



Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae

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Funding information

Marie Curie Actions FP7-2013-IOF, Grant/Award Number: 622716; Swiss National Science Foundation (SNSF), Grant/Award Number: Early Postdoc Mobility grant P2SKP3_121277; Spanish Agencia Estatal de Investigación (AEI) and European Regional Development Fund (ERDF), Grant/Award Number: CGL2016-76322-P (AEI/FEDER, UE)

Editor: Simone Fattorini

Abstract

Aim: Continents harbour unique faunas, and only a small percentage of species naturally inhabit more than a single continent. This pattern is most evident in the insects, a morphologically small and extremely diverse group. Nevertheless, 12 species of ants have traditionally been recognized as native to both North America and Eurasia, the Holarctic region. Since intercontinental dispersal is presumably rare in ants, allopatric speciation in the absence of gene flow can be expected over evolutionary time. Here, we reassess the existence of Holarctic ant species and reconstruct their biogeographical history.

Location: The Holarctic.

Taxon: All known ant species with purportedly Holarctic distributions.

Methods: We reconstructed the phylogenetic relationships, biogeographical history and reassessed the taxonomic status of all known ants with Holarctic distributions using genetic data based on one mitochondrial and three nuclear genes and an ancestral area reconstruction of 310 specimens and 73 species (the 12 Holarctic species plus outgroup taxa).

Results: Contrary to the currently accepted hypothesis, only three ant species have Holarctic native ranges, while six taxa separate into distinct Palearctic and Nearctic species. Four species are shown to be recent introductions from Europe to North America by human activity, one of which was thought to be native. Genetic diversity is considerably higher within the North American than within European species as currently defined.

Main conclusions: The Formicidae have repeatedly dispersed through Beringia, during and after land bridge formation, and in both directions between the Palearctic and Nearctic regions. However, only three cold-tolerant species crossed the Bering Strait in relatively recent time. Our results highlight the potential existence of many unknown Nearctic ant taxa. Reliance on an evolutionarily labile morphological character, erect hairs, seems to have obscured species delimitation in these ant taxa. Based on our investigation, the typical time for speciation in allopatry for ants is 2–5 Ma.

KEY WORDS

Beringia, Camponotus, DNA-barcoding, Formica, Hymenoptera, Lasius, Leptothorax, Nearctic, phylogeography

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1 | INTRODUCTION

The Holarctic is the largest biogeographical realm on earth. It connects the Nearctic (North America) and the Palearctic (northern Eurasia and North Africa) subregions through Beringia (Hopkins, 1967), which share important faunistic and floristic similarities. These subregions, nevertheless, also contain differences (Li, 1952) and have therefore been considered two realms by some and a single realm by others. Several studies have addressed Holarctic biogeographical patterns at the generic and higher levels, aiming to explain the present distributions of organisms through reconstructing past dispersal between continents (reviewed in Enghoff, 1995). While insights from ancient long-range dispersal estimates are useful, they are often speculative due to the long and complex history of tectonic and climatic changes. Studies at the species level using DNA markers, however, can be valuable additions to our understanding of transcontinental dispersal. There are numerous instances of Holarctic species among the invertebrates such as spiders (Marusik & Koponen, 2005), beetles (Larson & Nilsson, 1985) and Lepidoptera (Landry et al., 2013; Vila et al., 2011). Maintaining a Holarctic range over time presupposes continuous or frequent gene flow between the two continents in present or recent time. The former land bridge of Beringia, connecting Eurasia and North America, has been inferred as the main dispersal route in the Holarctic fauna and flora. Due to the high latitudinal position of Beringia, recent dispersal of organisms is restricted to species adapted to boreal climates (Hopkins, 1967; Vila et al., 2011).

Ants are among the most ecologically and economically important, abundant and diverse animals on land (Hölldobler & Wilson, 1990). Out of 2,695 known Palearctic and 812 known Nearctic ant species (Shattuck, 2017), only 12 are considered to have Holarctic range distributions: *Camponotus herculeanus* (Linnaeus, 1758), *Formica fusca* Linnaeus, 1758, *F. gagatoides* Ruzsky, 1904, *Lasius alienus* (Förster, 1850), *La. flavus* (Fabricius, 1782), *La. niger* (Linnaeus, 1758), *La. umbratus* (Nylander, 1846), *Leptothorax acervorum* (Fabricius, 1793), *Le. muscorum* (Nylander, 1846), *Myrmica rubra* (Linnaeus, 1758), *Tetramorium immigrans* Santschi, 1927, and *T. atratum* (Schenck, 1852) (AntWeb 2015; Francoeur, 1997). Three of these species have presumably been introduced to North America by humans: *T. immigrans* (Schlick-Steiner et al., 2006; Wagner et al., 2017), along with its social parasite *T. atratum* in the 19th century or earlier (Brown, 1957) and *M. rubra* around the year 1900 (Ellison, Gotelli, Farnsworth, & Alpert, 2012). The other nine species are ideal candidates to study evolutionary processes through large distributional ranges, and historical intercontinental dispersals.

Here, we present the first genetic reassessment of the status of all known ant species considered to have naturally Holarctic distributions (including three uncertain cases of species that have presumably been introduced by humans in the past) and review the biogeographical links between the Old and the New World. Using a molecular dataset of 310 specimens belonging to 73 extant taxa from both biogeographical subregions, we aim to resolve

transcontinental dispersal events and subsequent diversification in ant genera containing species with putative Holarctic distributions. Specifically, we will investigate the following questions:

1. To what extent do Holarctic species exist in the Formicidae? Do Nearctic and Palearctic populations form a monophyletic group and, if they do, is their degree of divergence within the range of what is typically found within species?
2. What is the history of intercontinental colonization events that have shaped current distributions? What are the routes, times, and directions of these events?

2 | MATERIALS AND METHODS

2.1 | Specimen collection, PCR, and sequencing

Sampling was designed to pool a comprehensive dataset for the 12 Holarctic ant species across their entire distributional range. In order to test for monophyly, 61 outgroup species (Supporting Information Appendix S1, Table S1) were examined. For each focal species we aimed to include the closest related species in the Nearctic and Palearctic regions as outgroups based on current morphological and/or molecular evidence (Jansen, Savolainen, & Vepsäläinen, 2010; Schlick-Steiner et al., 2006; Steiner et al., 2004; Talavera, Espadaler, & Vila, 2015). Additionally, DNA-barcoding (mitochondrial gene cytochrome c oxidase I, COI) data for all available species with similar morphology was used to identify the closest relative in each region. In case of uncertainty, all suspected sister species-candidates were included in the dataset. Within-taxon sampling was optimized to capture a comprehensive geographical coverage. Specimens were collected manually and stored in 70%–100% ethanol. A total of 310 specimens from several collections were gathered (Supporting Information Appendix S1, Table S2). DNA was extracted from whole bodies or legs using either a 10% Chelex protocol, DNeasy® Blood & Tissue Kit (Qiagen, Inc.) or an AutoGenprep 965 extraction robot. The barcode region of the COI was sequenced for all specimens using published primers and PCR conditions (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994; Talavera et al., 2015). Three nuclear genes were sequenced for one of each species or major DNA-barcoding clade: wingless (Wg), topoisomerase 1 (Top1), and rDNA 28S (Abouheif & Wray, 2002; Saux, Fisher, & Spicer, 2004; Ward & Sunnicht, 2012) (see Supporting Information Appendix S1, Table S2 for sequencing coverage). The resulting DNA sequences were edited and aligned in Geneious 10.0.3 (Kearse et al., 2012). Nearctic representatives of *La. niger* of Palearctic origin, *F. gagatoides*, and *T. atratum* were retrieved from the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert, 2007), after searching for close matches with sequences obtained from Palearctic representatives. Thus, COI sequences for two Nearctic specimens of *La. niger* of Palearctic origin (BOLD code: HPPPH364-13 and SMTPM2984-15), *F. gagatoides* (codes CNIVC571-14 and MHANT242-07 identified as “*F. fusca-complex*” and “*F. neorufibarbis* 2”) and one of *T. atratum*



(CNPPA3045-12 identified as "Anergates," the previous genus name of which *T. atratum* was the only known species) were downloaded. Finally, we also downloaded a COI sequence of *Myrmica wheeleri* from Genbank (GQ255195).

2.2 | Phylogeny and dating

Maximum likelihood (ML) and Bayesian inference (BI) were employed to estimate evolutionary relationships, using a combination of all available genes and specimens. ML was run in RAxML (Stamatakis, 2014) (Figure 1, Supporting Information Appendix S2), through the raxmlGUI interface (Silvestro & Michalak, 2012). The GTRGAMMA nucleotide substitution model was chosen. To test for a potential conflict between mitochondrial and nuclear DNA, ML trees were also inferred for mtDNA (Supporting Information Appendix S3), and nuclear DNA (Wg+Top1+28S) alone (Supporting Information Appendix S4). ML trees were repeated in IQ-TREE 1.6.1 (Minh, Nguyen, & von Haeseler, 2013) to assess clade support using ultrafast likelihood bootstrap with 1,000 replicates and to test for consistency between programs. Best-fitting nucleotide substitution models for each partition/gene (COI: TIM2+F+R5, Wg: HKY+I+G, Tp: TN+F+I, 28S: TIM3+F+R2) were inferred within IQ-TREE. BI in BEAST 1.8.0 (Drummond, Suchard, Xie, & Rambaut, 2012) was used to estimate divergence times for dispersal between the Holarctic subregions and phylogenetic relationships (Supporting Information Appendix S5). Best-fitting substitution models were determined in jMODELTEST 2.1.4 (Darriba, Taboada, Doallo, & Posada, 2012) and following the corrected Akaike information criterion. A strict clock model and a constant population size under a coalescent model were established as priors. Following ages in the Formicinae estimated by Blaimer et al. (2015), divergence analyses were calibrated by placing priors on three crown groups representing genera in our phylogeny: (a) *Camponotus*: 23.1 (15.2, 31.0) Ma, (b) *Formica* 28.6 (14.0, 43.2) Ma, (c) *Lasius* 21.9 (9.8, 32.8) Ma. Two independent chains were run for 50 million generations each, sampling values every 1,000 steps. Trace files were analyzed in TRACER 1.5 to assess chain convergence and burnin. Independent runs were combined in LogCombiner 1.8.0 and tree topologies were assessed using Treeannotator 1.8.0 to generate a maximum-clade-credibility tree. FigTree 1.4.0 was used to visualize the consensus tree along with node ages, age deviations and node posterior probabilities. Resulting phylogenies of the complete dataset are summarized in Figure 1, and fully displayed in the Supporting Information Appendix S2 (ML) and Supporting Information S5 (BI). Using a conservative approach, we only argue in favour of taxonomic splitting if allopatric populations were poly- or paraphyletic.

2.3 | Biogeography

Ancestral geographical ranges were estimated to infer intercontinental dispersal events underlying current distributions. Only phylogenies for those genera containing truly Holarctic species were considered for these analyses: *Camponotus*, *Leptothorax*, and *Formica*. The likelihoods of different dispersal scenarios were assessed in the

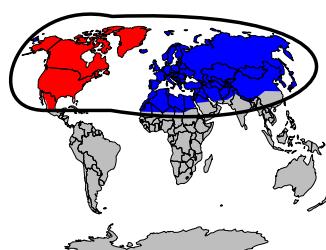
R package BioGeoBEARS 0.2.1 (Matzke, 2013). This package allows probabilistic inference of both historical biogeography (ancestral geographical ranges on a phylogeny) as well as comparison of different models of range evolution. The Holarctic was divided into four areas: West Palearctic (WPa), East Palearctic (EPa), West Nearctic (WNa), and East Nearctic (ENa) (Enghoff, 1995). Tests were performed for a dispersal-extinction-cladogenesis (DEC) model (Ree & Smith, 2008), a maximum-likelihood version of the dispersal-vicariance model (DIVALIKE) (Ronquist, 1997) and a Bayesian biogeographical inference (BAYAREALIKE) (Landis, Matzke, Moore, & Huelsenbeck, 2013). DEC specifies instantaneous transition rates between discrete states (ranges) along phylogenetic branches and estimates likelihoods of ancestral states (range inheritance scenarios) at cladogenesis events (Ree & Smith, 2008). DIVALIKE uses a three-dimensional step matrix based on a simple biogeographical model in which speciation is assumed to subdivide the ranges of widespread species into vicariant components with optimal ancestral distributions minimizing the number of implied dispersal and extinction events (Ronquist, 1997). Finally, BAYAREALIKE uses a Bayesian approach for inferring biogeographical history (Landis et al., 2013). The three models were also tested allowing for founder-effect speciation (j) (Matzke, 2014). A dispersal multiplier matrix was coded (Supporting Information Appendix S1, Table S3), maximum range size was fixed to two areas and an adjacency matrix was set to restrict the WPa-WNa or EPa-ENa pairs to occur simultaneously as ancestral states, scenarios that are biologically implausible. Likelihood ratio tests and AIC scores were used to detect the best-performing models in each phylogeny (Supporting Information Appendix S1, Table S4).

3 | RESULTS

3.1 | Phylogenetics and dating

Seven nodes were identified as common ancestors between lineages in the Palearctic and the Nearctic subregions, with ages ranging from 1.7 (0.9, 2.7) Ma to 8.7 (5.2, 12.1) Ma (Figure 3). The inferred trees support monophyly of the subfamilies (Formicinae and Myrmicinae) and genera (*Camponotus*, *Lasius*, *Leptothorax*, *Myrmica*, and *Tetramorium*). All three subgenera in the genus *Lasius* Fabricius, 1804 defined by Wilson (1955) were monophyletic: *Cauolasius*, *Chthonolasius* and *Lasius* s. str., with the exception of *La. pallitarsis*, which had a basal phylogenetic position within *Lasius* not clearly assignable to any subgenus (Figure 1). Six of the 12 tested species show monophyly and low to moderate degrees of genetic divergence. These six species are inferred to be truly Holarctic in distribution: *C. herculeanus* (*sensu lato*), *F. gagatoides*, *Le. acervorum*, *M. rubra*, *T. immigrans*, and *T. atratum*. The other five putatively Holarctic species, *La. alienus*, *La. flavus*, *La. umbratus*, *Le. muscorum*, and *F. fusca* show notable genetic divergence between the Nearctic and Palearctic populations, with COI-divergence much greater than 3% (Figure 1, Table 1). *Lasius niger*, however, is unique because our analyses identified two populations of the Palearctic *La. niger* recently introduced to Canada by humans (Supporting Information Appendix S2, Schär et al. in prep.). The natural

Maximum likelihood



Nearctic
Palearctic
Holarctic (natural)
Holarctic (introduced)

n=310

Bayesian inference

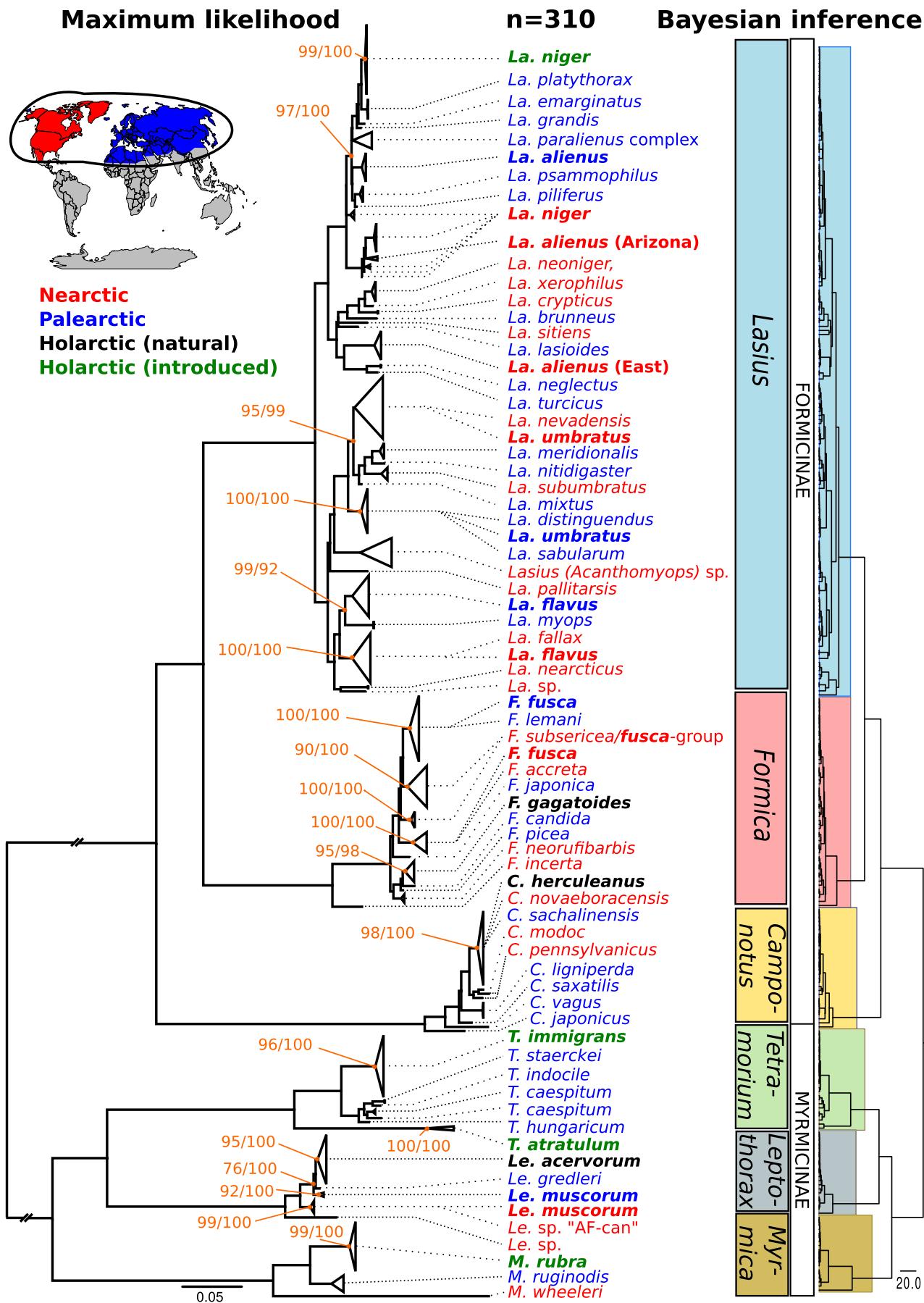




FIGURE 1 RAxML maximum likelihood tree of the samples used in this study based on COI (mtDNA) and three nuclear genes (Wg+Top1+28S) for 12 ants distributed in the Holarctic. Some clades consisting primarily of outgroups and all samples of the same species have been collapsed for simplicity. Relative divergence within collapsed clades is shown by the horizontal length of the triangles. Colours indicate the geographical origin of samples (red: Nearctic, blue: Palearctic, black: Holarctic, native, green: Holarctic, introduced). Support values of nodes of particular importance regarding taxonomical conclusions for the Holarctic species (orange numbers) are given in percentage ultrafast likelihood bootstraps/posterior probability (BI). The higher-level taxonomy (genera and subfamilies) is shown to the right, as is the topology of the tree for all specimens retrieved by BEAST. Scale units are substitutions per site. The full tree can be accessed via the supplementary information (Supporting Information Appendix S2)

North American populations identified as *La. niger* are not conspecific and belong to a currently undescribed species. We found no support for the distinction of *C. herculeanus* and *C. sachalinensis* Forel, 1904 syn. nov. (Bolton, 1995). Presently, *C. herculeanus* has a disjunct distribution in the Western Palearctic and Nearctic but is replaced in eastern Asia by *C. sachalinensis* syn. nov. We found low genetic diversity (<1.7% COI-divergence) and monophyly for both mitochondrial and nuclear DNA markers in Holarctic samples of *C. herculeanus* including Asian *C. sachalinensis* syn. nov. (Table 1, Figure 1, Supporting Information Appendices S2–S5). A dated tree including divergence times is shown in Supporting Information Appendix S5.

Disagreements between phylogeny and current taxonomy were more common in Nearctic versus Palearctic taxa: 52% (11/21) of the studied Nearctic species with $n > 1$ were polyphyletic (Palearctic samples excluded), while polyphyly was observed in only 15% (4/27) of the Palearctic taxa, excluding Nearctic samples (Supporting Information Appendix S2). DNA-barcode sharing occurred in 6 out of 21 (29%) Nearctic and 5 out of 27 (19%) Palearctic taxa ($n > 1$) (Supporting Information Appendix S3).

3.2 | Disparities between mitochondrial (COI) and nuclear phylogenetic trees

The COI sequences (DNA-barcodes) of some specimens of *C. herculeanus* and *C. novaeboracensis* were identical (Supporting Information Appendix S3), making *C. herculeanus* relaxed monophyletic (Figures 1 and 3, Table 1). However, the two species were clearly separated by the nuclear markers sequenced in this study (Supporting Information Appendix S4). Based on nuclear DNA, morphological and ecological differences, we therefore conclude that *C. herculeanus* and *C. novaeboracensis* are two separate species, but that they apparently share DNA-barcodes. Similarly, nuclear DNA, but no clear mitochondrial DNA differentiation, was also found between *La. fallax* and *La. brevicornis* stat. nov., *La. nevadensis*, and *La. aphidicola* stat. nov. and *F. fusca* and *F. lemani* (Supporting Information Appendices S3 and S4). However, the morphological characters used for distinguishing the latter two taxa are ambiguous and population genetic data cast doubt regarding their reproductive isolation (Seppä et al., 2011).

TABLE 1 Summary of the results from this study for the 12 ant species with previously recognized Holarctic distribution ranges. For each species, the minimum and maximum divergence (COI) between samples collected in the Palearctic and the Nearctic regions, the type of relationship in mitochondrial and nuclear phylogenies, and the revised distribution range is given. The three valid species with naturally Holarctic distribution according to our conclusions are highlighted in bold

Species name	Minimum intercontinental divergence (COI)	Maximum intercontinental divergence (COI)	Intercontinental relationship (mitochondrial, COI)	Intercontinental relationship (nuclear, Wg+Top1+28S)	Distribution range
<i>Camponotus herculeanus</i> ^a	0.8%	1.7%	Relaxed monophyletic	Monophyletic	Holarctic
<i>Formica fusca</i>	3.8%	4.9%	Polyphyletic	Polyphyletic	Palearctic
<i>F. gagatoides</i>	0.5%	2.1%	Monophyletic	Unknown	Holarctic
<i>Lasius alienus</i>	6.0%	9.1%	Polyphyletic	Polyphyletic	Palearctic
<i>La. flavus</i>	4.6%	6.4%	Polyphyletic	Polyphyletic	Palearctic
<i>La. niger</i>	0.0%	7.0%	Monophyletic/polyphyletic	Monophyletic/polyphyletic	Palearctic, introduced to Nearctic
<i>La. umbratus</i>	5.4%	6.8%	Polyphyletic	Polyphyletic	Palearctic
<i>Leptothorax acervorum</i>	1.8%	2.1%	Monophyletic	Monophyletic	Holarctic
<i>Le. muscorum</i>	6.4%	6.7%	Paraphyletic	Monophyletic	Palearctic
<i>Myrmica rubra</i>	0.0%	1.5%	Monophyletic	Monophyletic	Palearctic, introduced to Nearctic
<i>Tetramorium atratum</i>	4.9%	4.9%	Monophyletic	Unknown	Palearctic, introduced to Nearctic
<i>T. immigrans</i>	0.0%	1.2%	Monophyletic	Monophyletic	Palearctic, introduced to Nearctic

^asensu lato, including the former synonym *C. sachalinensis*.



3.3 | Taxonomic changes

While additional molecular markers and wider taxon inclusion may change phylogenetic relationships and biogeographical reconstruction, the results of this study have significant implications for ant taxonomy. Taxa in our study in which New and Old World populations are not monophyletic represent separate species. However, if the allopatric populations are monophyletic, the interpretation becomes slightly more complex primarily because a certain degree of genetic divergence between geographically isolated populations is expected, regardless of whether allopatric speciation has already been completed or not. Here, we take a conservative approach, arguing that there is no need to split monophyletic species based on genetic data until speciation can be demonstrated under sympatric conditions.

Based on our new understanding of our study species, the following nomenclatural changes are warranted:

1. The names *La. alienus*, *La. flavus*, *La. umbratus*, *Le. muscorum*, and *F. fusca* are restricted to Palearctic populations of these taxa. *Lasius niger* is also naturally Palearctic in distribution, except for the two recently introduced populations identified in Canada.
2. *Camponotus sachalinensis* Forel, 1904 stat. nov.: This taxon has long been regarded a synonym of *C. herculeanus* (Collingwood, 1976; Kupyanskaya, 1990; Radchenko, 1996) but was raised to the rank of species (Bolton, 1995; Collingwood, 1981) without clear justification for this change in status. Our results support the hypothesis of synonymy with *C. herculeanus*. *Camponotus herculeanus* and *C. sachalinensis* form a young clade (~1.8 Ma, Figure 3) with a continuous distribution throughout the Holarctic. *Camponotus sachalinensis* represents the link between European and North American populations of *C. herculeanus* (Figures 2 and 3, Supporting Information Appendices S2 and S4). The current view of *C. herculeanus* occupying a disjunct distribution in the Western Palearctic and North America while being replaced by a distinct species, *C. sachalinensis*, in the Eastern Palearctic is biogeographically not realistic. *Camponotus sachalinensis* is therefore here returned to synonymy with *C. herculeanus*. Its junior synonyms *Camponotus herculeanus altaica* Ruzsky, 1915 and *Camponotus herculeanus jacuticus* Karavaiev, 1929 are also placed in synonymy with *C. herculeanus*.
3. *Lasius americanus* Emery, 1893 stat. nov. was originally described as a subspecies of *La. niger*, and was then raised to species in 1945 by Gregg. Creighton (1950) treated it as a subspecies of *La. alienus*, and then Wilson (1955) finally placed it in synonymy with *La. alienus*. Our results support the species-level status of this

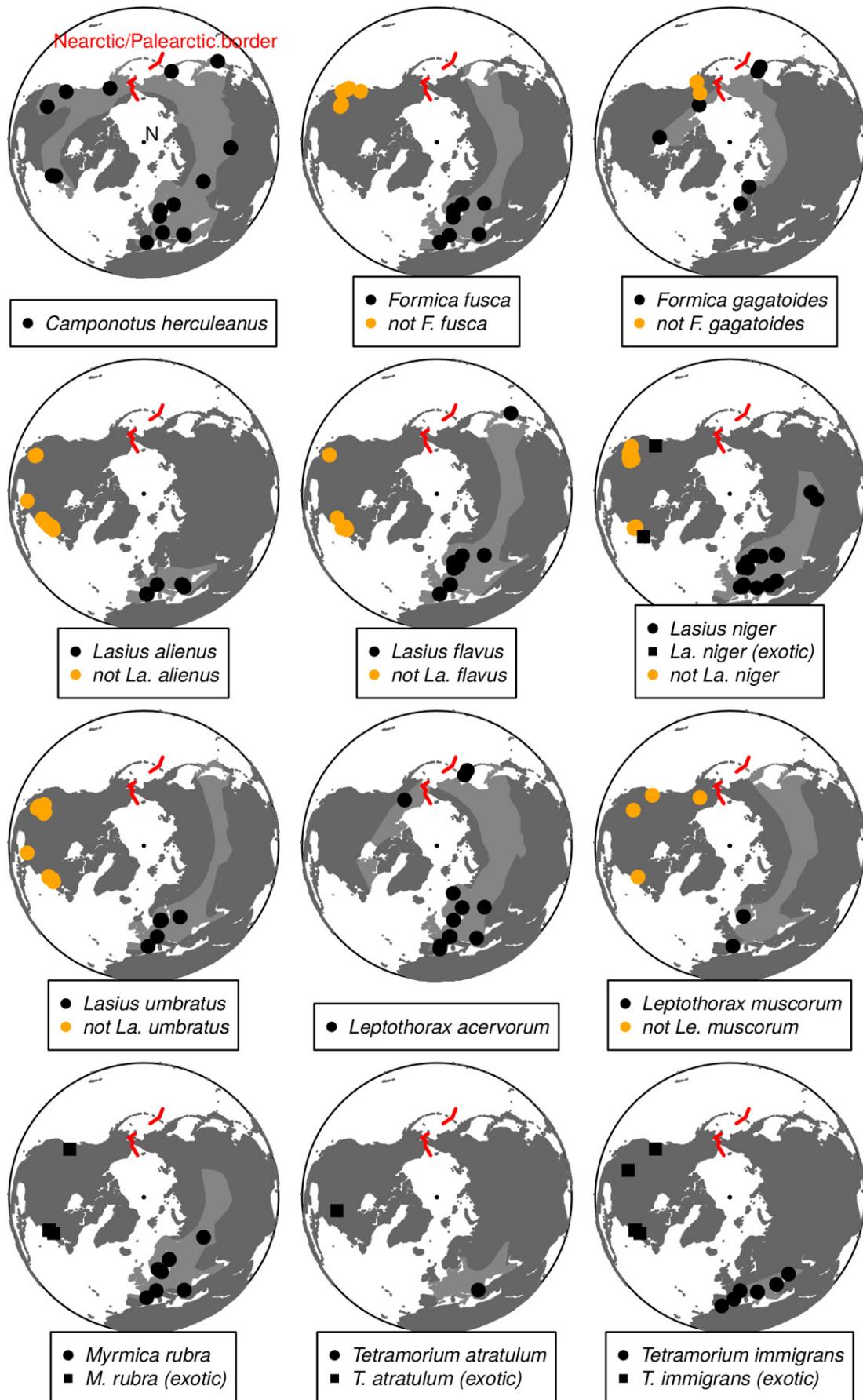
taxon as suggested by Gregg (1945). Therefore *La. americanus* is removed from synonymy with *La. alienus* and treated as a full species.

4. *Lasius brevicornis* Emery, 1893 stat. nov. was treated as a full species until it was placed in synonymy with *La. flavus* by Wilson (1955). This study supports its removal from synonymy and treatment as a full species. The junior synonyms *Lasius brevicornis microps* Wheeler, 1917, *Lasius flavus claripennis* Wheeler, 1917 and *Lasius helvus* Cook, 1953 should be transferred from synonymy with *Lasius flavus* and treated as junior synonyms of *Lasius brevicornis*.
5. *Lasius aphidiculus* (Walsh, 1863) stat. nov. was treated as a junior synonym of *La. flavus* by Mayr (1886), a subspecies of *La. umbratus* by Wheeler (1908) and most recently as a junior synonym of *La. umbratus* by Wilson (1955). Our results suggest that *Lasius aphidiculus* (Walsh, 1863) should be treated as a valid species. The junior synonym *Lasius umbratus epinotalis* Buren, 1944 should be transferred from synonymy with *L. umbratus* to synonymy with *La. aphidiculus*.
6. *Leptothorax canadensis* Provancher, 1887 stat. nov. was considered a subspecies of *Le. acervorum* by André (1887), a full species by Emery (1895) and a junior synonym of *Le. muscorum* by Brown (1955). Our findings support the hypothesis of *Le. canadensis* representing a full species, and it is therefore removed from synonymy with *Le. muscorum*. The following junior synonyms should be moved from *Le. muscorum* to *Le. canadensis*: *Leptothorax canadensis obscurus* Viereck, 1903, *Leptothorax canadensis yankee* Emery, 1895, *Leptothorax muscorum septentrionalis* Wheeler, 1917, *Leptothorax muscorum sordidus* Wheeler, 1903, and *Leptothorax yankee kincaidi* Pergande, 1900. However, the North American members of the genus *Leptothorax* are a difficult species complex whose taxonomy remains unresolved.
7. *Formica subaenescens* Emery, 1893: our study suggests that *F. fusca* is restricted to the Palearctic region and supports the already widely accepted view that North American ants currently described as *F. fusca* belong to *F. subaenescens* Emery, 1893 (Bolton, 1995; Francoeur, 1977). As with other Nearctic ant species, *F. subaenescens* may represent a species complex. Until this question is resolved, we regard the North American *F. fusca marcida* Wheeler, 1913, currently considered a subjective junior synonym of *F. fusca*, as a subjective junior synonym of *F. subaenescens*.

3.4 | Biogeographical analysis of dispersal events

The DIVALIKE with founder-event speciation (*j*) parameters model for the three genera analyzed was better than all other models

FIGURE 2 Distribution of the 12 putatively Holarctic ant species based on DNA samples used in this study. The maps are projected so that North is in the centre and 0° longitude is at the bottom of each map. Black dots show samples of the 12 focal, putatively Holarctic species identified by DNA; orange dots show populations that have erroneously been assigned to those species so far. Black squares indicate exotic populations in the Nearctic. The lighter shaded areas show the inferred range of each focal species combining the results from this study with distribution ranges given in (Czechowski, Radchenko, & Czechowska, 2002; Dlussky, 1965; Janicki et al., 2016)



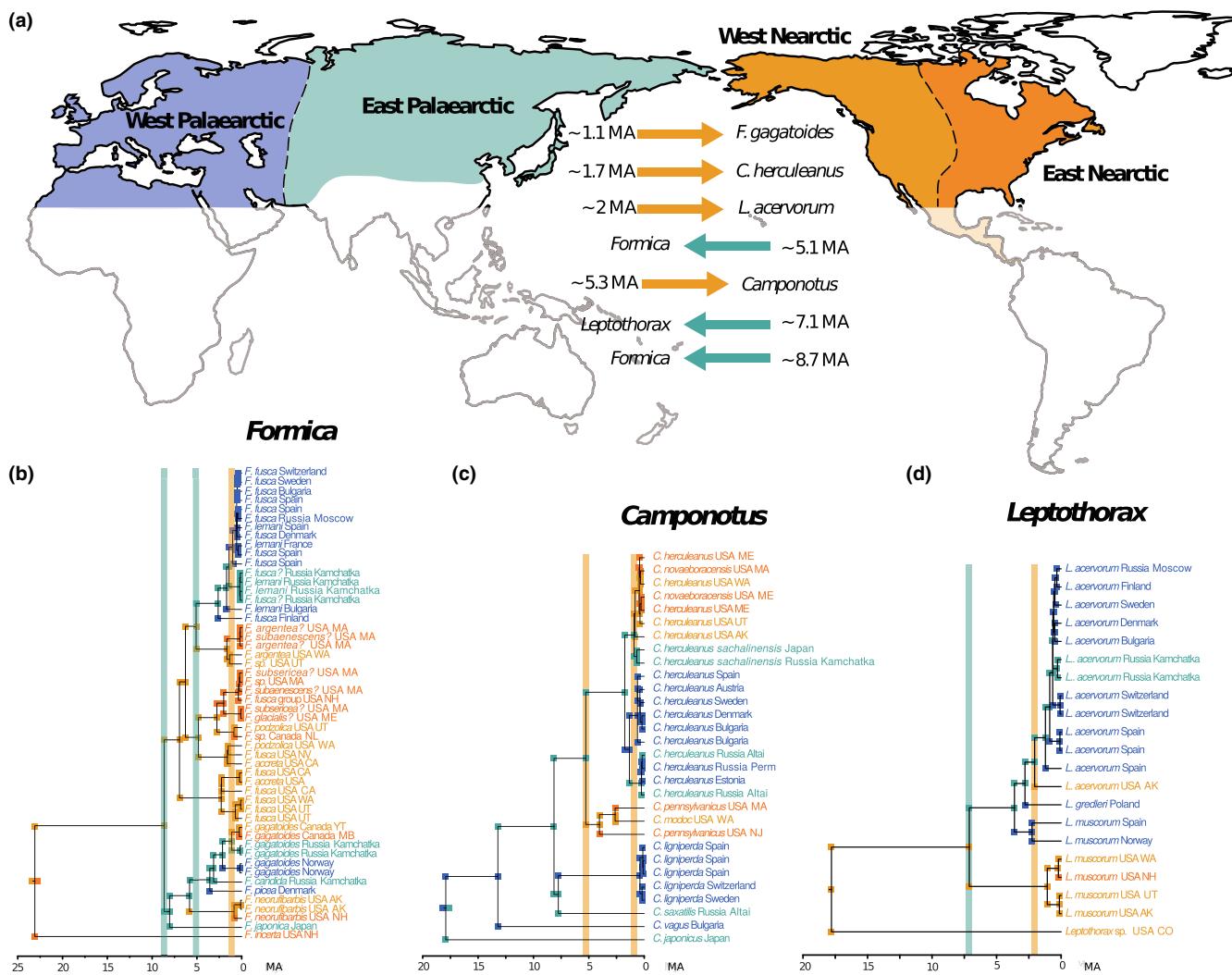


FIGURE 3 Results from the biogeographical analysis for the three ant genera containing native Holarctic species, summarizing recent intercontinental dispersal through the Beringian land bridge in ants. Panel a shows a map of the Holarctic divided into four areas (West and East Palearctic, West, and East Nearctic). The dated phylogenetic trees for each genus in panels b–d are at the level of specimens and colours indicate the geographical area-membership of each specimen. The colour at each node indicates the area-membership of ancestors of extant specimens inferred by the R package BioGeoBEARS 0.2.1. Vertical lines show dispersal events through Beringia (orange: Palearctic to Nearctic, blue: Nearctic to Palearctic)

tested with BioGeoBEARS (Supporting Information Appendix S1, Table S4). Seven dispersal events are identified between the Palearctic and the Nearctic, occurring in both directions. The three most recent intercontinental events were colonisations from EPa to WNa: (a) *F. gagatoides* (~1.1 Ma), (b) *C. herculeanus* (~1.7 Ma), and (c) *Le. acervorum* (~2 Ma) (Figure 3). Older Beringia crossings occurred in lineages of *Formica* (~5.1 Ma) from WNa to EPa, *Camponotus* (~5.3 Ma) from EPa to WNa, and in *Leptothorax* and *Formica* (~7.1 and ~8.7 Ma) from WNa to EPa (Figure 3).

4 | DISCUSSION

Our results suggest that seven out of 12 tested ant species do indeed have Holarctic distributions. Of these, three are native to the

Holarctic region (*C. herculeanus*, *Le. acervorum* and *F. gagatoides*) and the other four have been introduced from Eurasia to North America by human activity (*La. niger*, *M. rubra*, *T. atratulum*, and *T. immigrans*). The other taxa (*Lasius* sp., *Le. muscorum*, and *F. fusca*) clearly separate into distinct species of which the Nearctic forms are currently unrecognised and remain to be raised to species level. All three naturally Holarctic ant species have likely dispersed from the Palearctic to the Nearctic region within the past 2 Ma. Complete speciation, on the other hand, did result through historic movement of ants across the Bering Strait (≥ 5 Ma).

4.1 | Taxonomic changes

Our results are based on four genetic markers, among which the mitochondrial COI gene is the most widely sampled and informative.



Similar to Jansen, Savolainen, and Vepsäläinen (2009), we found that DNA-barcoding is in high congruence with ant taxonomy currently in use for the Palearctic region. Additionally, the investigated nuclear genes support our conclusions. Furthermore, evidence such as sequence data for more genes, cuticular hydrocarbons, microsatellites, and morphometrics would be required to reach more definitive conclusions. Our study cannot replace a comprehensive taxonomic revision of the studied ant genera in North America, including a detailed investigation of all available type material. The nomenclatural changes proposed are a provisional solution until formal taxonomic revisions can be conducted. Nevertheless, these taxonomic changes provide a more accurate reflection of the phylogenetic relationships than is currently known and establish the groundwork for forthcoming taxonomic revisions (Boer, 2016; Schär et al., in prep.).

4.2 | Niche limits and distribution ranges in ants

The natural ranges of the species investigated vary considerably. In general, range sizes correlated with species' cold tolerance. All three species with a natural Holarctic distribution are exceptionally cold-resistant, tolerating temperatures below -40°C (Berman, Alfimov, Zhigulskaya, & Leirikh, 2010; Ellison et al., 2012; Seifert, 2007), which allows for survival in areas too cold for other ant competitors. Remarkably, *Camponotus herculeanus* seems to have the largest natural distribution of all extant ant species (Figure 2). This species is widespread throughout boreal and montane habitats in the Holarctic while *Le. acervorum* and *F. gagatoides* seem to be limited to the far northern regions of North America (Figure 2). The exceptional ecological success of *Camponotus herculeanus* may be due to its extreme cold tolerance and the ability of using a widely available resource: it nests in trunks of living trees as well as in and under rotten logs (Ellison et al., 2012). While *C. herculeanus* shares this niche with a number of locally adapted congeneric species, it appears to have a competitive advantage in places with the lowest minimum temperatures.

4.3 | Intercontinental dispersal events and allopatric speciation

A total of three recent dispersal events between Asia and North America were estimated in the Formicidae. The most recent dispersal event took place in the subarctic endemic *F. gagatoides* (~1.1 Ma), followed by *C. herculeanus* (~1.7 Ma) and *Le. acervorum* (~2 Ma) (Figure 3). Although the genetic divergence between Holarctic populations of these species lies within the typical range of intraspecific divergence (<3% in COI), lineage sorting between populations from both continents seems nearly complete in the studied cases (Figure 3). However, *F. gagatoides*, *C. herculeanus*, and *Le. acervorum* presently occur on both sides of the Bering Strait (Janicki, Narula, Ziegler, Guénard, & Economo, 2016), raising the possibility of continued intercontinental gene flow. Interestingly, the existence of a landbridge, which disappeared in Beringia ~5 Ma (Marincovich & Gladkov, 1999), does not seem to be a prerequisite for ant dispersal. All

three expansions most likely took place from the Palearctic to the Nearctic rather than vice versa (Figure 3). However, *Formica* and *Leptothorax* have also dispersed from the Nearctic to the Palearctic >5 Ma, as well as from the Palearctic to the Nearctic (*Camponotus*, Figure 3). Allopatric speciation in those lineages in both Holarctic subregions is complete (Figures 1 and 3). Thus, 2–5 Ma may be the typical time-scale for allopatric speciation in ants. The three genera containing Holarctic species are diverse (*Camponotus*: 1,024 species; *Formica*: 176 species; *Leptothorax* 19 species, AntWeb 2015) and not all taxa were sampled. Although the selection of closely related out-group taxa was based on current morphological and phylogenetic evidence, it is possible that an unsampled species may be phylogenetically nested within the ingroups of the Holarctic populations of these Holarctic taxa. However, due to the low molecular divergences found among the populations of the ingroup taxa (Table 1), such cases could be interpreted as new synonymies of the Holarctic species rather than a conflicting interpretation of our results. Due to the incomplete taxon sampling, our results other than those concerned with the Holarctic species may or may not be congruent with more complete phylogenies and should therefore be interpreted with caution. Similarly, the intercontinental relationship of *Le. muscorum* may change from para- to polyphyletic if more North American *Lepthothorax* are included in the study or if the already informally recognized morphospecies *Le. "AF-can"* (Ellison et al., 2012) is described as a species in the future.

4.4 | Human introductions

Our results agree with previously documented recent introductions of Palearctic species to North America (Ellison et al., 2012; Schlick-Steiner et al., 2006). In particular, this concerns *T. atratum*, *T. immigrans*, *M. rubra* and *La. niger* (Schär et al., in prep.). In all these cases, specimens from both continents displayed a minimum genetic divergence of 0% at the level of the markers investigated. An exception was *T. atratum* for which an unusually high divergence in COI of 4.9% was found between a sample from each continent (Figure 1, Table 1). However, since this is an obligatory social parasite of the Old World endemic genus *Tetramorium*, the joint introduction together with *T. immigrans* seems to be the only plausible explanation for its occurrence in North America. In addition, parasites are known for their accelerated rates of molecular evolution compared to free living species (Dowton & Austin, 1995). Alternatively, the high divergence in COI within *T. atratum* could represent a numt artefact.

4.5 | Morphological similarity

Classical morphological taxonomy can suggest markedly different classifications than molecular evidence. Among the ants studied here, this is most evident in the genus *Lasius*. For example, ants historically recognized as *La. alienus* comprise representatives of the most distantly related clades within the subgenus *Lasius* s. str. (Figure 1). Traditionally, dark *Lasius* ants with erect hairs on the antennal scapes and tibiae were classified as *La. niger*, whereas those *Lasius*



lacking such hairs were called *La. alienus* (Wilson, 1955). However, our phylogenies show that this character is evolutionarily labile and may reflect a homoplasy (Figure 1), making it unreliable as a primary morphological trait for species delimitation. Convergent evolution may be caused by similar selective pressures, such as adaptation to similar habitats. However, the habitats of European and American representatives of *La. alienus* s.l. differ strongly (Ellison et al., 2012). Thus, ecological selection is not a likely explanation for the similar features observed between these groups. Alternatively, the lack of erect setae could represent an ancestral state within *Lasius* s. str. that has been conserved independently in different clades. This hypothesis could be supported by the fact that the most abundant *Lasius* species in Eocene amber, *La. schiefferdeckeri*, "resembles modern *L. alienus*" (Dlussky, 2011).

4.6 | State of knowledge of the North American (ant) fauna

The genetic diversity in North American populations of purportedly Holarctic ants is surprisingly high. For example, *La. americanus* stat. nov. consists of at least two deeply divergent lineages, well separated from one another by other *Lasius* species and with allopatric distribution, one in Eastern North America and the other in Arizona (Figure 1). Less extreme, but still striking is the divergence within native North American *La. aphidiculus* stat. nov., *La. brevicornis* stat. nov. and "*La. niger*," each comparable with that of a clade containing eight described species of *Lasius* s. str. in Europe. In addition, all those taxa are polyphyletic (Figure 1, Supporting Information Appendix S2). The provisional names resurrected here may be best understood as representing tentative species-complexes. The observed higher correlation of morphological and molecular taxonomy in Europe is not completely unexpected, because the European fauna has been the focus of intense effort over a longer historical period. For example, >40 *Lasius* species are currently catalogued in Europe (Borowiec, 2014), 16 more than recognized for the entire Palearctic in Wilson's revision in 1955 (Wilson, 1955) (+150%). However, the number of Nearctic *Lasius* species has remained nearly the same since the publication of Wilson's revision (Wilson, 1955). This contrast is also evident in the number of taxonomic revisions involving *Lasius* since 1955: 34 in Europe versus 12 in North America (AntWeb 2015). Contributing to this difference is the fact that North American and European ant taxonomy have diverged methodologically over the last 30 years. European ant taxonomy has increasingly relied on multivariate analyses of fine scale morphometric data in recent years (Seifert, Ritz, & Csósz, 2014), while most modern revisions of North American ants rely on classical morphological characters. This disparity in taxonomic methodologies reflects unresolved differences between European and North American taxonomists concerning the measurement and interpretation of morphological variation. While one approach may lead to overlooked cryptic diversity, the other could lead to taxonomic over-splitting. Molecular evidence has not been fully implemented as an essential tool in either of the two cases. Regardless of methodology, the results published

here indicate that additional work will be needed to further explore cryptic diversity in the North American ant fauna.

ACKNOWLEDGEMENTS

Special thanks to Steven O. Shattuck for important help with the taxonomic section of the paper. We are also very grateful to Lech Borowiec, Robert J. Higgins, Kathryn Daly, Vlad Dincă, André Francoeur, Alexander Kzutilin, John T. Longino, Frode Ødegaard, Derek S. Sikes, Robert A. Stevenson, Raluca Vodă and Philip S. Ward for contributing specimens to the study. This study was funded by the Swiss National Science Foundation (SNSF) via the Early Post-doc.Mobility grant P2SKP3_161677 to S. Schär, the Marie Curie Actions FP7-PEOPLE-2013-IOF (project 622716) to G. Talavera, and by CGL2016-76322-P (AEI/FEDER, UE) to R. Vila.

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BIOSKETCH

The authors are a team of evolutionary biologists, ecologists and taxonomists with a special focus on insects and ants in particular. Author contributions: Conceived of study: S.S., G.T.; performed laboratory work: S.S., G.T., J.R.; analysed data: S.S., G.T.; all authors contributed with DNA-material and to writing the manuscript.

SUPPORTING INFORMATION

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

How to cite this article: Schär S, Talavera G, Espadaler X, et al. Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae. *J Biogeogr*. 2018;00:1–12. <https://doi.org/10.1111/jbi.13380>

Supplementary Information to "Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae"

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Table S1. List of the twelve suspected Holarctic ants and their potential sister taxa in the Nearctic and Palearctic subregions included in this study. The Holarctic region was divided in four areas (West and East of each subregion). The outgroup taxa were included in order to test for monophyly of the Holarctic species.

Related species included in this study to test for monophyly

Suspected Holarctic taxon	Palearctic		Nearctic	
	West	East	West	East
<i>Camponotus herculeanus</i>	<i>C. ligniperda</i>	<i>C. japonicus</i>	<i>C. modoc</i>	<i>C. novaeboracensis</i>
	<i>C. saxatilis</i>	<i>C. sachalinensis</i>		<i>C. pennsylvanicus</i>
	<i>C. vagus</i>			
<i>Formica fusca</i>	<i>F. lemani</i>	<i>F. japonica</i>	<i>F. accreta</i>	<i>F. glacialis?</i>
		<i>F. lemani</i>	<i>F. argentea</i>	<i>F. incerta</i>
			<i>F. podzolica</i>	<i>F. subaenescens?</i>
				<i>F. subsericea?</i>
<i>F. gagatoides</i>	<i>F. picea</i>	<i>F. candida</i>	<i>F. neorufibarbis</i>	<i>F. neorufibarbis</i>
<i>Lasius alienus</i>	<i>La. bombycinus</i>		<i>La. crypticus</i>	<i>La. neoniger</i>
	<i>La. brunneus</i>		<i>La. niger</i>	
	<i>La. lasiooides</i>		<i>La. sitiens</i>	
	<i>La. neglectus</i>		<i>La. xerophilus</i>	
	<i>La. paralienus</i>			
	<i>La. piliferus</i>			
	<i>La. psammophilus</i>			
	<i>La. turcicus</i>			

<i>La. flavus</i>	<i>La. myops</i>	<i>La. fallax</i>	<i>La. nearcticus</i>
<i>La. niger</i>	<i>La. emarginatus</i>	<i>La. alienus</i>	<i>La. neoniger</i>
	<i>La. grandis</i>	<i>La. crypticus</i>	
	<i>La. platythorax</i>	<i>La. pallitarsis</i>	
		<i>La. xerophilus</i>	
<i>La. umbratus</i>	<i>La. distinguendus</i>	<i>La. arizonicus</i>	<i>La. claviger</i>
	<i>La. meridionalis</i>	<i>La. nevadensis</i>	<i>La. latipes</i>
	<i>La. mixtus</i>	<i>La. subumbratus</i>	<i>La. subumbratus</i>
	<i>La. nitidigaster</i>		
	<i>La. sabularum</i>		
<i>Leptothorax acervorum</i>	<i>Le. gredleri</i>	<i>Le. muscorum</i>	<i>Le. sp. "AF-can"</i>
	<i>Le. muscorum</i>	<i>Le. sp.</i>	
<i>Le. muscorum</i>	<i>Le. acervorum</i>	<i>Le. acervorum</i>	<i>Le. sp. "AF-can"</i>
	<i>Le. gredleri</i>	<i>Le. sp.</i>	
<i>Myrmica rubra</i>	<i>M. ruginodis</i>	<i>M. wheeleri</i>	
<i>Tetramorium atratum</i>	<i>T. caespitum</i>	<i>T. immigrans</i>	<i>T. immigrans</i>
	<i>T. hungaricum</i>		
	<i>T. immigrans</i>		
	<i>T. indocile</i>		
	<i>T. staercke</i>		
<i>T. immigrans</i>	<i>T. atratum</i>		<i>T. atratum</i>
	<i>T. caespitum</i>		
	<i>T. hungaricum</i>		
	<i>T. indocile</i>		

T. staercke

Table S2. Collection data, sequencing coverage and Genbank Accession numbers of samples used in this study. Collection abbreviations: MCZ = Museum of Comparative Zoology, Harvard University, RVcoll = Roger Vila research collection, Institut de Biología Evolutiva (CSIC-UPF), JTL = John T. Longino ant collection, The University of Utah, PSW = Philip S. Ward collection, University of California, UAM = University of Alaska Museum of the North, BOLD = Barcoding of Life Data Systems, NA15010 = a vial located at MCZ containing *Camponotus herculeanus* samples, labelled "Talkeetna Mountains, leg. 2003". RSA = samples of Robert A. Stevenson, located at MCZ.

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
	NA15010	<i>Camponotus herculeanus</i>	United States	62.00	-147.90	LT977357			
	RVcoll13S130	<i>Camponotus herculeanus</i>	United States	45.90	-69.05	LT977351	LT977283	LT977110	LT977195
JTL6130-3		<i>Camponotus herculeanus</i>	United States	43.92	-70.04	LT977356			
JTL8890		<i>Camponotus herculeanus</i>	United States	40.72	-110.19	LT977355			
JTL6931		<i>Camponotus herculeanus</i>	United States	46.91	-123.07	LT977354			
RVcoll13S199		<i>Camponotus herculeanus</i>	Austria	47.10	11.81	LT977349			
RVcoll17S542		<i>Camponotus herculeanus</i>	Bulgaria	42.26	23.30	LT977344			
RVcoll17S202		<i>Camponotus herculeanus</i>	Bulgaria	41.73	23.52	LT977352			
RVcoll17S554		<i>Camponotus herculeanus</i>	Denmark	56.05	12.04	LT977345			
RVcoll17S576		<i>Camponotus herculeanus</i>	Estonia	59.34	25.69	LT977346			
RVcoll13T062		<i>Camponotus herculeanus</i>	Russia	50.16	86.31	LT977343			
RVcoll17S522		<i>Camponotus herculeanus</i>	Russia	50.12	86.20	LT977348	LT977275	LT977102	LT977192
RVcoll17S363		<i>Camponotus herculeanus</i>	Russia	58.26	56.57	LT977353			
RVcoll13S189		<i>Camponotus herculeanus</i>	Spain	42.25	1.70	LT977350	LT977322	LT977149	LT977194
RVcoll17S384		<i>Camponotus herculeanus</i>	Sweden	58.78	13.74	LT977347			
RVcoll17S288		<i>Camponotus japonicus</i>	Japan	36.08	137.66	LT977358	LT977307	LT977134	LT977249
RVcoll13S161		<i>Camponotus ligniperda</i>	Spain	42.22	1.89	LT977360			
RVcoll13Y201		<i>Camponotus ligniperda</i>	Spain	42.31	1.73	LT977363			
RVcoll13Y206		<i>Camponotus ligniperda</i>	Spain	42.28	1.50	LT977362			
RVcoll13T044		<i>Camponotus ligniperda</i>	Sweden	56.84	16.65	LT977359			
RVcoll13S181		<i>Camponotus ligniperda</i>	Switzerland	46.11	7.94	LT977361	LT977302	LT977129	LT977198
RVcoll17S392		<i>Camponotus modoc</i>	United States	47.56	-121.14	LT977364	LT977319	LT977146	LT977196
RVcoll13T041		<i>Camponotus novae-boracensis</i>	United States	42.51	-72.21	LT977365			
RVcoll13T064		<i>Camponotus novae-boracensis</i>	United States	44.38	-68.20	LT977366	LT977332	LT977159	LT977216
RVcoll13T061		<i>Camponotus pennsylvanicus?</i>	United States	42.51	-72.21	LT977368	LT977320	LT977147	LT977200
RVcoll17S580		<i>Camponotus pennsylvanicus</i>	United States	40.71	-74.46	LT977367	LT977270	LT977097	LT977248
RVcoll17S525		<i>Camponotus sachalinensis</i>	Russia	55.93	158.67	LT977369	LT977337	LT977163	LT977193

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
	RVcoll16B132	<i>Camponotus sachalinensis</i>	Japan	36.11	137.60	LT977370			
	RVcoll17S571	<i>Camponotus saxatilis</i>	Russia	50.10	86.46	LT977371	LT977277	LT977104	LT977197
	RVcoll17S553	<i>Camponotus vagus</i>	Bulgaria	43.60	22.67	LT977372	LT977284	LT977111	LT977199
	PSW15462	<i>Formica acreta</i>	United States	41.05	-122.88	LT978303			
	PSW17349	<i>Formica acreta</i>	United States	39.38	-122.76	LT977373	LT977334	LT977161	LT977215
	RSA004	<i>Formica argentea?</i>	United States	42.38	-71.11	LT977376			
	RSA005	<i>Formica argentea?</i>	United States	42.38	-71.11	LT977375			
	JTL5951	<i>Formica argentea</i>	United States	47.55	-120.76	LT977374	LT977276	LT977103	LT977210
	RVcoll17S211	<i>Formica candida</i>	Russia	53.19	158.48	LT977377	LT977340	LT977166	
	JTL7126	<i>Formica fusca</i>	United States	37.53	-118.20	LT977393	LT977331	LT977158	LT977214
	PSW15950	<i>Formica fusca</i>	United States	40.47	-121.51	LT977394			
	PSW16378	<i>Formica fusca</i>	United States	39.32	-119.90	LT977395			
	JTL8008	<i>Formica fusca</i>	United States	40.85	-111.71	LT977391			
	JTL8971	<i>Formica fusca</i>	United States	40.56	-110.29	LT977392			
	JTL7831	<i>Formica fusca</i>	United States	47.48	-123.74	LT977390			
	RVcoll17S569	<i>Formica fusca</i>	Bulgaria	41.82	24.58	LT977378			
	RVcoll13T065	<i>Formica fusca</i>	Denmark	55.47	12.15	LT977379			
	RVcoll17S389	<i>Formica fusca</i>	Finland	60.45	22.29	LT977383	LT977304	LT977131	LT977206
	RVcoll17S368	<i>Formica fusca</i>	Russia	55.12	37.69	LT977386			
	RVcoll13S162	<i>Formica fusca</i>	Spain	42.22	1.89	LT977385	LT977286	LT977113	LT977209
	RVcoll13Y203	<i>Formica fusca</i>	Spain	42.30	1.67	LT977388			
	RVcoll13Y209	<i>Formica fusca</i>	Spain	42.27	1.60	LT977387			
	RVcoll13Y213	<i>Formica fusca</i>	Spain	42.33	1.71	LT977389			
	RVcoll17S373	<i>Formica fusca</i>	Sweden	58.78	13.75	LT977382			
	RVcoll13S192	<i>Formica fusca</i>	Switzerland	46.13	7.63	LT977384			
	RVcoll17S536	<i>Formica fusca?</i>	Russia	53.19	158.36	LT977380			
	RVcoll17S209	<i>Formica fusca?</i>	Russia	55.93	158.67	LT977381			
BOLD: MHANT242-07		<i>Formica gagatoides</i>	Canada	58.62	-93.82	FJ413256			
BOLD: CNIVC571-14		<i>Formica gagatoides</i>	Canada	69.17	-140.17	KR798307			
	RVcoll16B242	<i>Formica gagatoides</i>	Norway	69.01	23.10	LT977396			
	RVcoll17S279	<i>Formica gagatoides</i>	Norway	62.58	9.59	LT977398	LT977339	LT977165	
	RVcoll17S206	<i>Formica gagatoides</i>	Russia	53.16	158.13	LT977399	LT977338	LT977164	
	RVcoll17S548	<i>Formica gagatoides</i>	Russia	55.95	158.65	LT977397			
	RVcoll13T069	<i>Formica glacialis?</i>	United States	44.38	-68.20	LT977400	LT977336	LT977162	
	RVcoll13S116	<i>Formica incerta</i>	United States	43.08	-71.17	LT977401			
	RVcoll16B055	<i>Formica japonica</i>	Japan	35.34	138.80	LT977402	LT977333	LT977160	LT977207
	RVcoll17S589	<i>Formica lemani</i>	Bulgaria	42.21	23.32	LT977403			
	RVcoll13S185	<i>Formica lemani</i>	France	42.69	1.94	LT977406			
	RVcoll17S210	<i>Formica lemani</i>	Russia	55.94	158.68	LT977405			
	RVcoll17S208	<i>Formica lemani</i>	Russia	53.18	158.36	LT977404			
	RVcoll13S183	<i>Formica lemani</i>	Spain	42.31	1.91	LT977407	LT977287	LT977114	LT977208
UAM:Ento:309087	2011DSS031	<i>Formica neorufibarbis</i>	United States	59.63	-151.05	LT977408			
UAM:Ento:142050		<i>Formica neorufibarbis</i>	United States	64.96	-148.38	LT977410	LT977342	LT977168	
	RVcoll17S207	<i>Formica neorufibarbis</i>	United States	44.28	-71.28	LT977409	LT977341	LT977167	
	RVcoll17S203	<i>Formica picea</i>	Denmark	55.83	12.56	LT977411			
	JTL8344	<i>Formica podzolica</i>	United States	40.69	-111.67	LT977412			

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
	JTL5885	<i>Formica podzolica</i>	United States	48.42	-122.88	LT977413	LT977300	LT977127	LT977205
	RVcoll17S294	<i>Formica sp.</i>	Canada	~53.00	~-67.00	LT977416	LT977335		
MCZ:Ent:662287	SPC7523	<i>Formica sp.</i>	United States	41.81	-70.66	LT977415			
	RVcoll13S118	<i>Formica sp.</i>	United States	43.08	-71.17	LT977414			
MCZ:Ent:662100	SPC7695	<i>Formica sp.</i>	United States	38.71	-111.95	LT977417			
	RSA006	<i>Formica subaenescens?</i>	United States	42.38	-71.11	LT977419			
	RSA007	<i>Formica subaenescens?</i>	United States	42.38	-71.11	LT977418			
	RVcoll13T067	<i>Formica subsericea?</i>	United States	42.39	-71.11	LT977421	LT977285	LT977112	LT977259
	RVcoll13T068	<i>Formica subsericea?</i>	United States	42.51	-72.21	LT977420			
MCZ:Ent:654617	SPC6233	<i>Lasius alienus</i>	United States	32.65	-109.81	LT977426			
MCZ:Ent:655585	SPC8139	<i>Lasius alienus</i>	United States	31.92	-109.26	LT977439	LT977278	LT977105	LT977222
MCZ:Ent:655952	SPC8507	<i>Lasius alienus</i>	United States	30.58	-86.54	LT977433			
	RVcoll13T071	<i>Lasius alienus</i>	United States	42.51	-72.21	LT977437	LT977292	LT977119	LT977250
	RVcoll13T098	<i>Lasius alienus</i>	United States	42.42	-70.99	LT977424			
	RVcoll16B017	<i>Lasius alienus</i>	United States	39.58	-76.18	LT977427			
	RVcoll13T072	<i>Lasius alienus</i>	United States	44.38	-68.20	LT977425			
MCZ:Ent:654998	SPC7069	<i>Lasius alienus</i>	United States	44.30	-70.03	LT977436			
	RVcoll13T073	<i>Lasius alienus</i>	United States	40.71	-74.46	LT977432			
	RVcoll13T056	<i>Lasius alienus</i>	United States	40.76	-73.98	LT977434			
	RVcoll17S560	<i>Lasius alienus</i>	Bulgaria	41.52	23.40	LT977429			
	RVcoll17S529	<i>Lasius alienus</i>	Bulgaria	43.61	22.67	LT977430			
	RVcoll17S562	<i>Lasius alienus</i>	Bulgaria	42.62	23.30	LT977428			
	RVcoll13S164	<i>Lasius alienus</i>	Spain	42.22	1.89	LT977435			
	RVcoll13S179	<i>Lasius alienus</i>	Spain	42.22	1.89	LT977423			
	RVcoll13Y207	<i>Lasius alienus</i>	Spain	42.26	1.61	LT977422			
	RVcoll13Y212	<i>Lasius alienus</i>	Spain	42.28	1.50	LT977438	LT977308	LT977135	LT977225
	RVcoll13T075	<i>Lasius alienus</i>	Switzerland	47.47	8.32	LT977431			
MCZ:Ent:662036	SPC7600	<i>Lasius arizonicus</i>	United States	31.51	-110.69	LT977440			
	RVcoll17S539	<i>Lasius bombycinus</i>	Bulgaria	41.82	23.57	LT977442	LT977297	LT977124	LT977220
	RVcoll17S700	<i>Lasius bombycinus</i>	Bulgaria	41.53	23.39	LT977441			
	RVcoll17S551	<i>Lasius brunneus</i>	Switzerland	47.40	8.38	LT977443			
	RVcoll13R117	<i>Lasius claviger</i>	United States	43.08	-71.17	LT977444			
MCZ:Ent:655102	SPC7250	<i>Lasius crypticus</i>	United States	39.70	-108.80	LT977445	LT977317	LT977144	LT977230
MCZ:Ent:662231	SPC7866	<i>Lasius crypticus</i>	United States	38.37	-109.20	LT977553			
	RVcoll17S558	<i>Lasius distinguendus</i>	Bulgaria	43.60	22.78	LT977447	LT977267	LT977094	LT977213
	RVcoll17S523	<i>Lasius distinguendus</i>	Bulgaria	41.63	24.35	LT977446			
	RVcoll17S585	<i>Lasius emarginatus</i>	Switzerland	46.20	6.15	LT977448			
MCZ:Ent:655314	SPC7903	<i>Lasius fallax</i>	United States	36.53	-112.15	LT977451	LT977280	LT977107	LT977242
MCZ:Ent:655840	SPC8394	<i>Lasius fallax</i>	United States	33.98	-109.37	LT977452	LT977281	LT977108	LT977241
MCZ:Ent:655166	SPC7354	<i>Lasius fallax</i>	United States	37.79	-109.51	LT977450			
MCZ:Ent:655582	SPC8135	<i>Lasius fallax</i>	United States	39.11	-111.38	LT977449			
MCZ:Ent:654750	SPC6530	<i>Lasius flavus</i>	United States	32.66	-109.86	LT977467	LT977316	LT977143	LT977243
	RVcoll13Y279	<i>Lasius flavus</i>	United States	41.93	-70.07	LT977453			
MCZ:Ent:654999	SPC7072	<i>Lasius flavus</i>	United States	44.31	-70.02	LT977468	LT977315	LT977142	LT977260
MCZ:Ent:662069	SPC7655	<i>Lasius flavus</i>	United States	44.38	-68.29	LT977465			
	RVcoll13T076	<i>Lasius flavus</i>	United States	40.80	-76.41	LT977460			

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
MCZ:Ent:654933	SPC6973	<i>Lasius flavus</i>	United States	34.20	-109.72	LT977471			
MCZ:Ent:654996	SPC7063	<i>Lasius flavus</i>	United States	44.30	-70.03	LT977472			
MCZ:Ent:655337	SPC 7929	<i>Lasius flavus</i>	United States	37.48	-112.56	LT977470			
	RVcoll13S112	<i>Lasius flavus</i>	United States	43.08	-71.17	LT977461			
	RVcoll13T077	<i>Lasius flavus</i>	Denmark	55.71	12.58	LT977454			
	RVcoll17S372	<i>Lasius flavus</i>	Finland	60.45	22.29	LT977455			
	RVcoll16B133	<i>Lasius flavus</i>	Japan	36.18	137.49	LT977469			
	RVcoll17S365	<i>Lasius flavus</i>	Russia	55.12	37.69	LT977462			
	RVcoll13Y202	<i>Lasius flavus</i>	Spain	42.31	1.73	LT977464			
	RVcoll13Y210	<i>Lasius flavus</i>	Spain	42.30	1.50	LT977463			
	RVcoll17S532	<i>Lasius flavus</i>	Sweden	56.57	16.61	LT977456			
	RVcoll17S388	<i>Lasius flavus</i>	Sweden	58.78	13.75	LT977458			
	RVcoll17S579	<i>Lasius flavus</i>	Sweden	55.72	13.46	LT977457			
	RVcoll13T078	<i>Lasius flavus</i>	Switzerland	47.47	8.31	LT977459			
	RVcoll17S586	<i>Lasius flavus</i>	Switzerland	47.39	8.55	LT977466	LT977296	LT977123	LT977237
	RVcoll17S374	<i>Lasius grandis</i>	Spain	36.72	-4.41	LT977473			
MCZ:Ent:656108	SPC8660	<i>Lasius interjectus</i>	United States	40.88	-73.08	LT977474			
	RVcoll17S535	<i>Lasius lasiooides</i>	Croatia	43.59	15.92	LT977475			
MCZ:Ent:656118	SPC8670	<i>Lasius latipes</i>	United States	40.85	-72.65	LT977476			
	RVcoll17S530	<i>Lasius meridionalis</i>	Denmark	56.01	11.99	LT977477			
	RVcoll17S397	<i>Lasius meridionalis</i>	Denmark	55.48	12.20	LT977478			
	RVcoll17S545	<i>Lasius meridionalis</i>	Denmark	55.55	12.24	LT977481	LT977328	LT977155	LT977233
	RVcoll17S390	<i>Lasius meridionalis</i>	Sweden	55.72	13.47	LT977480			
	RVcoll17S524	<i>Lasius meridionalis</i>	Sweden	55.72	13.47	LT977479			
	RVcoll17S570	<i>Lasius mixtus</i>	Switzerland	47.40	8.39	LT977482	LT977303	LT977130	LT977238
	RVcoll13Y208	<i>Lasius myops</i>	France	42.48	2.94	LT977483			
	RVcoll13T007	<i>Lasius myops</i>	Italy	46.64	11.57	LT977485	LT977298	LT977125	LT977240
	RVcoll13Y211	<i>Lasius myops</i>	Spain	41.85	2.12	LT977484			
MCZ:Ent:657204	SPC8695	<i>Lasius nearcticus</i>	United States	42.40	-71.52	LT977487			
	RVcoll13S113	<i>Lasius nearcticus</i>	United States	43.08	-71.17	LT977486	LT977321	LT977148	LT977232
	RVcoll17S549	<i>Lasius neglectus</i>	Bulgaria	43.41	28.16	LT977488			
	RVcoll17S366	<i>Lasius neglectus</i>	Russia	44.80	37.41	LT977489			
	RVcoll13S137	<i>Lasius neoniger</i>	United States	42.38	-71.12	LT977491			
	RVcoll13T055	<i>Lasius neoniger</i>	United States	43.86	-76.2	LT977490			
	RVcoll13T054	<i>Lasius neoniger</i>	United States	45.00	-73.34	LT977492	LT977327	LT977154	LT977231
MCZ:Ent:655445	SPC8047	<i>Lasius nevadensis</i>	United States	36.26	-115.64	LT977493	LT977309	LT977136	LT977236
BOLD: SMTPM2984-15		<i>Lasius niger</i>	Canada	49.22	-123.02	MG339722			
BOLD: HPPPH364-13		<i>Lasius niger</i>	Canada	44.62	-63.57	KR807345			
MCZ:Ent:654847	SPC6809	<i>Lasius niger</i>	United States	36.61	-112.35	LT977519			
MCZ:Ent:651486	SPC6811	<i>Lasius niger</i>	United States	36.61	-112.35	LT977522	LT977323	LT977150	LT977224
MCZ:Ent:654865	SPC6840	<i>Lasius niger</i>	United States	36.68	-112.22	LT977514			
MCZ:Ent:655309	SPC7897	<i>Lasius niger</i>	United States	36.74	-112.22	LT977517			
MCZ:Ent:655107	SPC7261	<i>Lasius niger</i>	United States	39.07	-108.09	LT977518			
	RSA001	<i>Lasius niger?</i>	United States	42.68	-70.75	LT977524			
	RSA002	<i>Lasius niger?</i>	United States	41.53	-70.68	LT977525			
	RSA003	<i>Lasius niger?</i>	United States	41.28	-70.04	LT977526			

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
MCZ:Ent:655446	SPC8048	<i>Lasius niger</i>	United States	36.26	-115.64	LT977521	LT977314	LT977141	LT977219
MCZ:Ent:655496	SPC8051	<i>Lasius niger</i>	United States	36.31	-115.61	LT977512			
MCZ:Ent:656017	SPC8571	<i>Lasius niger</i>	United States	40.66	-109.48	LT977508			
MCZ:Ent:656037	SPC8590	<i>Lasius niger</i>	United States	40.70	-109.49	LT977507			
MCZ:Ent:655169	SPC7358	<i>Lasius niger</i>	United States	37.83	-109.51	LT977523	LT977313	LT977140	LT977227
MCZ:Ent:655182	SPC7378	<i>Lasius niger</i>	United States	38.37	-109.17	LT977516			
MCZ:Ent:655293	SPC7826	<i>Lasius niger</i>	United States	38.51	-109.32	LT977494			
MCZ:Ent:655344	SPC7938	<i>Lasius niger</i>	United States	37.44	-112.53	LT977513			
MCZ:Ent:655574	SPC8127	<i>Lasius niger</i>	United States	39.09	-111.31	LT977515			
MCZ:Ent:655427	SPC8028	<i>Lasius niger</i>	United States	36.34	-115.65	LT977549			
	RVcoll17S377	<i>Lasius niger</i>	Bulgaria	43.36	28.08	LT977495			
	RVcoll17S556	<i>Lasius niger</i>	Bulgaria	42.71	23.32	LT977496			
	RVcoll17S538	<i>Lasius niger</i>	Croatia	43.80	15.95	LT977497			
	RVcoll17S557	<i>Lasius niger</i>	Denmark	56.46	10.04	LT977520	LT977282	LT977109	LT977223
	RVcoll17S587	<i>Lasius niger</i>	Estonia	58.92	26.28	LT977499			
	RVcoll17S578	<i>Lasius niger</i>	Estonia	59.34	25.69	LT977498			
	RVcoll17S383	<i>Lasius niger</i>	Finland	60.45	22.29	LT977500			
	RVcoll13T084	<i>Lasius niger</i>	Russia	50.16	86.31	LT977501			
	RVcoll17S282	<i>Lasius niger</i>	Russia	53.40	91.17	LT977502			
	RVcoll17S364	<i>Lasius niger</i>	Russia	55.12	37.69	LT977510			
	RVcoll17S201	<i>Lasius niger</i>	Russia	55.96	37.40	LT977511			
	RVcoll17S375	<i>Lasius niger</i>	Sweden	58.89	14.01	LT977504			
	RVcoll17S590	<i>Lasius niger</i>	Sweden	55.70	13.47	LT977503			
	RVcoll13S193	<i>Lasius niger</i>	Switzerland	46.13	7.63	LT977509			
	RVcoll17S575	<i>Lasius niger</i>	Switzerland	47.39	8.55	LT977506			
	RVcoll17S533	<i>Lasius niger</i>	Switzerland	46.21	6.15	LT977505			
	RVcoll17S543	<i>Lasius nitidigaster</i>	Bulgaria	43.60	22.67	LT977527	LT977274	LT977101	LT977211
MCZ:Ent:655816	SPC8370	<i>Lasius occidentalis</i>	United States	34.24	-109.52	LT977528			
	RVcoll17S394	<i>Lasius pallitarsis</i>	United States	47.55	-121.13	LT977529			
	RVcoll17S391	<i>Lasius paralienus</i>	Bulgaria	43.57	27.83	LT977531	LT977325	LT977152	LT977218
	RVcoll17S574	<i>Lasius paralienus</i>	Switzerland	47.47	8.31	LT977530			
	RVcoll17S577	<i>Lasius paralienus</i>	Bulgaria	43.60	22.67	LT977532			
	RVcoll17S303	<i>Lasius piliferus</i>	Spain	40.39	-0.65	LT977533	LT977288	LT977115	LT977226
	RVcoll17S559	<i>Lasius platythorax</i>	Bulgaria	43.60	22.78	LT977535			
	RVcoll13T046	<i>Lasius platythorax</i>	Denmark	55.83	12.56	LT977539	LT977272	LT977099	LT977221
	RVcoll17S380	<i>Lasius platythorax</i>	Finland	60.48	22.32	LT977536			
	RVcoll13S147	<i>Lasius platythorax</i>	Spain	42.22	1.96	LT977538			
	RVcoll13S177	<i>Lasius platythorax</i>	Spain	42.22	1.89	LT977537			
	RVcoll13T085	<i>Lasius platythorax</i>	Switzerland	47.40	8.39	LT977534			
	RVcoll17S537	<i>Lasius psammophilus</i>	Bulgaria	42.26	23.30	LT977544	LT977273	LT977100	LT977228
	RVcoll13T086	<i>Lasius psammophilus</i>	Denmark	55.55	12.25	LT977540			
	RVcoll13Y205	<i>Lasius psammophilus</i>	Denmark	56.01	11.99	LT977543			
	RVcoll17S540	<i>Lasius psammophilus</i>	Estonia	58.91	22.13	LT977541			
	RVcoll13T043	<i>Lasius psammophilus</i>	Sweden	56.64	16.57	LT977542			
	RVcoll17S520	<i>Lasius sabularum</i>	Finland	60.51	22.28	LT977545			
	RVcoll17S971	<i>Lasius sabularum</i>	Switzerland	47.40	8.39	LT977546			

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
	RVcoll17S378	<i>Lasius sabularum</i>	Switzerland	47.40	8.38	LT977547	LT977324	LT977151	LT977234
MCZ:Ent:655524	SPC8078	<i>Lasius sitiens</i>	United States	38.84	-111.74	LT977548			
MCZ:Ent:655821	SPC8375	<i>Lasius sp.</i>	United States	34.24	-109.52	LT977550			
MCZ:Ent:655507	SPC8062	<i>Lasius sp.</i>	United States	36.34	-115.65	LT977551			
MCZ:Ent:655521	SPC8075	<i>Lasius sp.</i>	United States	38.65	-111.96	LT977552			
MCZ:Ent:655595	SPC8150	<i>Lasius sp.</i>	United States	31.91	-109.24	LT977554			
MCZ:Ent:651407	SPC6528	<i>Lasius subumbratus</i>	United States	32.66	-109.86	LT977555			
MCZ:Ent:654938	SPC6980	<i>Lasius subumbratus</i>	United States	34.03	-109.77	LT977556			
MCZ:Ent:655290	SPC7579	<i>Lasius subumbratus</i>	United States	31.91	-109.27	LT977557			
MCZ:Ent:655754	SPC8309	<i>Lasius subumbratus</i>	United States	44.35	-68.07	LT977558	LT977310	LT977137	LT977239
	RVcoll17S550	<i>Lasius turcicus</i>	Bulgaria	43.60	22.78	LT977559			
MCZ:Ent:651585	SPC6631	<i>Lasius umbratus</i>	United States	33.52	-109.31	LT977570			
MCZ:Ent:654942	SPC6984	<i>Lasius umbratus</i>	United States	34.29	-110.86	LT977571			
MCZ:Ent:655810	SPC8364	<i>Lasius umbratus</i>	United States	34.19	-109.73	LT977563			
MCZ:Ent:655811	SPC8365	<i>Lasius umbratus</i>	United States	34.19	-109.73	LT977572			
MCZ:Ent:651542	SPC7271	<i>Lasius umbratus</i>	United States	39.15	-107.93	LT977575			
MCZ:Ent:655120	SPC7282	<i>Lasius umbratus</i>	United States	39.17	-107.95	LT977580	LT977311	LT977138	LT977246
MCZ:Ent:655970	SPC8524	<i>Lasius umbratus</i>	United States	30.56	-86.56	LT977573			
	RVcoll13T087	<i>Lasius umbratus</i>	United States	42.51	-72.21	LT977578	LT977291	LT977118	LT977245
MCZ:Ent:655063	SPC7190	<i>Lasius umbratus</i>	United States	42.4	-71.52	LT977581	LT977318	LT977145	LT977247
MCZ:Ent:655613	SPC8168	<i>Lasius umbratus</i>	United States	42.46	-71.31	LT977574			
	RVcoll13T088	<i>Lasius umbratus</i>	United States	44.38	-68.20	LT977568			
MCZ:Ent:651520	SPC7070	<i>Lasius umbratus</i>	United States	44.3	-70.03	LT977562			
MCZ:Ent:651535	SPC7242	<i>Lasius umbratus</i>	United States	39.56	-108.80	LT977576			
MCZ:Ent:655292	SPC7824	<i>Lasius umbratus</i>	United States	38.51	-109.32	LT977579	LT977312	LT977139	LT977244
MCZ:Ent:655336	SPC7928	<i>Lasius umbratus</i>	United States	37.48	-112.56	LT977561			
	RVcoll17S367	<i>Lasius umbratus</i>	Belarus	53.97	26.89	LT977569			
	RVcoll13T090	<i>Lasius umbratus</i>	Denmark	55.46	12.18	LT977577	LT977269	LT977096	LT977235
	RVcoll17S588	<i>Lasius umbratus</i>	Denmark	55.83	12.57	LT977564			
	RVcoll17S521	<i>Lasius umbratus</i>	Denmark	55.70	12.56	LT977565			
	RVcoll13T096	<i>Lasius umbratus</i>	Spain	42.02	2.44	LT977560			
	RVcoll17S531	<i>Lasius umbratus</i>	Sweden	55.72	13.46	LT977566			
	RVcoll13T092	<i>Lasius umbratus</i>	Switzerland	47.41	8.37	LT977567			
MCZ:Ent:655333	SPC7925	<i>Lasius xerophilus</i>	United States	37.16	-112.59	LT977582	LT977293	LT977120	LT977229
UAM:Ento:306588		<i>Leptothorax acervorum</i>	United States	65.57	-144.84	LT977594	LT977330	LT977157	LT977202
	RVcoll17S544	<i>Leptothorax acervorum</i>	Bulgaria	42.22	23.30	LT977583			
	RVcoll17S546	<i>Leptothorax acervorum</i>	Denmark	55.83	12.56	LT977584			
	RVcoll17S376	<i>Leptothorax acervorum</i>	Finland	60.51	22.27	LT977585			
	RVcoll17S564	<i>Leptothorax acervorum</i>	Russia	53.16	158.14	LT977587			
	RVcoll17S591	<i>Leptothorax acervorum</i>	Russia	55.94	158.69	LT977586			
	RVcoll17S371	<i>Leptothorax acervorum</i>	Russia	55.12	37.70	LT977593			
	RVcoll17S285	<i>Leptothorax acervorum</i>	Spain	42.30	1.92	LT977595	LT977301	LT977128	LT977201
	RVcoll17S284	<i>Leptothorax acervorum</i>	Spain	40.52	-1.65	LT977588			
	RVcoll17S283	<i>Leptothorax acervorum</i>	Spain	40.52	-1.65	LT977589			
	RVcoll16C436	<i>Leptothorax acervorum</i>	Sweden	68.32	18.86	LT977590			
	RVcoll17S972	<i>Leptothorax acervorum</i>	Switzerland	47.47	7.78	LT977592			

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
	RVcoll17S973	<i>Leptothorax acervorum</i>	Switzerland	47.40	8.36	LT977591			
	RVcoll17S280	<i>Leptothorax gredleri</i>	Poland	51.15	17.08	LT977596	LT977290	LT977117	LT977212
	PSW15435	<i>Leptothorax muscorum</i>	United States	65.22	-148.06	LT977601			
	JTL8969	<i>Leptothorax muscorum</i>	United States	40.56	-110.29	LT977600	LT977268	LT977095	LT977203
	JTL6936	<i>Leptothorax muscorum</i>	United States	46.91	-123.08	LT977598			
	RVcoll17S278	<i>Leptothorax muscorum</i>	Norway	59.13	9.67	LT977597			
	Rvcoll13Y258	<i>Leptothorax muscorum</i>	Spain	42.27	1.70	LT977599	LT977326	LT977153	LT977204
	Rvcoll17S395	<i>Leptothorax</i> sp. "AF-can"	United States	44.30	-71.28	LT977603			
MCZ:Ent:654826	SPC6763	<i>Leptothorax</i> sp.	United States	38.18	-107.62	LT977602			
	RVcoll17S300	<i>Myrmica rubra</i>	Canada	49.30	-123.10	LT977612			
	RVcoll13T099	<i>Myrmica rubra</i>	United States	42.39	-71.00	LT977616	LT977295	LT977122	LT977253
	RVcoll17S568	<i>Myrmica rubra</i>	United States	42.42	-70.99	LT977611			
	RVcoll17S581	<i>Myrmica rubra</i>	United States	42.42	-71.00	LT977610			
	RVcoll17S565	<i>Myrmica rubra</i>	United States	42.39	-70.99	LT977609			
	RVcoll13T052	<i>Myrmica rubra</i>	United States	44.38	-68.20	LT977605			
	RVcoll17S582	<i>Myrmica rubra</i>	Bulgaria	41.82	23.55	LT977615	LT977289	LT977116	LT977252
	RVcoll13T048	<i>Myrmica rubra</i>	Denmark	57.31	11.11	LT977606			
	RVcoll17S387	<i>Myrmica rubra</i>	Finland	60.45	22.29	LT977607			
	RVcoll17S369	<i>Myrmica rubra</i>	Russia	58.02	56.33	LT977613			
	RVcoll13Y214	<i>Myrmica rubra</i>	Spain	42.25	1.61	LT977614			
	RVcoll17S396	<i>Myrmica rubra</i>	Sweden	55.70	13.50	LT977608			
	RVcoll13S198	<i>Myrmica rubra</i>	Switzerland	46.13	7.63	LT977604			
	RVcoll17S566	<i>Myrmica ruginodis</i>	Bulgaria	42.25	23.30	LT977621	LT977279	LT977106	LT977251
	RVcoll13T057	<i>Myrmica ruginodis</i>	Denmark	56.33	8.48	LT977617			
	RVcoll17S386	<i>Myrmica ruginodis</i>	Finland	60.48	22.32	LT977618			
	RVcoll13Y204	<i>Myrmica ruginodis</i>	Spain	42.33	1.71	LT977620			
	RVcoll17S385	<i>Myrmica ruginodis</i>	Sweden	55.72	13.46	LT977619			
MCZ:Ent:654700	SPC6413	<i>Myrmica wheeleri</i>	United States	31.94	-109.30			LT978304	
	GJ498	<i>Myrmica wheeleri</i>				GQ255195			
BOLD: CNPPA3045-12		<i>Tetramorium atratum</i>	Canada	41.94	-82.52	KJ167194			
	RVcoll17S555	<i>Tetramorium atratum</i>	Bulgaria	41.64	24.06	LT977622			
	RVcoll13T091	<i>Tetramorium caespitum</i>	Switzerland	47.40	8.38	LT977624			
	RVcoll17S552	<i>Tetramorium caespitum</i>	Bulgaria	41.71	23.51	LT977625	LT977329	LT977156	LT977217
	RVcoll17S398	<i>Tetramorium caespitum</i>	Sweden	55.72	13.47	LT977623			
	RVcoll17S528	<i>Tetramorium hungaricum</i>	Bulgaria	41.63	24.37	LT977626	LT977271	LT977098	LT977258
	RVcoll13T095	<i>Tetramorium immigrans</i>	Canada	49.03	-123.08	LT977627			
MCZ:Ent:550546	JDCRC04	<i>Tetramorium immigrans</i>	United States	39.98	-105.11	LT977640			
	RVcoll17S583	<i>Tetramorium immigrans</i>	United States	42.39	-70.99	LT977634			
	RVcoll17S534	<i>Tetramorium immigrans</i>	United States	42.38	-71.10	LT977637			
	RVcoll17S572	<i>Tetramorium immigrans</i>	United States	42.39	-71.12	LT977636			
	RVcoll17S561	<i>Tetramorium immigrans</i>	United States	42.38	-71.12	LT977642	LT977294	LT977121	LT977256
	RVcoll17S592	<i>Tetramorium immigrans</i>	United States	42.39	-71.11	LT977635			

Specimen code	Collection code	Species	Country	Lat.	Lon.	COI	Wg	28S	Top1
RVcoll13T097		<i>Tetramorium immigrans</i>	United States	44.39	-68.21	LT977638			
RVcoll17S304		<i>Tetramorium immigrans</i>	Bulgaria	43.40	28.17	LT977641	LT977306	LT977133	LT977257
RVcoll17S541		<i>Tetramorium immigrans</i>	Croatia	43.52	16.25	LT977628			
RVcoll17S526		<i>Tetramorium immigrans</i>	Croatia	43.51	16.19	LT977629			
RVcoll17S370		<i>Tetramorium immigrans</i>	Russia	44.80	37.41	LT977639			
RVcoll17S382		<i>Tetramorium immigrans</i>	Spain	36.72	-4.41	LT977630			
RVcoll17S547		<i>Tetramorium immigrans</i>	Spain	41.39	2.20	LT977631			
RVcoll17S567		<i>Tetramorium immigrans</i>	Switzerland	46.20	6.15	LT977633			
RVcoll17S584		<i>Tetramorium immigrans</i>	Switzerland	46.21	6.15	LT977632			
RVcoll13T094		<i>Tetramorium indocile</i>	Russia	50.16	86.31	LT977643	LT977299	LT977126	LT977254
RVcoll17S379		<i>Tetramorium staerckeii</i>	Bulgaria	43.60	22.67	LT977645	LT977305	LT977132	LT977255
RVcoll17S563		<i>Tetramorium staerckeii</i>	Bulgaria	42.69	23.41	LT977644			

Table S3. Manual dispersal multipliers coded in the BIOGEOBEARS analyses to weight likelihood of dispersal events. The Holarctic region was divided into four subregions (areas): Western Palaearctic (WPa), Eastern Palaearctic (EPa), Western Nearctic (WNa) and Eastern Palaearctic (ENa). Rows are areas of origin and columns areas of destination. Probabilities are identical in both directions (thus probabilities above and below the diagonal are identical). The probability to remain in an area is 1 (diagonal).

from \ to	WPa	EPa	WNa	ENa
WPa	1	1	0	0.01
EPa		1	1	0.8
WNa	0	0.8	1	1
ENa	0.01	0	1	1

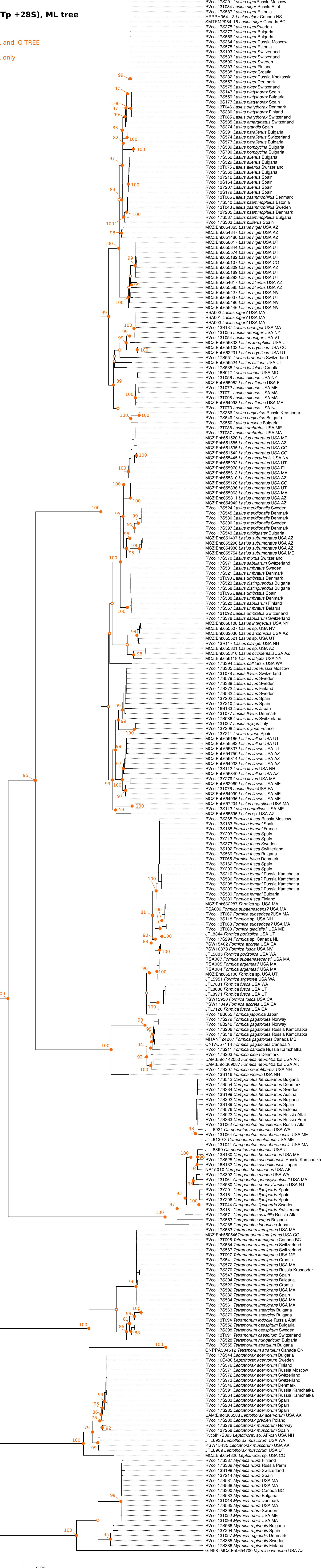
Table S4. Model testing output parameters for the BIOGEOBEARS analysis for the genera *Camponotus*, *Leptothorax* and *Formica*. Best resulting models are shaded.

Clade	Model	LnL	d.f.	<i>d</i>	<i>e</i>	<i>j</i>	AIC	Δ AIC
<i>Formica</i>	DEC	-59.72	2	0.0756	0.0111	0.0000	123.4	25.89
	DEC+J	-46.19	3	1e-12	1e-12	0.1272	98.39	0.88
	DIVALIKE	-55.66	2	0.0961	0.0119	0.0000	115.3	17.79
	DIVALIKE+J	-45.75	3	1e-12	1e-12	0.1225	97.51	0
	BAYAREALIKE	-84.57	2	0.0592	0.1729	0.0000	173.2	75.69
	BAYAREALIKE+J	-46.74	3	1e-07	1e-07	0.1201	99.5	1.99
<i>Camponotus</i>	DEC	-46.23	2	0.1563	0.0560	0.0000	96.47	34.53
	DEC+J	-28.19	3	1e-12	1e-12	0.27	62.38	0.44
	DIVALIKE	-46.68	2	0.2069	0.0444	0.0000	97.37	35.43
	DIVALIKE+J	-27.96	3	1e-12	1e-12	0.2671	61.94	0
	BAYAREALIKE	-67.59	2	0.2635	0.2641	0.0000	139.2	77.26
	BAYAREALIKE+J	-28.67	3	1e-07	1e-07	0.2412	63.35	1.41
<i>Leptothorax</i>	DEC	-30.17	2	0.1489	0.0331	0.0000	64.35	12.45
	DEC+J	-23.22	3	1e-12	1e-12	0.2925	52.45	0.55
	DIVALIKE	-34.03	2	0.1969	0.0453	0.0000	72.07	20.17
	DIVALIKE+J	-22.94	3	1e-12	1e-12	0.2817	51.9	0
	BAYAREALIKE	-39.99	2	0.1602	0.1401	0.0000	84	32.1
	BAYAREALIKE+J	-23.37	3	1e-07	1e-07	0.2418	52.74	0.84

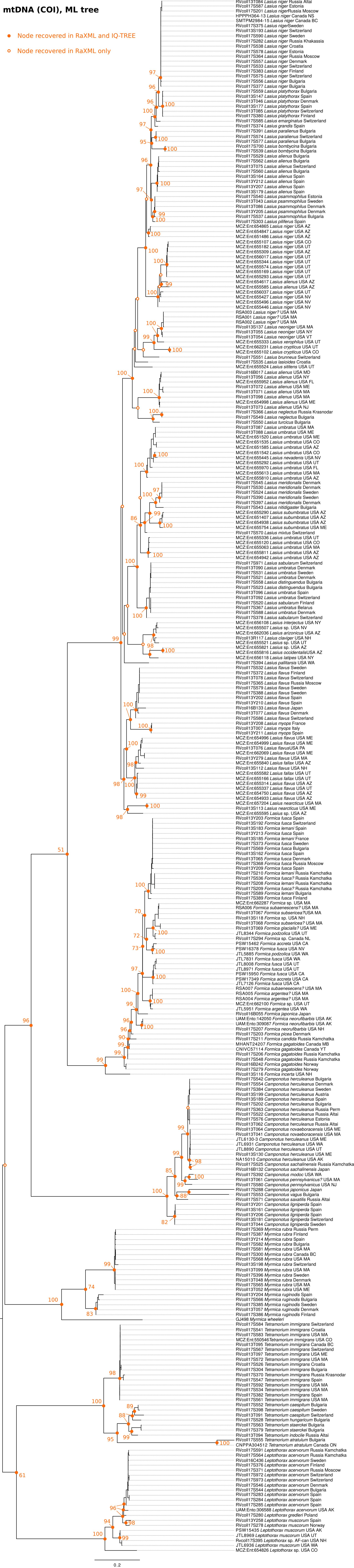
All genes (COI + Wg + Tp +28S), ML tree

● Node recovered in RaXML and IQ-TREE

○ Node recovered in RaXML only



mtDNA (COI), ML tree



Nuclear genes only (Wg + Tp + 28S)



