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

Sämi Schär^{1,*} | Gerard Talavera^{1,2,*}  | Xavier Espadaler³ | Jignasha D. Rana^{2,4} | Anne Andersen Andersen⁵ | Stefan P. Cover² | Roger Vila¹

¹Institut de Biologia Evolutiva (CSIC-UPF), Barcelona, Spain

²Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts

³CREAF and Unitat d'Ecologia, Universitat Autònoma de Barcelona, Bellaterra, Spain

⁴Department of Biological Sciences, The George Washington University, Washington, District of Columbia

⁵Centre for Social Evolution, University of Copenhagen, Copenhagen O, Denmark

Correspondence

Sämi Schär, Institut de Biologia Evolutiva (CSIC-UPF), Passeig Marítim de la Barceloneta, 37, 08003 Barcelona, Spain. Email: saemi.schaer@gmail.com

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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.

KEYWORDS

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

*These authors should be considered joint first authors.

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 dispersal.

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 & Sumnicht, 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. atratulum* 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) (Engelhoff, 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

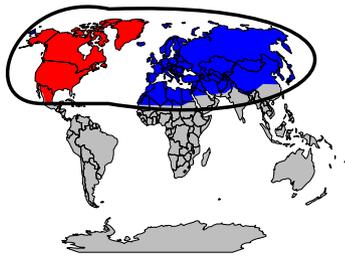
3.1 | Phylogenetics and dating

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: *Cautolasius*, *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. atratulum*. 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

n=310

Bayesian inference



Nearctic
Palearctic
Holarctic (natural)
Holarctic (introduced)

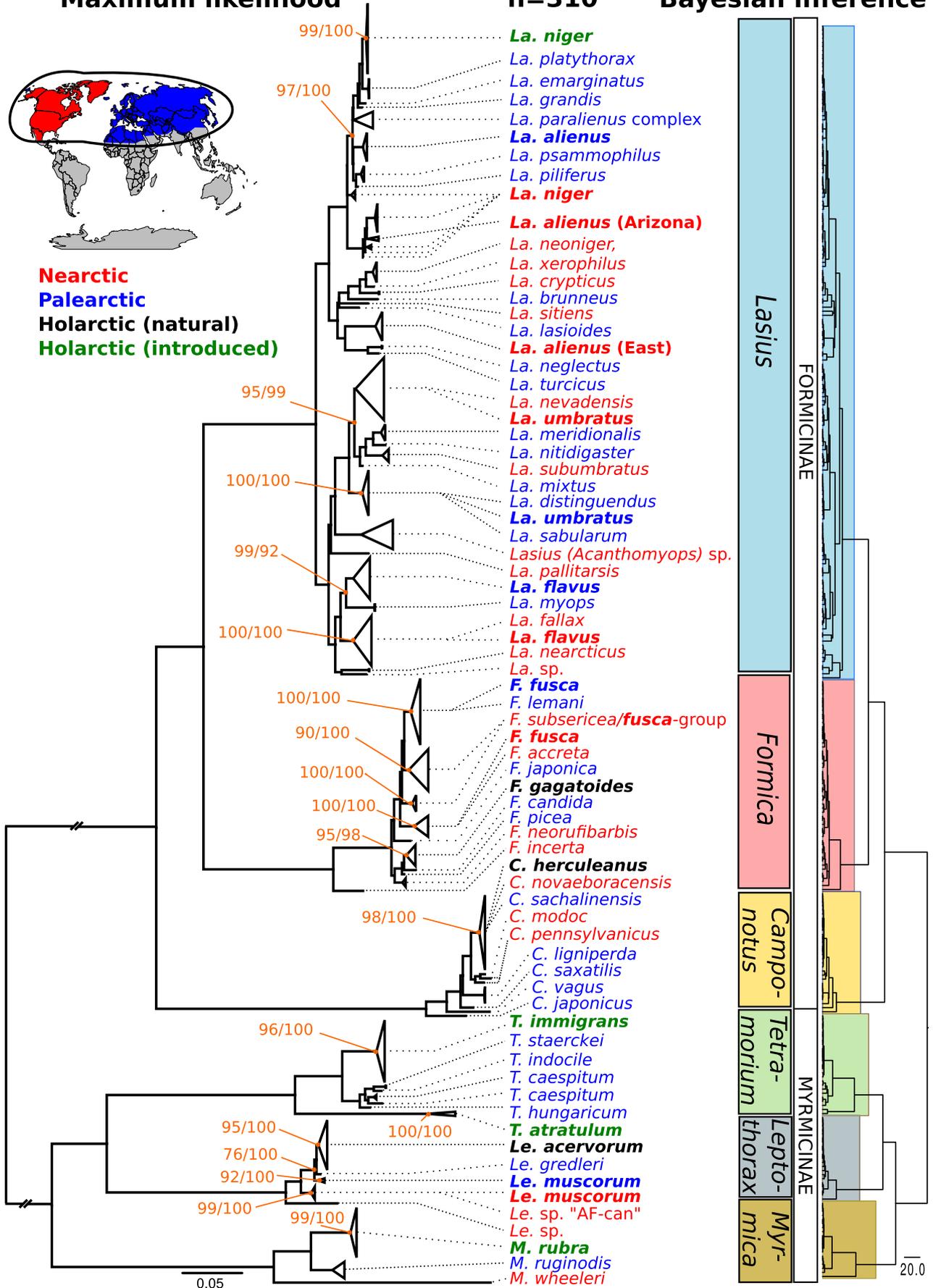


FIGURE 1 RAxML maximum likelihood tree of the samples used in this study based on COI (mtDNA) and three nuclear genes (Wg+Top1+28S) for 12 ants distributed in the Holarctic. Some clades consisting primarily of outgroups and all samples of the same species have been collapsed for simplicity. Relative 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. aphidicolus* 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.

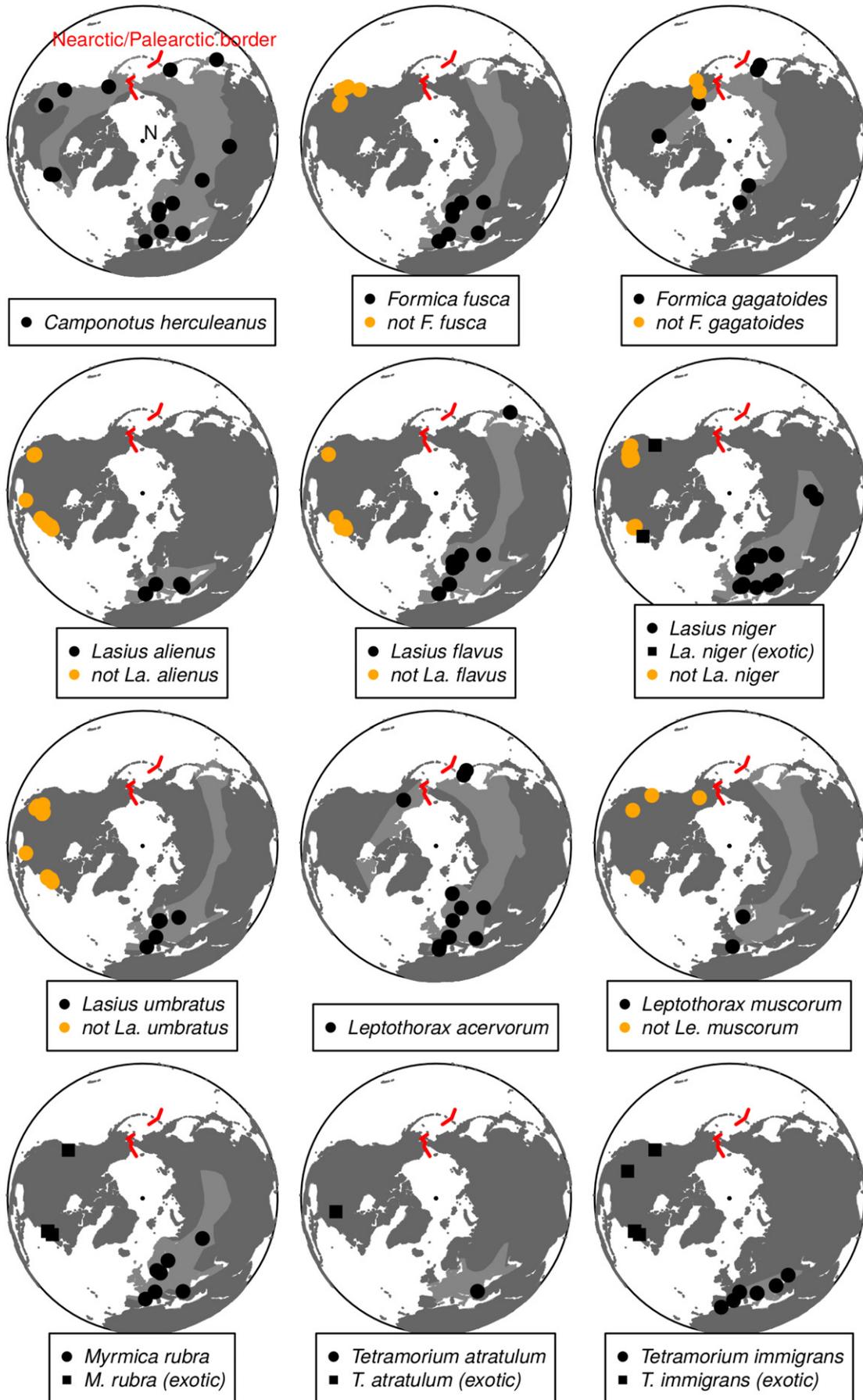
Based on our new understanding of our study species, the following nomenclatural changes are warranted:

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 syn. 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 aphidicolus* (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 aphidicolus* (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. aphidicolus*.
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)



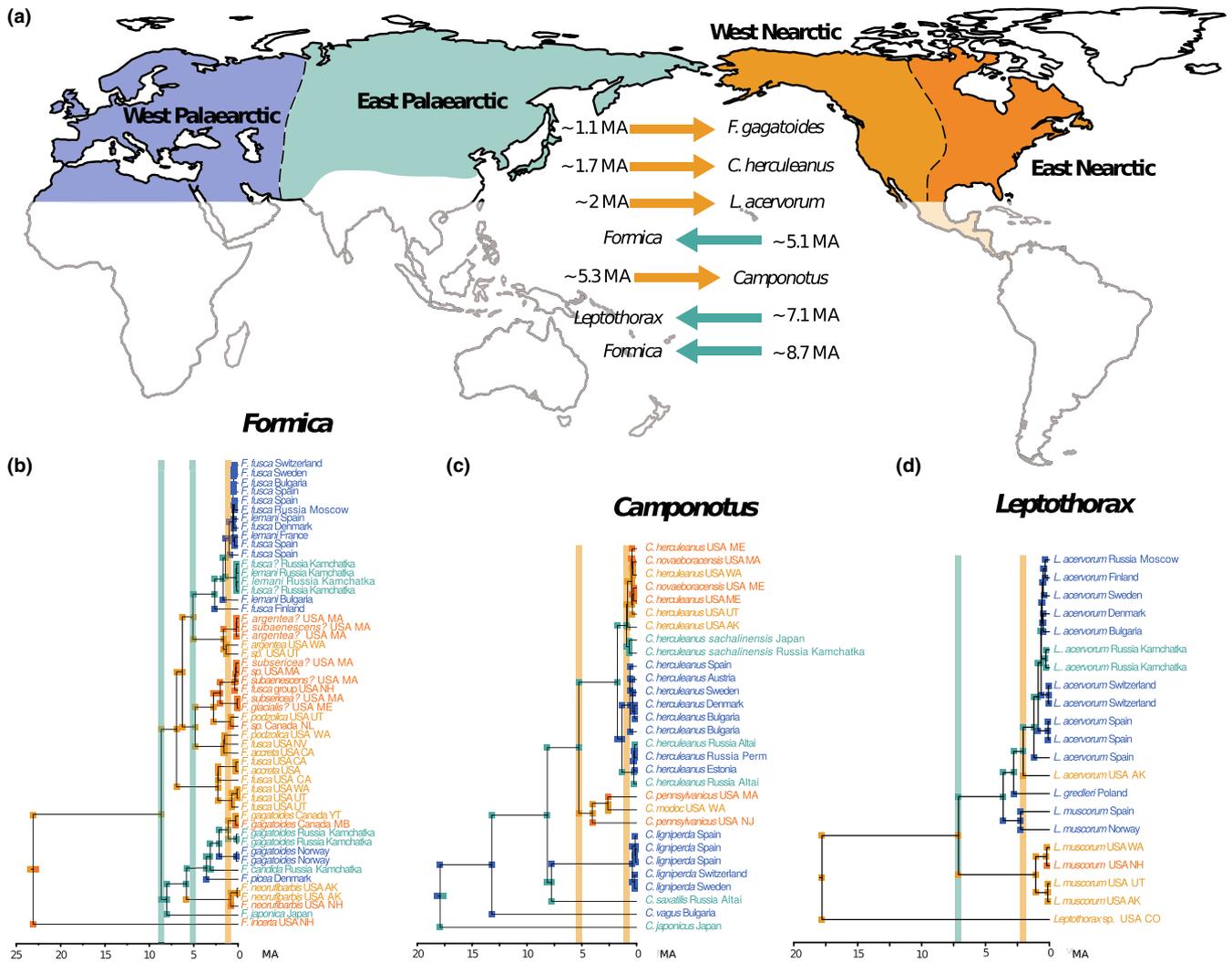


FIGURE 3 Results from the biogeographical analysis for the three ant genera containing native Holarctic species, summarizing recent intercontinental dispersal through the Beringian land bridge in ants. Panel a shows a map of the Holarctic divided into four areas (West and East Palearctic, West, and East 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, Zhigul'skaya, & 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 land bridge, which disappeared in Beringia ~5 Ma (Marincovich & Gladenkov, 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 outgroup 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 *Leptothorax* 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. aphidicolus* 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, & Csösz, 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.

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ORCID

Gerard Talavera  <http://orcid.org/0000-0003-1112-1345>

REFERENCES

- Abouheif, E., & Wray, G. A. (2002). Evolution of the gene network underlying wing polyphenism in ants. *Science*, 297(5579), 249–252. <https://doi.org/10.1126/science.1071468>
- André, E. (1887). Description de quelques fourmis nouvelles ou imparfaitement connues. *Revue d'Entomologie (Caen)*, 6, 280–298.
- AntWeb. (2015). Retrieved from <https://www.antweb.org/>.
- Berman, D. I., Alfimov, A. V., Zhigulskaia, Z. A., & Leirikh, A. N. (2010). *Overwintering and cold-hardiness of ants in the north of Asia*. Sofia-Moscow: Pensoft.
- Blaimer, B. B., Brady, S. G., Schultz, T. R., Lloyd, M. W., Fisher, B. L., & Ward, P. S. (2015). Phylogenomic methods outperform traditional multi-locus approaches in resolving deep evolutionary history: A case study of formicine ants. *BMC Evolutionary Biology*, 15(1), 271. <https://doi.org/10.1186/s12862-015-0552-5>
- Boer, P. (2016). Retrieved from http://www.nlmieren.nl/IMAGES/Nearctic%20Lasius_species.pdf.
- Bolton, B. (1995). *A new general catalogue of the ants of the world*. Cambridge, MA: Harvard University Press.
- Borowiec, L. (2014). Catalogue of ants of Europe, the Mediterranean Basin and adjacent regions (Hymenoptera: Formicidae). *Genus*, 1(25), 1–340.
- Brown, W. L. (1955). The ant *Leptothorax muscorum* (Nylander) in North America. *Entomological News*, 66, 43–50.
- Brown, W. L. Jr. (1957). Is the ant genus *Tetramorium* native in North America? *Breviora*, 72, 1–8.
- Collingwood, C. A. (1976). Ants (Hymenoptera: Formicidae) from North Korea. *Annales Historico-Naturales Musei Nationalis Hungarici*, 68, 295–309.
- Collingwood, C. A. (1981). Ants (Hymenoptera: Formicidae) from Korea. 2. *Folia Entomologica Hungarica*, 42(34), 25–30.
- Creighton, W. (1950). The ants of North America. *Bulletin of the Museum of Comparative Zoology*, 104, 1–585.
- Czechowski, W., Radchenko, A., & Czechowska, W. (2002). *The ants (Hymenoptera: Formicidae) of Poland*. Warsaw, Poland: Museum and Institute of Zoology, Polish Academy of Sciences.
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9(8), 772–772. <https://doi.org/10.1038/nmeth.2109>



- Dlussky, G. M. (1965). Ants of the genus *Formica* L. of Mongolia and north-east Tibet (Hymenoptera, Formicidae). *Annales Zoologici*, 23, 15–43.
- Dlussky, G. M. (2011). The ants of the genus *Lasius* (Hymenoptera, Formicidae) from Late Eocene European ambers. *Vestnik zoologii*, 45 (3), 209–222.
- Dowton, M., & Austin, A. D. (1995). Increased genetic diversity in mitochondrial genes is correlated with the evolution of parasitism in the Hymenoptera. *Journal of Molecular Evolution*, 41(6), 1432–1432.
- Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29(8), 1969. <https://doi.org/10.1093/molbev/mss075>
- Ellison, A., Gotelli, N., Farnsworth, E., & Alpert, G. (2012). *A field guide to the ants of New England*. New Haven, CT: Yale University Press.
- Emery, C. (1895). Beiträge zur Kenntniss der nordamerikanischen Ameisenfauna. *Zoologische Jahrbücher Abteilung für Systematik, Geographie und Biologie der Tiere*, 8, 257–360.
- Engel, H. (1995). Historical biogeography of the holarctic: Area relationships, ancestral areas, and dispersal of non-marine animals. *Cladistics*, 11(3), 223–263. [https://doi.org/10.1016/0748-3007\(95\)90014-4](https://doi.org/10.1016/0748-3007(95)90014-4)
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294–299.
- Francoeur, A. (1977). Synopsis taxonomique et économique des fourmis du Québec (Formicidae, Hymenoptera). *Annales de la Société Entomologique du Québec*, 22, 205–212.
- Francoeur, A. (1997). Ants (Hymenoptera: Formicidae) of the Yukon. *Biological Survey of Canada Monograph Series*, 2, 901–910.
- Gregg, E. (1945). A statistical study of taxonomic categories in ants (Formicidae: *Lasius neoniger* and *Lasius americanus*). *Annals of the Entomological Society of America*, 38(4), 529–548. <https://doi.org/10.1093/aesa/38.4.529>
- Hölldobler, B., & Wilson, E. (1990). *The ants*. Cambridge, MA: Harvard University Press. <https://doi.org/10.1007/978-3-662-10306-7>
- Hopkins, D. M. (1967). *The Bering land bridge*. Stanford, CA: Stanford University Press.
- Janicki, J., Narula, N., Ziegler, M., Guénard, B., & Economo, E. P. (2016). Visualizing and interacting with large-volume biodiversity data using client–server web-mapping applications: The design and implementation of antmaps. *Ecological Informatics*, 32, 185–193. <https://doi.org/10.1016/j.ecoinf.2016.02.006>
- Jansen, G., Savolainen, R., & Vepsäläinen, K. (2009). DNA barcoding as a heuristic tool for classifying undescribed Nearctic *Myrmica* ants (Hymenoptera: Formicidae). *Zoologica Scripta*, 38(5), 527–536. <https://doi.org/10.1111/j.1463-6409.2009.00386.x>
- Jansen, G., Savolainen, R., & Vepsäläinen, K. (2010). Phylogeny, divergence-time estimation, biogeography and social parasite–host relationships of the Holarctic ant genus *Myrmica* (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution*, 56(1), 294–304. <https://doi.org/10.1016/j.ympev.2010.01.029>
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Drummond, A., et al. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kupianskaya, A. N. (1990). *Ants of the Far Eastern USSR*. Vladivostok, Russia: Akademiya Nauk SSSR.
- Landis, M. J., Matzke, N. J., Moore, B. R., & Huelsenbeck, J. P. (2013). Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology*, 62(6), 789. <https://doi.org/10.1093/sysbio/syt040>
- Landry, J.-F., Nazari, V., Dewaard, J. R., Mutanen, M., Lopez-Vaamonde, C., Huemer, P., & Hebert, P. D. (2013). Shared but overlooked: 30 species of Holarctic Microlepidoptera revealed by DNA barcodes and morphology. *Zootaxa*, 3749(1), 1–93. <https://doi.org/10.11646/zootaxa.3749.1.1>
- Larson, D. J., & Nilsson, A. N. (1985). The Holarctic species of *Agabus* (*sensu lato*) Leach (Coleoptera: dytiscidae). *The Canadian Entomologist*, 117(1), 119–130. <https://doi.org/10.4039/Ent117119-1>
- Li, H.-L. (1952). Floristic relationships between eastern Asia and eastern North America. *Transactions of the American Philosophical Society*, 42 (2), 371–429. <https://doi.org/10.2307/1005654>
- Marincovich, L., & Gladenkov, A. Y. (1999). Evidence for an early opening of the Bering Strait. *Nature*, 397(6715), 149–151. <https://doi.org/10.1038/16446>
- Marusik, Y. M., & Koponen, S. (2005). A survey of spiders (Araneae) with holarctic distribution. *The Journal of Arachnology*, 33(2), 300–305. <https://doi.org/10.1636/04-115.1>
- Matzke, N. (2013). BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R scripts, CRAN: The Comprehensive R Archive Network. cran. r-project. Retrieved from org/web/packages/BioGeoBEARS/index.html.
- Matzke, N. J. (2014). Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology*, 63(6), 951. <https://doi.org/10.1093/sysbio/syu056>
- Mayr, G. (1886). Die Formiciden der Vereinigten Staaten von Nordamerika. *Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien*, 39, 419–464.
- Minh, B. Q., Nguyen, M. A. T., & von Haeseler, A. (2013). Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30(5), 1188–1195. <https://doi.org/10.1093/molbev/mst024>
- Radchenko, A. G. (1996). A key to the ant genus *Camponotus* (Hymenoptera, Formicidae) in Palearctic Asia. *Zoologicheskii Zhurnal*, 75, 1195–1203.
- Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data system (<http://www.barcodinglife.org>). *Molecular Ecology Notes*, 7(3), 355–364. <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, 57(1), 4–14. <https://doi.org/10.1080/10635150701883881>
- Ronquist, F. (1997). Dispersal–vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology*, 46(1), 195–203. <https://doi.org/10.1093/sysbio/46.1.195>
- Saux, C., Fisher, B. L., & Spicer, G. S. (2004). Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae). *Molecular Phylogenetics and Evolution*, 33(2), 457–468. <https://doi.org/10.1016/j.ympev.2004.06.017>
- Schlick-Steiner, B. C., Steiner, F. M., Moder, K., Seifert, B., Sanetra, M., Dyreson, E., ... Christian, E. (2006). A multidisciplinary approach reveals cryptic diversity in Western Palearctic *Tetramorium* ants (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution*, 40 (1), 259–273. <https://doi.org/10.1016/j.ympev.2006.03.005>
- Seifert, B. (2007). *Die Ameisen Mittel- und Nordeuropas*. Tauer: Lutra Verlags- und Vertriebsgesellschaft.
- Seifert, B., Ritz, M., & Csösz, S. (2014). Application of exploratory data analyses opens a new perspective in morphology-based alpha-taxonomy of eusocial organisms. *Myrmecological News*, 19, 1–15.
- Seppä, P., Helanterä, H. O., Trontti, K., Punttila, P., Chernenko, A., Martin, S. J., & Sundström, L. (2011). The many ways to delimit species: Hairs, genes and surface chemistry. *Myrmecological News*, 15, 31–41.
- Shattuck, S. (2017). AntWiki. Retrieved from <http://www.antwiki.org/>.
- Silvestro, D., & Michalak, I. (2012). raxmlGUI: A graphical front-end for RAXML. *Organisms Diversity & Evolution*, 12(4), 335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Steiner, F. M., Schlick-Steiner, B. C., Schödl, S., Espadaler, X., Seifert, B., Christian, E., & Stauffer, C. (2004). Phylogeny and bionomics of *Lasius*



- austriacus* (Hymenoptera, Formicidae). *Insectes Sociaux*, 51, 24–29. <https://doi.org/10.1007/s00040-003-0699-8>
- Talavera, G., Espadaler, X., & Vila, R. (2015). Discovered just before extinction? The first endemic ant from the Balearic Islands (*Lasius balearicus* sp. nov.) is endangered by climate change. *Journal of Biogeography*, 42(3), 589–601. <https://doi.org/10.1111/jbi.12438>
- Vila, R., Bell, C. D., Macniven, R., Goldman-Huertas, B., Ree, R. H., Marshall, C. R., ... Pierce, N. E. (2011). Phylogeny and palaeoecology of *Polyommatus* blue butterflies show Beringia was a climate-regulated gateway to the New World. *Proceedings of the Royal Society of London B: Biological Sciences*, 278(1719), 2737–2744. <https://doi.org/10.1098/rspb.2010.2213>
- Wagner, H. C., Arthofer, W., Seifert, B., Muster, C., Steiner, F. M., & Schlick-Steiner, B. C. (2017). Light at the end of the tunnel: Integrative taxonomy delimits cryptic species in the *Tetramorium caespitum* complex (Hymenoptera: Formicidae). *Myrmecological News*, 25, 95–129.
- Ward, P., & Sumnicht, T. (2012). Molecular and morphological evidence for three sympatric species of *Leptanilla* (Hymenoptera: Formicidae) on the Greek island of Rhodes. *Myrmecological News*, 17, 5–11.
- Wheeler, W. M. (1908). Comparative ethology of the European and North American ants. *Journal für Psychologie und Neurologie*, 13, 404–435.
- Wilson, E. (1955). A monographic revision of the ant genus *Lasius*. *Bulletin of the Museum of Comparative Zoology*, 113, 1–201.

BIOSKETCH

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SUPPORTING INFORMATION

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