; Makarova, 2013). According to the phylogenetic approach of cluster identification (IQtree analysis), this singleton is closely related to the clade of *P. monospinosus* (Figure S2).

4.4 | The European clusters: *P. carabi s.s.*, *P. necrophori*, and a new clade

We found three clusters that are almost exclusively distributed in Europe and are closely related. We can unequivocally assign the clusters Europe-1 and Europe-2 to the species *P. carabi s.s.* and *P. necrophori* because we tested the host preference of some mites before killing them (Schwarz, 1996). Based on their association with different beetle species, these mite clusters appear to either prefer or avoid certain hosts, which is in agreement with observations on the host range of the two reproductively isolated mite species (Müller & Schwarz, 1990; Nehring et al., 2017; Schwarz, 1996; Schwarz et al., 1998). Several *Nicrophorus* species occur sympatrically in Europe, and most of them overlap in their seasonal reproductive period but differ in seasonal and diel activity and habitat use (Dekeirsschieter et al., 2011; Esh & Oxbrough, 2021; Majka, 2011; Müller & Eggert, 1987; Schwarz & Koulianos, 1998; Scott, 1998). Thus, as early work has already suggested (Schwarz, 1996), the European mite generalist (Europe-1 = *P. carabi s.s.*) exploits several host species with different life history traits, while *P. necrophori* (= Europe-2) is specialized on *N. vespillo*, which prefers open area habitats where other *Nicrophorus* species are less abundant (Esh & Oxbrough, 2021).

We found a third cluster of mites from across Europe: Europe-3. This cluster is a curious case because it is widespread across Europe but was not found in Germany, where most of our samples were collected. The three European clusters were found in sympatry in some locations (France, Austria), indicating that they do not necessarily competitively exclude each other. Sun and Kilner (2019) described a *P. carabi s.l.* population from the United Kingdom that differs in its phenotype from *P. carabi s.s.* While this population may correspond to *P. necrophori*, it is tempting to speculate that it is in fact our Europe-3 cluster, given that we did not find any *P. necrophori* among the 16 mites from the UK.

4.5 | American samples of *P. carabi s.l.*

The ecology and behaviour of the North American *P. carabi s.l.* is well studied, although not to the same extent as the European populations. Brown and Wilson (1992) reported two reproductively isolated populations from Michigan that differed in morphology, and their preference for *Nicrophorus* species. We were not able to obtain any reference samples from Michigan but our analysis confirms the occurrence of at least two genetic clusters in Northeastern America (USA-1, North America), in addition to *P. monospinosus*. We also found evidence for further clusters from Canada and South America.

The North America cluster was the most diverse one in terms of host species numbers. The members of this cluster did not appear to prefer any specific host species among those occurring across its distribution range. Such a broad host range increases the independence of host abundance, seasonal and diel activity, and other life history traits. In comparison, USA-1 was found almost exclusively on *N. orbicollis* and *N. tomentosus* in the northeastern USA, which might be an indication of local specialization on two host species. Being a local specialist on two sympatric *Nicrophorus* beetles with different seasonal activity (Brown & Wilson, 1992; Keller et al., 2019; Scott, 1998; Wilson, 1982) could expand the reproductive period of the mites.

Some previously described populations of *P. carabi s.l.* from Michigan indeed reproduced successfully using various host species, but others were local specialists (Brown & Wilson, 1992, 1994; Wilson, 1982). In our data, we found no evidence of any clusters being strict specialists for either *N. orbicollis* or *N. tomentosus*, as had been reported by Brown and Wilson (1992) for the Michigan populations. While it is possible that these Michigan populations belong to genetic clusters that we did not sample, it may also be that genetic clusters are specialists in one community and less strict in their host choice in another (Brown & Wilson, 1992).

4.6 | Asian diversity of *P. carabi s.l.*

Our Asian samples cover a great number of sampling locations and host species, but we could only analyse one or few individuals for most of the genetic clusters. Thus, any ecological inference is impossible and we may have only scratched the surface of the biodiversity of *Poecilochirus* mites that use *Nicrophorus* as hosts in Asia.

4.7 | Phylogenetic inference

Our phylogeny provides a basic overview of the relationships among *Poecilochirus* mites. We applied different branch support methods (SH-aLRT, UFBoot, SBS, PP) as their accuracy is debated and confidence levels can vary (Anisimova et al., 2011; Pyron et al., 2011). Relationships between clusters are resolved at deeper levels and in some derived clades, as indicated by well-supported branches across all methods. Medium ranged support values occur mainly in more derived relationships and reveal higher values for posterior probabilities than for bootstrap approximations. Such deviations occur because bootstrap values are a more conservative support measure than Bayesian posterior probabilities which can produce a higher false-positive rate (Anisimova et al., 2011; Cummings et al., 2003; Erixon et al., 2003). According to the variation of support values, certain phylogenetic relationships between European, Asian, and North American clusters should be interpreted with caution. Low support values could result from inconsistencies between the gene trees as we used a concatenated supermatrix of COI, ITS, and LSU, with a partitioning approach. Branch lengths are analogous across the analyses and express an adequate amount of genetic change between internal nodes.

However, the congruent tree topology inferred by all analyses, the medium to high support values, the appropriate branch lengths, and the exclusive dichotomy all indicate a high degree of robustness for this phylogeny. The genes we concatenated for this phylogenetic reconstruction already provided sufficient genetic information individually to distinguish between morphologically described species in other groups of mites (Lehmitz & Decker, 2017; Lv et al., 2014; Schäffer & Koblmüller, 2020). In general, the mites showed low morphological variability, despite their high genetic divergence, but the combined approach of molecular and morphological techniques helps us to better understand the species boundaries and cryptic diversity in this unique group of mites.

4.8 | Evolutionary history and biogeography

Our data suggest a split between the ancestors of *P. carabi s.l.* and the *P. monospinosus* clade during the Eocene/Oligocene and a further radiation within *P. carabi s.l.* in the Miocene. During this period, most of the *Nicrophorus* diversity already existed (Sikes & Venables, 2013). Although the geographic origin of their common ancestor cannot be stated with certainty, the ancestral area of the *P. monospinosus* clade is clearly the North American continent. *Poecilochirus carabi s.l.* might have originated in Eurasia with an early dispersal to the New World (USA-1). The likelihood proportions of the most likely ancestral areas differ only slightly at this cladogenesis event (Figure S5), but regardless of ancestral area, the mites moved between the New and Old World during the Eocene/Oligocene. The Miocene diversification of *P. carabi s.l.* took place in Eurasia with at least one colonization of the New World that is less debatable in terms of dispersal direction (resulting in the North America clade). In both the Eocene/Oligocene and the Miocene, a connection between Eurasia and North America by the Bering and North Atlantic Land Bridges is assumed (Brikiatis, 2014; Denk et al., 2010; Graham, 2018; Jiang et al., 2019; Tiffney, 1985). Although the Bering Land Bridge is often considered the only relevant connection between the continents for floral and faunal migration (Lee et al., 2020; Wen et al., 2016), the assumption that the mites used both land bridges fits our data better. Hence, a closer look at the phylogenetic relationships of beetles and mites occurring near the North Atlantic (e.g., Western Europe; Eastern Canada) would be useful in assessing the role of a North Atlantic Land Bridge and its suitability for the dispersal of small organisms. Regardless of the routes on which the mites migrated between continents, Europe might be a pivotal starting point for their dispersal during the Miocene.

In Southern Asia, mites colonized multiple areas. As this region experienced several geological and climatic changes since the early Miocene that could have resulted in the origin of new geographical barriers (e.g., sea level changes and aridification: Bird et al., 2005; Miao et al., 2012; Zhisheng et al., 2001), vicariant speciation might have contributed to the scattered pattern of Asian clusters.

We would like to emphasize that models including the "jump dispersal" parameter were most likely in all biogeographic scenarios, which highlights the importance of founder-event speciation for the evolution of *Poecilochirus* mites. Furthermore, the results of the tested biogeographic model types DEC+J and DIVALIKE+J deviate just slightly among all analyses. This indicates that dispersal with extinction and vicariance are key processes for understanding the historical biogeography of this species complex.

4.9 | Drivers of speciation

Coevolution between symbionts is generally seen as an important driver of speciation. Symbionts can either cospeciate with each other, or one of the symbionts can radiate when it specializes on local populations or species of the other symbiont. Based on our

analysis, co-speciation of mites with the host beetles can be largely ruled out for *Poecilochirus*, since phylogenetic relationships between the mite clusters did not match those of the host beetles and the main mite diversification happened at least 40 million years after the radiation of the burying beetles (~75 Ma; Sikes & Venables, 2013). Similarly, the specialization on certain beetle species did not play a significant role on a global scale. Instead, geographic separation is likely to be responsible for the divergence among the major *P. carabi s.l.* lineages. Globally, spatial separation between continents can explain the deep splits between clades relatively well, although it is clear that the mites migrated back and forth between continents. Because the mites are small and cannot fly, migrations between the widely separated distribution areas of the mite clusters are likely due to beetle mobility rather than mite mobility. The holarctic species *N. vespilloides* and *N. investigator* are of particular interest in this respect, as both species dispersed either from the New to the Old World (*N. vespilloides*) or vice versa (*N. investigator*) (Sikes et al., 2008; Sikes & Venables, 2013). Mites carried by these beetle species appear in multiple genetic clusters, some of which are also closely related (e.g. Eurasia and North America cluster). This suggests that both *Nicrophorus* species could have played a major part in the dispersal and evolution of *P. carabi s.l.*.

Within continents, further radiation may have been driven by spatial separation on smaller geographical scales that we cannot track or by ecological factors like adaptation to certain host species. In addition to mite clusters that have a broad host range (e.g., Europe-1, North America), host specialists (e.g., USA-2, Europe-2), were also identified. As both types occur in the same geographic area, their evolution in sympatry might be driven by ecological adaptation either directly to local hosts or to the biotic environment the host lives in. For example, the hosts of the European populations occur in different microhabitats. *Nicrophorus vespillo*, host to the specialized *P. necrophori*, is more common in meadows, while *N. vespilloides* is more abundant in forested areas in Germany, the UK, and Alaska (Majka, 2011; Scott, 1998; Sikes et al., 2016). Since meadows are more sun-exposed than wooded habitats, *N. vespillo* and its *P. necrophori* mites may have been adapted to warmer temperatures.

However, when kept at the same temperature, *N. vespilloides* develops quicker than *N. vespillo*, which may be the result of counter gradient variation across the two species (Conover & Schultz, 1995; Müller & Schwarz, 1990). The carried mites track this difference in their own developmental time. This could be a direct adaptation to the host species, because the mite development needs to be completed before the beetle development for optimal dispersal (Müller & Schwarz, 1990; Nehring et al., 2017; see Brown & Wilson, 1992 for a similar effect in American populations). Selection on mite developmental time (e.g. in the event of a host switch) can lead to rapid adaptation in this trait and correlated changes in other traits through hitchhiking or pleiotropy (Schedwill et al., 2018). These effects could cause reproductive isolation among the differentially selected mite populations (Nosil & Harmon, 2009), and thus host specialization can drive genetic divergence. Indeed, a relationship between genetic divergence and host specificity has also been reported for other parasites, such as the honey bee parasite *Varroa* (Beaurepaire et al., 2015) and *Macrocheles* species that are associated with *Nicrophorus* beetles (Knee, 2017). Genetic clustering driven by host adaptation is also found in feather mites that are phoretic on seabirds (Stefan et al., 2018). Host specificity is often seen as a species-level trait. However, it should be considered that local populations of one and the same species could encounter different host communities and may thus specialize on different hosts, an excellent subject for future studies (Brown & Wilson, 1992; Korallo-Vinarskaya et al., 2009; Thompson, 2009).

5 | CONCLUSIONS

Our global analysis of the *P. carabi* species complex revealed a surprisingly high genetic diversity and supports previous ecologically and morphologically defined species clades for *P. necrophori*, *P. carabi s.s.*, *P. monospinosus*, and *P. cf. austroasiaticus*. In addition, there is a complex of several cryptic species.

In *Poecilochirus*, drivers of genetic diversification differ depending on the geographic scale. Spatial separation among continents probably caused early separation among the *P. carabi s.l.* mites. However, the close interaction with their beetle hosts seems to have shaped further divergent evolution among *Poecilochirus* clades. Overall, our study suggests that spatial separation, ecological selection, and coevolution can interact at different geographical scopes to shape biodiversity patterns of mites and other species.

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AUTHOR CONTRIBUTIONS

Julia Canitz conducted bioinformatic analyses, conceptualized and drafted the manuscript.

Anne-Katrin Eggert, Wayne Knee, Derek S. Sikes edited the manuscript. Julia Canitz, Petra Haftaro, Wayne Knee, Martin Nave and Nadine Steinmetz conducted molecular wet laboratory work and initial sequence analyses. Julia Baumann identified and measured mite specimens. Wenbe Hwang, Petra Haftaro, Wayne Knee, Volker Nehring, Nadine Steinmetz, Derek S. Sikes contributed mite specimens. Volker Nehring conceived and supervised the project and cowrote the manuscript.

DATA AVAILABILITY STATEMENT

Mite vouchers have been deposited in the Sikes Research Collection at the University of Alaska Fairbanks; the Canadian National Collection of Insects, Arachnids, and Nematodes; and the Acarological Collection at the University of Graz. Sampling information is listed in Table S1, and raw morphological measurements in Table S5 of this study. DNA sequences are available on GenBank (NCBI) with the following accessions numbers: MW890765 – MW890966 (COI); MW893012 – MW893060 and MW893063 – MW893153 (ITS); and MW893154 – MW893193 and MW893196 – MW893239 (LSU).

ORCID

Volker Nehring https://orcid.org/0000-0002-0494-1428

REFERENCES

Anderson, R. S. (1982). Resource partitioning in the carrion beetle (Coleoptera: Silphidae) fauna of southern Ontario: ecological and evolutionary considerations. *Canadian Journal of Zoology*, *60*, 1314–1325. https://doi.org/10.1139/z82-178

Anisimova, M., Gil, M., Dufayard, J. F., Dessimoz, C., & Gascuel, O. (2011). Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology*, *60*(5), 685–699. https://doi.org/10.1093/sysbio/syr041

Baker, A. S., & Schwarz, H. H. (1997). Morphological differences between sympatric populations of the *Poecilochirus carabi* complex (Acari Mesostigmata: Parasitidae) associated with burying beetles (Silphidae: *Nicrophorus*). *Systematic Parasitology*, *37*, 179–185. https://doi.org/10.1023/A:1005822702267

Beaurepaire, A. L., Truong, T. A., Fajardo, A. C., Dinh, T. Q., Cervancia, C., & Moritz, R. F. A. (2015). Host specificity in the honeybee parasitic mite, *Varroa spp*. in *Apis mellifera* and *Apis cerana*. *PLoS One*, *10*(8), 1–12.

Bird, M. I., Taylor, D., & Hunt, C. (2005). Palaeoenvironments of insular Southeast Asia during the Last Glacial Period: a savanna corridor in Sundaland? *Quaternary Science Review*, *24*(20–21), 2228–2242. https://doi.org/10.1016/j.quascirev.2005.04.004

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*(4), 1–28. https://doi.org/10.1371/journal.pcbi.1006650

Brikiatis, L. (2014). The de geer, thulean and beringia routes: Key concepts for understanding early cenozoic biogeography. *Journal of Biogeography*, *41*(6), 1036–1054. https://doi.org/10.1111/jbi.12310.

Brown, J. M. (1989). *Specialization in* Poecilochirus carabi, *a phoretic mite*. Michigan State University. (Unpublished doctoral dissertation).

Brown, J. M., & Wilson, D. S. (1992). Local specialization of phoretic mites on sympatric carrion beetle hosts. *Ecology*, *73*(2), 463–478. https://doi.org/10.2307/1940753

Brown, J. M., & Wilson, D. S. (1994). *Poecilochirus carabi*: Behavioral and life-history adaptations to different hosts and the consequences of geographical shifts in host communities. In M. A. Houck (Ed.), *Mites: Ecological and Evolutionary Analyses of Life-History Patterns* (1st ed., pp. 3–22). Capman & Hall Inc.

Burke, K., Wettlaufer, J., Beresford, D., & Martin, P. (2020). Habitat use of co-occurring burying beetles (genus *Nicrophorus*) in southeastern Ontario. *Canadian Journal of Zoology*, *98*(9), 591–602.

Conover, D. O., & Schultz, E. T. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution*, *10*(6), 248–252. https://doi.org/10.1016/S0169-5347(00)89081-3

Cummings, M. P., Handley, S. A., Myers, D. S., Reed, D. L., Rokas, A., & Winka, K. (2003). Comparing bootstrap and posterior probability values in the four-taxon case. *Systematic Biology*, *52*(4), 477–487. https://doi.org/10.1080/10635150309311

De Gasperin, O., & Kilner, R. M. (2015). Friend or foe: Interspecific interactions and conflicts of interest within the family. *Ecological Entomology*, *40*(6), 787–795. https://doi.org/10.1111/een.12259

Dekeirsschieter, J., Verheggen, F., Lognay, G., & Haubruge, E. (2011). Large carrion beetles (Coleoptera, Silphidae) in Western Europe: A review. *Biotechnologie, Agronomie, Société Et Environnement*, *15*(3), 435–447.

DeMarco, K. V. B., & Martin, P. R. (2020). A case of a pustulated carrion beetle (*Nicrophorus pustulatus*, Coleoptera: Silphidae) burying live tree swallow (*Tachycineta bicolor*, Passeriformes: Hirundinidae) nestlings under the nest. *The Canadian Field-Naturalist*, *134*(3), 217–221.

Denk, T., Grímsson, F., & Zetter, R. (2010). Episodic migration of oaks to iceland: Evidence for a North Atlantic "Land Bridge" in the latest Miocene. *American Journal of Botany*, *97*(2), 276–287. https://doi.org/10.3732/ajb.0900195

Erixon, P., Svennblad, B., Britton, T., & Oxelman, B. (2003). Reliability of bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology*, *52*(5), 665–673. https://doi.org/10.1080/10635150390235485

Esh, M., & Oxbrough, A. (2021). Macrohabitat associations and phenology of carrion beetles (Coleoptera: Silphidae, Leiodidae: Cholevinae). *Journal of Insect Conservation*, *25*(1), 123–136. https://doi.org/10.1007/s10841-020-00278-4

García-Varela, M., De León, G. P. P., Aznar, F. J., & Nadler, S. A. (2011). Erection of Ibirhynchus gen. nov. (Acanthocephala: Polymorphidae), based on molecular and morphological data. *Journal of Parasitology*, *97*(1), 97–105.

Graham, A. (2018). The role of land bridges, ancient environments, and migrations in the assembly of the North American flora. *Journal of Systematics and Evolution*, *56*(5), 405–429.

Grossman, J. D., & Smith, R. J. (2008). Phoretic mite discrimination among male burying beetle (*Nicrophorus investigator*) hosts. *Annals of the Entomological Society of America*, *101*(1), 266–271.

Hatch, M. H. (1927). Studies on the silphinae. *Journal of the New York Entomological Society*, *35*, 331–370.

Hoberg, E. P., Brooks, D. R., & Siegel-Causey, D. (1997). Host-parasite co-speciation: History, principles, and prospects. *Host–parasite Evolution: General Principles and Avian Models*, 212–235.

Hyatt, K. H. (1980). Mites of the subfamily Parasitinae (Mesostigma: Parasitidae) in the British Isles. *Bulletin of the British Museum (Natural History). Zoology*, *38*(5), 237–378.

Jiang, Y., Gao, M., Meng, Y., Wen, J., Ge, X.-J., & Nie, Z.-L. (2019). The importance of the North Atlantic land bridges and eastern Asia in the post-boreotropical biogeography of the Northern Hemisphere as revealed from the poison ivy genus (*Toxicodendron*, Anacardiaceae). *Molecular Phylogenetics and Evolution*, *139*. https://doi.org/10.1016/j.ympev.2019.106561

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., Von Haeseler, A., & Jermiin, L. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*, 587–589. https://doi.org/10.1038/nmeth.4285

Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., & Flouri, T. (2017). Multi-rate poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics*, *33*(11), 1630–1638. https://doi.org/10.1093/bioinformatics/btx025

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Keller, M. L., Howard, D. R., & Hall, C. L. (2019). Spatiotemporal niche partitioning in a specious silphid community (Coleoptera: Silphidae, *Nicrophorus*). *The Science of Nature*, *106*(11–12), https://doi.org/10.1007/s00114-019-1653-6

Knee, W. (2017). New *Macrocheles* species (Acari, Mesostigmata, Macrochelidae) associated with burying beetles (Silphidae, *Nicrophorus*) in North America. *ZooKeys*, *721*, 1–32. https://doi.org/10.3897/zookeys.721.21747

Knee, W., Beaulieu, F., Skevington, J. H., Kelso, S., Cognato, A. I., & Forbes, M. R. (2012). Species boundaries and host range of tortoise mites (Uropodoidea) phoretic on bark beetles (Scolytinae), using morphometric and molecular markers. *PLoS One*, *7*(10), 1–15. https://doi.org/10.1371/journal.pone.0047243

Knee, W., Beaulieu, F., Skevington, J. H., Kelso, S., & Forbes, M. R. (2012). Cryptic species of mites (Uropodoidea: *Uroobovella spp.*) associated with burying beetles (Silphidae: *Nicrophorus*): The collapse of a host generalist revealed by molecular and morphological analyses. *Molecular Phylogenetics and Evolution*, *65*(1), 276–286.

Korallo-Vinarskaya, N. P., Krasnov, B. R., Vinarski, M. V., Shenbrot, G. I., Mouillot, D., & Poulin, R. (2009). Stability in abundance and niche breadth of gamasid mites across environmental conditions, parasite identity and host pools. *Evolutionary Ecology*, *23*(3), 329–345. https://doi.org/10.1007/s10682-007-9229-x

Korn, W. (1982). Zur Fortpflanzung von *Poecilochirus carabi* G. und R. Canestrini 1882 (syn. *P. necrophori* Vitzt.) und *P. austroasiaticus* Vitzthum 1930 (Gamasina, Eugamasidae). *SPIXIANA*, *5*(3), 261–288.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lee, G. E., Condamine, F. L., Bechteler, J., Pérez-Escobar, O. A., Scheben, A., Schäfer-Verwimp, A., Pócs, T., & Heinrichs, J. (2020). An ancient tropical origin, dispersals via land bridges and Miocene diversification explain the subcosmopolitan disjunctions of the liverwort genus *Lejeunea*. *Scientific Reports*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-71039-1

Lehmitz, R., & Decker, P. (2017). The nuclear 28S gene fragment D3 as species marker in oribatid mites (Acari, Oribatida) from German peatlands. *Experimental and Applied Acarology*, *71*(3), 259–276. https://doi.org/10.1007/s10493-017-0126-x.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L., & Lin, X. (2014). Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasites & Vectors*, *7*(1), 93. https://doi.org/10.1186/1756-3305-7-93

Magalhães, S., Forbes, M. R., Skoracka, A., Osakabe, M., Chevillon, C., & McCoy, K. D. (2007). Host race formation in the Acari. *Experimental and Applied Acarology*, *42*(4), 225–238. https://doi.org/10.1007/s10493-007-9091-0

Majka, C. G. (2011). The Silphidae (Coleoptera) of the maritime provinces of Canada. *Journal of the Acadian Entomological Society*, *7*.

Makarova, O. L. (2013). Gamasid mites (Parasitiformes, Mesostigmata) of the European arctic and their distribution patterns. *Entomological Review*, *93*(1), 113–133. https://doi.org/10.1134/S0013873813010156

Mašán, P. (1999). Mites (Acarina) associated with burying and carrion beetles (Coleoptera, Silphidae) and description of *Poecilochirus mrciaki sp. n.* (Mesostigmata, Gamasina). *Biologia*, *Bratislava*, *54*, 515–524.

Matzke, N. J. (2014). Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology*, *63*(6), 951–970. https://doi.org/10.1093/sysbio/syu056

Miao, Y., Herrmann, M., Wu, F., Yan, X., & Yang, S. (2012). What controlled mid-late miocene long-term aridification in Central Asia? - Global cooling or Tibetan Plateau uplift: A review. *Earth-Science Reviews*, *112*(3–4), 155–172. https://doi.org/10.1016/j.earscirev.2012.02.003

Minh, B. Q., Nguyen, M. A. T., & Von Haeseler, A. (2013). Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, *30*(5), 1188–1195. https://doi.org/10.1093/molbev/mst024

Müller, J. K., & Eggert, A. K. (1987). Effects of carrion-independent pheromone emission by male burying beetles (Silphidae: *Necrophorus*). *Ethology*, *76*(4), 297–304. https://doi.org/10.1111/j.1439-0310.1987.tb00690.x

Müller, J. K., & Schwarz, H. H. (1990). Differences in carrier-preference and evidence of reproductive isolation between mites of *Poecilochirus carabi* (Acari, parasitidae) living phoretically on two sympatric *Necrophorus species* (Coleoptera, silphidae). *Zoologische Jahrbücher: Abteilung Für Systematik, Geographie Und Biologie Der Tiere*, *117*(1), 23–30.

Nehring, V., Müller, J. K., & Steinmetz, N. (2017). Phoretic *Poecilochirus* mites specialize on their burying beetle hosts. *Ecology and Evolution*, *7*(24), 10743–10751. https://doi.org/10.1002/ece3.3591

Nehring, V., Teubner, H., & König, S. (2019). Dose-independent virulence in phoretic mites that parasitize burying beetles. *International Journal for Parasitology*, *49*(10), 759–767. https://doi.org/10.1016/j.ijpara.2019.05.011

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Nosil, P., & Harmon, L. J. (2009). Niche dimensionality and ecological speciation. *Speciation and Patterns of Diversity*, 127–154.

Paterson, S., Vogwill, T., Buckling, A., Benmayor, R., Spiers, A. J., Thomson, N. R., Quail, M., Smith, F., Walker, D., Libberton, B., Fenton, A., Hall, N., & Brockhurst, M. (2010). Antagonistic coevolution accelerates molecular evolution. *Nature*, *464*, 275–278. https://doi.org/10.1038/nature08798

Pyron, R. A., Burbrink, F. T., Colli, G. R., de Oca, A. N. M., Vitt, L. J., Kuczynski, C. A., & Wiens, J. J. (2011). The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. *Molecular Phylogenetics and Evolution*, *58*(2), 329–342. https://doi.org/10.1016/j.ympev.2010.11.006

Peck, S. B., & Anderson, R. S. (1985). Taxonomy, phylogeny and biogeography of the carrion beetles of Latin America (Coleoptera: Silphidae). *Quaestiones Entomologicae*, *21*, 247–317.

Perotti, M. A., & Braig, H. R. (2009). Phoretic mites associated with animal and human decomposition. *Experimental and Applied Acarology*, *49*(1–2), 85–124. https://doi.org/10.1007/s10493-009-9280-0

Ramaraju, K., & Madanlar, N. (1998). Four new *Poecilochirus* G. & R. Canestrini (Acarina: Parasitidae) species from Turkey. *Türkiye Entomoloji Dergisi*, *22*(1), 3–17.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542.

Satou, A., Nisimura, T., & Numata, H. (2000). Reproductive competition between the burying beetle *Nicrophorus quadripunctatus* without phoretic mites and the blow fly *Chrysomya pinguis*. *Entomological Science*, *3*(2), 265–268.

Schäffer, S., & Koblmüller, S. (2020). Unexpected diversity in the host-generalist oribatid mite *Paraleius leontonychus* (Oribatida, Scheloribatidae) phoretic on Palearctic bark beetles. *PeerJ*, *8*, https://doi.org/10.7717/peerj.9710

Schedwill, P., Geiler, A. M., & Nehring, V. (2018). Rapid adaptation in phoretic mite development time. *Scientific Reports*, *8*(1), 1–10. https://doi.org/10.1038/s41598-018-34798-6

Schedwill, P., Paschkewitz, S., Teubner, H., Steinmetz, N., & Nehring, V. (2020). From the host's point of view: Effects of variation in burying beetle brood care and brood size on the interaction with parasitic mites. *PLoS One*, *15*(1), e0228047. https://doi.org/10.1371/journal.pone.0228047

Schwarz, H. H. (1996). Host range and behavioral preferences in german sibling species of the *Poecilochirus carabi* complex (Acari: Mesostigmata: Parasitidae). *International Journal of Acarology*, *22*(2), 135–140.

Schwarz, H. H., & Koulianos, S. (1998). When to leave the brood chamber? Routes of dispersal in mites associated with burying beetles. *Experimental and Applied Acarology*, *22*(11), 621–631.

Schwarz, H. H., & Müller, J. K. (1992). The dispersal behaviour of the phoretic mite *Poecilochirus carabi* (Mesostigmata, Parasitidae): Adaptation to the breeding biology of its carrier *Necrophorus vespilloides* (Coleoptera, Silphidae). *Oecologia*, *89*(4), 487–493. https://doi.org/10.1007/BF00317154

Schwarz, H. H., Starrach, M., & Koulianos, S. (1998). Host specificity and permanence of associations between mesostigmatic mites (Acari: Anactinotrichida) and burying beetles (Coleoptera: Silphidae: *Nicrophorus*). *Journal of Natural History*, *32*(2), 159–172.

Scott, M. P. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology*, *43*, 595–618. https://doi.org/10.1146/annurev.ento.43.1.595

Sikes, D. S. (1996). The natural history of *Nicrophorus nigrita*, a western Nearctic species. *Pan-pacific Entomologist*, *72*, 70–81.

Sikes, D. S., Madge, R. B., & Newton, A. F. (2002). A catalog of Nicrophorinae (Coleoptera: Silphidae) of the world. *Zootaxa*, *65*. https://doi.org/10.11646/zootaxa.65.1.1

Sikes, D. S., Trumbo, S. T., & Peck, S. B. (2016). Cryptic diversity in the New World burying beetle fauna: *Nicrophorus hebes* Kirby; new status as a resurrected name (Coleoptera: Silphidae: Nicrophorinae). *Arthropod Systematics and Phylogeny*, *74*(3), 299–309. https://www.senckenberg.de/wp-content/uploads/2019/08/05_asp_74_3_sikes_299-309.pdf

Sikes, D. S., Vamosi, S. M., Trumbo, S. T., Ricketts, M., & Venables, C. (2008). Molecular systematics and biogeography of *Nicrophorus* in part-The *investigator* species group (Coleoptera: Silphidae) using mixture model MCMC. *Molecular Phylogenetics and Evolution*, *48*(2), 646–666. https://doi.org/10.1016/j.ympev.2008.04.034

Sikes, D. S., & Venables, C. (2013). Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). *Molecular Phylogenetics and Evolution*, *69*(3), 552–565. https://doi.org/10.1016/j.ympev.2013.07.022

Smith, G., Trumbo, S. T., Sikes, D. S., Scott, M. P., & Smith, R. L. (2007). Host shift by the burying beetle, *Nicrophorus pustulatus*, a parasitoid of snake eggs. *Journal of Evolutionary Biology*, *20*(6), 2389–2399. https://doi.org/10.1111/j.1420-9101.2007.01404.x

Springett, B. P. (1968). Aspects of the relationship between burying beetles, *Necrophorus spp*. and the mite, *Poecilochirus necrophori* Vitz. *The Journal of Animal Ecology*, 417–424. https://doi.org/10.2307/2957

Stadler, T. (2010). Sampling-through-time in birth-death trees. *Journal of Theoretical Biology*, *267*(3), 396–404. https://doi.org/10.1016/j.jtbi.2010.09.010

Stadler, T., Gavryushkina, A., Warnock, R. C. M., Drummond, A. J., & Heath, T. A. (2018). The fossilized birth-death model for the analysis of stratigraphic range data under different speciation modes. *Journal of Theoretical Biology*, *447*, 41–55. https://doi.org/10.1016/j.jtbi.2018.03.005

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stefan, L. M., Gómez-Díaz, E., Mironov, S. V., González-Solís, J., & McCoy, K. D. (2018). "More than meets the eye": Cryptic diversity and contrasting patterns of host-specificity in feather mites inhabiting seabirds. *Frontiers in Ecology and Evolution*, *6*(JUL), 1–16. https://doi.org/10.3389/fevo.2018.00097

Sun, S.-J., Horrocks, N. P. C., & Kilner, R. M. (2019). Conflict within species determines the value of a mutualism between species. *Evolution Letters*, *3*(2), 185–197. https://doi.org/10.1002/evl3.109

Sun, S.-J., & Kilner, R. (2019). Cryptic host specialisation within *Poecilochirus carabi* mites explains population differences in the extent of co-adaptation with their burying beetle *Nicrophorus vespilloides* hosts. *BioRxiv*, *1223*(3), 641936.

Thompson, J. N. (2009). The coevolving web of life. *American Naturalist*, *173*(2), 125–140. https://doi.org/10.1086/595752

Tiffney, B. H. (1985). The Eocene North Atlantic land bridge: Its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *Journal of the Arnold Arboretrum*, *66*, 243–274. https://doi.org/10.5962/bhl.part.13183

Wen, J., Nie, Z. L., & Ickert-Bond, S. M. (2016). Intercontinental disjunctions between eastern Asia and western North America in vascular plants highlight the biogeographic importance of the Bering land bridge from late Cretaceous to Neogene. *Journal of Systematics and Evolution*, *54*(5), 469–490. https://doi.org/10.1111/jse.12222

Wettlaufer, J. D., Burke, K. W., Schizkoske, A., Beresford, D. V., & Martin, P. R. (2018). Ecological divergence of burying beetles into the forest canopy. *PeerJ*, *2018*(11), 1–18. https://doi.org/10.7717/peerj.5829

Wilson, D. S. (1982). Genetic polymorphism for carrier preference in a phoretic mite. *BioScience*, *32*(6), 537. https://doi.org/10.2307/1308907

Wilson, D. S., & Knollenberg, W. G. (1987). Adaptive indirect effects: The fitness of burying beetles with and without their phoretic mites. *Evolutionary Ecology*, *1*, 139–159. https://doi.org/10.1007/BF02067397

Wise, G. U., Hennessey, M. K., & Axtell, B. C. (1988). A new species of manure-inhabiting mite in the genus *Poecilochirus* (Acari: Mesostigmata: Parasitidae) predacious on house fly eggs and larvae. *Entomological Society of America*, *81*(2), 209–224. https://doi.org/10.1093/aesa/81.2.209

Yoder, J. B., & Nuismer, S. L. (2010). When does coevolution promote diversification? *American Naturalist*, *176*(6), 802–817. https://doi.org/10.1086/657048

Zhang, G., Basharat, U., Matzke, N., & Franz, N. M. (2017). Model selection in statistical historical biogeography of Neotropical insects

- The *Exophthalmus* genus complex (Curculionidae: Entiminae). *Molecular Phylogenetics and Evolution*, *109*, 226–239. https://doi.org/10.1016/j.ympev.2016.12.039

Zhisheng, A., Kutzbatch, J. E., Prell, W. L., & Porder, S. C. (2001). Evolution of Asian monsoons and phased uplift of the Himalaya- Tibetan plate since Late Miocene times. *Nature*, *411*, 62–66.

SUPPORTING INFORMATION

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