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

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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. A taxon for which this holds true are the 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* and *P. austroasiaticus.* 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. 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.

Introduction

Parasites are known for their high biodiversity (García-Varela et al., 2011; Huyse et al., 2005; Magalhães et al., 2007). In all organisms, geographic separation can lead to allopatric speciation, and ecological factors may cause disruptive selection and lead to reproductive isolation in sympatry (Butlin et al., 2012). Ecological effects are amplified by the interaction of parasites with their hosts: Any divergence in host populations can potentially alter selection on the parasites and lead to divergence and even co-speciation with their hosts (Hoberg et al., 1997). Further, reciprocal selection between hosts and parasites may speed up evolution and lead to higher diversification rates among populations (Paterson et al., 2010; Thompson, 2009; Yoder & Nuismer, 2010). These processes can result in many parasite populations or species each specialized on just one host species or population (García-Varela et al., 2011; Hunter & Rosario, 1988; Huyse et al., 2005; Magalhães et al., 2007; Perotti & Braig, 2009). The diversity of parasites is often cryptic, as they tend to live hidden lives and morphological adaptations to parasitism may be similar for all hosts (García-Varela et al., 2011). Hence, the diversity of parasites is likely underestimated.

The mite genus *Poecilochirus* G. & R. Canestrini, 1882 (Mesostigmata: Parasitidae) consists of 20 morphologically described species (Hyatt, 1980; Perotti & Braig, 2009; Ramaraju & Madanlar, 1998). Several species are phoretic as immatures (deutonymphs). They attach to adult burying beetles (Silphidae: *Nicrophorus*) for dispersal (Farish & Axtell, 1971; Schwarz & Koulianos, 1998). The beetles bury small vertebrate carcasses, on which they raise their offspring. While the parental beetles provide brood care, *Poecilochirus* mites dismount from their host beetles, feed on microorganisms, fly eggs, and sometimes beetle eggs, develop into adults, and reproduce (Brown & Wilson, 1992; Meierhofer et al., 1999; Schwarz & Müller 1992).

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63   Deutonymphal mites disperse from the carcass by attaching to the parental beetles or

64   their adult offspring (Schwarz & Koulianos, 1998; Schwarz & Müller, 1992). At larger

65   carcasses, multiple beetle species can co-occur and mites can switch between host

66   individuals. Mites recognize their main host by olfactory cues and some prefer specific

67   *Nicrophorus* species over others (Korn, 1982; Müller & Schwarz, 1990). If the preferred

68   host species is not available, the mites will mount other host species, but their fitness

69   may be reduced when they reproduce along with the 'wrong' host species (Brown &

70   Wilson, 1994; Nehring et al., 2017). Such host switches may counteract host

71   specialization (Thompson, 2009).

72   There is no evidence that the mites affect host beetle fitness during the phoretic

73   dispersal. However, some observations suggest that mites may affect beetle fitness

74   during the reproduction phase. Depending on the environmental conditions, the mites

75   can either directly reduce beetle brood weight and offspring number (De Gasperin &

76   Kilner, 2015; Nehring et al., 2019; Schedwill et al., 2020), or have positive effects on

77   beetle fitness by helping to fend off other competitors such as blowflies, nematodes, or

78   other beetles (Springett, 1968; Sun et al., 2019; Wilson & Knollenberg, 1987). In any

79   case, whether the mites are parasites or mutualists, they have likely coevolved with the

80   host beetles.

81   *Poecilochirus* species can be distinguished morphologically based on body size, the

82   presence or absence of setae, the form and pattern of setae and the sternal shield, the

83   morphology of the chelicerae, and genital structures (Baker & Schwarz, 1997; Hyatt,

84   1980; Ramaraju & Madanlar, 1998; Wise et al., 1988). However, the taxonomy of the

85   genus is poorly understood, at least partly because morphological differences between

86   species are small (Mašán, 1999). The best-studied species is *Poecilochirus carabi*, but it

87   has been shown that this species actually consists of at least two reproductively isolated

88   populations that occur sympatrically in Central Europe and that have been named *P.*

89   *carabi sensu stricto* and *P. necrophori* (Baker & Schwarz, 1997; Hyatt, 1980; Müller &

90   Schwarz, 1990). Field and laboratory studies indicate that *P. necrophori* is a host

91   specialist primarily found on *Nicrophorus vespillo*, while *P. carabi s.s.* is prevalent on

92   at least three different *Nicrophorus* species (*N. vespilloides, N. investigator,* and *N.*

93   *humator*), but rarely on *N. vespillo* (Schwarz, 1996). Furthermore, two reproductively

94   isolated populations of *P. carabi* are specialized on two sympatric North American

95   *Nicrophorus* species (*N. tomentosus* and *N. orbicollis*) and differ in morphology

96   (Brown, 1989; Brown & Wilson, 1992), but their relationship to the European species is

97   unknown. Mites of these morphologically very similar but reproductively isolated and

98   ecologically well-defined populations are considered to belong to a cryptic species

99   complex, termed *P. carabi sensu lato* (Baker & Schwarz, 1990; Masan, 1999).

100   One might expect that *P. carabi s.l.* consists of more than these four cryptic species,

101   given that the holarctic genus *Nicrophorus* includes more than 60 species (Sikes et al.,

102   2002, 2016). Burying beetles originated in the Cretaceous (99–127 Ma) in Asia/Eurasia,

103   colonized the Western hemisphere and have likely back-migrated to Eurasia more than

104   once (Hatch, 1927; Peck & Anderson, 1985; Sikes & Venables, 2013). Today, most

105   species are restricted to single continents, and at most locations, multiple species occur

106   in sympatry (e.g. Brown & Wilson, 1992; Dekeirsschieter et al., 2011). Only *N.*

107   *vespilloides* and *N. investigator* occur in both the Western and Eastern hemispheres

108   (Sikes et al., 2008; Sikes et al., 2016). *Nicrophorus* species vary in their habitat

109   preferences. Some species inhabit woodlands and others open areas, and differences in

110   the diel activity and reproductive season have been reported for species on both

111   continents (Anderson 1982, Burke et al., 2020; Esh & Oxbrough, 2021; Majka, 2011;

112   Scott, 1998).

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113　Molecular, phylogenetic, and divergence time analyses have been conducted for

114　*Nicrophorus* (Sikes et al., 2008, 2016; Sikes & Venables, 2013), but are missing for

115　*Poecilochirus* species. The scant genetic data that are available are primarily from

116　broader studies on the diversity and phylogenetics of Mesostigmata or Acari, with many

117　samples only identified to the genus level (Klompen et al., 2007; Rueda-Ramírez et al.,

118　2019; Young et al., 2019). The lack of molecular data on the diversity of this genus

119　prevents further insight into its ecology and evolutionary history.

120　In this study, we want to understand the evolutionary history of *Poecilochirus* mites that

121　are phoretic on *Nicrophorus* beetles, and *P. carabi s.l.* in particular, using molecular

122　analyses. Given the biodiversity and wide distribution of the host genus, and the

123　independent descriptions of reproductively isolated mite populations in Europe and

124　North America, we predicted that genetic diversity within the genus *Poecilochirus* is

125　currently underestimated. Based on the results of previous studies (Brown & Wilson,

126　1992; Müller & Schwarz, 1990), host specificity is expected to be evident in mite

127　genetics, as much of the mites' reproductive isolation is suggested to be caused by

128　diverging host preferences. We sequenced three genetic markers of *Poecilochirus* mites

129　collected with their *Nicrophorus* hosts on four different continents. We documented

130　their genetic diversity, reconstructed the phylogenetic relationships, and estimated

131　evolutionary divergence times to better understand the drivers of speciation in this mite

132　clade.

Materials and Methods

We collected *Poecilochirus* mites from burying beetles from North and South America, Europe and Asia and used nuclear and mitochondrial DNA sequences to identify genetic clusters. We then reconstructed phylogenetic relationships, and analyzed the biogeography and host specificity of the main mite clusters.

Sampling

We focused on mites from burying beetles that morphologically resemble *P. carabi* in their habitus and body size (body length ca. 1 mm; 218 individuals). Samples originated from 43 different locations ranging from Alaska (USA), through Europe, Central Asia, Japan and Melanesia (Figure S1). They were collected together with their host beetles, including 31 *Nicrophorus* species and one carabid beetle (*Pterostichus melanarius*). Specimens were sampled from the wild between 1998 and 2020, and were preserved either in 96% ethanol, propylene glycol, or kept dry (Supplement Table S1). Several specimens of the German populations (Mooswald, Freiburg) could be identified as *P. necrophori* or *P. carabi s.s.* by host preference tests following the description of Nehring et al. (2017).

In addition, specimens identified as *P. subterraneus* (n = 12) and *Macrocheles* sp. (n = 2) were added to the data set to serve as the outgroup in phylogenetic analyses (Supplement 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.

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Molecular Methods

We extracted DNA from 232 mites. Two different methods were used for DNA extraction. We applied either the Chloroform/Isopropanol method where the whole individual was ground up in liquid nitrogen, or a non-destructive approach using the DNeasy Blood and Tissue Kit (Qiagen). For the non-destructive method, we incubated the entire specimen in 50µl lysis buffer (ATL buffer) and 10µl Proteinase K for approximately 24h at 56°C. After 6–8h, additional 5µl Proteinase K were added. Subsequently, we removed the undamaged specimens and followed the default protocol of the DNeasy Kit. Mite voucher specimens following DNA extraction were preserved in absolute ethanol.

A partial sequence of the Cytochrome Oxidase I gene (COI) was amplified using the universal primer pair LCO1490 and HCO2198 (Folmer et al., 1994). Furthermore, the ribosomal Internal Transcribed Spacer gene (ITS) was amplified. The primer pair used for the ITS amplification was based on the sequences published by Navajas et al. (1999), but we modified them slightly for better annealing (forward: 5'- AGTCGTAACAAGGTTTCCGTAG-3'; reverse: 5'- GGGGGTAATCGCACTTGA TTTC-3'). Additionally, the gene coding for the Large Subunit of the rRNA (LSU) was amplified partly using the universal primer pair LSU-D1D2.fw2 and LSU-D1D2.rev2 (Sonnenberg et al., 2007). Amplification was performed with 30 thermocycler cycles. PCR products were purified either by a polyethylene glycol (PEG)-based method or enzymatically with ExoSAP-IT™ (ThermoFisher). We sent PCR products to Macrogen Europe Inc. (Amsterdam, The Netherlands) for forward and/or reverse Sanger sequencing.

Identification of genetic clusters

The COI, ITS, and/or LSU sequences of 230 *Poecilochirus* samples were aligned gene-wise using default settings of the Geneious Prime implementation MAFFT v.7.450 (Katoh & Standley, 2013). Alignments were concatenated to a supermatrix in which sequences of "N" symbolized missing data/genes. The supermatrix served as input for a phylogenetic analysis with IQtree multicore version 1.6.12 (Nguyen et al., 2015). IQtree estimates maximum-likelihood (ML) phylogenies using a fast and effective stochastic search algorithm. 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 analysis ran with 10,000 bootstrap replicates using the ultra-fast bootstrap approximation for branch support (Minh et al., 2013), and with a parametric approximate likelihood-ratio test (SH-aLTR) testing the null hypothesis that assumes inferred branches with a length of 0 (Anisimova et al., 2011). We set *P. subterraneus* as outgroup. The phylogenetic tree was visualized with FigTree version 1.4.4 and edited using Inkscape 1.0.1. We also used the "Poisson Tree Process" (PTP) model implemented in the tool multi-rate (m)PTP version 0.2.4 (Kapli et al., 2017). The model is a tree-based approach for species delimitation. It suggests the most likely number of species based on maximum likelihood and a Markov Chain Monte Carlo (MCMC) algorithm to provide support values for each putative species clade. Initially, the minimum branch length was calculated (--minbr_auto) based on the input alignment. In a second step the resulting values and the phylogenetic tree were input to the main mPTP analysis with the following parameters: --mcmc 100,000,000; --mcmc_sample 10,000; --mcmc_burnin 500,000; --mcmc_runs 4; --mcmc_startrandom. To illustrate the geographic distribution of the identified genetic clusters, the R packages maptools v1.0-2 and scatterpie v0.1.5 (R Core Team, 2020) were used to plot

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203 the relative abundance of the different clusters at each locality on a world map. To

204 support cluster delineation, uncorrected p-distances within and between clusters were

205 calculated based on the COI alignment using MEGA version 10.1.7 (Kumar, Stecher,

206 Li, Knyaz & Tamura, 2018).

Morphological identification

208 Mite specimens that were not destroyed during DNA extraction (n = 95) were mounted

209 and clarified in Heinze-PVA medium and stored in an oven at 50°C until total

210 clarification. Morphological and morphometric analyses of mites were performed using

211 differential interference contrast in a compound microscope (Reichert Diavar, Vienna).

212 Identification of mites was based on the key by Hyatt (1980) and the description of *P.*

213 *monospinosus* by Wise et al. (1988).

Host specificity

215 Host specificity was calculated as Shannon-Wiener Diversity (H') and Evenness. We

216 performed these calculations for three European and three North American clusters that

217 contained enough samples and host species. In addition, we used a χ^2-test to investigate

218 if the frequency of host species occupied by a mite cluster deviates from the overall host

219 species frequency in the same geographical area. The host species frequency was

220 derived from the number of mites that we sequenced from each beetle species;

221 whenever possible, we had selected mites from different beetle individuals.

222 Subsequently, the χ^2 value of each cluster ($\chi^2_{(cluster)}$) was set in relation to the theoretical

223 χ^2 maximum of the respective cluster ($\chi^2_{(max)}$). A high quotient of $\chi^2_{(cluster)}/\chi^2_{(max)}$ suggests

224 host specificity of the mite clusters for that area. Calculations for the Shannon-Wiener

225 Index and Evenness were performed using the open source spreadsheet tool provided by

226 LibreOffice version 6.2.8.2., and χ^2 values were calculated with the R Stats package

227 v3.6.2.

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Phylogenetic inference

We used 38 samples for which sequences of all three genes were available for phylogenetic analyses. These samples covered 16 of the clusters previously identified. We applied Maximum Likelihood (ML) and Bayesian Inference (BI) approaches and used four different methods for assigning confidence levels to branches - 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 generated with Geneious Prime. All analyses were conducted with the data partitioned by gene. Best-fitting substitution models were found using IQtree. Models were adjusted to the most similar substitution model RaxML and MrBayes can integrate. 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) to perform 10,000 bootstrap replicates and ML searches at once (-# 10,000; -T 20). For our Bayesian approach, all prior parameters of MrBayes except for the model settings were kept at default. We started MrBayes with 1,000,000 generations (ngen=1,000,000 samplefreq=5,000; printfreq=5,000; diagnfreq=1,000). Afterwards, parameter values like effective sample size (ESS) were summarized and checked for reliability with Tracer v. 1.7.1 (Rambaut et al., 2018). Trees were summarized with a burn-in of 10%. All phylogenies and their support values were read and plotted with FigTree. Illustrations and modifications were conducted with InkScape. In all analyses, *P. subterraneus* specimens were used as the outgroup.

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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 dataset 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 (Supplement Table S2). The analysis was conducted with Beast v. 2.6.3 (Bouckaert et al., 2019). Beast2 includes the Fossilized-Birth-Death Process model (FBD) which is an extended version of the Birth-Death Process (Stadler, 2010; Stadler et al., 2018). Both models assume that every living lineage can experience speciation at rate λ or go extinct at rate μ, but the FBD model allows the treatment of known fossil calibration points as part of the tree prior at node times. Such priors can be set with the graphical user interface Beauti2. We ran Beast2 under the FBD model using fossil data available for five taxa (Supplement 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 model 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. Trees were stored every 100 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) using a burn-in of

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277   10% and the maximum clade credibility as target tree type. Results were plotted using

278   FigTree and edited with InkScape.

Biogeography and ancestral-area estimation

280   The divergence time reconstruction was the basis for a biogeographical analysis. We

281   used the R package BioGeoBears (Matzke, 2014) which performs inferences of

282   biogeographic histories on phylogenies. With BioGeoBears, different models of how

283   biogeography may evolve on phylogenies can be tested on a given dated tree. Currently

284   the package includes the Dispersal-Extinction-Cladogenesis model (DEC), a likelihood

285   version of the Dispersal-Vicariance model (DIVALIKE) and the Bayesian Analysis of

286   Biogeography (BAYAREALIKE). Moreover, it provides an extended version for the

287   models by the consideration of additional free parameters like 'j' ("jump dispersal") or

288   'x' (geographical distances) while modeling. The "jump dispersal" parameter simulates

289   the founder-event speciation. It describes that at the time of cladogenesis one daughter

290   lineage inherits the ancestral range, and the other lineage occupies a new area through a

291   rare, long-distance colonization event and founds an instantly genetically isolated

292   population (Matzke, 2014; Zhang et al., 2017). Since the biogeography of *Poecilochirus*

293   is our focus, we pruned the dated tree to a subset containing only *Poecilochirus*

294   specimens (excl. *P. subterraneus*) and used it for the BioGeoBears analysis. We divided

295   the Northern Hemisphere in 6 areas: Western North America (W), Eastern North

296   America (N), Europe (E), Northern Asia + Japan (A), Southern Asia (S), and South East

297   Asia ranging to the Solomon Islands (I). In an initial analysis, we tested whether the

298   existence of only the Bering Land Bridge (BLB) or the North Atlantic Land Bridge

299   (NALB) and the BLB might fit the data better. Three model types were tested in three

300   different versions for each scenario (M0=DEC, DIVALIKE, BAYAREALIKE;

301   M1=DEC+J, DIVALIKE+J, BAYAREA+J, and M2=DEC+J+X, DIVALIKE+J+X,

302   BAYAREA+J+X), and the likelihood and Akaike Information Criterion with sample

303   correction (AICc) were compared between both scenarios. We continued with the

304   scenario showing the highest likelihood (lowest negative log likelihood value: -lnL) and

305   lowest AICc values in most of the models and compared nested models using a

306   Likelihood Ratio Test (same model type: M0 vs. M1 and M1 vs. M2). The AICc was

307   used to compare among model types (DEC vs. DIVALIKE).

308   A more likely scenario is obtained by running the biogeographical models under a time-

309   stratified analysis. In such an analysis, BioGeoBears can take into account geographical

310   changes and different difficulty levels for dispersal occurring over time. Our time-

311   stratified analysis included three time slices. We tried to represent the geographic

312   conditions of the Eocene/Oligocene, the Miocene, and present conditions. For this

313   scenario we ran the models DEC/DEC+J and DIVALIKE/DIVALIKE+J.

Results

Sequence data

We obtained 429 DNA sequences after editing and quality-checking. Of these, 193 COI, 136 ITS, and 79 LSU sequences belonged to mites that resemble *P. carabi,* 10 COI, 6 ITS, and 3 LSU sequences belonged to *P. subterraneus,* and one COI and one LSU sequence were generated from two *Macrocheles* specimens (Supplement 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 are 655bp, 509bp and 645bp, respectively. All sequences that were blasted against the NCBI database matched published sequences from either *Poecilochirus* or Parasitidae, with low E-values and a sequence coverage > 70%. *Macrocheles* samples matched with sequences from *M. nataliae.*

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, Supplement Figure S2). Of these, 3 were clusters in 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).

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Table 1: List of genetic clusters of *Poecilochirus*. 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.

	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			
PH-N.apo	1	*N. apo*	Philippines
IND-N.ins	1	*N. insularis*	Indonesia (Bali)
SI-N.kie	1	*N. kieictus*	Solomon Islands
IDN-N.cha	1	*N. charon*	Indonesia (Sulawesi)
CH-N.jap	1	*N. japonicus*	China (Liaoning)
RUS-N.mor	1	*N. morio*	Russia
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)

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We named the clusters according to their main distribution area. All clusters but Asia-2 were represented by reliable branch support values (SH-aLRT > 80%; UFBoot > 95%). The separation of Asia-1, IDN-N.ins and Asia-2 from the remaining singletons resulted in less confident values (SH-aLRT=26%; UFBoot=53%). However, the close relationships among Asia-1, Asia-2, IDN-N.ins, PH-N.apo and SI-N.kie were well supported as a monophyletic group (SH-aLRT=81.6%; UFBoot=95%). Its sister group is a clade formed by the North America and Eurasia clusters but with lower branch support values (SH-aLRT=71.8%; UFBoot=91%). Branch support values of deeper phylogenetic splits varied highly (SH-aLRT: 20.7--99.9%; UFBoot: 67-100%) indicating a more fragile tree topology (Supplement Figure S2).

The mPTP species delimitation results were similar to those of the IQtree approach. The four independent MCMC runs yielded the highest frequencies for species numbers between 22 and 26 with the highest likelihood score for a multi-coalescent rate (-lnL=1099.35) calculated for 23 species (including 3 *P. subterraneus* clusters, Supplement Figure S3).

Mites in the three *P. subterraneus* clusters were collected in Europe (2 clusters) or North America (Supplement Table S1). The remaining mite clusters were each restricted to one of three major geographical regions (the European, Asian, and American continent). In North America (USA and Canada), five different clusters occurred. While the North America 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 cluster was only present in Illinois and Ohio (Middle-Eastern USA), and the USA-3 cluster was restricted to Florida (South-Eastern USA). The Canada cluster only appeared in Calgary/Alberta. In South America the Ecuador cluster was found (Figure 1, Supplement Table S1).
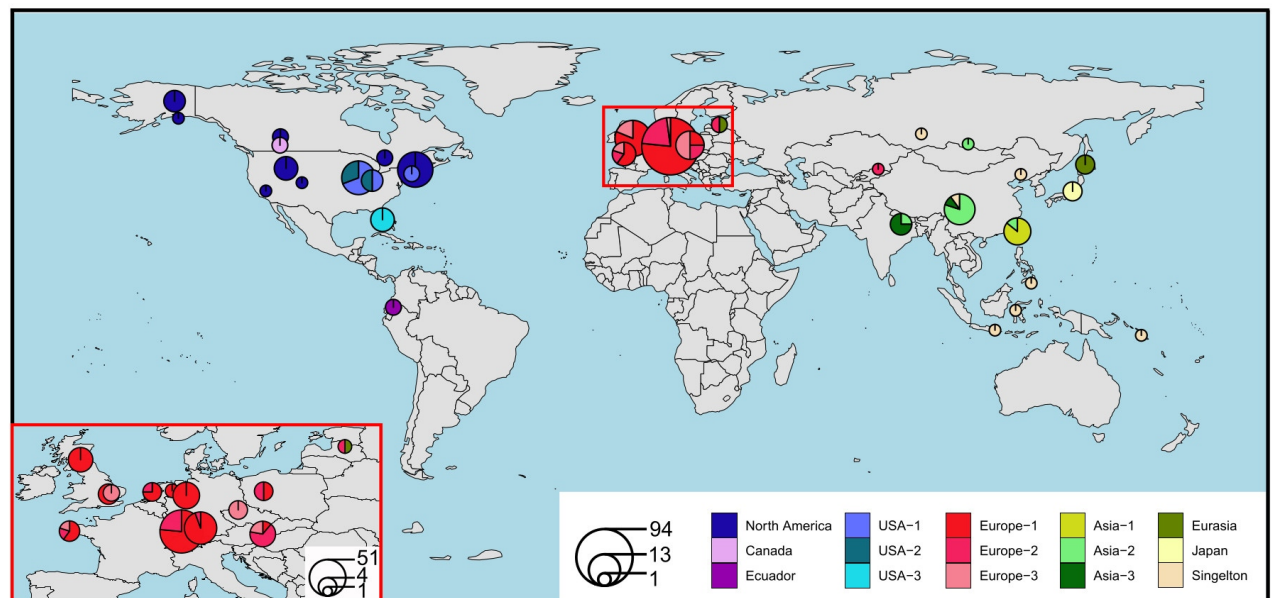
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Figure 1: Distribution of the Poecilochirus clusters without P. subterraneus. Pie charts show the relative proportion of the different clusters at each location. Each cluster is represented by another color (except singletons) and pie size reflects the sample size at each location. The European distribution is enlarged in the left bottom corner (red rectangle).

The clusters Europe-1, Europe-2, and Europe-3 were distributed across Europe whereby 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. We identified a distinct Japan cluster in addition to the Eurasia cluster on the Japanese Islands (Figure 1).

The mean uncorrected p-distance 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/IND-N.ins) and 21.06% (Psub-GER1/USA-2). The 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%.

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Morphological identification

We morphologically identified 95 large *Poecilochirus* specimens 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 by having a weakly sclerotized body and long podosomal and opisthosomal shields (Supplement Tables S1 and S4).

Five specimens were identified as other species: The single intact specimen of the USA-2 cluster (sample ID: oh-pus2) corresponded to the description of *Poecilochirus monospinosus* Wise, Hennessey & Axtell, 1988. All individuals of the USA-3 cluster resembled *P. monospinosus* as well but differ in the setal pattern. The CH-N.con singleton 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, CH-N.con, and the three *P. subterraneus* clusters.

Host specificity

We focused on six clusters that contained more than six mite specimens each and that were found in more than one location. Among the three European clusters, Europe-1 and Europe-3 were each associated with five, and Europe-2 with four *Nicrophorus* species, respectively (Table 2; Figure 2).
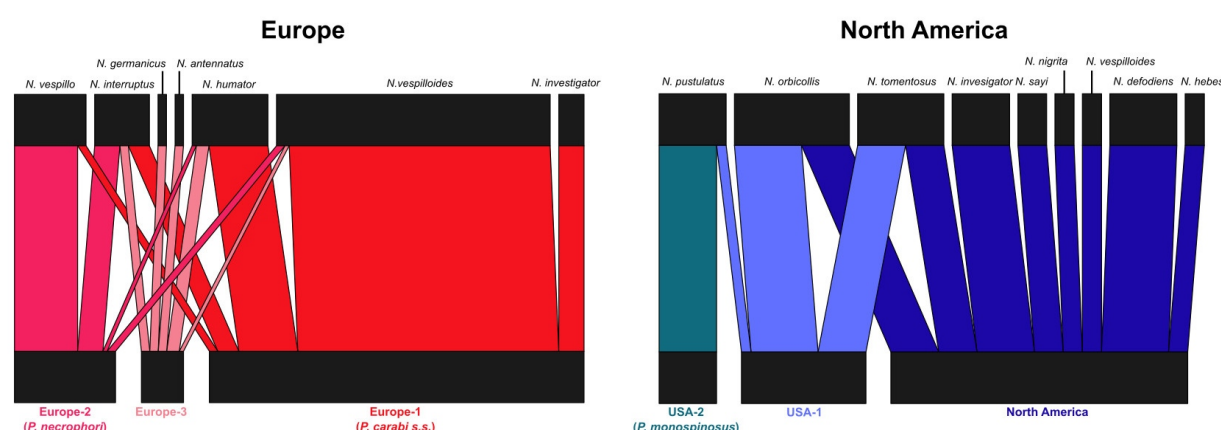
Figure 2: Association network between host species and the six genetic clusters tested for host specificity. The map illustrates the weighted association between mite clusters and *Nicrophorus* species for the clusters Europe-1, Europe- 2 and Europe-3, as well as the clusters USA-1, USA-2 and North America. The thicker the bars the more mite individuals are associated with the respective host species.

392   Both Shannon-Wiener Diversity Index and Evenness were highest for the cluster

393   Europe-1 and lowest for Europe-3 (Table 2). The association between the three North

394   American clusters and their host species is clearer, even though more host species were

395   involved. The clusters USA-2, USA-1 and North America were found on one, three and

396   eight *Nicrophorus* species, respectively (Table 2; Figure 2). Furthermore, a similar

397   pattern for both statistical indices was shown in North America where both values

398   decreased from the North America cluster over USA-1 to USA-2. Furthermore, the χ^2

399   ratio ranged from 0.003 to 0.082 in Europe and from 0.014 to 0.256 in North America

400   (Table 2). The higher the quotient, the more the samples from a cluster were

401   concentrated on specific host species.

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.

Cluster	Host species †							H'	Even-ness	$\chi^2_{(cluster)}$	$\chi^2_{(cluster)}/\chi^2_{(max)}$
	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

Cluster	N. def	N. heb	N. inv	N. nig	N. orb	N. pus	N. say	N. tom	N. vs	H'	Even-ness	$\chi^2_{(cluster)}$	$\chi^2_{(cluster)}/\chi^2_{(max)}$
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

402 * significant: $\chi^2_{(cluster)}$ > critical value of 12.59 (df=6; α=0.05) for the European clusters;
403 $\chi^2_{(cluster)}$ > critical value of 15.51 (df=8; α=0.05) for the North American clusters

404 † N. vs = *N. vespilloides*, N. vo = *N. vespillo*, N. hum = *N. humator*, N. int = *N. interruptus*, N. inv
405 = *N. investigator*, N. ant = *N. antennatus*, N. ger = *N. germanicus*, N. def = *N. defodiens*, N. heb
406 = *N. hebes*, N. nig = *N. nigrita,* N. orb = *N. orbicollis*, N. pus = *N. pustulatus,* N. say = *N. sayi,* N.
407 tom = *N. tomentosus*

408 **Phylogenetic inference**

409 The tree topologies inferred by the ML (IQtree and RaxML) and BI (MrBayes) analyses

410 were consistent. The phylogeny comprised 37 mite individuals covering 16 genetic

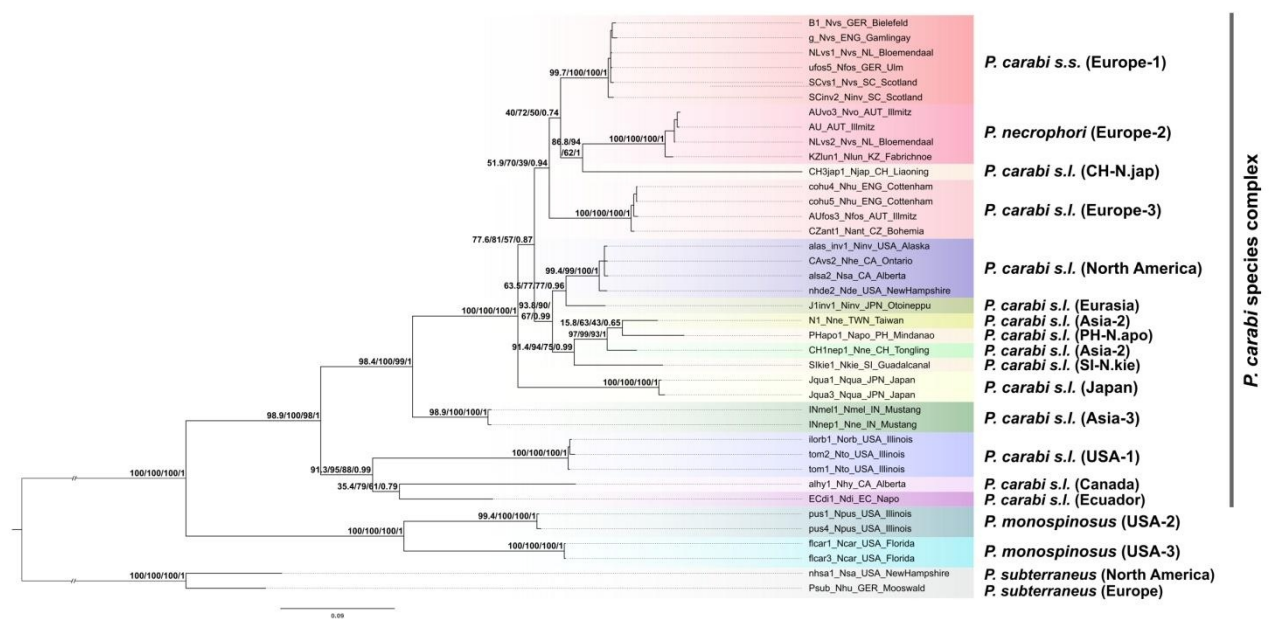411 clusters. Two *P. subterraneus* samples served as the outgroup (Figure 3).

Figure 3: The phylogeny of *Poecilochirus carabi s.l.* 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 colors. Basal branches are trimmed and the scale indicates the estimated substitutions per site. Species names are those of the best-fitting species description.

412 Monophyly of the previously defined genetic clusters was confirmed by SH-aLRT,

413 UFBoot; SBS, and PP values. The topology depicted a basal separation into two clades

414 (PP=1; aLRT/UFBoot/SBS=100). One clade consisted of mites identified likely as *P.*

415 *monospinosus* (USA-2 and USA-3), while the other one included all clusters of *P.*

416 *carabi s.l.*. Within this *P. carabi s.l.* clade, the most recent common ancestor of the

417 USA-1, Canada, and Ecuador clusters split off first but the close relationship between

418 the Canada and Ecuador cluster showed lower branch support (aLRT=35.4;

419 UFBoot=79; SBS=61; PP=0.79). Subsequently, our tree shows first the Asia-3 and then

420 the Japan cluster branching off, both with high support values (PP=1;

421 aLRT/UFBoot/SBS>98). The remaining clusters diverged into two clades but this

422 separation was less confident (aLRT=77.6; UFBoot=81; SBS=57; PP=0.87). While the

423 close relationship between the North America/Eurasia and the Asian cluster had

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424 consistent support through all but one branch value (aLRT=93.8; UFBoot=90; SBS=67;

425 PP=0.99), values within the European/CH-N.jap/RUS-N.mor clade were low and varied

426 among analyses (Figure 3).

Divergence time analysis

428 The divergence time analysis was based on the COI and LSU gene alignment of 40

429 specimens covering 10 genetic clusters of *P. carabi s.l.,* 2 clusters from each *P.*

430 *monospinosus* and *P. subterraneus*, respectively, and 15 additional Mesostigmata taxa.

431 Certain taxa were represented by chimeric sequences, meaning that the COI and LSU

432 sequences did not originate from the same individual but from the same genus or family

433 (Supplement Table S2).

434 The phylogenetic tree generated by Beast2 had high support values at all but two

435 branches (split between Phytoseiidae and Podocinidae: PP=0.79, and split among Asia-

436 2 samples: PP=0.81) (Supplement Figure S4). The relaxed-clock model suggested an

437 origin of the Mesostigmata in the Late Jurassic (156.2 Ma; 95% credibility intervals

438 [CI] 77 – 272 Ma; Supplement Figure S4) and the divergence into Parasitiae and

439 Dermanyssiae occurred approximately 40 million years later (114.0 Ma; 95% CI 66 –

440 189 Ma). Within the Parasitiae clade the first diversification occurred in the early

441 Eocene (53.2 Ma; 95% CI 26 – 90 Ma). The segregation of *P. subterraneus* was

442 suggested to occur in the mid Eocene (43.9 Ma; 95% CI 23 – 74 Ma). The *P.*

443 *monospinosus* clade branched off during the transition from the Eocene to the Oligocene

444 (34.7 Ma; 95% CI 18 – 59 Ma). Diversification of the *P. carabi s.l.* clade started in the

445 Oligocene with the separation of USA-1 (29.5 Ma; 95% CI 15 – 50 Ma). All remaining

446 divergence events occurred during the late Oligocene/Miocene (~ 5 – 25 Ma;

447 Supplement Figure S4).

448 Biogeography and ancestral-area estimation

449 Regarding the first two scenarios we tested (NALB/BLB vs. BLB-only), the

450 NALB/BLB scenario received higher log-likelihood and lower AICc values than the

451 BLB-only scenario in six out of nine models (Supplement Table S5). Hence, our data

452 better fit the assumption that mites dispersed via both the North Atlantic and Bering

453 Land Bridge. As the BAYAREALIKE model type yielded the lowest percentage value

454 of weighted AICc in both of these scenarios (< 4%; Supplement Table S5), we excluded

455 this model type from further analyses.

456 Within the NALB/BLB scenario, p-values of the LRT were significant when comparing

457 M0 and M1 (DEC: p=0.04; DIVALIKE: p=0.04), but were non-significant for the M1

458 and M2 comparison (DEC: p=0.06; DIVALIKE: p=0.08). Hence, the more complex

459 model including the parameter "x" which described the different levels of dispersal

460 difficulties (M2 model) was rejected for both model types.

461 Regarding the weighted AICc values, DEC+J and DIVALIKE+J yielded the highest

462 percentage with 39% and 45%, respectively (Supplement Table S6). In the third, the

463 time-stratified scenario, the comparison of nested models resulted in an acceptance of

464 the M1 model in all cases ($p_{(LRT)}<0.05$). The AICc and weighted AICc values were

465 lowest and highest, respectively, for the DIVALIKE+J model (Table 3).

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%

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466   A dispersal rate of d<0.001, an extinction rate of e=0.55, and a relative per-event weight

467   of founder-event speciation of j=1.28 was estimated. The results suggest that dispersal

468   and vicariance as well as founder-event speciation played an important role for the

469   biogeographic pattern of the mites. The most likely ancestral distribution areas of the

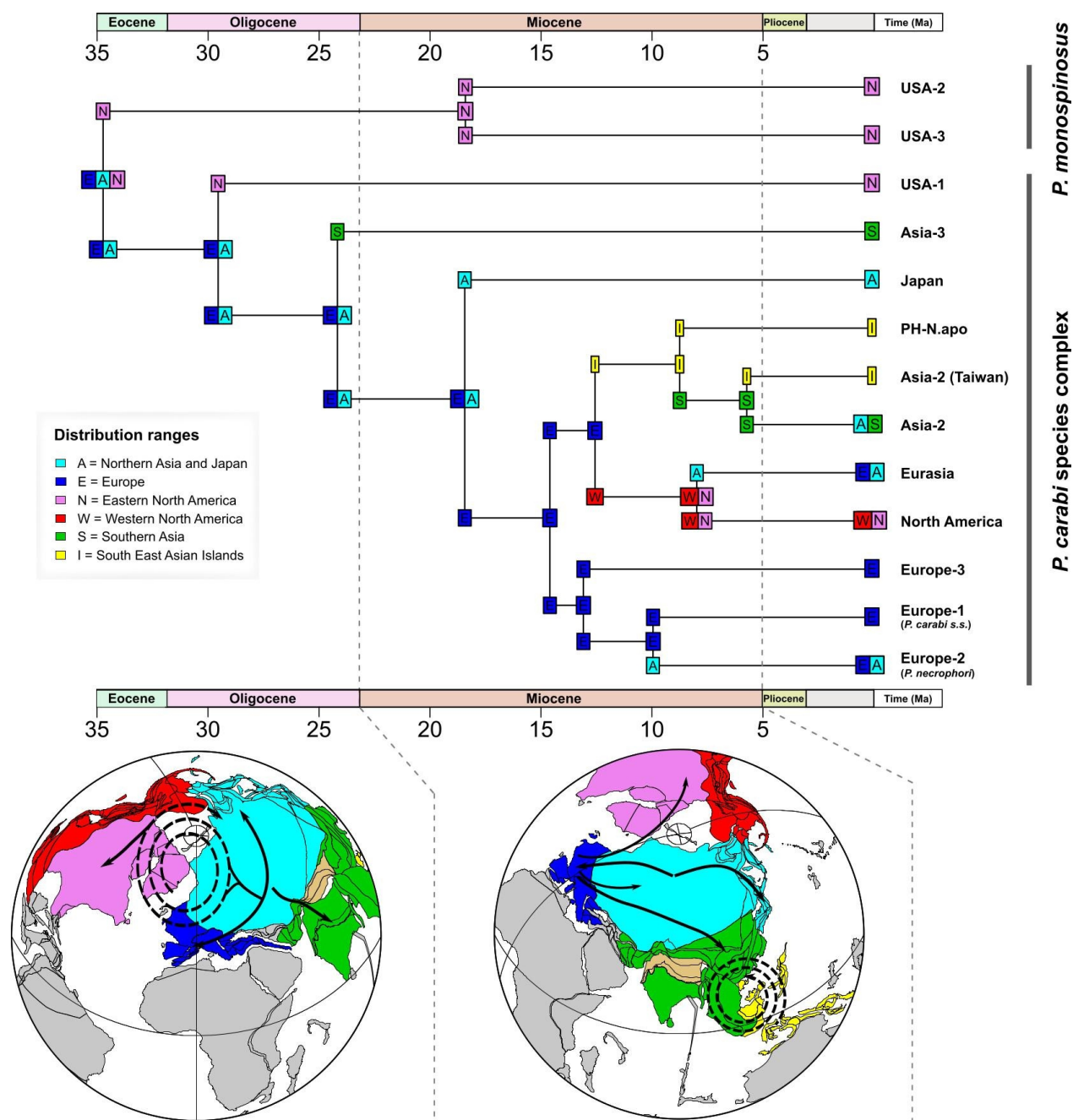470   time-stratified DIVALIKE+J model are visualized in Figure 4.

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.

471   The distribution of the last common ancestor of *P. cabi s.l.* and *P. monospinosus* was

472   estimated to range from Eurasia (E and A) to Eastern North America (N). Vicariance

473   was inferred in the branching off of the common ancestor of the USA-2 and the USA-3

474   cluster (EAN → EA+N), and of the ancestor of the Japan cluster (EA → E+A). Six

475   long-distance dispersals with founder-event speciation were suggested to explain the

476   origin of both the USA-1 and the Asia-3 cluster (EA → EA+N; EA → EA+S), the

477   divergence of the Europe-1/Europe-2 clusters (E → E+A) and the North

478   America/Eurasia clusters (WN → WN+A), and two cladogenesis events within the

479   Asian clade (I → I+S; S → S+I). However, the proportion of the most likely ancestral

480   states deviated just slightly at several cladogenesis events (Supplement Figure S5).

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Discussion

481 Our study identified 24 distinct genetic *Poecilochirus* clusters including mites that

483 likely represent four different named species: *P. subterraneus, P. monospinosus, P.*

484 *carabi s.s.*, and *P. necrophori*. The phylogenetic and species delimitation analyses

485 indicate that many of the genetic clusters found within these morphological species are

486 likely to be cryptic species, most of which are to our knowledge not formally described.

487 We cannot infer with certainty the geographical origin of *Poecilochirus* with our data

488 set, but mites appear to have migrated more than once between Asia, Europe and North

489 America. We also found indication that some mite clusters are specialized on particular

490 *Nicrophorus* species, which may have driven speciation, but this pattern appears to be

491 largely concealed by the effects of multiple migrations between continents. It is difficult

492 to separate these interwoven factors in the evolution of mite species, obfuscating our

493 understanding of the importance of coevolution with hosts and sympatric speciation in

494 *Poecilochirus*. However, we can state with certainty that all speciation events we could

495 infer happened more than five million years ago, with no indication of recent speciation

496 events or ongoing segregation among extant populations.

Cryptic diversity and host specificity of *Poecilochirus* mites

498 Cryptic species have been uncovered by molecular investigations across many mite

499 groups (Beaurepaire et al., 2015; Knee, Beaulieu, Skevington, Kelso, & Forbes, 2012;

500 Knee et al., 2012; Schäffer & Koblmüller, 2020) and based on our molecular analyses,

501 we propose that *P. carabi s.l.* consists of at least 17 genetic clusters. Genetically,

502 samples within each cluster are very similar (p-distance < 2%), but clusters differ

503 clearly and consistently from each other with a COI divergence of at least 6% between

504 clusters. Given that the well-studied biological species *P. necrophori* and *P. carabi s.s.*

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505 (Baker & Schwarz, 1997; Müller & Schwarz, 1990; Nehring et al., 2017) are relatively

506 closely related within *P. carabi s.l.* and diverge by 10.21% in their COI sequences, we

507 suggest that most of the genetic clusters that we document, at least those for which we

508 sequenced enough replicates (e.g. Europe-3 or North America), represent separate

509 biological species, most of which are undescribed.

Poecilochirus subterraneus

511 *Poecilochirus subterraneus* served as the outgroup in our study. The species has

512 previously been observed in Europe (mostly on different *Nicrophorus* species; Hyatt,

513 1980; Korn, 1983), North America (*N. investigator, N. nigrita*; Grossman & Smith,

514 2008; Sikes et al., 1996), and Asia (*N. quadripunctatus*, Satou et al., 2000). Here, we

515 sequenced American and European mites resembling the *P. subterraneus* description

516 and found that while mites from both continents clustered together in our dataset, they

517 segregated into three distinct clusters, one from North America and two from Europe.

518 While species delimitation may be unreliable because of limited sampling among the *P.*

519 *subterraneus* clusters, our data indicate that *P. subterraneus* might be more diverse than

520 previously thought.

The clades *P. monospinosus* (USA-2 & USA-3) and *P. cf. austroasiaticus* (CH-N.con)

523 The mites that morphologically resemble the description of *P. monospinosus* fall into

524 two separate genetic clusters that we found on two different host species. USA-3 (n=5)

525 have been sampled from *N. carolina* in Florida only. We did not have any samples

526 available from other beetle species in Florida, thus we cannot speculate about any

527 potential specialization on *N. carolina*.

528 We found USA-2 mites only associated with *N. pustulatus*. This host association

529 persisted across two locations. In both, *N. pustulatus* occurs sympatrically with other

530 host species (*N. orbicollis*, *N. tomentosus*). In our data set, only one mite from another

531 genetic cluster was found on *N. pustulatus*. USA-2 was also the cluster with the second

532 lowest Shannon-Wiener index and evenness, and by far the highest χ^2 ratio, a measure

533 that takes into account the sampling data quality of the cluster in question. USA-2 thus

534 appears to be a strict monospecific host specialist on *N. pustulatus*. *Nicrophorus*

535 *pustulatus* has a unique ecology; it has been reported to reproduce on snake eggs and in

536 bird nests on dead nestlings, and it occurs predominantly in the forest canopy, while

537 other *Nicrophorus* species are typically found near the ground (DeMarco & Martin,

538 2020; Smith et al., 2007; Wettlaufer et al., 2018). *Nicrophorus pustulatus* thus occupies

539 a distinct ecological niche that may isolate the mites, and possibly select for adaptations

540 specific to this niche. Other families of phoretic mites associated with *N. pustulatus*

541 showed no apparent host specificity for this beetle species (Knee, 2017; Knee et al.,

542 2012), indicating that this beetle's unique niche has not caused mite divergence in every

543 case. Previously, *P. monospinosus* had only been described from poultry manure,

544 preying on fly eggs and larvae – it has not been documented on beetle hosts (Wise et al.,

545 1988). This begs the question whether mites of the original description are an aberrant

546 lineage without beetle association or whether *P. monospinosus* is more general in its

547 host usage and occurs with and without beetles.

548 The mite individual from *N. concolor* found in Central China (Sichuan Province) is

549 particularly interesting because morphologically it resembles *P. austroasiaticus* more

550 than *P carabi s.s.* or any other described species. A discovery of this species in Central

551 China and the association with *N. concolor* is an unexpected observation as so far *P.*

552 *austroasiaticus* has only been recorded in Siberia/Northwestern China and Europe on

553 animal corpses, or in association with silphid beetles including *N. investigator* (Hyatt,

554 1980; Makarova, 2013). According to the phylogenetic approach of cluster

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555   identification (IQtree analysis), this singleton is closely related to the clade of *P.*

556   *monospinosus* (Supplement Figure S2).

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

558   We found three clusters that are almost exclusively distributed in Europe and are closely

559   related. We can unequivocally assign the clusters Europe-1 and Europe-2 to the species

560   *P. carabi s.s.* and *P. necrophori* because we tested the host preference of some mites

561   before killing them (Schwarz, 1996). Based on their association with different beetle

562   species, these mite clusters appear to either prefer or to avoid certain hosts, which is in

563   agreement with observations on the host range of the two reproductively isolated mite

564   species (Müller & Schwarz, 1990; Nehring et al., 2017; Schwarz, 1996; Schwarz et al.,

565   1998). Several *Nicrophorus* species occur sympatrically in Europe, and most of them

566   overlap in their seasonal reproductive period but differ in seasonal and diel activity and

567   habitat use (Dekeirsschieter et al., 2011; Esh & Oxbrough, 2021; Majka, 2011; Müller

568   & Eggert, 1987; Schwarz & Koulianos, 1998; Scott, 1998). Thus, as earlier work

569   already suggested (Schwarz, 1996), the European mite generalist (Europe-1 = *P. carabi*

570   *s.s.*) exploits several host species with different life history traits, while *P. necrophori*

571   (= Europe-2) is specialized on *N. vespillo*, which prefers open area habitats where other

572   *Nicrophorus* species are less abundant (Esh & Oxbrough, 2021).

573   We found a third cluster of mites from across Europe: Europe-3. At the moment, this

574   cluster is a curious case because it is widespread across Europe but was not found in

575   Germany, where most of our samples were collected. The three European clusters were

576   found in sympatry in some locations (France, Austria), indicating that they do not

577   necessarily competitively exclude each other. Sun and Kilner (2019) described a *P.*

578   *carabi s.l.* population from the United Kingdom that differs in its phenotype from *P.*

579   *carabi s.s.*. While this population may correspond to *P. necrophori*, it is tempting to

580 speculate that it is in fact our Europe-3 cluster, given that we did not find any *P.*

581 *necrophori* among the 16 mites from the UK.

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

583 The ecology and behavior of the North American *P. carabi s.l.* is quite well studied,

584 although not to the same extent as the European populations. Brown & Wilson (1992)

585 reported two reproductively isolated populations from Michigan that differed in

586 morphology, and their preference for host species of *Nicrophorus*. We were not able to

587 obtain any reference samples from Michigan but our analysis confirms the occurrence

588 of at least two genetic clusters from Northeastern America (USA-1, North America) in

589 addition to *P. monospinosus*. We also found evidence for further clusters from Canada

590 and South America.

591 The North America cluster was the most diverse in terms of host species numbers, and

592 the cluster did not appear to prefer any specific host species among those occurring

593 across its distribution range. Such a broad host range increases the independence of host

594 abundance, seasonal and diel activity, and other life history traits. In comparison, USA-

595 1 was found almost exclusively on *N. orbicollis* and *N. tomentosus* in the northeastern

596 USA, which might be an indication of local specialization on two host species. Being a

597 local specialist on two sympatric *Nicrophorus* beetles with different seasonal activity

598 (Brown & Wilson, 1992; Keller et al., 2019; Scott, 1998; Wilson, 1982) could expand

599 the reproductive period of the mites.

600 Some previously described populations of *P. carabi s.l.* from Michigan indeed

601 reproduced successfully using various host species, but others were local specialists

602 (Brown & Wilson, 1992, 1994; Wilson, 1982). In our data, we found no evidence of any

603 clusters being strict specialists for either *N. orbicollis* or *N. tomentosus*, as had been

604 reported by Brown and Wilson (1992) for the Michigan populations. While it is possible

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605   that these Michigan populations belong to genetic clusters that we did not sample, it

606   may also be that genetic clusters are specialists in one community and less strict in their

607   host choice in another (Brown & Wilson, 1992).

Asian diversity

609   Our Asian samples cover a great number of sampling locations and host species, but we

610   could only analyze a few or one replicate individuals for most of the genetic clusters.

611   Thus, any ecological inference is impossible and we may have only scratched the

612   surface of the biodiversity of *Poecilochirus* mites that use *Nicrophorus* as hosts in Asia.

Phylogenetic inference

614   Our phylogeny provides a basic overview of the relationships among *Poecilochirus*

615   mites. Maximum likelihood and Bayesian Inference analyses reveal congruent tree

616   topologies without polytomous relationships among the clusters. We applied different

617   branch support methods (SH-aLRT, UFBoot, SBS, PP) as their accuracy is debated and

618   confidence levels can vary (Anisimova et al., 2011; Pyron et al., 2011). Relationships

619   between clusters are resolved at deeper levels and in some derived clades, as indicated

620   by well-supported branches across all methods. Support values of a medium range (50 <

621   SH-aLRT/UFBoot/SBS < 75; 0.5 < PP < 0.75) occur mainly in more derived

622   relationships and reveal higher values for posterior probabilities than for bootstrap

623   approximations. Such deviations occur because bootstrap values are a more

624   conservative support measure than Bayesian posterior probabilities which can produce a

625   higher false-positive rate (Anisimova et al., 2011; Cummings et al., 2003; Erixon et al.,

626   2003). According to the variation of support values at some branches, certain

627   phylogenetic relationships between European, Asian, and North American clusters

628   should be interpreted with caution. Low support values could result from

629   inconsistencies between the gene trees as we used a concatenated supermatrix of COI,

630   ITS, and LSU, with a partitioning approach. Branch lengths are analogous across the

631   analyses and express an adequate amount of genetic change between internal nodes.

632   Certain branches are in fact relatively long (e.g., USA-2, USA-3 and CH-N.jap). This

633   high level of genetic change could be due to an accelerated rate of evolution, a high

634   extinction rate leaving only one member of a radiation, or an underrepresented diversity

635   caused by incomplete taxon sampling.

636   However, the congruent tree topology inferred by all analyses, the medium to high

637   support values, the appropriate branch lengths, and the exclusive dichotomy all indicate

638   a high degree of robustness for this phylogeny. The genes we concatenated for this

639   phylogenetic reconstruction already provided sufficient genetic information individually

640   to distinguish between morphologically described species in other groups of mites

641   (Lehmitz & Decker, 2017; Lv et al., 2014; Schäffer & Koblmüller, 2020). In general,

642   the mites showed low morphological variability, despite their high genetic divergence,

643   but the combined approach of molecular and morphological techniques helps us to

644   better understand the species boundaries and cryptic diversity in this unique group of

645   mites.

646   **Evolutionary history and biogeography**

647   Our data suggest a split between the ancestors of *P. carabi s.l.* and the *P. monospinosus*

648   clade during the Eocene/Oligocene and a further radiation within *P. carabi s.l.* in the

649   Miocene. During this period, most of the *Nicrophorus* diversity already existed (Sikes

650   & Vernables, 2013). Although the geographic origin of their common ancestor cannot

651   be stated with certainty, the ancestral area of the *P. monospinosus* clade is clearly the

652   North American continent. *Poecilochirus carabi s.l.* might have originated in Eurasia

653   with an early dispersal to the New World (USA-1). The likelihood proportions of the

654   most likely ancestral areas differ only slightly at this cladogenesis event (Supplementary

655 Figure S5), but regardless of ancestral area, the mites moved between the New and Old

656 World during the Eocene/Oligocene. The Miocene diversification of *P. carabi s.l.* took

657 place in Eurasia with at least one colonization of the New World that is less debatable in

658 terms of dispersal direction (resulting in the North America clade). In both epochs

659 (Eocene/Oligocene and Miocene), a connection between Eurasia and North America by

660 the Bering and North Atlantic land bridges is assumed (Brikiatis, 2014; Denk et al.,

661 2010; Graham, 2018; Jiang et al., 2019; Tiffney, 1985). Although the Bering Land

662 Bridge is often considered the only relevant connection between the continents for floral

663 and faunal migration (Lee et al., 2020; Wen et al., 2016), the assumption that the mites

664 used both land bridges fits our data better. Hence, a closer look at the phylogenetic

665 relationships of beetles and mites occurring near the North Atlantic (e.g., Western

666 Europe; Eastern Canada) would be useful in assessing the role of a North Atlantic Land

667 Bridge and its suitability for the dispersal of small organisms. Regardless of the routes

668 on which the mites migrated between continents, Europe might be a pivotal starting

669 point for their dispersal during the Miocene.

670 In Southern Asia, mites colonized multiple areas. As this region experienced several

671 geological and climatic changes since the early Miocene that could have resulted in the

672 origin of new geographical barriers (e.g., sea level changes and aridification: Bird et al.,

673 2005; Miao et al., 2012; Zhisheng et al., 2001), vicariant speciation might have

674 contributed to the scattered pattern of Asian clusters.

675 We would like to emphasize that models including the "jump dispersal" parameter were

676 most-likely in all biogeographic scenarios, which highlights the importance of founder-

677 event speciation for the evolution of *Poecilochirus* mites. Furthermore, the results of the

678 tested biogeographic model types DEC+J and DIVALIKE+J deviate just slightly among

679 all analyses. This indicates that dispersal with extinction and vicariance are key

680 processes for understanding the historical biogeography of this species complex.

681 **Drivers of speciation**

682 Based on our phylogenetic analyses, geographic separation is responsible for the main

683 divergence among *P. carabi s.l.* lineages, while the specialization on certain beetle

684 species did not play a significant role on a global scale. Although host-parasite

685 relationships are seen as an important driver of sympatric speciation, co-speciation with

686 the host beetles can be largely ruled out for the *Poecilochirus* in our study since the

687 main mite diversification happened at least 40 million years after the radiation of the

688 burying beetles (~ 75 Ma, Sikes & Venables, 2013).

689 Because the mites are small and cannot fly, the widely separated distribution areas of

690 the mite clusters are likely due to beetle mobility rather than mite mobility. This may

691 have also facilitated founder events since beetles typically carry several mites, thus

692 increasing the probability that individuals arriving in new areas will be able to find a

693 mate. The holarctic species *N. vespilloides* and *N. investigator* are of particular interest

694 as both species dispersed either from the New to the Old World (*N. vespilloides*) or vice

695 versa (*N. investigator*) (Sikes et al., 2008; Sikes & Venables, 2013). Mites carried by

696 these beetle species appear in multiple genetic clusters, some of which are also closely

697 related (e.g. Eurasia and North America cluster). This suggests that both *Nicrophorus*

698 species played a major part in the dispersal and evolution of *P. carabi s.l.*.

699 Within the continents, further radiation seems to have taken place, with some lineages

700 specializing on certain hosts (e.g USA-2, Europe-2) and others having a broader host

701 range (Europe-1, North America). In this context, European populations may have

702 radiated in sympatry. Their hosts use different microhabitats to which the mites may

703 have adapted. For example, *N. vespillo*, host to the specialized *P. necrophori*, is more

704 common in meadows, while *N. vespilloides* is more abundant in forested areas in

705 Germany, the UK, and Alaska (Majka, 2011; Scott, 1998, Sikes et al., 2016). This may

706 have caused genetic divergence among the mites, both through drift after spatial

707 separation and ecological adaptation. Since meadows are more sun-exposed, *N. vespillo*

708 and its mites may be adapted to warmer temperatures. When kept at the same

709 temperature, *N. vespilloides* develops quicker than *N. vespillo*, which may be the result

710 of countergradient variation across the two species (Conover & Schultz, 1995; Müller &

711 Schwarz, 1990). The mites they carry track this difference in their own development

712 time, which may either be a direct adaptation to temperature or an indirect one - because

713 mite development needs to be completed before beetle development for optimal

714 dispersal (Müller & Schwarz, 1990; Nehring et al 2017; see Brown & Wilson, 1992 for

715 a similar effect in American populations). Selection on mite development time (e.g. in

716 the event of a host switch) can lead to rapid adaptation in development time and

717 correlated changes in other traits through hitchhiking or pleiotropy (Schedwill et al.,

718 2018). These effects could cause reproductive isolation among the differentially

719 selected mite populations (Nosil & Harmon, 2009), and thus host specialization can

720 drive genetic divergence. Indeed, a relationship between genetic divergence and host

721 specificity has also been reported from other parasites, such as the honey bee parasite

722 *Varroa* (Beaurepaire et al., 2015) and *Macrocheles* species that are associated with

723 *Nicrophorus* beetles (Knee, 2017). Genetic clustering driven by host adaptation is also

724 found in feather mites that are phoretic on seabirds (Stefan et al., 2018). Host specificity

725 is often seen as a species-level trait. However, it should be considered that local

726 populations of one and the same species could encounter different host communities and

727 may thus specialize on different hosts, an excellent subject for future studies (Brown &

728 Wilson, 1992; Korallo-Vinarskaya et al., 2009; Thompson, 2009).

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Conclusions

Our global analysis of the *P. carabi* species complex revealed a surprisingly high genetic diversity. Our data support previous ecologically and morphologically defined species clades for *P. necrophori*, *P. carabi s.s.*, *P. monospinosus,* and *P. cf. austroasiaticus*. In general, phylogenetic relationships between the mite clusters did not match those of the beetles (Sikes & Venables, 2013).

Drivers of genetic diversification differ depending on the geographic scale. Globally, spatial separation between continents can explain the deep splits between clades relatively well, although back-migrations among continents are obvious. Within continents, further diversification appears to have occurred that was independent of separation by oceans (e.g. among Europe 1-3). While this may have happened due to spatial separation on smaller scales that we cannot track, it may have also been driven by ecological factors like adaptation to different host species. Separate mite clusters may evolve in sympatry by ecological adaptation directly to local hosts or to the abiotic environment the hosts live in. The former is most likely for the apparent host specialists that we have identified in our dataset. It is still uncertain in which cases the mites may adapt to the host species or just take any opportunity for dispersal and/or reproduction. However, the close association and occurrence with their beetle hosts shaped the evolution of *Poecilochirus* mites, and putative drivers depend on the host communities as well as the features of their biotic and abiotic environment.A taxonomic revision of the genus in future investigations would greatly facilitate our understanding of speciation and biogeographic processes in this species complex and genus.

Acknowledgments

Supplemental information

Supplement Table S1: Overview of single samples with cluster assignment, sampling time and location, host species, country, storage, morphological affiliation, and cluster affiliation.

Supplement Table S2: Downloaded COI and LSU sequences of the Mesostigmata taxa with accession number and references. Sequences are used for the divergence time analysis.

Supplement Table S3: Fossil data used in the divergence time analysis. Listed are the taxonomic state (superfamily, family and/or genus), age in million years ago [Ma].

Supplement Table S4: Morphometric measurements of specimens underwent DNA extraction. Measurements are listed for the podosomal length and width, sternal plate length, opithosomal length, the z1, s1, s2, r3, st4, the macroseta leg IV, and the length of digitus mobilis. All measurements are in µm.

Supplement Table S5: Comparison table of likelihood and sample corrected Akaike Information Criteria values (AICc) for the NALB/BLB and BLBonly scenarios inferred by the BioGeoBears analysis.

Supplement Table S6: Statistics of the BioGeoBear analysis to test for the best fitting biogeographic model considering the scenario NALB/BLB. Log likelihood values for each model are calculated and nested models (M0 vs. M1 and M1 vs. M2) are tested using the Likelihood ratio test (LRT) with its p-values. Non-nested model comparisons are evaluated by the sample corrected Akaike Information Criteria (AICc).

Supplement Figure S1: Sample distribution. Coloration of the world map indicates the annual temperature obtained from WorldClim data (Bioclimatic variable BIO1 – Annual Mean Temperature, www.worldclim.org). Green shaded areas represent the current distribution area of *Nicrophorus* and each dot represents one sample locality of the mite sample set.

794 **Supplement Figure S2:** Phylogenetic reconstruction of the entire *Poecilochirus* dataset
795 obtained with IQtree. Branch labels represent SH-aLRT and ultrafast bootstrap values in
796 percentage. High branch support is suggested with SH-aLRT > = 80% and UFBoot > =
797 95%. Clades which show short branch length and/or polytomy have been collapsed.
798 Clade/Cluster labels are assigned to the right.

799 **Supplement Figure S3:** Species delimitation analysis performed with the mPTP tool.
800 The analysis is based on the entire *Poecilochirus* data set and its resulting IQtree
801 phylogeny. The analysis inferred a total of 23 species (including the 3 *P. subterraneus*
802 species). The diagram shows the likelihood distribution of the 4 MCMC runs over 10
803 million iterations.

804 **Supplement Figure S4:** Time-calibrated tree of the Mesostigmata taxa with the focus
805 on divergence times of the *P. carabi* species complex generated by Beast2. Represented
806 branch labels indicate posterior probabilities below 0.9, branches without labeling have
807 support values above 0.9. The chronogram tree shows mean node ages and node bars
808 represent a 95% credibility interval. Time scale on the bottom refers to millions of years
809 ago. Red circles depict fossil calibration points.

810 **Supplement Figure S5:** Illustration of the ancestral state estimation with proportions of
811 all ancestral states at each node depicted as pie charts. Values are calculated with the R
812 package BioGeoBears and represent the results of the DIVALIKE+J model conducted
813 by the time-stratified analysis. The legend shows all possible ancestral state
814 combinations allowed by the analysis settings.

Author contributions

816 JC conducted bioinformatic analyses, conceptualized and drafted the manuscript.

817 AKE, WK, DSS edited the manuscript

818 JC, PH, WK, MN, NS conducted molecular wet lab work and initial sequence analyses

819 JB identified and measured mite specimens

820 WH, PH, WK, VN, NS, DSS contributed mite specimens

821 VN conceived and supervised the project, co-wrote the manuscript

Data Accessibility

DNA sequences: Genbank accessions MW890765 – MW890966 (COI), MW893012 – MW893060 and MW893063 – MW893153 (ITS), and MW893154 – MW893193 and MW893196 – MW893239 (LSU) are provided at NCBI.

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