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Phoretic or not? Phylogeography of the pseudoscorpion *Chernes hahnii* (Pseudoscorpiones: Chernetidae)

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Abstract. An organism's ability to respond to ecological changes at its currently inhabited location, and to colonize a new one, is particularly important for organisms inhabiting ephemeral habitats. Phoresy, which involves attaching of a non-vagile individual to a selected carrier of a different species, is used by a wide variety of taxa, but surprisingly little is known about the genetic structure of phoretic species. A better understanding of their genetic structure would help elucidate the efficacy of this manner of dispersal. In this study, we analyse the phylogeographic patterns of the pseudoscorpion *Chernes hahnii* (C.L. Koch, 1839) across a 1830 km range, encompassing most of the species' distribution range in Europe. The lack of geographic structure and low divergences within the two main clades suggest that *C. hahnii* disperses by phoresy. Individuals shared haplotypes at localities 350 km apart and very little divergence was detected between localities over 1450 km away from each other, indicating that phoresy is a very efficient manner of dispersal in this species. We also detected highly divergent populations within *C. hahnii*; however, more material and additional data would be necessary in order to evaluate the potential existence of cryptic diversity within this species.

Keywords: Arachnida; dispersal; Europe; lack of geographic structure; phoresy

An organism's dispersal capability plays a key role in colonizing new habitats and is one of the main factors behind the distribution patterns of individual taxa (Bernatchez & Wilson 1998; Michalak et al. 2010; Gittenberger 2012; Wahlberg & Johanson 2014). The ability to leave a currently inhabited site and find a new one after unfavourable ecological changes or habitat degradation is particularly important for organisms living in temporary habitats such as decomposing wood, animal carrion, faeces, ephemeral streams, vernal pools, or host organisms as in the case for parasites. Many organisms with low dispersal capability evolved different strategies of colonizing new habitats by means of passive transportation (Hulsmans et al. 2007; Macchioni 2007; Vanschoenwinkel et al. 2008). Phoresy (Beier 1948) is a type of passive transportation that involves attachment of a non-vagile individual to a selected carrier from a different species. Phoresy is used by a wide variety of animal groups; e.g., nematodes (Okumura & Yoshiga 2014; Pimentel et al. 2014), crustaceans (Brochet et al. 2010; Sabagh et al. 2011), bryozoans (Brochet et al. 2010), mollusks (Gittenberger 2012), insects (Bartlow et al. 2016; Hastriter et al. 2017) and arachnids. Among arachnids it is particularly common in mites (Barton et al. 2014; Hastriter & Bush 2014; Keum et al. 2016; Pfammatter et al. 2016) and pseudoscorpions (Muchmore 1971; Poinar et al. 1998), but there are also records for spiders (Camargo et al. 2015) and ticks (Saloña-Bordas et al. 2015). Surprisingly, there is very little information about the genetic structure of phoretic organisms (Wilcox et al. 1997; Zeh et al. 2003; Pfeiler et al. 2009; Harvey et al. 2015); however, this information would help evaluate the efficacy of this manner of dispersal.

Phoresy is known from at least 10 pseudoscorpion families (Poinar et al. 1998), where the most common hosts are Coleoptera and Diptera (Poinar et al. 1998). There are also records of phoretic dispersal of pseudoscorpions on vertebrate carriers such as birds (Harvey et al. 2015) and bats (Finlayson et al. 2015), which may play an important role in colonization of cave systems (Moulds et al. 2007). The carrier host specificity varies depending on the species. Most species use multiple carriers, for example a variety of different beetle or fly species, or a combination of both (Poinar et al. 1998), but some species appear to be ecologically linked to a specific carrier host (Zeh & Zeh 1992a, b). Pseudoscorpions usually attach themselves to their carrier's appendages (e.g., legs, wing bases, antennae) (Poinar et al. 1998) or crawl underneath the elytra in the case of beetles (Zeh & Zeh 1992b). Phylogeographic patterns have been studied in several phoretic pseudoscorpion species (Wilcox et al. 1997; Ranius & Douwes 2002; Zeh et al. 2003; Pfeiler et al. 2009). In some species, the populations showed significant structuring (Wilcox et al. 1997; Zeh et al. 2003; Pfeiler et al. 2009), while other species displayed very little genetic variation across populations (Ranius & Douwes 2002; Harvey et al. 2015). However, it is difficult to make assumptions about the efficacy of phoretic dispersal from these results. Deep structuring in some species was likely caused by the presence of cryptic diversity (Wilcox et al. 1997; Zeh et al. 2003), a relatively small area was sampled (Ranius & Douwes 2002), the species naturally occurs in a specific type of habitat that likely limits the species' distribution (Pfeiler et al. 2009), or the study involved only a couple of populations (Harvey et al. 2015).

Chernes hahnii (C.L. Koch, 1839) (Fig. 1) belongs to the family Chernetidae, one of the most diverse pseudoscorpion families with a cosmopolitan distribution (Harvey 2013). Its representatives can be found in a large variety of terrestrial habitats, and many species are specialized to patchy ephemeral habitats such as tree hollows or decaying plant material (Weygoldt 1969). *Chernes hahnii* is a medium sized pseudoscorpion (2.2 – 2.7 mm) with a wide distribution, ranging from Central Europe, northern parts of southern Europe to Caucasus and Central Asia, with sporadic records from eastern China (Fig. 1) (Harvey 2013). There are few records in the literature suggesting an even wider distribution; however, these may correspond to the congeneric species *C. cimicoides* (Fabricius, 1793) that is morphologically very similar to *C. hahnii* and misidentifications were likely until

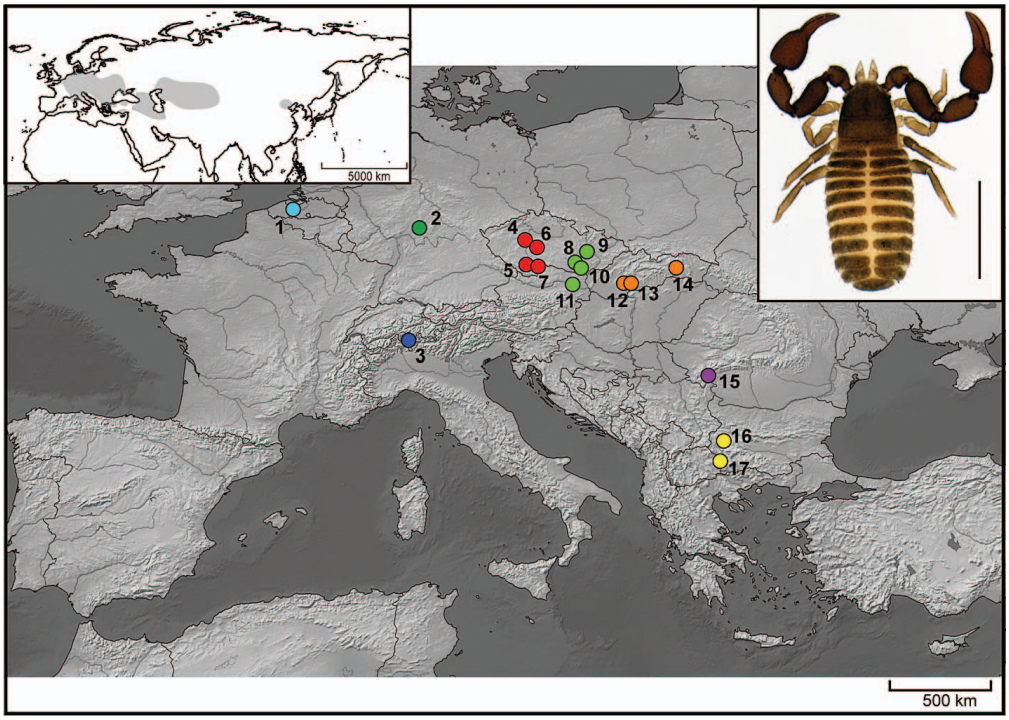

Figure 1.—Map showing sampling locations of *C. hahnii*. Upper left corner insert shows the distribution of species according to the Pseudoscorpions of the World catalogue (Harvey 2013), only references after species redescription by Beier (1960) are considered. Both maps were created with the help of online version of SimpleMappr (Shorthouse 2010). Upper right corner insert shows adult male of *C. hahnii* (bar = 1mm).

diagnostic characters were redefined in both species (Beier 1960). *Chernes hahnii* is mostly found under the bark of various deciduous trees, in tree hollows, in bird nests and nest boxes (Koch 1873; Beier 1948; Christophoryová et al. 2017). There is little information available about phoresy in *C. hahnii* and the earliest record (Schiner 1872) is possibly a misidentification (Beier 1948). However, the combination of the wide distribution range of the species and its presence under tree bark, in tree hollows and nests; i.e., the habitat typical for phoretic species, suggests that *C. hahnii* uses phoresy as a manner of dispersal. Alternatively, *C. hahnii* could also represent a species-complex harbouring multiple cryptic lineages that are geographically isolated, but unrecognizable on the basis of morphological characters. In this study, we analyse the phylogeographic patterns of *C. hahnii* from Central and Eastern Europe using mitochondrial data in order to establish whether the species uses phoretic dispersal, and to test its efficiency.

METHODS

Taxonomic sampling, PCR amplification, sequencing and sequence alignment.—The 33 individuals of *Chernes hahnii* used in this study were hand collected from under tree bark at 17 different locations across Europe, covering most of the species' known distribution range in this region (Fig. 1). *Chernes nigrimanus* Ellingsen, 1897 and *Dinocheirus panzeri* (C.L. Koch, 1837) (Chernetidae) were used as outgroups in the phylogenetic analyses. The outgroups were selected based on the results of an ongoing project focusing on diversity of European chernetids. In this project, we did not detect any ancestrally shared haplotypes/alleles between *C. hahnii* and any other *Chernes* taxa (Opatova et al. unpublished). Detailed locality information and Genbank accession numbers are provided in Table 1. The vouchers were deposited in the collections of the Department of Zoology, Faculty of Science, Charles University in Prague, Czech Republic.

Whole genomic DNA was extracted from the samples using the DNeasy Tissue Kit (Qiagen), according to the manufacturer's guidelines. A partial fragment of the mitochondrial gene Cytochrome oxidase I (*cox1*) (the animal barcode) was amplified for all individuals with the primer pair C1-J-1490/C1-N-2198 (Folmer et al. 1994). Partial fragments of two nuclear genes—28S rDNA (*28S*) and Histone H3 (*H3*)—were amplified for a small number of *C. hahnii* individuals (4 and 2 respectively) from different localities in order to assess the amount of variability of the gene fragments and their potential informativeness for the phylogenetic analyses. The fragments were amplified with the following primer combinations: 28SpsF1/28SpsR1 (Murienne et al. 2008) for *28S*, and H3a F/H3a R (Colgan et al. 1998) for *H3*. PCR conditions followed the protocol described in Opatova & Arnedo (2014), the PCR products were then purified using MinElute PCR Purification Kit (Qiagen) and sequenced in both directions by Macrogen Inc. (Seoul, South Korea). The chromatograms were assembled and edited in Geneious v. 5.3.6 (Drummond et al. 2010). The alignment of all three fragments (*cox1*, *28S*, *H3*) was trivial since no length polymorphism due to indel mutations was observed.

Phylogenetic analysis.—The best partitioning scheme and evolutionary models for each codon position of the *cox1* dataset were selected using the greedy algorithm in the

Table 1.—Locality data and GenBank accession numbers for the specimens sequenced in this study.

Species	Locality Name (Number)	Country	Lat/Long	Sample code	*cox1*
Dinocheirus panzeri	Hlohovec	Czech Republic	48.7738N 16.7822E	DINP1	MF538664
Chernes nigrimanus	Cervene Blato	Czech Republic	48.8560N 14.8016E	CHERN1	MF538665
Chernes hahnii	Litovelske Pomoravi I (9)	Czech Republic	49.7781N 16.9712E	8581	MF538666
Chernes hahnii	Litovelske Pomoravi II (9)	Czech Republic	49.7102N 17.0468E	8584	MF538667
Chernes hahnii	Litovelske Pomoravi III (9)	Czech Republic	49.7196N 17.0298E	8585	MF538668
Chernes hahnii	Magadino (3)	Switzerland	46.1549N 08.8629E	AlpenF1	MF538669
Chernes hahnii	Magadino (3)	Switzerland	46.1549N 08.8629E	AlpenF2	MF538670
Chernes hahnii	Magadino (3)	Switzerland	46.1549N 08.8629E	AlpenF3	MF538671
Chernes hahnii	Lilyanovo (17)	Bulgaria	41.6135N 23.3133E	BG2	MF538672
Chernes hahnii	Sofia (16)	Bulgaria	42.6960N 23.3280E	BG22	MF538673
Chernes hahnii	Prague I (4)	Czech Republic	50.0689N 14.4290E	Ch53	MF538674
Chernes hahnii	Prague I (4)	Czech Republic	50.0689N 14.4290E	Ch54	MF538675
Chernes hahnii	Prague I (4)	Czech Republic	50.0689N 14.4290E	Ch55	MF538676
Chernes hahnii	Prague II (4)	Czech Republic	50.0734N 14.4247E	Ch58	MF538677
Chernes hahnii	Prague II (4)	Czech Republic	50.0734N 14.4247E	Ch59	MF538678
Chernes hahnii	Brno (8)	Czech Republic	49.2515N 16.5750E	Ch60	MF538679
Chernes hahnii	Ceske Budejovice (5)	Czech Republic	48.974N 14.47817E	Ch64	MF538680
Chernes hahnii	Ceske Budejovice (5)	Czech Republic	48.974N 14.47817E	Ch65	MF538681
Chernes hahnii	Konopiste (6)	Czech Republic	49.7803N 14.6619E	CHERH1	MF538682
Chernes hahnii	Bad Hersfeld (2)	Germany	50.8684N 09.7011E	CHERH3	MF538683
Chernes hahnii	Modry kamen – Riecky (12)	Slovakia	48.2774N 19.3150E	GSK336	MF538684
Chernes hahnii	Filakovske Kovace (13)	Slovakia	48.2816N 19.7962E	GSK337	MF538685
Chernes hahnii	Kosice (14)	Slovakia	48.7224N 21.2641E	GSK338	MF538686
Chernes hahnii	Kosice (14)	Slovakia	48.7224N 21.2641E	GSK339	MF538687
Chernes hahnii	Kosice (14)	Slovakia	48.7224N 21.2641E	GSK358	MF538688
Chernes hahnii	Vienna (11)	Austria	48.2047N 16.3626E	M1	MF538689
Chernes hahnii	Vienna (11)	Austria	48.2047N 16.3626E	M2	MF538690
Chernes hahnii	Konopiste (6)	Czech Republic	49.7803N 14.6619E	M3	MF538691
Chernes hahnii	Beile Herculane (15)	Romania	44.8819N 22.4143E	M117	MF538692
Chernes hahnii	Ghent (1)	Belgium	51.0389N 03.7240E	M124	MF538693
Chernes hahnii	Ghent (1)	Belgium	51.0389N 03.7240E	M125	MF538694
Chernes hahnii	Lednice na Morave (10)	Czech Republic	48.8044N 16.8077E	M174	MF538695
Chernes hahnii	Lednice na Morave (10)	Czech Republic	48.8044N 16.8077E	M175	MF538696
Chernes hahnii	Bad Hersfeld (2)	Germany	50.8684N 09.7011E	M178	MF538697
Chernes hahnii	Straz nad Nezarkou (7)	Czech Republic	49.0692N 14.9094E	M184	MF538698

program PartionFinder v. 1.0.1 (Lanfear et al. 2012). The Bayesian inference (BI) analyses were conducted in MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) and run remotely at the CIPRES Science gateway v 3.3 (Miller et al. 2010). An independent evolutionary model (see Results) was defined for each codon position. Two independent runs of $5x10^7$ generations with 8 MCMC (Markov Chain Monte Carlo) chains each, starting from random trees and resampling each 1000 generations, were run simultaneously. The first 20% of the generations were discarded as a *burn-in* for the analyses. Convergence and chain mixing was assessed by standard deviation of split frequencies (<0.01) and ESS values summarized in TRACER v. 1.5 (Rambaut & Drummond 2009). Maximum Likelihood (ML) analyses were conducted in RaxML v.7.4.2 (Stamatakis 2006). An independent GTR+G substitution model was assigned to each codon position. The best maximum likelihood tree was selected from 1000 searches, while support of the nodes was assessed with 1000 replicates of bootstrap resampling. All trees were visualized and manipulated with the program FigTree v. 1.3.1 (Rambaut 2009).

Estimation of divergence times.—Divergence times were estimated for the full *C. hahnii cox1* dataset using the program BEAST v. 1.8.4. (Drummond et al. 2012). Because of the lack of relevant biogeographic events and no fossil record for the genus *Chernes*, we used the general arthropod mitochondrial substitution rate of 2.3% (Brower 1994). A lognormal prior was assigned to the ucld.mean parameter of the lognormal relaxed clock for *cox1* with an initial and mean value of 0.0115, each. A Coalescent – constant size model was set as a tree prior. A corresponding evolutionary model selected by PartionFinder was assigned to the *cox1* dataset, which was treated as a single partition.

Genetic divergence and haplotype network.—Genetic distances were calculated for the *cox1* dataset in MEGA 7 (Kumar et al. 2016) via both the uncorrected p-distance and the Tamura – Nei distance model (Tamura & Nei 1993; Tamura & Kumar 2002). Standard genetic diversity indices, including the nucleotide (π) and haplotype diversity (Hd) indices, were calculated in DnaSP 5.10.1 (Librado & Rozas 2009). The genetic distances and diversity indices were calculated for the entire *C. hahnii* dataset, the two main clades recovered in the Bayesian analyses and between them. Genetic distances using the Tamura – Nei distance model were also calculated among all sampled localities. The haplotype network was constructed with the program PopART (available online at http://popart.otago.ac.nz/) using the TCS method (Clement et al. 2000). Geographic distances among sampled locations were obtained in Geographic Matrix

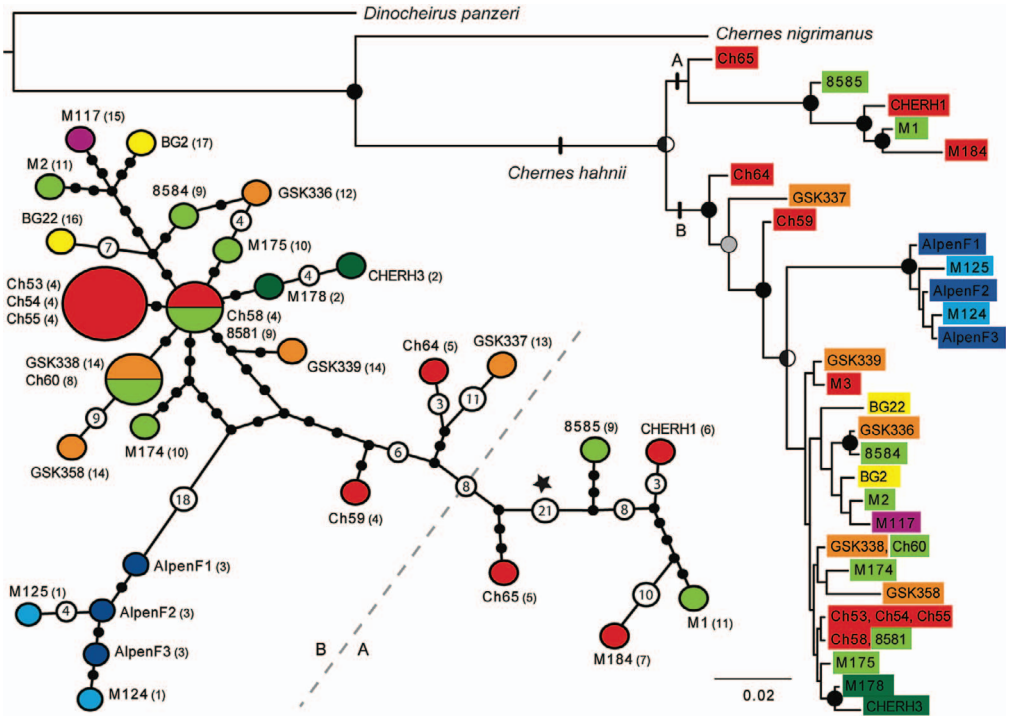

Figure 2.—Upper right corner shows topology obtained in the Bayesian analyses. Dots on nodes denote support as follows: right semi-circles are Bayesian posterior probabilities (PP) and left semi-circles are maximum likelihood bootstraps, black= PP> 0.95, ML bootstrap support > 70%, grey= same topology but with lower support values than thresholds above, white= topology not recovered in ML, nodes without dots= topology not recovered in ML and BI support lower than threshold. Left corner shows the haplotype network obtained by the TCS method. Four and more mutational steps between haplotypes or reticulation are depicted as numbers. Doted grey line corresponds to the clades recovered in BI analyses. Star marks the division in ML analyses. Haplotypes are colour-corded according to the sampling locations in Fig. 1 in both cases.

Distance Generator v 1.2.3. (Ersts, available online at http://biodiversityinformatics.amnh.org/open_source/gdmg).

RESULTS

A total of 35 specimens (33 *C. hahnii* samples, 2 outgroups) was used in this study (Table 1), sampled from the localities shown in Fig. 1. The sequence length for each gene fragment was as follows: *cox1:* 608bp *28S*: 1126bp and *H3:* 371bp. Thirty-one unique haplotypes (including outgroups) identified within the *cox1* dataset were used in ML and BI analyses. No variability was detected in the *28S* and *H3* datasets, therefore these gene fragments were not amplified for the entire *C. hahnii* sample and were not included in the analyses. Independent nucleotide substitution models selected by PartitionFinder for each codon position within the *cox1* dataset in BI were defined as follows: HKY+G was assigned to both 1st and 3rd positions and F81+I to 2nd position. HKY+G model was assigned to *cox1* in divergence time estimation analyses in BEAST.

In both maximum likelihood (-lnL=2121.852) and Bayesian analyses (Fig. 2), a large portion of the tree lacked support. Overall BI yielded support for a higher number of nodes. The topology was similar between the two methods in supported nodes, but differed in unsupported ones. Both methods recovered the monophyly of *C. hahnii* and supported its division into two main clades (clade A and clade B). The placement of the individual Ch65 differed between the analyses. In the BI, the individual belonged to clade A (as shown in Fig. 2), while in the ML it was placed as sister to all individuals in clade B. Successive branching of the individuals Ch64, GSK337, Ch59 at the base of clade B was recovered by both methods. Bayesian analyses further supported a division between a lineage comprising five individuals from Belgium and Switzerland (AlpenF1-F3, M124, M125) and all the remaining *C. hahnii* individuals with unresolved relationships. Haplotypes were shared by individuals from different localities in two instances (see details below), while individuals from both A and B clades co-occurred at three localities: individuals M1, M2 at loc. 11 in Austria, and loc. 6 (individuals CHERH1, M3) and loc. 9 (individuals 8584, 8585) in the Czech Republic.

Divergence time estimation analyses conducted in BEAST also yielded a poorly supported tree (Fig. 3). The overall topology was similar to the topology obtained in BI and the topology of the supported clades was similar to both BI and ML results. The basal split between the two *C. hahnii* clades was dated approximately to 0.09 Million years ago (Ma) (95% highest posterior density (HPD): 0.5644 – 0.0017). Clade A started diversifying at 0.035 Ma (0.23 – 0.0006), although this node was not supported, two supported nodes from clade A dated back to 0.018 Ma (0.122 – 0.0003) and 0.008 Ma (0.05 – 0.001). Clade B started diversifying at 0.044 Ma (0.257 – 0.0009) (unsupported node), the only supported deeper node from clade B dated to 0.027 Ma (0.171 – 0.0004).

We observed a lack of geographic structure in the haplotype network (Fig. 2). Nine individuals from Central Europe

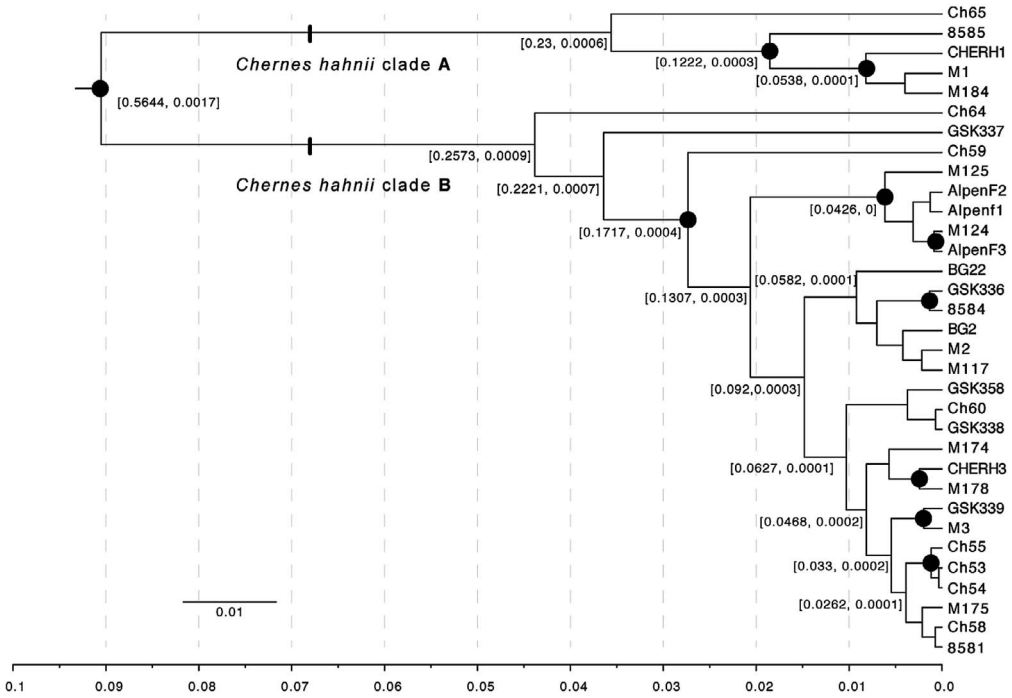

Figure 3.—Chronograms obtained in BEAST analyses. Dots on nodes denote Bayesian posterior probabilities above 0.95. Numbers in square brackets indicate the 95% HPD confidence intervals of the divergence time. The x-axis is time in million years (My).

presented very similar haplotypes. The centrally connected haplotype involving individuals Ch58 and 8581 from localities 4 and 9 in Czech Republic (185 km apart), respectively, connected with up to three mutational steps to another shared haplotype by individuals Ch60 and GSK338 from Czech Republic (loc. 8) and eastern Slovakia (loc. 14, 348 km apart), respectively. Furthermore, other individuals from Germany, Czech Republic and Slovakia were also connected with up to three different mutational steps. Haplotypes 7–9 steps away from the central haplotype belonged to samples that were either geographically distant (e.g., individuals from Bulgaria and Romania) or proximate (e.g., individuals from Austria and Slovakia). Individuals from Belgium and Switzerland were connected to the central haplotype by 24 – 31 mutational steps, while the distantly related samples that were placed into the same clade as the central haplotype (clade B) in the phylogenetic inference were up to 28 steps away. The samples from Czech Republic and Austria placed into clade A were 26 – 68 (BI) and 48 – 68 (ML) steps away from the central haplotype. The values of the diversity indices and haplotype distances within the *C. hahnii* dataset are reported in Table 2; distances calculated among the localities are reported in Table 3.

DISCUSSION

Pseudoscorpions can be found practically in all types of terrestrial habitats, but they are particularly abundant in leaf litter, tree hollows and under tree bark (Weygoldt 1969). Their dispersal capability by walking is very low and species that do not use phoresy for their dispersal, typically those inhabiting leaf litter, tend to have limited distributions and show tendencies to short-range endemism (Gardini 2013; Gardini 2014; Harrison et al. 2014; Cosgrove et al. 2016), where chromosomal differentiation within one population may constitute a reproductive barrier even on a relatively small geographic scale (Kotrbová et al. 2016). On the other hand, pseudoscorpions inhabiting temporary habitats, or those found under tree bark, compensate for their low vagility by dispersal via phoresy (Poinar et al. 1998). These species tend to have wider distributions (Harvey 2013) and previously conducted studies that employed molecular markers found in some cases relatively subtle population structuring (Ranius & Douwes 2002; Harvey et al. 2015) or haplotype sharing among localities (Zeh et al. 2003). Deep phylogeographic breaks were detected in some phoretic species (Wilcox et al. 1997; Zeh et al. 2003); however, the divergences were mostly attributed to the previously unrecognized cryptic diversity within these taxa (Wilcox et al. 1997; Zeh et al. 2003).

In this study, we sequenced *C. hahnii* individuals across an approximately 1830 km range, encompassing most of the European species' distribution in order to establish whether this species uses phoresy and how efficient is this manner of dispersal in arthropods. We uncovered surprisingly high haplotype diversity, but no clear geographic patterns were detected within the sampled area. Unfortunately, low supports of most of the internal nodes hamper the interpretation of the results of the phylogenetic analyses; however, fine-grain relationships among individuals can still be assessed from the haplotype network.

We detected shared haplotypes between Central European localities that were up to 350 km apart, and when calculating the distances among all combination pairs of sampled localities, we found less than 2% divergence in 35 pairs, which constitutes 28% of the whole. Populations that were furthest away from each other (1465 km) were from Bad Hersfeld (loc.

Table 2.—Uncorrected p-distances, Tamura – Nei genetic distances and diversity indices within *C. hahnii* dataset and the main clades recovered in Bayesian Analyses. N_{ind}: number of individuals π: nucleotide diversity, H: number of haplotypes, Hd: Haplotype diversity, S.E: Standard error, S.D: Standard deviation

C. hahnii	N_{ind}	p-distance [S.E]	Tamura – Nei [S.E]	π [S.D]	h	Hd [S.D]
Overall *cox1* dataset	33	0.039 [0.004]	0.038 [0.005]	0.036 [0.005]	29	0.991 [0.011]
Clade A	5	0.035 [0.005]	0.036 [0.006]	0.035 [0.008]	5	1.0 [0.126]
Clade B	28	0.025 [0.003]	0.026 [0.004]	0.023 [0.004]	23	0.987 [0.014]
Clade A vs. B	—	0.042 [0.006]	0.047 [0.007]	—	—	—

2) in Germany and Lilyanovo (loc. 17) in Bulgaria. Similar divergences that were considered "low" (2.6%), were found over the span of 1200 km between *Cordylochernes scorpioides* (Linnaeus, 1758) populations from Trinidad and French Guiana (Wilcox et al. 1997), shared haplotypes and low divergences were detected in another *C. scorpioides* lineage from Panama that was sampled across a 418 km span (Zeh et al. 2003). Even less divergence (1.1%) has been found between two populations of newly described pseudoscorpion species *Sociochelifer metoecus* (Harvey, 2015) (Cheliferidae) which has been collected in plumage and nests of the sociable weaver bird *Philetairus socius* (Latham, 1790) over a 440 km span in southern Africa (Harvey et al. 2015). Little geographic structuring was also detected in 2 allozyme loci in pseudoscorpion species *Allochernes wideri* (L.C. Koch, 1843) (Chernetidae) and *Larca lata* (Hansen, 1884) (Larcidae) sampled across 900 km in southern Sweden (Ranius & Douwes 2002). The patterns observed in the mitochondrial data of *C. hahnii* do not show the deep structuring typical for non-vagile pseudoscorpions (Harrison et al. 2014; Cosgrove et al. 2016), or the general lack of the haplotype diversity within populations documented from other sedentary arachnids (Opatova & Arnedo 2014). On the other hand, our results show similarities with the patterns reported from known phoretic species, such as low divergences among geographically distant localities (Wilcox et al. 1997; Ranius & Douwes 2002; Harvey et al. 2015). The combination of the above-mentioned features substantiates our hypothesis, that *C. hahnii* is not sedentary and likely disperses via phoresy.

The observed patterns (i.e., wide distribution, lack of geographic structure and haplotype sharing) could also be produced by accidental human-mediated introduction. Cases of introduction of other arachnids casually transported with trees are known from the Mediterranean (Pantini & Isaia 2008) and trading of infested timber, or its usage for packaging, can result in alien species' introduction over long distances (Meng et al. 2015). However, the ecological preferences of *C. hahnii* make the species an unlikely candidate for human-mediated dispersal. *Chernes hahnii* is known to prefer large deciduous trees in parks or open landscape (Beier 1960; Šťáhlavský 2001, 2011; Šťáhlavský & Chytil 2013) that are not of primary interest of commercial logging. The different structure and the absence of bark layering in young trees, essential for pseudoscorpions inhabiting the tree bark, also makes the species unlikely to be present on young trees (Šťáhlavský & Hörweg, pers. obs.) that are primarily used for park or urban landscaping. The wood of large deciduous trees was certainly logged in the past, but the means of transporting lumber were not as efficient as in modern day. The travelling time is one of the key aspects determining the success of a potential introduction. As logs become dryer over time, the survival of organisms associated with them decreases (Kobelt & Nentwig 2008). Successful introduction would also have to be followed by finding a new suitable microhabitat either by walking or hitchhiking on locally available carriers, which

Table 3.—Pairwise Tamura – Nei genetic distances based on cox1 mtDNA (above diagonal) and standard errors (below diagonal) among the sampled localities.

	Loc. 1	Loc. 2	Loc. 3	Loc. 4	Loc. 5	Loc. 6	Loc. 7	Loc. 8	Loc. 9	Loc. 10	Loc. 11	Loc. 12	Loc. 13	Loc. 14	Loc. 15	Loc. 17	Loc. 16
Loc. 1		0.051	0.006	0.044	0.057	0.079	0.078	0.045	0.06	0.043	0.073	0.047	0.071	0.049	0.047	0.045	0.047
Loc. 2	0.01		0.049	0.01	0.037	0.061	0.089	0.008	0.037	0.011	0.06	0.015	0.043	0.014	0.019	0.015	0.021
Loc. 3	0.002	0.009		0.042	0.055	0.078	0.076	0.043	0.059	0.042	0.072	0.047	0.068	0.046	0.047	0.043	0.045
Loc. 4	0.009	0.003	0.008		0.031	0.057	0.085	0.005	0.033	0.007	0.056	0.011	0.035	0.01	0.015	0.011	0.016
Loc. 5	0.01	0.007	0.01	0.006		0.053	0.061	0.029	0.04	0.035	0.054	0.035	0.029	0.031	0.042	0.039	0.039
Loc. 6	0.011	0.009	0.011	0.008	0.007		0.06	0.056	0.057	0.061	0.059	0.061	0.06	0.058	0.067	0.063	0.069
Loc. 7	0.013	0.014	0.013	0.014	0.011	0.009		0.089	0.072	0.085	0.054	0.089	0.075	0.086	0.098	0.093	0.096
Loc. 8	0.009	0.003	0.009	0.002	0.006	0.008	0.014		0.032	0.006	0.055	0.01	0.033	0.007	0.013	0.01	0.015
Loc. 9	0.009	0.006	0.009	0.005	0.006	0.008	0.011	0.005		0.034	0.057	0.032	0.046	0.035	0.038	0.036	0.041
Loc. 10	0.009	0.003	0.009	0.002	0.007	0.009	0.014	0.002	0.005		0.059	0.009	0.038	0.011	0.014	0.013	0.018
Loc. 11	0.01	0.009	0.01	0.008	0.007	0.008	0.008	0.008	0.008	0.009		0.057	0.059	0.057	0.06	0.058	0.064
Loc. 12	0.009	0.005	0.009	0.004	0.007	0.009	0.014	0.004	0.005	0.003	0.008		0.041	0.016	0.01	0.01	0.015
Loc. 13	0.012	0.009	0.012	0.008	0.007	0.009	0.013	0.008	0.007	0.008	0.008	0.009		0.034	0.044	0.041	0.047
Loc. 14	0.009	0.004	0.009	0.002	0.006	0.008	0.014	0.002	0.005	0.003	0.008	0.004	0.008		0.019	0.016	0.021
Loc. 15	0.009	0.005	0.009	0.005	0.008	0.009	0.015	0.005	0.006	0.004	0.009	0.004	0.009	0.005		0.007	0.019
Loc. 17	0.009	0.005	0.009	0.004	0.007	0.009	0.015	0.004	0.006	0.004	0.009	0.004	0.008	0.004	0.003		0.019
Loc. 16	0.009	0.006	0.009	0.005	0.008	0.011	0.016	0.005	0.007	0.005	0.01	0.005	0.01	0.006	0.006	0.006	

further limits the potential survival of the individuals. Human mediated introduction thus seems unlikely to play an important role in shaping the current distribution of *C. hahnii*.

There are differences in geographic patterns or distribution sizes among phoretic species (Wilcox et al. 1997; Zeh et al. 2003; Pfeiler et al. 2009) that could be related to the dispersal ability of the carrier species or its ecological preferences (Pfeiler & Markow 2011). In this case, the widest ranges and most homogeneous populations would be expected in species phoretic on birds. *Chernes hahnii* is known to inhabit bird boxes and nests (Turienzo et al. 2010; Christophoryová et al. 2011, 2017) and using birds as carriers could offer a better explanation for the species' reported presence in eastern China and Sakhalin Island (Schawaller 1995a, b), rather than hitchhiking on flies or beetles. However, there is no record about the species' presence in bird plumage so far. The results of this study suggest that the phoretic dispersal in *C. hahnii* is similarly or slightly more effective than in other species that hitchhike on beetles or flies (Wilcox et al. 1997; Ranius & Douwes 2002; Zeh et al. 2003; Pfeiler et al. 2009). In this respect, a thorough analysis of phylogeographic patterns of bird-phoretic species like *S. metoecus* would allow us to evaluate if the efficacy of phoretic dispersal could be as variable as for example in the aerial dispersal (i.e., ballooning) of spiders (Pedersen & Loeschcke 2001; Krehenwinkel & Tautz 2013; Opatova et al. 2016).

The phylogenetic analyses supported the division of *C. hahnii* into two main clades (clade A and B) with a sympatric distribution separated by a mean genetic distance (*d*) between 4.2% (uncorrected p-distance) and 4.7% (Tamura – Nei distance). The pseudoscorpions are a taxonomically challenging group and some widely distributed species may actually constitute cryptic species complexes that could be characterized by deep molecular divergences but otherwise display morphological stasis (Wilcox et al. 1997). Intraspecific divergences reported in the literature for putative cryptic species in two chernetid pseudoscorpions range from 2.6% in *Dinocheirus arizonensis* (Banks, 1901), where the main clades are referred to as "independent lineages" (Pfeiler et al. 2009) up to 13.4% in *C. scorpioides* species complex (Wilcox et al. 1997; Zeh et al. 2003). The sympatric occurrence and deeper divergence between clades A and B may be indicative of the presence of unrecognized diversity within *C. hahnii*, despite the fact that no morphological differences were observed between the two clades. The distances between some pairs of localities belonging to the different clades were particularly high. The highest divergences were found between Straz nad Nezarkou (loc. 7) in Czech Republic and localities in Bulgaria and Romania: Sofia (loc. 16, 9.6%, 962 km from Straz nad Nezarkou), Lilyanovo (loc. 17, 9.3%, 1057 km from Straz nad Nezarkou) and Biele Herculane (loc. 15, 9.8%, 735 km from Straz nad Nezarkou). Experimental crosses among populations of *C. scorpioides* revealed a postzygotic reproductive barrier among populations that showed sequence divergence of 11% (Zeh & Zeh 1994; Wilcox et al. 1997), which is much higher than the overall divergence value between clade A and B, but relatively close to the values of divergence among the above-mentioned populations. It is possible that individuals from these particular localities may potentially experience some degree of reproductive isolation as part of the speciation processes (Wilcox et al. 1997), while the reproduction between individuals from other localities could be unaffected. However, delimiting species solely based on *cox1* genetic divergence would most likely lead to taxa over splitting (Kekkonen & Hebert 2014). Additional material as well as ecological and behaviour data (or potentially crossbreeding experiments) would be necessary to confirm the presence of cryptic diversity.

Despite the poorly supported tree recovered in the divergence time analyses, the basal split of *C. hahnii* (dated to approximately 90,000 years ago (ya)), and the two oldest supported nodes in both clades (Clade A, 18,000 ya and Clade B, 27,000 ya) date to the most recent Quaternary glaciation. This glaciation began approximately 110,000 ya, reached its maximum in Europe around 22,000–25,000 ya and ended 11,500 ya, marking the beginning of the Holocene, which was characterized by sporadic local glacial advancements (Ivy-Ochs et al. 2009; Federici et al. 2017; Zens et al. 2017). The cold and dry climate during glaciation would cause thermophilic taxa to retreat to refugia with more suitable climatic conditions, typically located in the Mediterranean region and Caucasus (Hewitt 1996). Furthermore, the long-term isolation and smaller sizes of populations would facilitate fixation of genetic differences by genetic drift (Hewitt 1996, 2000, 2004; Bálint et al. 2008; Scheel & Hausdorf 2012). In the case of *C. hahnii*, it is possible that the species survived glaciation in multiple refugia and subsequently colonized the currently inhabited area. Similar cases are known from both vertebrate (Bernatchez & Wilson 1998; Taberlet et al. 1998) and invertebrate taxa (Habel et al. 2005). There is also an increasing amount of evidence that some taxa (including invertebrates) were able to survive glaciations in small local refugia outside of the Mediterranean region (Pauls et al. 2006; Provan & Bennett 2008; Tzedakis et al. 2013; Copilaş-Ciocianu et al. 2017). Dispersal via phoresy from multiple refugia across Europe and partial interbreeding among the formerly isolated population could potentially explain the lack of geographic structure we observe in the *C. hahnii cox1* data and the lack of variability in nuclear markers (data not shown, but see Results). A similar scenario has been put forward to explain the phylogeographic patterns observed in the pseudoscorpion *D. arizonensis*. In this case, the glacial cycles could have isolated the population on the Baja California Peninsula from the mainland one and the current overlap of their lineages could be a result of a secondary contact after area expansion via phoresy (Pfeiler et al. 2009).

CONCLUSIONS

The lack of geographic structuring and low divergences across a large portion of the *C. hahnii* distribution suggests that *C. hahnii* is a phoretic species and that phoresy in pseudoscorpions can be a very efficient manner of dispersal on larger geographic scales. Despite the poorly supported results of divergence time analyses, most of the deeper splits of *C. hahnii* dated back to the last glaciation. We hypothesize that the species survived the glaciation in multiple refugia and subsequently colonized the currently inhabited region, which could explain the existence of some highly divergent populations. However, more material and additional data concerning the biology of these populations is necessary in order to

evaluate the potential existence of cryptic diversity within *C. hahnii*.

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LITERATURE CITED

Bálint, M., P. Barnard, T. Schmitt, L. Ujvárosi & O. Popescu. 2008. Differentiation and speciation in mountain streams: a case study in the caddisfly *Rhyacophila aquitanica* (Trichoptera). Journal of Zoological Systematics and Evolutionary Research 46:340–345.

Bartlow, A.W., S.M. Villa, M.W. Thompson & S.E. Bush. 2016. Walk or ride? Phoretic behaviour of amblyceran and ischnoceran lice. International Journal for Parasitology 46:221–227.

Barton, P.S., H.J. Weaver & A.D. Manning. 2014. Contrasting diversity dynamics of phoretic mites and beetles associated with vertebrate carrion. Experimental and Applied Acarology 63:1–13.

Beier, M. 1948. Phoresie and Phagophilie bei Pseudoscorpionen. Österreichische Zoologische Zeitschrift 1:441–497.

Beier, M. 1960. *Chernes cimicoides* (F.) und *Chernes hahni* (L.C. Koch), zwei gut unterschiedene Arten. Zeitschrift der Arbaitsgemeinschaft Österreichischer Entomologen 12:100–102.

Bernatchez, L. & C.C. Wilson. 1998. Comparative phylogeography of nearctic and palearctic fishes. Molecular Ecology 7:431–452.

Brochet, A.-L., M. Gauthier-Clerc, M. Guillemain, H. Fritz, A. Waterkeyn, Á. Baltanás et al. 2010. Field evidence of dispersal of branchiopods, ostracods and bryozoans by teal (*Anas crecca*) in the Camargue (southern France). Hydrobiologia 637:255.

Brower, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences, USA 91:6491–6495.

Camargo, R.d.S., L.C. Forti, C.A.O. de Matos & A.D. Brescovit. 2015. Phoretic behaviour of *Attacobius attarum* (Roewer, 1935) (Araneae: Corinnidae: Corinninae) dispersion not associated with predation? Journal of Natural History 49:1653–1658.

Christophoryová, J., D. Grul'a & K. Krajčovičová. 2017. New records of pseudoscorpions (Arachnida: Pseudoscorpiones) associated with animals and human habitats in Slovakia and the Czech Republic. Arachnologische Mitteilungen 53:67–76.

Christophoryová, J., Z. Krumpálová, J. Krištofík & Z. Országhová. 2011. Association of pseudoscorpions with different types of bird nests. Biologia 66:669–677.

Clement, M., D. Posada & K.A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1660.

Colgan, D.J., A. McLauchlan, G.D.F. Wilson, S.P. Livingston, G.D. Edgecombe, J. Macaranas et al. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46:419–437.

Copilaş-Ciocianu, D., T. Rutová, P. Pařil & A. Petrusek. 2017. Epigean gammarids survived millions of years of severe climatic fluctuations in high latitude refugia throughout the Western Carpathians. Molecular Phylogenetics and Evolution 112:218–229.

Cosgrove, J.G., I. Agnarsson, M.S. Harvey & G.J. Binford. 2016. Pseudoscorpion diversity and distribution in the West Indies: sequence data confirm single island endemism for some clades, but not others. Journal of Arachnology 44:257–271.

Drummond, A., B. Ashton, M. Cheung, J. Heled, M. Kearse, R. Moir et al. 2010. Geneious v.5.3. Available online at http://www.geneious.com/

Drummond, A.J., M.A. Suchard, D. Xie & A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29:1969–1973.

Ersts, P.J.A. Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. Available online at http://biodiversityinformatics.amnh.org/open_source/gdmg Accessed on 2017-5-6.

Federici, P.R., A. Ribolini & M. Spagnolo. 2017. Glacial history of the Maritime Alps from the Last Glacial Maximum to the Little Ice Age. Geological Society, London, Special Publications 433:137–159.

Finlayson, G., G. Madani, G. Dennis & M. Harvey. 2015. First reported observation of phoresy of pseudoscorpions on an endemic New Zealand mammal, the lesser short-tailed bat, *Mystacina tuberculata*. New Zealand Journal of Zoology 42:298–301.

Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294–299.

Gardini, G. 2013. A revision of the species of the pseudoscorpion subgenus *Chthonius* (*Ephippiochthonius*) (Arachnida, Pseudoscorpiones, Chthoniidae) from Italy and neighbouring areas. Zootaxa 3655:1–151.

Gardini, G. 2014. The species of the *Chtonius heterodactylus* group from the eastern Alps and the Carpathians. Zootaxa 3887:101–137.

Gittenberger, E. 2012. Long-distance dispersal of molluscs: 'Their distribution at first perplexed me much'. Journal of Biogeography 39:10–11.

Habel, J.C., T. Schmitt & P. Müller. 2005. The fourth paradigm pattern of post-glacial range expansion of European terrestrial species: the phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera). Journal of Biogeography 32:1489–1497.

Harrison, S.E., M.T. Guzik, M.S. Harvey & A.D. Austin. 2014. Molecular phylogenetic analysis of Western Australian troglobitic chthoniid pseudoscorpions (Pseudoscorpiones: Chthoniidae) points to multiple independent subterranean clades. Invertebrate Systematics 28:386–400.

Harvey, M.S. 2013. Pseudoscorpions of the World, version 3.0. Western Australian Museum, Perth. Available online at http://www.museum.wa.gov.au/catalogues/pseudoscorpions

Harvey, M.S., P.C. Lopes, G.R. Goldsmith, A. Halajian, M.J. Hillyer & J.A. Huey. 2015. A novel symbiotic relationship between sociable weaver birds (*Philetairus socius*) and a new cheliferid pseudoscorpion (Pseudoscorpiones: Cheliferidae) in southern Africa. Invertebrate Systematics 29:444–456.

Hastriter, M.W. & S.E. Bush. 2014. Description of *Medwayella independencia* (Siphonaptera, Stivaliidae), a new species of flea from Mindanao Island, the Philippines and their phoretic mites, and miscellaneous flea records from the Malay Archipelago. ZooKeys 408:107–123.

Hastriter, M.W., K.B. Miller, G.J. Svenson, G.J. Martin & M.F. Whiting. 2017. New record of a phoretic flea associated with earwigs (Dermaptera, Arixeniidae) and a redescription of the bat flea *Lagaropsylla signata* (Siphonaptera, Ischnopsyllidae). ZooKeys 657:67–79.

Hewitt, G. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58:247–276.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Hewitt, G. 2004. The structure of biodiversity - insights from molecular phylogeography. Frontiers in Zoology 1:4.

Hulsmans, A., K. Moreau, L. De Meester, B.J. Riddoch & L. Brendonck. 2007. Direct and indirect measures of dispersal in the fairy shrimp *Branchipodopsis wolfi* indicate a small scale isolation-by-distance pattern. Limnology and Oceanography 52:676–684.

Ivy-Ochs, S., H. Kerschner, M. Maisch, M. Christl, P.W. Kubik & C. Schlüchter. 2009. Latest Pleistocene and Holocene glacier variations in the European Alps. Quaternary Science Reviews 28:2137–2149.

Kekkonen, M. & P.D. Hebert. 2014. DNA barcode-based delineation of putative species: efficient start for taxonomic workflows. Molecular Ecology Resources 14:706–715.

Keum, E., G. Takaku, K. Lee & C. Jung. 2016. New records of phoretic mites (Acari: Mesostigmata) associated with dung beetles (Coleoptera: Scarabaeidae) in Korea and their ecological implication. Journal of Asia-Pacific Entomology 19:353–357.

Kobelt, M. & W. Nentwig. 2008. Alien spider introductions to Europe supported by global trade. Diversity and Distributions 14:273–280.

Koch, L. 1873. Uebersichtliche Darstellung der europäischen Chernetiden (Pseudoscorpione). Bauer & Raspe, Nürnberg.

Kotrbová, J., V. Opatova, G. Gardini & F. Šťáhlavský. 2016. Karyotype diversity of pseudoscorpions of the genus *Chthonius* (Pseudoscorpiones, Chthoniidae) in the Alps. Comparative Cytogenetics 10:325–345.

Krehenwinkel, H. & D. Tautz. 2013. Northern range expansion of European populations of the wasp spider *Argiope bruennichi* is associated with global warming–correlated genetic admixture and population-specific temperature adaptations. Molecular Ecology 22:2232–2248.

Kumar, S., G. Stecher & K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874.

Lanfear, R., B. Calcott, S.Y.W. Ho & S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29:1695–1701.

Librado, P. & J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Macchioni, F. 2007. Importance of phoresy in the transmission of Acarina. Parassitologia 49:17–22.

Meng, P., K. Hoover & M. Keena. 2015. Asian longhorned beetle (Coleoptera: Cerambycidae), an introduced pest of maple and other hardwood trees in North America and Europe. Journal of Integrated Pest Management 6:4. DOI: 10.1093/jipm/pmv003

Michalak, I., L.-B. Zhang & S.S. Renner. 2010. Trans-Atlantic, trans-Pacific and trans-Indian Ocean dispersal in the small Gondwanan Laurales family Hernandiaceae. Journal of Biogeography 37:1214–1226.

Miller, M.A., W. Pfeiffer & T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, pp. 1–8. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA.

Moulds, T.A., N. Murphy, M. Adams, T. Reardon, M.S. Harvey, J. Jennings et al. 2007. Phylogeography of cave pseudoscorpions in southern Australia. Journal of Biogeography 34:951–962.

Muchmore, W. 1971. On phoresy in pseudoscorpions. Bulletin of the British Arachnological Society 2:38.

Murienne, J., M.S. Harvey & G. Giribet. 2008. First molecular phylogeny of the major clades of Pseudoscorpiones (Arthropoda: Chelicerata). Molecular Phylogenetics and Evolution 49:170–184.

Okumura, E. & T. Yoshiga. 2014. Host orientation using volatiles in the phoretic nematode *Caenorhabditis japonica*. Journal of Experimental Biology 217:3197–3199.

Opatova, V. & M.A. Arnedo. 2014. Spiders on a hot volcanic roof: colonisation pathways and phylogeography of the Canary Islands endemic trap-door spider *Titanidiops canariensis* (Araneae, Idiopidae). PLoS ONE 9:e115078. doi:115010.111371/journal.pone.0115078.

Opatova, V., J.E. Bond & M.A. Arnedo. 2016. Uncovering the role of the Western Mediterranean tectonics in shaping the diversity and distribution of the trap-door spider genus *Ummidia* (Araneae, Ctenizidae). Journal of Biogeography 43:1955–1966.

Pantini, P. & M. Isaia. 2008. New records for the Italian spider fauna (Arachnida, Araneae). Arthropoda Selecta. 17:133–144.

Pauls, S.U., H.T. Lumbsch & P. Haase. 2006. Phylogeography of the montane caddisfly *Drusus discolor*: evidence for multiple refugia and periglacial survival. Molecular Ecology 15:2153–2169.

Pedersen, A.A. & V. Loeschcke. 2001. Conservation genetics of peripheral populations of the mygalomorph spider *Atypus affinis* (Atypidae) in northern Europe. Molecular Ecology 10:1133–1142.

Pfammatter, J.A., D.R. Coyle, K.J. Gandhi, N. Hernandez, R.W. Hofstetter, J.C. Moser et al. 2016. Structure of phoretic mite assemblages across subcortical beetle species at a regional scale. Environmental Entomology 45:53–65.

Pfeiler, E. & T.A. Markow. 2011. Phylogeography of the cactophilic *Drosophila* and other arthropods associated with cactus necroses in the Sonoran Desert. Insects 2:218–231.

Pfeiler, E., B.G. Bitler, S. Castrezana, L.M. Matzkin & T.A. Markow. 2009. Genetic diversification and demographic history of the cactophilic pseudoscorpion *Dinocheirus arizonensis* from the Sonoran Desert. Molecular Phylogenetics and Evolution 52:133–141.

Pimentel, C.S., M.P. Ayres, E. Vallery, C. Young & D.A. Streett. 2014. Geographical variation in seasonality and life history of pine sawyer beetles *Monochamus* spp: its relationship with phoresy by the pinewood nematode *Bursaphelenchus xylophilus*. Agricultural and Forest Entomology 16:196–206.

Poinar, G.O., B.P. Curcic & J.C. Cokendolpher. 1998. Arthropod phoresy involving pseudoscorpions in the past and present. Acta Arachnologica 47:79–96.

PopART. Available online at http://popart.otago.ac.nz/

Provan, J. & K. Bennett. 2008. Phylogeographic insights into cryptic glacial refugia. Trends in Ecology & Evolution 23:564–571.

Rambaut, A. 2009. FigTree v. 1.3.1

Rambaut, A. & A.J. Drummond. 2009. TRACER v.1.5. Available online at http://tree.bio.ed.ac.uk/software/tracer/ (accessed on December 1st, 2009).

Ranius, T. & P. Douwes. 2002. Genetic structure of two pseudoscorpion species living in tree hollows in Sweden. Animal Biodiversity and Conservation 25:67–74.

Ronquist, F. & J. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Sabagh, L.T., R.J.P. Dias, C.W. Branco & C.F. Rocha. 2011. News records of phoresy and hyperphoresy among treefrogs, ostracods, and ciliates in bromeliad of Atlantic forest. Biodiversity and Conservation 20:1837–1841.

Saloña-Bordas, M.I., P.B. de la Puebla, B.D. Martín, J. Sumner & M.A. Perotti. 2015. *Ixodes ricinus* (Ixodidae), an occasional phoront on necrophagous and coprophagous beetles in Europe. Experimental and Applied Acarology 65:243–248.

Schawaller, W. 1995a. Review of the pseudoscorpion fauna of China (Arachnida: Pseudoscorpionida). Revue Suisse de Zoologie 102:1045–1063.

Schawaller, W. 1995b. Review of the pseudoscorpion fauna of the Far

East of Russia (Arachnida Pseudoscorpionida). Arthropoda Selecta 3:123–126.

Scheel, B.M. & B. Hausdorf. 2012. Survival and differentiation of subspecies of the land snail *Charpentieria itala* in mountain refuges in the Southern Alps. Molecular Ecology 21:3794–3808.

Schiner, T. 1872. Miscellen. Vorkommen von Chelifer an Fliegen. Verhandlungen der K. K. Zoologischen-Botanischen Gesellschaft in Wien 22:75–76.

Shorthouse, D.P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. [Retrieved from http://www.simplemappr.net/ Accessed May 2017].

Šťáhlavský, F. 2001. Pseudoscorpions of Prague. In Czech, with English Summary. Klapalekiana 37:73–121.

Šťáhlavský, F. 2011. Pseudoscorpions (Arachnida: Pseudoscorpiones) of the Třeboňsko Protected Landscape Area and the adjacent area. In Czech, with English Summary. Klapalekiana 47:247–258.

Šťáhlavský, F. & J. Chytil. 2013. Štírci (Arachnida: Pseudoscorpiones) Biosférické rezervace Dolní Morava a okolí (Česká republika). In Czech, with English summary. Klapalekiana 49:73–88.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Taberlet, P., L. Fumagalli, A.G. Wust-Saucy & J.F. Cosson. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7:453–464.

Tamura, K. & S. Kumar. 2002. Evolutionary distance estimation under heterogeneous substitution pattern among lineages. Molecular Biology and Evolution 19:1727–1736.

Tamura, K. & M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512–526.

Turienzo, P., O. Di Iorio & V. Mahnert. 2010. Global checklist of pseudoscorpions (Arachnida) found in birds' nests. Revue Suisse de Zoologie 117:557–598.

Tzedakis, P., B. Emerson & G. Hewitt. 2013. Cryptic or mystic? Glacial tree refugia in northern Europe. Trends in Ecology & Evolution 28:696–704.

Vanschoenwinkel, B., S. Gielen, M. Seaman & L. Brendonck. 2008. Any way the wind blows-frequent wind dispersal drives species sorting in ephemeral aquatic communities. Oikos 117:125–134.

Wahlberg, E. & K.A. Johanson. 2014. The age, ancestral distribution and radiation of *Chimarra* (Trichoptera: Philopotamidae) using molecular methods. Molecular Phylogenetics and Evolution 79:433–442.

Weygoldt, P. 1969. The Biology of Pseudoscorpions. Harvard University Press, Cambridge, MA.

Wilcox, T.P., L. Hugg, J.A. Zeh & D.W. Zeh. 1997. Mitochondrial DNA sequencing reveals extreme genetic differentiation in a cryptic species complex of neotropical pseudoscorpions. Molecular Phylogenetics and Evolution 7:208–216.

Zeh, D.W. & J.A. Zeh. 1992a. Dispersal-generated sexual selection in a beetle-riding pseudoscorpion. Behavioral Ecology and Sociobiology 30:135–142.

Zeh, D.W. & J.A. Zeh. 1992b. On the function of harlequin beetle-riding in the pseudoscorpion, *Cordylochernes scorpioides* (Pseudoscorpionida: Chernetidae). Journal of Arachnology 20:47–51.

Zeh, D.W. & J.A. Zeh. 1994. When morphology misleads - interpopulation uniformity in sexual selection masks genetic divergence in harlequin beetle-riding pseudoscorpion populations. Evolution 48:1168–1182.

Zeh, J., D. Zeh & M. Bonilla. 2003. Phylogeography of the harlequin beetle-riding pseudoscorpion and the rise of the Isthmus of Panamá. Molecular Ecology 12:2759–2769.

Zens, J., C. Zeeden, W. Römer, M. Fuchs, N. Klasen & F. Lehmkuhl. 2017. The Eltville Tephra (Western Europe) age revised: Integrating stratigraphic and dating information from different Last Glacial loess localities. Palaeogeography, Palaeoclimatology, Palaeoecology 466:240–251.

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