



The evolution of social parasitism in *Formica* ants revealed by a global phylogeny

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Studying the behavioral and life history transitions from a cooperative, eusocial life history to exploitative social parasitism allows for deciphering the conditions under which changes in behavior and social organization lead to diversification. The Holarctic ant genus *Formica* is ideally suited for studying the evolution of social parasitism because half of its 172 species are confirmed or suspected social parasites, which includes all three major classes of social parasitism known in ants. However, the life history transitions associated with the evolution of social parasitism in this genus are largely unexplored. To test competing hypotheses regarding the origins and evolution of social parasitism, we reconstructed a global phylogeny of *Formica* ants. The genus originated in the Old World ~30 Ma ago and dispersed multiple times to the New World and back. Within *Formica*, obligate dependent colony-founding behavior arose once from a facultatively polygynous common ancestor practicing independent and facultative dependent colony foundation. Temporary social parasitism likely preceded or arose concurrently with obligate dependent colony founding, and dulotic social parasitism evolved once within the obligate dependent colony-founding clade. Permanent social parasitism evolved twice from temporary social parasitic ancestors that rarely practiced colony budding, demonstrating that obligate social parasitism can originate from a facultative parasitic background in socially polymorphic organisms. In contrast to permanently socially parasitic ants in other genera, the high parasite diversity in *Formica* likely originated via allopatric speciation, highlighting the diversity of convergent evolutionary trajectories resulting in nearly identical parasitic life history syndromes.

brood parasitism | dulosis | Emery's rule | Formicidae | inquilinism

The complex societies of eusocial insects are vulnerable to exploitation by social parasites that depend on their host colonies for survival and reproduction without contributing to colony maintenance and brood care (1–4). Social parasitism is common among eusocial Hymenoptera and evolved independently in distantly related lineages, including bees, wasps, and ants (3–8). Many studies on social parasitism have focused on the evolution of cooperation and conflict in colonies of eusocial insects and on coevolutionary arms race dynamics between hosts and parasites (9–12). However, the evolutionary origins of social parasitism and the coevolutionary factors causing speciation and thereby contributing to the high diversity of social parasite species in eusocial insects are not well understood (13, 14). Comparative evolutionary studies of social parasites are promising, because they are expected to provide insights into the conditions associated with a behavioral change from cooperative eusociality to exploitative social parasitism as well as into the consequences of the life history transitions on speciation and biological diversification.

Social parasitism is a life history strategy that evolved at least 60 times in ants, and more than 400 socially parasitic species are known from six distantly related subfamilies (4). Despite the high diversity, three main life history strategies can be recognized across social parasites: 1) temporary, 2) dulotic, and 3) permanent social parasitism (1, 3, 15–21). The queens of

temporary socially parasitic ant species invade the host nest and kill the resident queen(s), and the host workers raise the parasite's offspring (16). In the absence of an egg-laying host queen, the host workforce is gradually replaced until the colony is composed solely of the temporary social parasite species. The queens of dulotic social parasites start their colony life cycle as temporary social parasites, and once sufficient parasitic workers have been reared, they conduct well-organized raids of nearby host nests to capture their brood (22). Some brood is eaten, but most workers eclose in the parasite's nest and contribute to the workforce of the colony. By contrast, most permanent social parasite (i.e., inquiline) species are tolerant of the host queen, allowing her to continuously produce host workers, whereas the inquiline queens focus their reproductive effort on sexual offspring (1, 13). Inquilines obligately depend on their hosts and most inquiline species lost their worker caste entirely (1, 18, 19, 23).

The evolutionary origins of social parasitism have been debated since Darwin's *On the Origin of Species by Means of Natural Selection* (24). Entomologists have long noticed that ant social parasites and their hosts are close relatives (15, 16, 25–29), an observation subsequently referred to as “Emery's rule” (30). Strictly interpreted, Emery's rule postulates a sister group relationship between host and parasite, whereas a less restrictive or “loose” interpretation signifies for example a congeneric, but not

Significance

Identifying the conditions associated with a life history transition from cooperative colony life to exploitative social parasitism is important for understanding how changes in behavior contribute to speciation. To explore the evolutionary origins of social parasitism, we reconstructed the evolutionary history of *Formica* ants because half of all species are social parasites and all socially parasitic life history syndromes known from eusocial insects are represented in this genus. We demonstrate that social parasites evolved from an ancestor that lost the ability to establish new colonies independently and that highly specialized parasites can evolve from less complex social parasite syndromes. Our findings emphasize that social parasite syndromes readily originate in socially polymorphic organisms and evolved convergently across the ant phylogeny.

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Table 1. Diversity of social parasites in the genus *Formica* compared to all other ants

	Temporary social parasites (%)	Dulotic social parasites (%)	Permanent social parasites (%)	Total social parasite diversity (%)
All ants ($n = 13,861$)	200 (1.4)	80 (0.6)	100 (0.7)	>400 (2.9)
<i>Formica</i> ($n = 172$)	68 (39.5)	14 (8.1)	2 (1.2)	84 (48.8)

Socially parasitic life histories are significantly overrepresented in *Formica* ants, except for inquilineism. The total social parasite diversity in ants is higher than the sum of species in individual life history categories because the biology of numerous social parasites remains unknown. The data are derived from published sources (1, 3, 4, 55).

necessarily a sister taxon relationship (13, 31–33). Consequently, two competing hypotheses were developed for explaining the speciation mechanisms of social parasites: 1) The interspecific hypothesis proposes that host and social parasite evolved reproductive isolation in allopatry, whereas 2) the intraspecific hypothesis postulates that the social parasite evolved directly from its host in sympatry (3, 13, 18, 20, 21, 31–37). Empirical studies of temporary, dulotic, and host queen-intolerant workerless ant social parasites generally provide support for the interspecific hypothesis (14, 38–48), whereas recent phylogenetic studies lend support to the intraspecific hypothesis for queen-tolerant inquilines (33, 36, 49–51). In some cases, host shifts, secondary speciation events of hosts and/or parasites, and extinctions obscure the original evolutionary conditions under which social parasitism originated (52–54).

To explore the origin and evolution of diverse socially parasitic life histories in eusocial insects, we reconstructed the evolutionary history of the Holarctic ant genus *Formica*. *Formica* ants are ideally suited for comparative studies of social parasitism because the genus has the highest number of social parasite species in any ant genus (84 of 172; Table 1), and all socially parasitic life history traits known from eusocial insects evolved in *Formica* ants (Fig. 1 and Tables 1 and 2). In addition, colonies of *Formica* species vary significantly in colony-founding behavior as well as in nest and colony structures, providing an opportunity to explore the interplay between colony organization and life history at the origin of social parasitism. Some *Formica* species use independent colony foundation (ICF), when new colonies are started by a single queen (i.e., haplometrosis) or a group of coop-

erating queens (i.e., pleometrosis). Queens of other species rely on dependent colony founding (DCF), cooperating with groups of conspecific workers to found a new colony (i.e., budding) or invading an existing heterospecific colony as a temporary social parasite (TSP) or a permanent social parasite (PSP) (Table 2) (1, 56–62). In contrast to other studies (63), we regard TSP as a form of DCF because the socially parasitic queen relies on the social environment of the host for colony founding and rearing of the first brood. Furthermore, *Formica* colonies can have a single or multiple functional queens (monogyny vs. polygyny) and comprise one (monodomous) or multiple (polydomous) to thousands of interconnected physical nests covering a large area (supercolonial) (64).

To infer the evolutionary origins of social parasitism and explore the behavioral transition from a social colony life to a socially parasitic life history, we reconstructed a global phylogeny for *Formica* ants and relevant outgroups from the formicine genera *Iberoformica*, *Polyergus*, *Proformica*, and *Rossomyrmex*, thus spanning the root node of the tribe Formicini (65). The comprehensive, time-calibrated phylogeny allows for 1) testing competing hypotheses regarding the origins and evolutionary transitions of social parasitism, 2) reconstructing the evolutionary and biogeographic history of the group, and 3) suggesting modifications to the internal classification of the genus.

Results and Discussion

***Formica* Originated in Eurasia during the Oligocene.** To infer the life history evolution of the diverse, Holarctic genus *Formica*, we inferred a comprehensive phylogeny for 101 *Formica* species



Fig. 1. Diversity of life history traits in the formicine ants. In clockwise direction: (A) members of the *F. fusca* group practicing independent colony founding; (B) *F. obscuripes*, representing the *Formica integra* group (Nearctic members of the paraphyletic “*rufa*” group), which practices dependent and temporary social parasitic colony founding; (C) *Formica gynocrates*, representing the facultatively dulotic species of the *F. sanguinea* group, with a worker of its *neogagates* group host species, *Formica vinculans*; (D) the highly modified worker of *Polyergus mexicanus*, representing the obligately dulotic formicine ants in the genera *Polyergus* and *Rossomyrmex*. All images courtesy of Alex Wild (www.alexanderwild.com).

Table 2. Diversity, taxonomy, life history, and evolutionary traits of *Formica* ants across currently recognized species groups, as well as of closely related formicine ants

<i>Formica</i> species group or genus	No. of described of species	Estimated no. of new species	Colony-founding behavior	Colony organization	Nest organization	Socially parasitic life history	Phylogenetic information	Geographic distribution
<i>F. dakotensis</i> gr.	2	Unknown	TSP; budding at low frequency	Monogynous, polygynous	Monodomous, polydomous	TSP, PSP(?)	Monophyletic	Nearctic
<i>F. difficilis</i> gr.	16	5 to 10	TSP; budding at low frequency	Monogynous, polygynous	Monodomous	TSP, PSP	Monophyletic	Nearctic
<i>F. exsecta</i> gr.	17	Unknown	TSP; budding at high frequency	Monogynous, polygynous	Monodomous, polydomous, supercolonial	TSP	Monophyletic	Nearctic and Palearctic
" <i>F. fusca</i> gr."	76	1 to 15	Haplo- and pleometrosis; budding rare, if present in a species, at low frequency	Monogynous, polygynous	Monodomous, polydomous, rarely supercolonial	Not socially parasitic	Paraphyletic	Nearctic and Palearctic
<i>F. integra</i> gr.	20	2 to 3	TSP; budding at low frequency	Monogynous, polygynous	Monodomous, polydomous, rarely supercolonial	TSP	Monophyletic	Nearctic
" <i>F. neogagates</i> gr."	8	2 to 3	Haplometrosis	Monogynous, polygynous	Monodomous	Not socially parasitic	Paraphyletic	Nearctic
<i>F. pallidefulva</i> gr.	5	Unknown	Haplo- and pleometrosis	Monogynous, polygynous	Monodomous	Not socially parasitic	Monophyletic	Nearctic
<i>F. rufa</i> gr.	13	None	TSP; budding at high frequency	Monogynous, polygynous	Monodomous, polydomous, supercolonial	TSP	Monophyletic	Paleartic
<i>F. sanguinea</i> gr.	14	3 to 5	TSP	Monogynous, polygynous	Monodomous, rarely polydomous	TSP, facultative and obligate dulosis	Monophyletic	Nearctic and Palearctic
<i>F. uralensis</i> gr.	1	None	TSP; budding at high frequency	Monogynous, polygynous	Monodomous, polydomous, supercolonial	TSP	Monotypic	Paleartic
<i>Iberoformica</i>	1	None	Haplometrosis	Monogynous	Monodomous	Not socially parasitic	Monotypic	Paleartic
<i>Polyergus</i>	14	Unknown	TSP	Monogynous	Monodomous	Obligat TSP, dulosis	Monophyletic	Nearctic and Palearctic

The former *F. rufa* group is divided into three clades, i.e., the *dakotensis*, *integra*, and *rufa* groups. The erstwhile *microgyna* group is properly referred to as the *difficilis* group based on name priority. Please refer to *SI Appendix, Table S2* for a detailed list of traits for individual species and references to original research. Total number of *Formica* species does not add to 172 because of one valid, poorly described species (*Formica graveleyi*) of uncertain group affinity.

representing all 10 currently recognized species groups (Table 2) across their wide geographic distribution in both the Old World (19 spp.) and the New World (82 spp.) and outgroups, using 2,242 ultraconserved element (UCE) loci per taxon. Our analyses recovered *Formica* as a strongly supported clade with the monotypic genus *Iberoformica* as its sister lineage (Fig. 2 and *SI Appendix, Fig. S1*). *Formica* and *Iberoformica* split from their sister genus *Polyergus* around 33 Ma ago (95% highest posterior density [HPD]: 27 to 39 Ma) and *Formica* diverged from a common ancestor with its sister lineage *Iberoformica subrufa* ~30 Ma ago (95% HPD: 24 to 35 Ma). The crown group age of extant *Formica* ants is ~26 Ma (95% HPD: 21 to 31 Ma). Therefore, modern *Formica* ants evolved recently and likely originated in Eurasia during the Oligocene after the global cooling following the Terminal Eocene Event (66). A similar evolutionary history was inferred for the species-rich Holarctic ant genus *Myrmica* (49).

Independent Colony Founding and Social Polymorphism Are Ancestral. To understand the evolution of social parasitism in *Formica* ants, it is necessary to recover the evolutionary origins of different life history strategies in the biologically diverse species groups. Character state reconstructions of life history traits

including nest structure, social organization of colonies, and colony-founding behaviors (Table 2 and *SI Appendix, Figs. S3–S5*) based on our phylogenomic tree (Fig. 2) show that facultative polygyny and polydomy originated early during *Formica* evolution. Purely monogynous species groups are found only outside of *Formica* (Table 2). Within *Formica*, the deepest node marks the divergence between the independent colony-founding species in the "*neogagates*" and *pallidefulva* groups plus *Formica gagates* on one side and the independently colony-founding species in the paraphyletic grade of the *Formica* "*fusca* group" on the other side of the bifurcation (Fig. 2). Accordingly, facultative polygyny, independent colony founding, and polydomy are ancestral traits that were likely present in the most recent common ancestor (MRCA) of all extant *Formica* species. The *fusca* group arises as a paraphyletic grade nested between these early diverging lineages and all other *Formica* species and consists of at least five monophyletic groups (Fig. 2). Facultative DCF via budding has been documented in certain species of the *fusca* group (*SI Appendix, Table S2*). The nesting behavior transitioned repeatedly between constructing monodomous, polydomous, and even supercolonial nests in the *fusca* group, which has important implications for population structure and population density of those species.

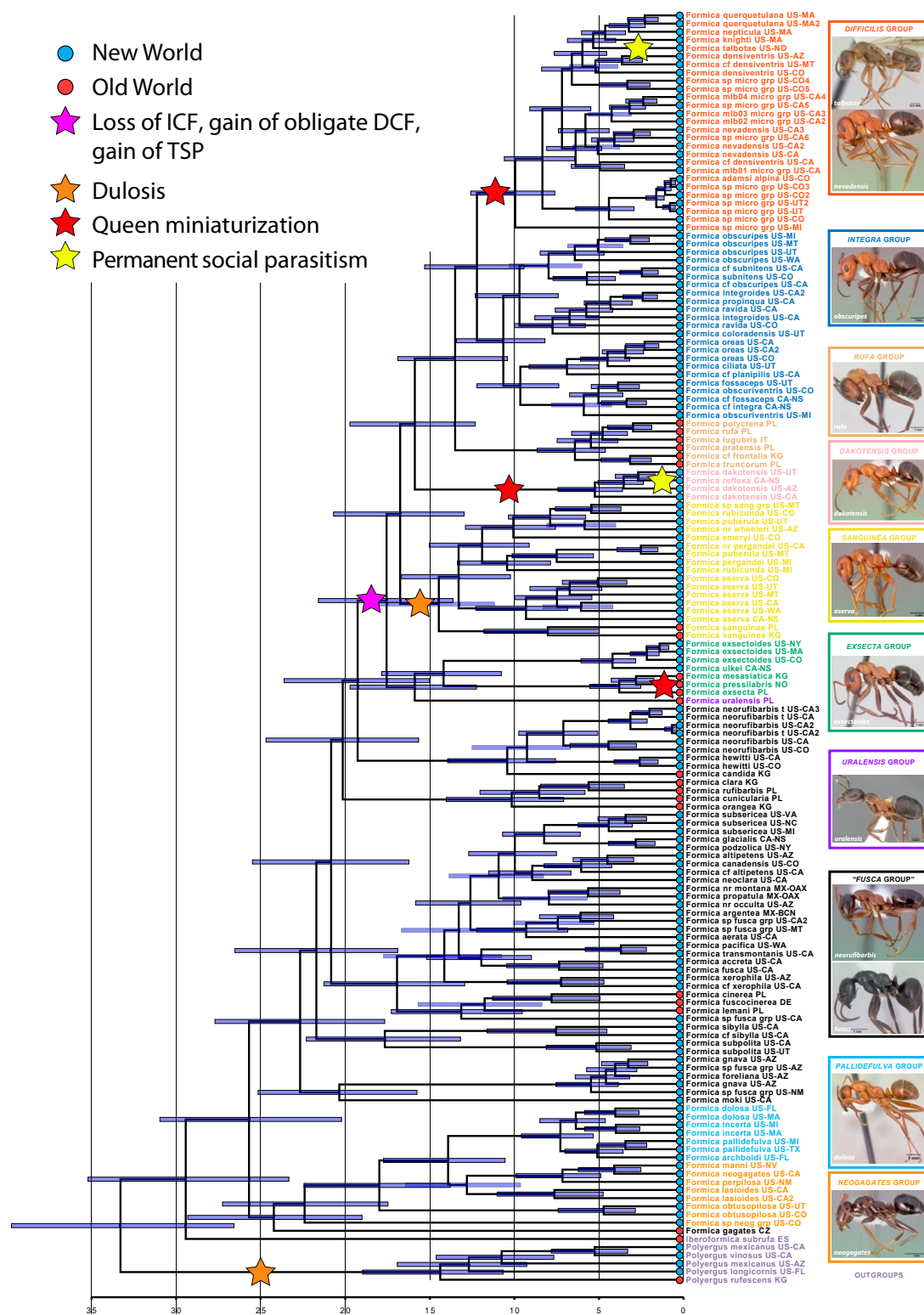


Fig. 2. A time-calibrated molecular phylogeny of *Formica*, *Iberoformica*, and *Polyergus*. Node bars indicate 95% highest posterior density. Scale is in millions of years. Abbreviations after taxa names indicate sample origin. Country codes follow the International Organization for Standardization (ISO) 3166: United States (US), Canada (CA), and Mexico (MX), in addition to noting state/province. Note single loss of independent colony foundation and gain of obligate DCF with inquilinism arising from within temporary parasites (yellow star). Taxon highlight colors signify species group membership; *neogagates* and *fusca* species groups are not monophyletic. Photographs are by April Nobile, Erin Prado, and Estella Ortega. Courtesy of <https://www.antweb.org/>.

ICF Was Lost Once, Leading to Obligate DCF. In *Formica*, obligate dependent colony founding evolved ~18 Ma ago (95% HPD: 14 to 21 Ma) (Fig. 2) when an ancestor practicing both

independent and facultative dependent colony founding via budding, as observed in some *fusca* group species, lost the capacity for independent founding. This loss was precipitated by or

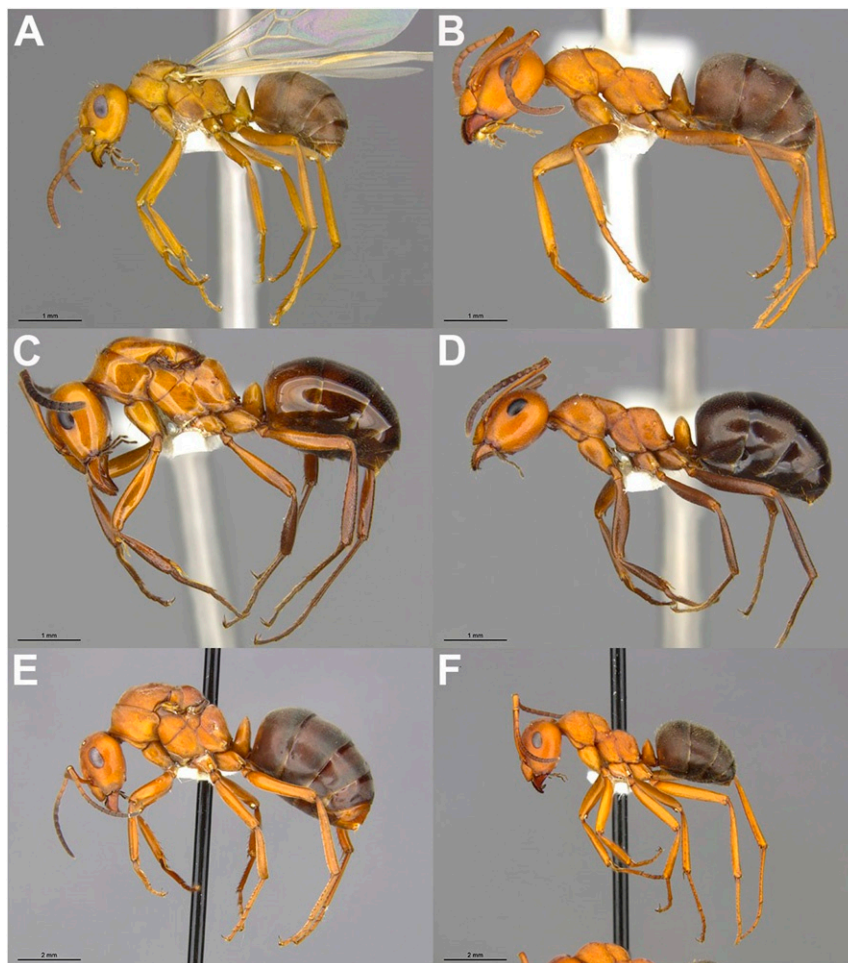


Fig. 3. Convergent evolution of queen miniaturization in temporary social parasitic *Formica* ants. Queen miniaturization evolved in the *F. difficilis* (A and B) and *dakotensis* (C and D) species groups. In comparison, queens in the *integra* group (E and F) show a pronounced queen–worker dimorphism typical for *Formica* ants. (A and B) Queen (A) and worker (B) of a hitherto undescribed *F. difficilis* group species showing one of the most extreme cases of queen size reduction known in ants. (C and D) Queen (C) and worker (D) of *F. reflexa* representing a second, independent evolutionary origin of queen miniaturization, which is less extreme than in the *difficilis* group species. (E and F) Queen (E) and worker (F) of *Formica ravidia* demonstrating a typically sized queen with morphological modifications related to wing bearing and reproduction that are absent from the worker. Note that the *F. ravidia* individuals are significantly larger than all other ants depicted here. (Scale bars, 1 mm in A–D and 2 mm in E and F.) Specimen identifiers are as follows: (A) MCZ 574034, (B) MCZ 574022, (C) MCZ 525288, (D) MCZ 525283, (E) MCZ 552096, and (F) MCZ 575163. Photographs are by Patrick McCormack. Images copyright President and Fellows of Harvard University.

coincided with using heterospecific workers for dependent colony founding (TSP), which likely evolved from budding, a strategy involving the use of conspecific workers for colony founding. This is suggested by the fact that there are no confirmed obligate dependent founding species that practice budding only, without the capacity for TSP.

The clade with obligate DCF and capacity for TSP includes species of the *exsecta*, *sanguinea*, *dakotensis*, *rufa*, *integra*, and *difficilis* groups, as well as *Formica uralensis*, a species of uncertain taxonomic affiliation that is here inferred to be the sister lineage to the *exsecta* group. Recently, Romiguier et al. (48) also recovered a single loss of ICF among Palearctic *Formica* species including representatives of 4 of the 10 species groups. Our global phylogenetic analysis confirms and significantly expands on this earlier conclusion and adds a temporal scale showing that the MRCA of this clade lived around 18 Ma ago. Furthermore, our analysis reveals that clades of socially parasitic species have secondarily transitioned to other parasitic life histories. Evolutionary reversals from social parasitism to independent colony founding were not recovered, suggesting that a transition to a socially parasitic lifestyle is irreversible. A similar pattern

was found in the ant genus *Lasius*, where temporary parasitism evolved twice but reversals to ICF are unknown (40).

Among the TSP species, two ecologically distinct life histories can be recognized. First, species in the *dakotensis*, *difficilis*, and *integra* groups are predominantly facultative temporary social parasites and practice colony budding at low frequency. Newly mated queens are unable to found new colonies independently via haplo- or pleometrosis, but instead, they seek adoption in heterospecific host colonies or readoption into conspecific colonies leading to secondary polygyny. In these species, colony budding seems to occur occasionally, which results in a characteristic population structure with smaller clusters of nests (usually less than five), whereas large, unicolonial populations are absent. In contrast, species in the *exsecta* and *rufa* groups are facultative temporary social parasites practicing colony budding at high frequency, which can result in highly polydomous and/or supercolonial populations (Fig. 2 and Table 2 and *SI Appendix, Table S2*).

The temporary social parasite species in the *Formica difficilis*, *integra*, *rufa*, and *dakotensis* groups shared a common ancestor ~16 Ma ago (95% HPD: 13 to 19 Ma), and they constitute the

sister group to the dulotic *sanguinea* group (Fig. 2). The *difficilis* group is also monophyletic and is sister to the *integra* group (Fig. 2). All species in the *difficilis* group have miniature queens not larger than their largest workers (Fig. 3 A and B), which is likely associated with the socially parasitic life history. For the *difficilis* group, we infer a single origin of queen miniaturization ~10 Ma ago (95% HPD: 8 to 12 Ma). It is important to note that most *difficilis* group species are rare and our knowledge about their biology is fragmentary at best. Therefore, the temporary social parasitic behavior remains to be observed for most species. However, the few existing direct observations on nest founding behavior, which include *F. difficilis* (16), *Formica densiventris* (67), *Formica impexa* (67), *Formica adamsi alpina* (S.P.C., personal observation), and *Formica* new species (S.P.C., personal observation), confirm temporary social parasitism.

Interestingly, queen miniaturization evolved repeatedly in *Formica*, including in the *exsecta* group, where it is present in several Palearctic species (68), and in the Nearctic *dakotensis* group. Because our sampling includes only one of the Palearctic *exsecta* group species with miniature queens (*Formica pressilabris*), we cannot ascertain whether miniaturization evolved once or multiple times in this group. In the temporary social parasite species of the Nearctic *Formica dakotensis* clade (Fig. 3), both species (*F. dakotensis* and *F. reflexa*) have small queens. *Formica dakotensis* is a facultative temporary social parasite (55) and fully independent colonies are common. In contrast, *Formica reflexa* is rare and was found only in association with *fusca* group host workers (69, 70) (S.P.C., personal observation), suggesting a unique life history including parasitic colony founding and potentially a life-long dependence on the host. Queen size reduction is frequently observed in inquiline social parasites (13, 71), but the independent origins of miniature queens in the *difficilis*, *dakotensis*, and *exsecta* groups imply that queen size reduction is adaptive for a temporary social parasitic life history syndrome in *Formica* ants.

Evolution of Dulosis. The dulotic species of the *Formica sanguinea* group are monophyletic (Fig. 2), suggesting that dulotic behavior evolved once some time prior to its inferred crown group age of ~14 Ma (95% HPD: 11 to 18 Ma). Thus, dulotic behavior and temporary social parasitism did not evolve simultaneously in *Formica*, but instead dulosis evolved secondarily from a temporary socially parasitic ancestor. The single origin of dulotic behavior in a diverse clade of temporary social parasite species supports the hypothesis that dulosis originates only under rare circumstances (3, 4, 22). In fact, the evolutionary origins of dulotic behavior in ants have been debated since Darwin's *On the Origin of Species by Means of Natural Selection* (24) and three not mutually exclusive hypotheses have been proposed to explain the origins of this highly specialized behavior: 1) predation, 2) brood transport, and 3) territorial competition (1, 3, 20, 22, 24, 35, 72–76).

Our phylogenetic results and behavioral observations indicate that the predatory behavior of temporary social parasites could lead to the evolution of facultative dulosis in *Formica*. Brood stealing would be favored by natural selection if the aid of heterospecific workers increased the parasite's fitness, although we are not aware of experimental studies demonstrating fitness benefits provided by stolen host workers to facultatively dulotic species. Additional biological factors that were associated with the evolutionary origins of dulosis, including polygyny, polydomy, brood transport, and territoriality (17, 22, 35, 77), can also be inferred for the common ancestor of the dulotic species in the *F. sanguinea* group.

It is important to note that dulosis evolved convergently and under different ecological conditions in distantly related, non-predatory ants, such as the omnivorous, scavenging species in the genera *Temnothorax* and *Tetramorium* (12, 41, 42, 44, 46, 78). This pattern suggests that alternative factors, such as territori-

ality and brood transport, likely play an important role in the origin of dulotic behavior in nonpredatory ants. Across the ant tree of life, dulosis originated at least nine times convergently in distantly related clades (22, 47, 79), including three origins in the Formicini (65) and six origins in the Crematogastrini (41, 45, 46).

Evolution of Inquiline Social Parasitism. The only confirmed workerless inquiline social parasite in the genus *Formica* is *Formica talbotae* (80, 81). *Formica talbotae* is phylogenetically nested within the *difficilis* clade (Fig. 2), suggesting that workerless permanent parasitism evolved once from a facultatively polygynous ancestor practicing temporary social parasitism. This is empirical evidence for an evolutionary transition from temporary to workerless inquiline social parasitism, a hypothesis earlier suggested by Wilson (18). *Formica dirksi* has also been repeatedly suggested to be a workerless social parasite of *Formica subaenescens* in Maine (3, 82, 83), but there are no natural history data substantiating this claim.

Formica talbotae is a distant relative of its *integra* group host, *Formica obscuripes*, with which it shared a common ancestor ~12 Ma ago (95% HPD: 10 to 15 Ma). The host–parasite relationship of *F. talbotae* and *F. obscuripes* is consistent with the “loose” interpretation of Emery's rule, where hosts and parasites can be congeners but not sister lineages, suggesting that *F. talbotae* evolved via the interspecific, allopatric route of social parasite evolution. This result contrasts with previous studies inferring workerless inquiline social parasites as directly evolving from free-living, closely related ancestors via the intraspecific, sympatric route of social parasite speciation (33, 36, 49, 50). However, and in contrast to many host queen tolerant inquiline social parasites, *F. talbotae* was found exclusively in queenless host colonies. It seems unlikely that the miniature *F. talbotae* queens assassinate the many and much larger host queens. Instead, it appears more likely that *F. talbotae* specializes on declining host colonies that lost their reproductive queen(s) (80, 81). Preferentially inhabiting queenless host colonies is a highly specialized and rare behavior that was described only for few social parasite species (84–87). It is important to note that the phylogenetic placement of *F. talbotae* was not unequivocal in our analysis, but it is important for understanding the evolution of workerless inquilinism in *Formica* ants. The museum specimens of *F. talbotae* available to us were collected in the 1950s, and from these specimens we recovered only fragments of 496 UCE loci (~8% of nucleotides in the full data matrix). Coalescent-based species tree estimation is known to suffer from missing data (88), and to test for incongruencies, we performed coalescent-based species tree estimations (89–92) using a reduced data matrix that included only the 67 loci for which at least 50% of the *F. talbotae* sequences were present. This analysis recovered *F. talbotae* as the sister lineage to the *difficilis* group (*SI Appendix*, Fig. S8), and statistical support was low across the species tree. The phylogenetic position of *F. talbotae* differs from the nested position obtained in concatenation (Fig. 2 and *SI Appendix*, Fig. S1), but the topology is more similar to the concatenated tree than the species tree analysis of the full dataset (*SI Appendix*, Fig. S6), suggesting that missing data do have a negative impact on the species tree analysis. A quartet sampling analysis (93) revealed topological conflict at the same nodes where the concatenated tree differed from the species tree, but strongly supported monophyly of the *difficilis* group including *F. talbotae* nested within (*SI Appendix*, Fig. S9).

In agreement with morphological evidence, which places *F. talbotae* within the *difficilis* group (80, 81), we also consider *F. talbotae* a member of the *difficilis* group. We interpret the placement outside of the *difficilis* group by species tree methods as an artifact caused by missing data.

Historical Biogeography. To infer the biogeographic conditions under which social parasitism evolved in the Formicini, we

conducted a historic biogeography analysis inferring repeated dispersal between the Old and the New World in the genera *Formica*, *Iberoformica*, and *Polyergus* (Fig. 4 and *SI Appendix*, Fig. S2). According to our biogeographical stochastic mapping analysis (94), at least 8 and more likely 9 or 10 such dispersal events occurred in this genus group (Fig. 4). All other genera classified in the Formicini are confined to the Old World. Therefore, early stem lineages of the genus *Formica* almost certainly lived in the Old World and subsequently started dispersing into the New World and back, likely via Beringia, which connected Eurasia and the Nearctic throughout most of the Paleogene (95). Recent trans-Beringian dispersal was also demonstrated for several Holarctic ant species, which include *Formica gagateoides* (96).

Nine fossil species of *Formica* are known from Eocene amber inclusions of Europe (47.8 to 33.9 Ma) (97–99). Several fossils have been compared to extant species in the *fusca* and *rufa* groups (100), and close examination suggests that this resemblance may be superficial (101, 102), which raises the question of whether the Eocene amber fossils indeed represent crown group *Formica*. Doubts about the correct identification of putative *Formica* fossils in Baltic amber currently limit the utility of Eocene amber fossils for calibrating divergence analyses in the genus.

Implications for *Formica* Taxonomy and Classification. The internal classification of species-rich genera, such as *Formica*, is important because the affiliation with a species group (or subgenus) can provide first clues regarding the life history and general biology of an unknown species. Of the 10 presently recognized *Formica* species groups (Table 2), the *pallidefulva* (= *Neoformica*), *exsecta*, *sanguinea* (= *Raptiformica*), and *difficilis* groups were recovered as monophyletic. In contrast, species traditionally classified in three species groups (or subgenera), the *fusca* (= *Serviformica*), *neogagates* (= *Proformica*), and *rufa* (= *Formica* s. str.) groups, form nonmonophyletic assemblages. The Palearctic species *F. gagateoides*, traditionally placed in the *fusca* species group, was inferred as the sister species to the Nearctic

clade comprising the *neogagates* and *pallidefulva* groups. The *neogagates* group itself forms a paraphyletic grade outside the *pallidefulva* group. The Holarctic *exsecta* group plus the problematic *F. uralensis*, placed in a monotypic group, are sisters to all other dependent colony-founding/temporary social parasite groups, instead of *F. uralensis* being part of either the *fusca* or the *rufa* group, as previously suggested (103, 104). The rest of species traditionally classified in the *fusca* group form a grade consisting of five clades progressively more closely related to the dependent colony-founding clade. Although our sampling did not include *Formica fusca*, prior work shows that it would be placed within the Palearctic clade containing *Formica cinerea*, *Formica fuscocinerea*, and *Formica lemmani* (61). More thorough taxon sampling and a careful morphological study are necessary for a stable classification of those species. The Nearctic *Formica obtusopilosa* is closely related to *Formica neogagates* and not a member of the *sanguinea* group (105, 106), as treated by some authors (107, 108). This classification is consistent with the biology of *F. obtusopilosa*, which is not dulotic and its queens found colonies independently, like other species in the *neogagates* group (105, 107) (S.P.C., personal observation). Hence, the clypeal notch, long thought to be diagnostic of *sanguinea* group species, evolved convergently in *F. obtusopilosa*. The traditional *rufa* group is recovered as paraphyletic because the Old World *rufa* group species (now the true *rufa* species group) form a clade that is sister to the Nearctic *integra* and *difficilis* groups, and the distinctive *F. dakotensis* and *F. reflexa* form a clade (here called the *dakotensis* group) sister to *rufa*, *integra*, and *difficilis* groups. Finally, according to custom and for consistency we refer to the erstwhile *microgyna* group as the *difficilis* group, after the oldest constituent species name. The *difficilis* group species are monophyletic and sister to the *integra* group, not nested within it as previously suggested (109).

Considering that all the traditional *Formica* subgenera are nested within *Formica* and that three of the four subgenera are paraphyletic, we suggest discontinuing the use of the subgeneric names and using species group names instead. While these results clarify the internal structure within the genus, much work remains to be done on the species level. The *fusca*, *integra*, *difficilis*, Nearctic *sanguinea*, and *neogagates* groups all need taxonomic revisions.

Conclusions

Our study provides a robust phylogenetic framework for studying the evolution of the diverse and ecologically important Holarctic ant genus *Formica* and allows for testing competing hypotheses regarding the origins and evolution of social parasitism in ants. We conclude that in the formicine genera *Formica*, *Polyergus*, and *Rossomyrmex*, social parasitism originated repeatedly and convergently. In the genus *Formica*, multiple transitions to increasingly more complex socially parasitic life histories evolved. First, the capacity for occasional DCF via budding evolved in facultatively polygynous species practicing ICF. Eventually the ability for ICF was lost in the ancestor of what is now a large clade of obligate dependent colony-founding species (clade marked by a purple star in Fig. 2). Temporary social parasitism either coincided with or preceded this loss of ICF and the transition to obligate DCF. Because all species of the obligate DCF clade appear capable of TSP, it is likely that the evolution of TSP precipitated the loss of ICF. Within this obligate DCF clade dulosis evolved once in the ancestor of the *sanguinea* group. Finally, the permanent social parasites, *F. reflexa* and the workerless *F. talbotae*, evolved independently from temporary social parasitic ancestors. Across species, *Formica* social parasites likely originated via the interspecific, allopatric speciation route of social parasite evolution, emphasizing that convergent evolutionary trajectories can lead to highly similar parasitic life history syndromes across eusocial insects.

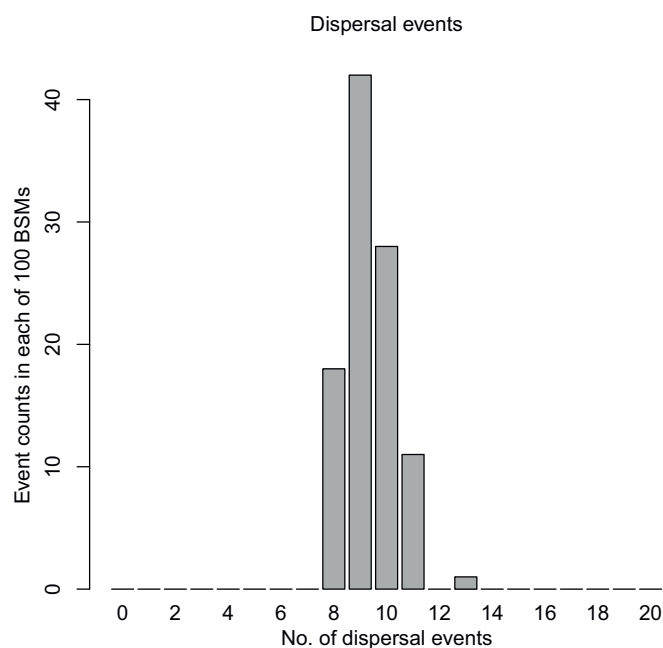


Fig. 4. Inferred dispersal events between the Old and the New World in *Formica*, *Iberoformica*, and *Polyergus* ants. Shown is a histogram of dispersal event counts from 100 biogeographic stochastic maps under the DEC+J model in BioGeoBEARS.

The inferred sequence for the evolution of dulosis lends empirical support to Charles Darwin's "predation hypothesis" for the origin of dulotic behavior in ants. Furthermore, our results suggest that the ancestor of the dulotic *Formica* species likely possessed all the traits associated with the evolution of dulotic behavior, namely territorial and predatory behavior, brood transport behavior among spatially distinct nests of polygynous colonies, and the capacity for parasitic and dependent colony founding. The origin of dulosis was then followed by secondary diversification into 14 species that today form the monophyletic *sanguinea* group.

Our study inferred the workerless social parasite *F. talbotae* as arising from a clade of temporary social parasites in the *difficilis* group, providing empirical evidence for a transition from temporary to workerless social parasitism. The example of *F. talbotae* underscores the importance of distinguishing between the different life history traits summarized under the umbrella term "inquiline social parasitism." Taking the evolutionary origins into account is important because the majority of queen-tolerant workerless inquiline parasites likely speciated directly from free-living ancestors, whereas most queen-intolerant workerless social parasites apparently transitioned to the workerless state from a dulotic or temporary social parasitic ancestor. *Formica talbotae* can be regarded as a queen-intolerant obligate temporary social parasite that preferentially inhabits queenless host colonies and secondarily lost its worker caste. The distant relatedness to its host, *F. obscuripes*, and its distinct life history traits suggest that *F. talbotae* also evolved via the allopatric, interspecific route of social parasite evolution, contrasting with the sympatric origins of some queen-tolerant inquiline social parasites.

We show that *Formica* evolved during the early Oligocene, representing a relatively young ant genus that diversified rapidly into a diverse, ecologically dominant group. During its evolutionary history *Formica* ants dispersed several times between the Old and the New World.

Our study outlines the life history changes associated with the transition from a cooperative eusocial to exploitative socially parasitic life history. Given the high diversity of social parasite species in the genus *Formica*, and considering the high degree of morphological and behavioral specialization, socially parasitic *Formica* species appear to be an ideally suited study organism for investigating caste determination and for exploring the genetic basis underlying behavioral and life history evolution.

Materials and Methods

Taxon Sampling. We newly sequenced 101 ingroup morphospecies from all 10 species groups of *Formica* ants that were recognized prior to our study and 8 outgroup species. Collection data associated with sequenced samples can be found in *SI Appendix, Table S1* and detailed voucher information is on Zenodo.

Molecular Data Generation. To obtain the genetic data we extracted DNA, prepared genomic libraries, and then enriched them using 9,446 custom-designed probes targeting 2,524 UCE loci in Hymenoptera (110). We submitted the enriched libraries to the University of Utah High Throughput Genomics Core Facility for sequencing on two Illumina HiSeq 125 Cycle Paired-End Sequencing v4 runs.

Data Processing. We processed the resulting reads using the Phyluce bioinformatics pipeline (111). Following alignment and trimming, we retained only individual locus alignments that had 110 or more taxa (70% of total), resulting in 2,242 loci on average 667 nt long. The resulting concatenated matrix was 1,497,044 nt long and contained 17.58% of missing data and gaps.

Phylogenetics. To infer the maximum-likelihood phylogeny, we used ModelFinder (112) as implemented in IQ-TREE (113) to select the best model for each UCE locus under the Akaike information criterion (AICc). These models were then used for by-locus partitioned analysis of the concatenated data matrix (114). To assess the robustness of this result to different analytics we performed an unpartitioned analysis and a quartet sampling analysis (93). In addition to concatenated analyses we performed coalescent-based species tree estimation using ASTRAL-III (92).

Divergence Time Analyses. For divergence time analyses we used a node dating approach, as implemented in MCMCTree, a part of the PAML package, v4.9e (115). We constrained our root node with soft bounds around a conservative maximum age estimate of 79 Ma, which corresponds to the lower bound of the 95% highest posterior density interval for that split in a previous phylogenomic study (65).

Biogeography. For biogeographic inference we used BioGeoBEARS (116). We discretized the distribution of *Formica* species into two regions, the New World and the Old World. We used 100 replicates of biogeographical stochastic maps (94) to estimate the number of times *Formica* dispersed between the Old and the New World.

Ancestral State Reconstruction. To investigate the evolution of nest structure, colony structure, and mode of colony foundation, we used stochastic character mapping (117) as implemented in the R package Phytools (118). We compared and selected best-fitting models of character evolution using GEIGER (119) with a time-calibrated tree pruned from distant outgroups and intraspecific samples as input. We based our character coding for each species (*SI Appendix, Table S2*) on literature records and 80 y of cumulative field research by ourselves and colleagues.

Data Availability. For detailed methods, see *SI Appendix*. Reads generated for this study are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject ID [PRJNA749764](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA749764)). Other files used in analyses are available on Zenodo (DOI: [10.5281/zenodo.4341310](https://doi.org/10.5281/zenodo.4341310)) (120).

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1

2 **Supplementary Information for**
3 **The evolution of social parasitism in *Formica* ants revealed by a global phylogeny**
4 **Marek L. Borowiec, Stefan P. Cover and Christian Rabeling**
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7 **This PDF file includes:**
8 Supplementary text
9 Figs. S1 to S9
10 Tables S1 to S2
11 References for SI reference citations

12 Supporting Information Text

13 Supplementary Methods

14 **Data availability.** Trimmed reads generated for this study are available at the NCBI Sequence Read Archive (BioProject ID
15 PRJNA749764). Detailed voucher collection information, assembled sequences, analyzed matrices, configuration files and
16 output of all analyses, and code used are available on Zenodo (DOI: [10.5281/zenodo.4341310](https://doi.org/10.5281/zenodo.4341310)).

17 **Taxon sampling.** For this study we gathered samples collected in the past ~60 years which were available as either ethanol-
18 preserved or point-mounted specimens. Taxon sampling comprises 101 newly sequenced ingroup morphospecies from all seven
19 species groups of *Formica* ants (1) that were recognized prior to our study and 8 outgroup species. Our sampling was guided
20 by previous taxonomic and phylogenetic work (1–17) and included representatives from both the New and the Old World.
21 Collection data associated with sequenced samples can be found in Table S1.

22 **Molecular data collection and sequencing.** We performed non-destructive extraction and preserved same-specimen vouchers
23 for each newly sequenced sample. We re-mounted all vouchers, assigned unique specimen identifiers (Table S1), and deposited
24 them in the ASU Social Insect Biodiversity Repository (contact: Christian Rabeling, christian.rabeling@asu.edu). Detailed
25 voucher collection data can be accessed at [10.5281/zenodo.4341310](https://doi.org/10.5281/zenodo.4341310). Briefly, we extracted DNA from all newly sequenced
26 specimens using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) and followed a library preparation protocol that
27 follows was slightly modified from (18). We used a KAPA Hyper Prep Library Kit (Kapa Biosystems, Inc., Wilmington, MA,
28 USA) with magnetic bead cleanup (19) and a SPRI substitute (20) as described in (21). We used TruSeq adapters (22) for
29 ligation followed by PCR amplification of the library using a mix of HiFi HotStart polymerase reaction mix (Kapa Biosystems),
30 Illumina TruSeq primers, and nuclease-free water.

31 We enriched each pool with 9,446 custom-designed probes (MYcroarray, Inc.) targeting 2,524 UCE loci in Hymenoptera
32 (23). We followed library enrichment procedures for the MYcroarray MYBaits kit (24) except we used a 0.1× of the standard
33 MYBaits concentration and added 0.7 μL of 500 μM custom blocking oligos designed against the custom sequence tags. We ran
34 the hybridization reaction for 24 h at 65 °C, subsequently bound all pools to streptavidin beads (MyOne C1; Life Technologies)
35 and washed bound libraries according to a standard target enrichment protocol (24). We used the with-bead approach for
36 PCR recovery of enriched libraries as described in (25).

37 We submitted pre-pooled libraries to the University of Utah High Throughput Genomics Core Facility for quality control
38 on Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and normalization. The pooled libraries were then
39 sequenced using one full and one partial lane of a HiSeq 125 Cycle Paired-End Sequencing v4 run. Quality-trimmed sequence
40 reads generated as part of this study are available from the NCBI Sequence Read Archive (to be submitted upon publication).

41 **Processing of UCE data.** We performed read cleaning, assembly, and matching of assembled contigs to UCE probes using
42 Phyluce bioinformatics pipeline (25). We trimmed the FASTQ data using Illumiprocessor, a wrapper around Trimmomatic
43 (26), with default settings (LEADING:5, TRAILING:15, SLIDINGWINDOW:4:15, MINLEN:40). Assemblies were done using
44 Trinity v20140717 (27) with the phyluce_assembly_assemble_trinity wrapper. We then assessed orthology by matching the
45 assembled contigs to enrichment probe sequences with phyluce_assembly_match_contigs_to_probes (min_coverage=50,
46 min_identity=80).

47 **Alignment.** We used phylogeny-aware UPP workflow (28) to align all UCE sequences. We used AMAS (29) for alignment
48 wrangling and obtaining summary statistics and AliView (30) for visualization. Although alignment trimming has been
49 criticized in the past (31), we decided to trim the alignments because of substantial computational burden associated with
50 analysis of untreated data with high proportion of gaps. We used trimAl (32) and its “gappyout” algorithm, which is a
51 relatively relaxed algorithm for removal of gappy sites. Visual inspection of alignments revealed that occasionally sequences
52 were misaligned towards flanks. To automatically identify and discard the misaligned sequences we wrote a custom R script
53 (R Core Team) that leveraged packages ape (33, 34), seqinr (35), doParallel, and plyr. The script first generates a matrix of
54 uncorrected p-distances from a UCE locus alignment and for each taxon it computes average p-distance to all other taxa. Then
55 it creates a distribution of average per-locus p-distances for each taxon and detects outliers defined as sequences that lay above
56 3 SD from the mean of that distribution. Once identified, the script removes outliers using AMAS. This procedure resulted in
57 removal of 0.97 % of all sequences. For downstream analyses we retained only alignments that had 110 or more taxa (70 % of
58 total), resulting in 2,242 loci on average 667 nt long. The resulting concatenated matrix was 1,497,044 nt long and contained
59 17.58 % of missing data and gaps.

60 **Partitioning.** We used ModelFinder (36) as implemented in IQ-TREE (37). For each UCE we selected the best model under
61 AICc. These models were then used for by-locus partitioned analysis of concatenated data matrix (38). We have also employed
62 the newly proposed strategy of partitioning UCE loci based on a sliding window approach that groups UCE sites with similar
63 entropies (39). Unfortunately, many partitions identified using this approach were saturated and caused numerical instability
64 in maximum likelihood analyses using IQ-TREE, resulting in unreasonably long tree lengths. We therefore proceeded with
65 downstream analyses using per-locus partitioning.

66 **Phylogenetic and concordance analyses using maximum likelihood.** We used IQ-TREE (37) for maximum likelihood inference
67 of phylogeny on single-locus alignments and concatenated data matrix. To test the robustness of the partitioned concatenated
68 analysis, we performed unpartitioned analysis under HKY+4G model, which was the most common model identified as best
69 under AICc for single loci. To assess the sensitivity of our results using measures other than bootstrap support we performed a
70 quartet sampling analysis with 500 maximum replicates (40). Results are summarized in Figure S7.

71 **Species tree analyses.** In addition to concatenated analyses we performed coalescent-based species tree estimation using
72 ASTRAL-III (41). Because summary coalescence methods such as ASTRAL have been shown to be negatively impacted
73 by error in estimated gene trees (42) we used the weighted statistical binning pipeline (43, 44). We collapsed all nodes
74 with ultrafast bootstrap (45) support below 95 for the binning pipeline, which resulted in identification of 1,733 supergenes
75 containing from one to three UCE loci. We then used IQ-TREE to estimate supergene trees under a fully partitioned model
76 (i.e. with branch lengths unlinked across partitions). Because of recent criticism of the statistical binning pipeline (46) we also
77 performed an analysis where raw trees from individual locus analyses were used. We mapped all terminals to putative species
78 using morphology and the concatenated tree as guidance. Because some of the 101 species we recognized using morphology
79 were non-monophyletic on the concatenated tree, we mapped the terminals onto 113 total monophyletic lineages representing
80 putative species (Figures S4–S6). To test the effect of missing data (47) on the position of *Formica talbotae* we performed
81 additional analysis that used only the 67 loci which contained at least 50 % complete sequence for this taxon (Figure S6).

82 **Divergence time inference.** For divergence time analyses we used a node dating approach, as implemented in MCMCTree, a
83 part of the PAML package, v4.9e (48). MCMCTree utilized rapid approximate likelihood computation (49), which makes
84 it suitable for divergence dating of genome-scale data sets (50). We constrained our root node with soft bounds around a
85 conservative maximum age estimate of 79 Ma, which corresponds to the lower bound of the 95 % highest posterior density
86 interval for that split in (18). Although *Formica* has a rich fossil record, the affinity of these fossils is uncertain because, as this
87 study shows, morphology has thus far been misleading about phylogeny. Because of this, we conservatively constrained the
88 split of *Polyergus* and *Formica*+*Iberoformica* to be at least 34 Ma, or one of the younger estimates for the age of Baltic amber
89 (51, 52). We ran each analysis unpartitioned, under the HKY+4G model for 20 million generations. We examined each run's
90 statistics in Tracer.

91 **Biogeographic analyses.** For biogeographic inference we used BioGeoBEARS v1.1.2 (53). We discretized the distribution of
92 *Formica* species into two regions, the New World and Old World. Model selection implemented in BioGeoBEARS suggested
93 DEC+J (54) as the best-fitting model and we used it for all downstream analyses. We used 100 replicates of biogeographical
94 stochastic maps (55) to estimate the number of times *Formica* dispersed between the Old and New World. We used our
95 time-calibrated tree with taxon duplicates removed such that each species or putative species was represented by only one
96 terminal. Results are summarized in Figure S2.

97 **Ancestral state reconstruction.** In order to investigate the evolution of natural history traits in *Formica* and *Polyergus* we
98 used stochastic character mapping (56). We used the same time-calibrated tree as for biogeographic analyses but with distant
99 (*Rossomyrmex minuchae* and *Proformica mongolica*) outgroups and *Formica* taxa for which we had insufficient life history data
100 (see Table S2) pruned.

101 For each species we collected literature and field observation data on nest structure, colony structure, and colony foundation
102 mode. We discretized nest structure into three categories: i) monodomous, ii) polymorphic: either monodomous or polydomous,
103 and iii) polymorphic: monodomous, polydomous, or supercolonial. We discretized colony structure into i) monogynous or ii)
104 polygynous. We assigned species to the polygynous category if they have been observed to be either monogynous or polygynous.
105 We discretized colony founding mode into five categories: i) independent colony foundation via haplometrosis or pleometrosis,
106 queen readoption occurs, budding absent or at low frequency, ii) facultative temporary social parasitism with budding at low
107 frequency, iii) facultative temporary social parasitism with budding at high frequency, iv) obligate temporary social parasitism
108 and dulosis without budding, v) permanent social parasitism. The coding of character states for each species and references to
109 original research articles are available in Table S2.

110 We performed ancestral state reconstruction on each of the three characters (nest structure, colony structure, and colony
111 founding). We first compared three commonly-used variants of the discrete character evolution Mk model (57): all rates equal
112 (character state change rates are equal for all states), symmetric transition rates (character state change rates are different
113 for each pair of states), and all rates different (character state change rates are different for each transition). We also fit the
114 meristic model (assuming characters change in step-wise fashion). We fit these four models using "fit" functions the R package
115 GEIGER v2.0.6 (58) to all three characters. We used Akaike Information Criterion corrected for sample size (AICc) weights as
116 computed by the "aic.w" function in Phytools v0.6-44 (59) to see which model fit best. This approach identified the all rates
117 different model to be the best fit for nest and colony structure and the symmetric model was found best-fitting for colony
118 founding.

119 Ancestral reconstruction results are found in Figures S3-S5.

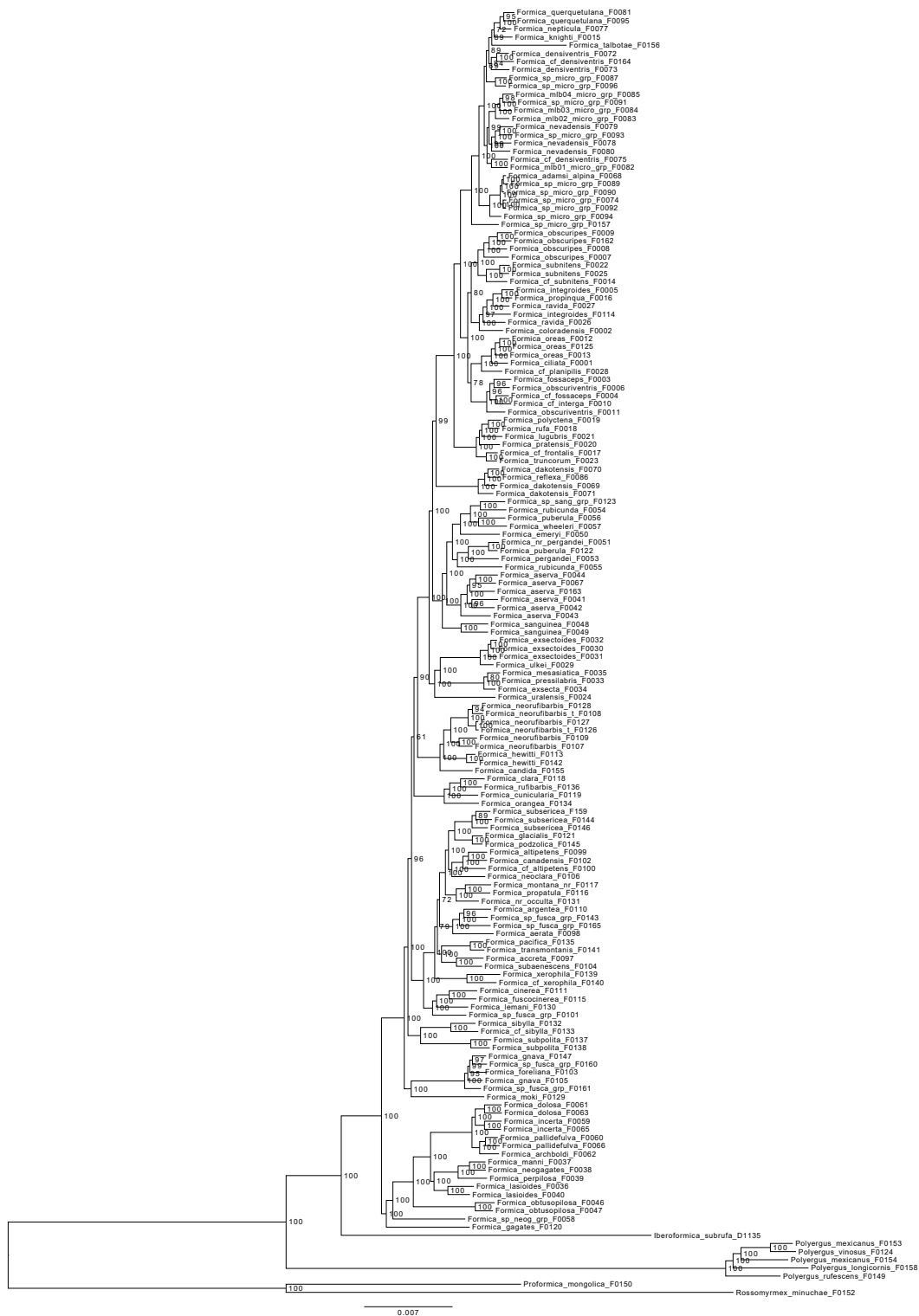


Fig. S1. Phylogram of *Formica* and outgroups from concatenated alignment inferred under maximum likelihood using IQ-TREE. Support in ultrafast bootstrap (values above 95 indicate strong support). Scale in substitutions per site.

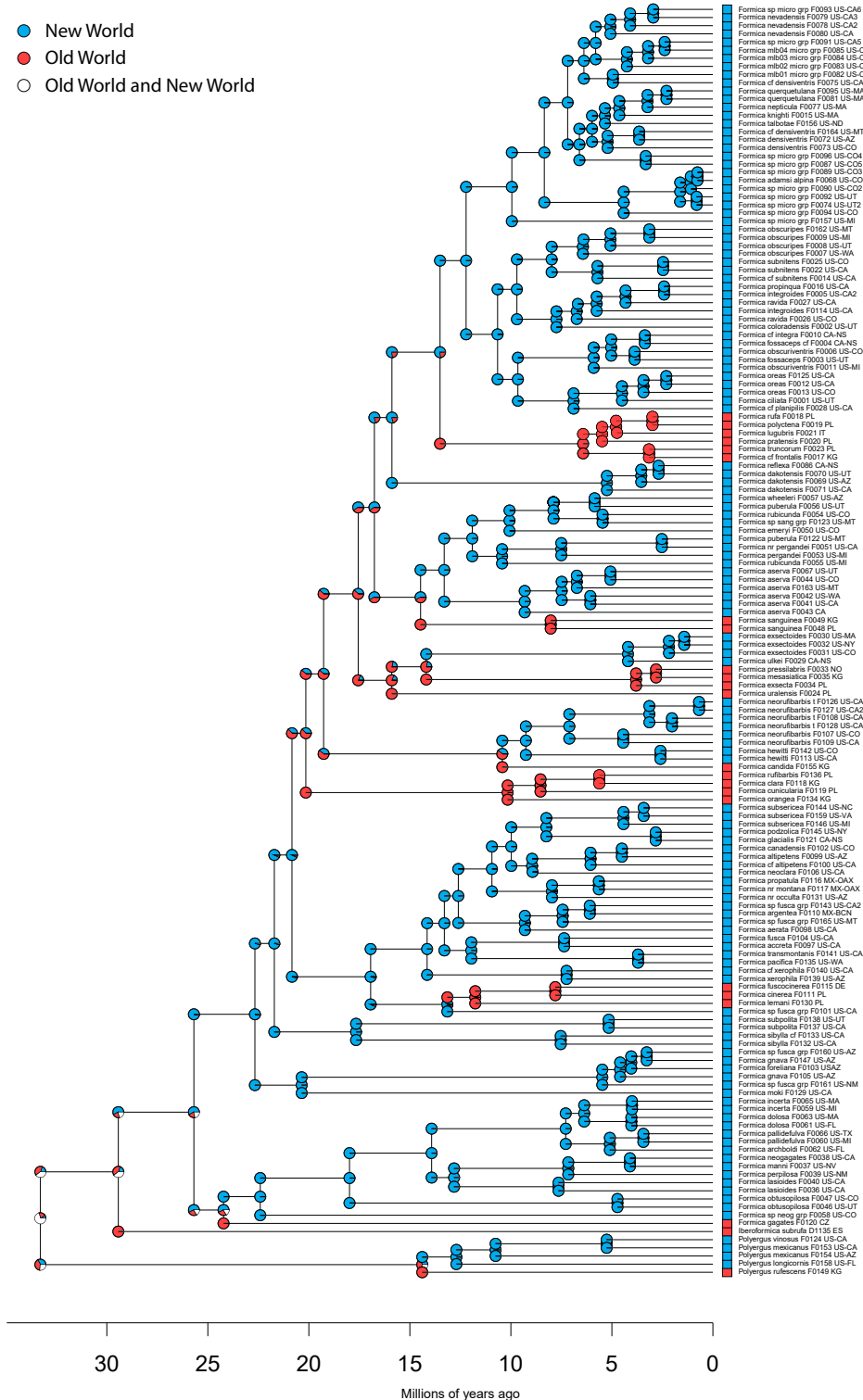


Fig. S2. Biogeographic history of *Formica*, *Iberofornica* and *Polyergus*. Pie charts depict relative likelihoods of range estimations from BioGeoBEARS under DEC+J. Pie charts at nodes correspond to ancestral state estimations and charts on the corners correspond to ranges immediately following speciation. Numbers preceded by the letter F indicate extraction code and correspond to identifiers in Table S1. Country and state/province codes as in main text Figure 1.

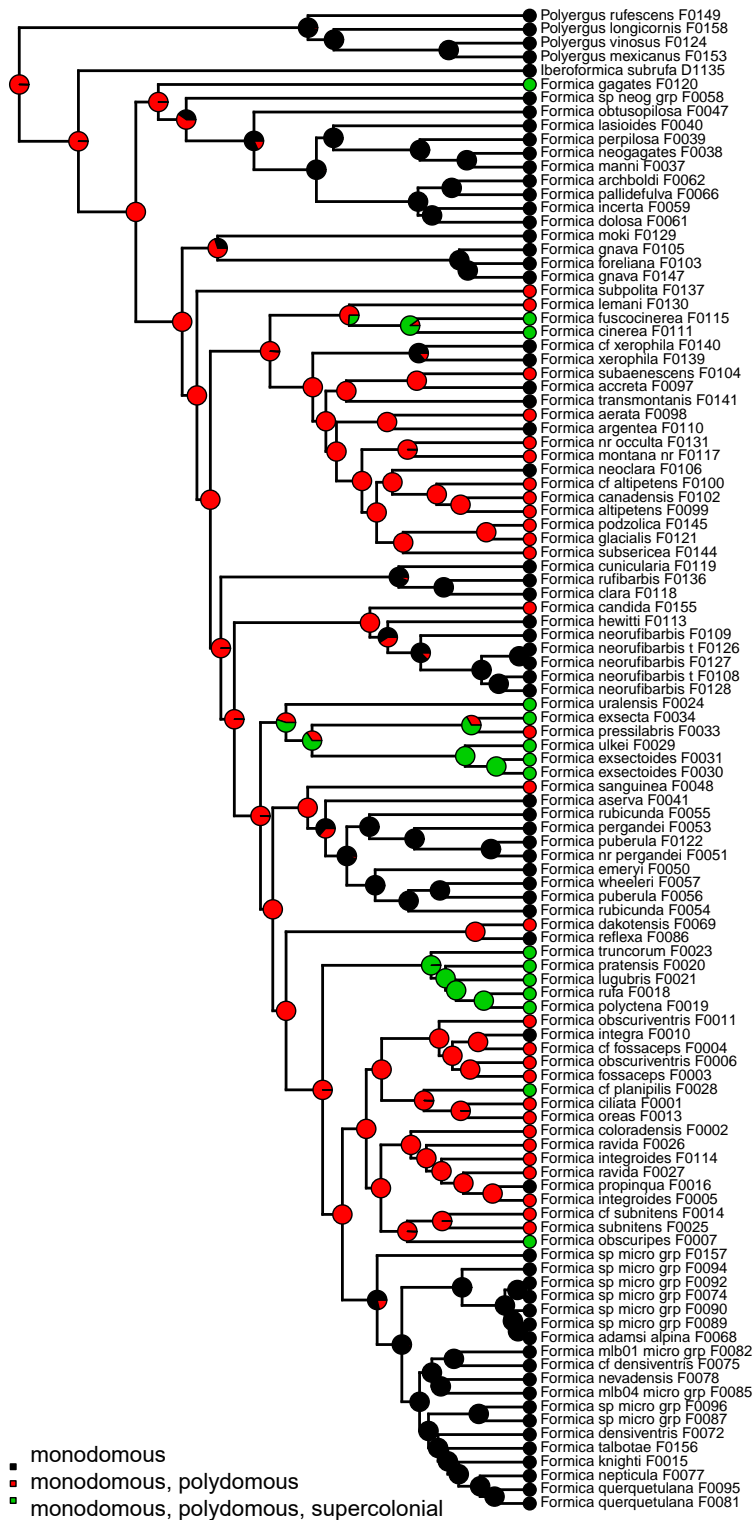


Fig. S3. Ancestral state reconstruction of nest structure using stochastic character mapping in Phytools of *Formica* nest structure under all rates different model. Pie charts at nodes correspond to ancestral state estimations and circles at tips correspond to states extant species. Numbers preceded by the letter F indicate extraction code and correspond to identifiers in Tables S1 and S2.

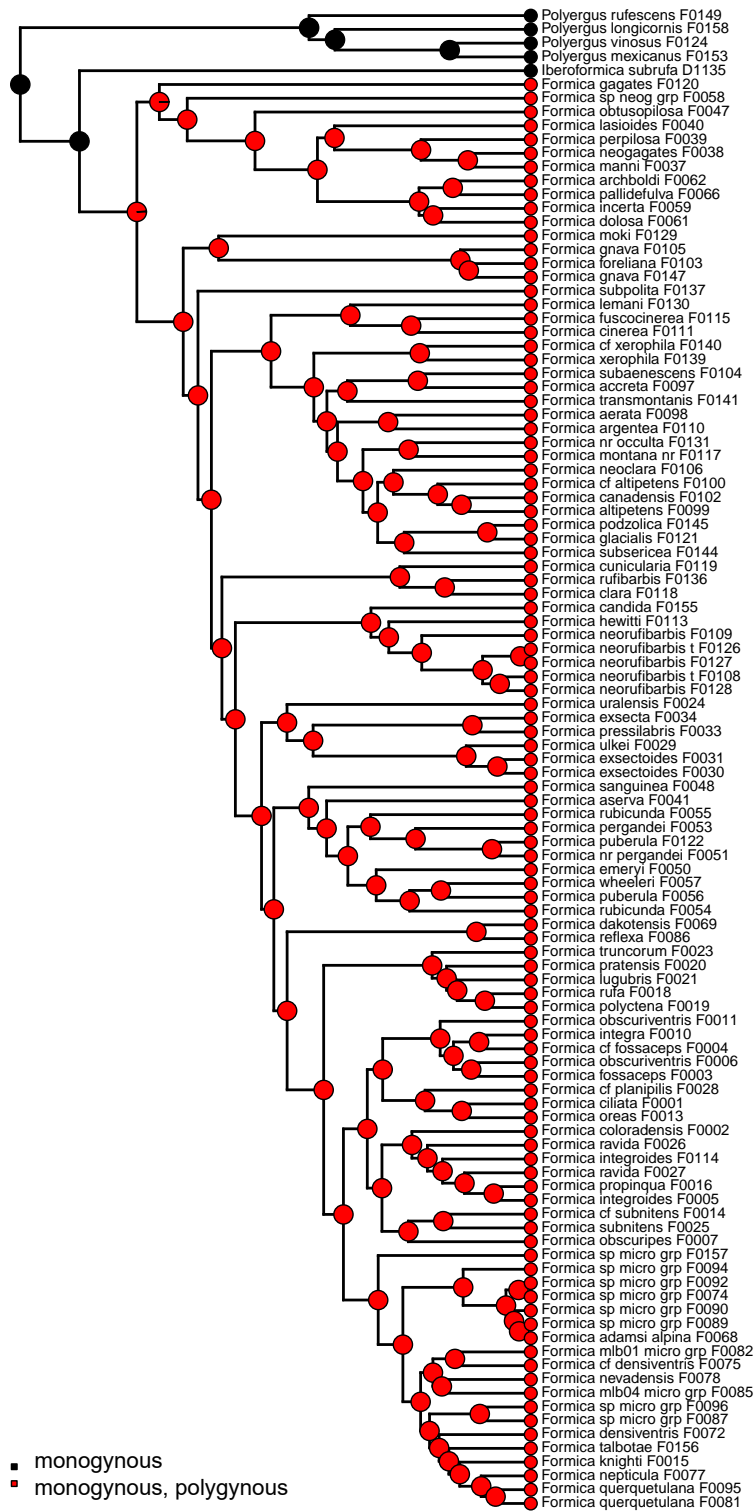


Fig. S4. Ancestral state reconstruction of colony structure using stochastic character mapping in Phytools of *Formica* colony structure under all rates different model. Pie charts at nodes correspond to ancestral state estimations and circles at tips correspond to states extant species. Numbers preceded by the letter F indicate extraction code and correspond to identifiers in Tables S1 and S2.

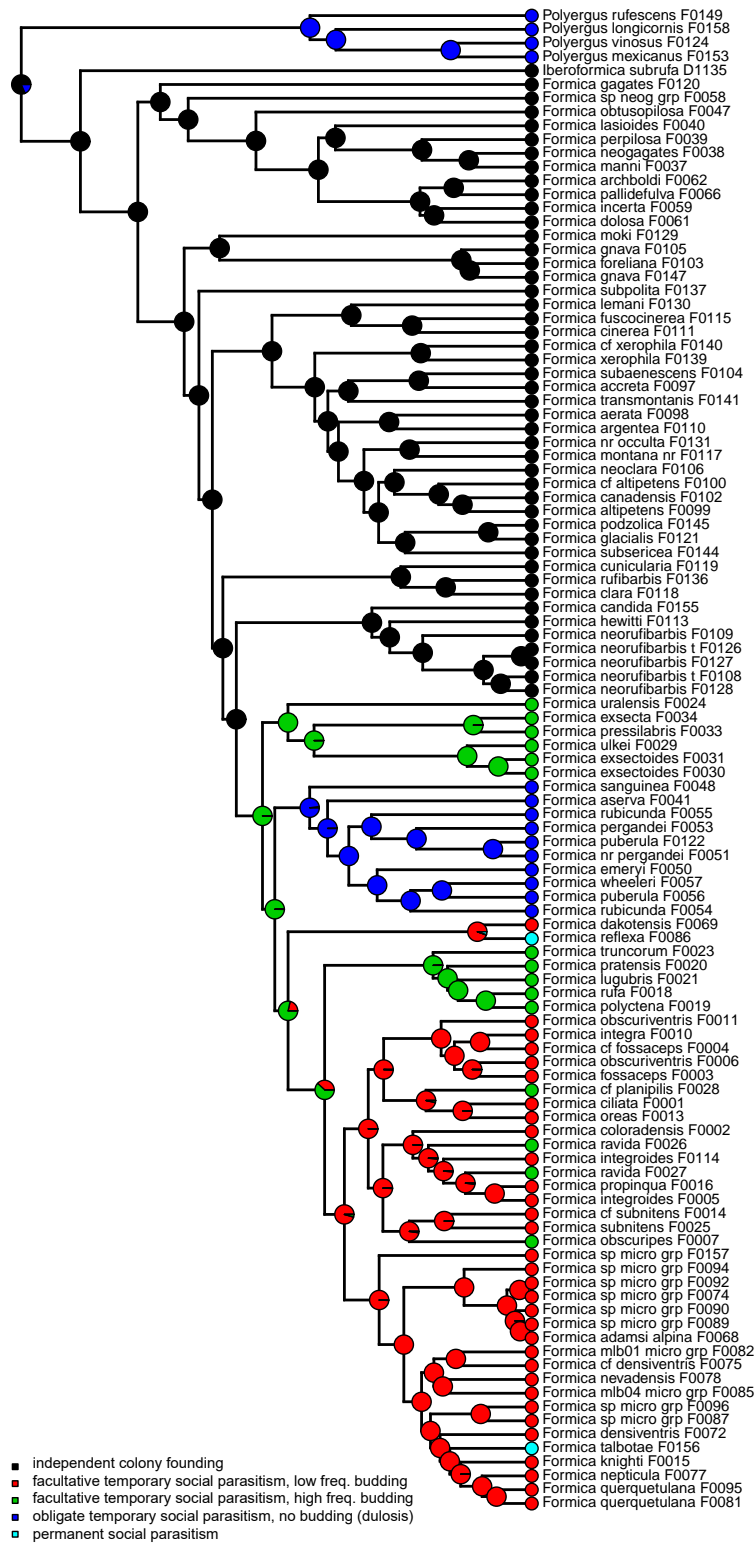


Fig. S5. Ancestral state reconstruction of colony founding mode using stochastic character mapping in Phytools of *Formica* colony founding under symmetrical rates model. Pie charts at the nodes correspond to ancestral state estimations and circles at tips correspond to states extant species. Numbers preceded by the letter F indicate extraction code and correspond to identifiers in Table S1 and S2.

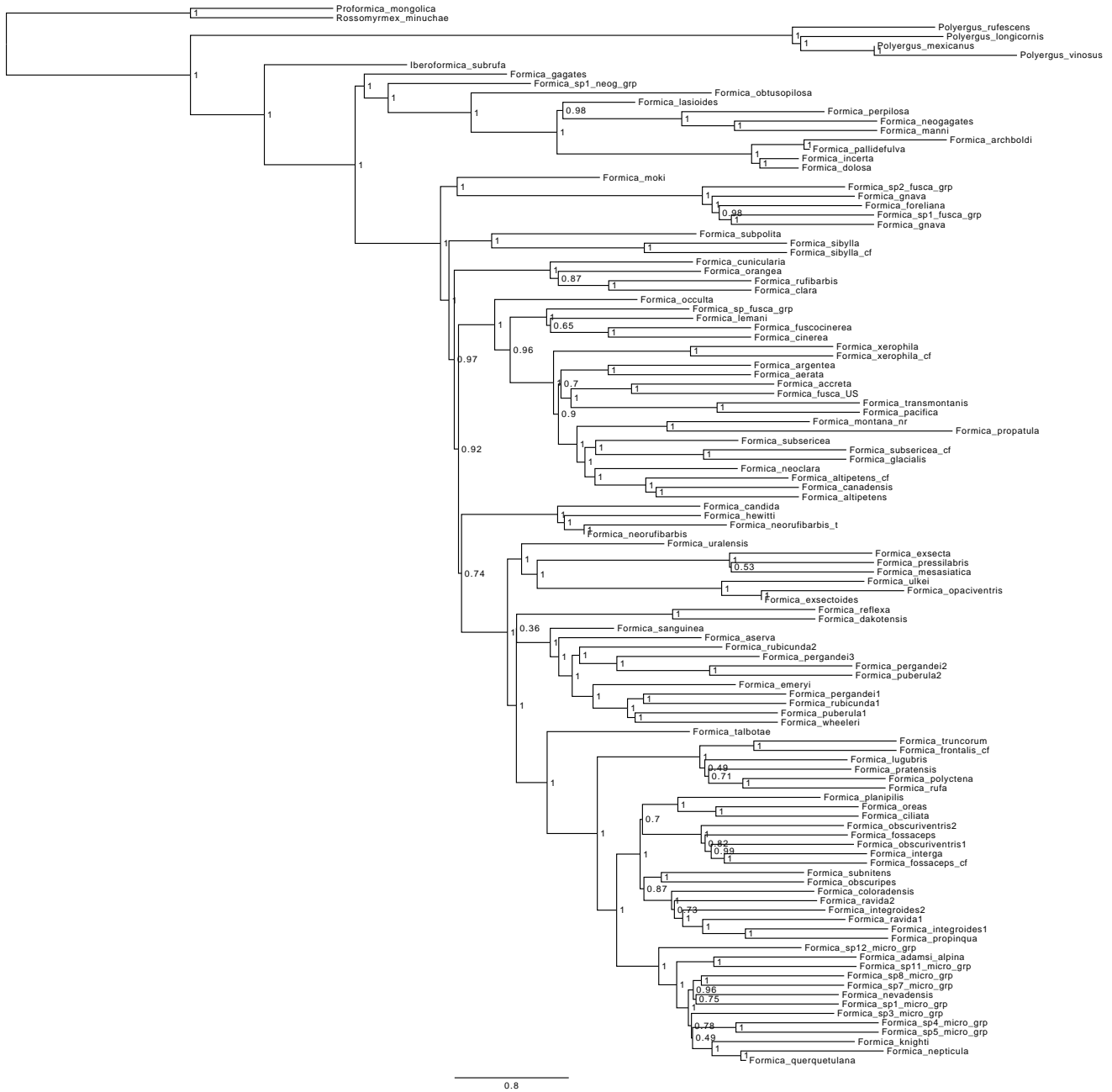


Fig. S6. Species tree inferred under coalescent using ASTRAL-III and 1,733 weighted supergene trees identified using statistical binning pipeline. Support is expressed in local posterior probability and branch lengths are in coalescent units.

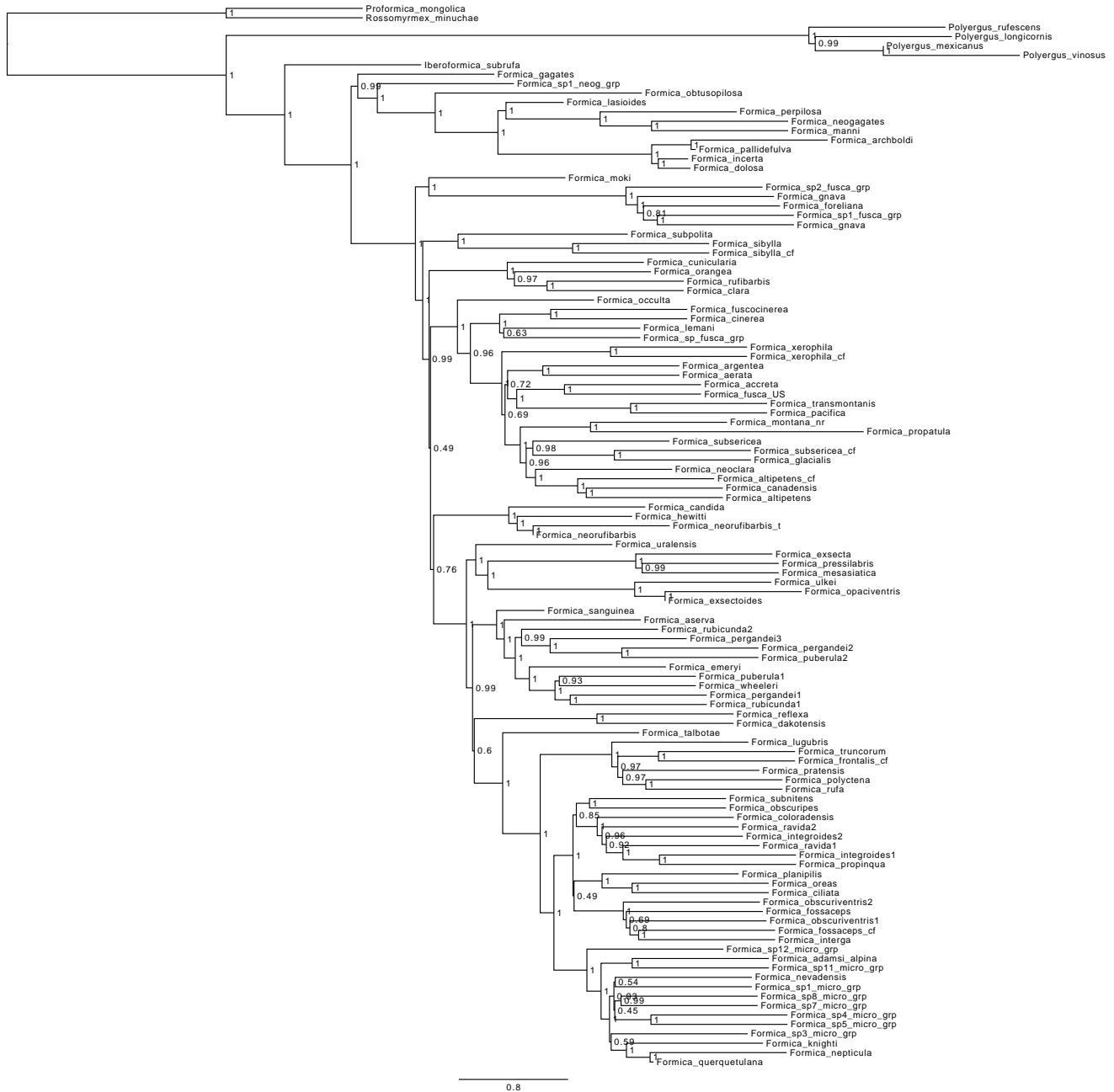


Fig. S7. Species tree inferred under coalescent using ASTRAL-III and all 2,242 individual UCE locus trees. Support is expressed in local posterior probability and branch lengths are in coalescent units.

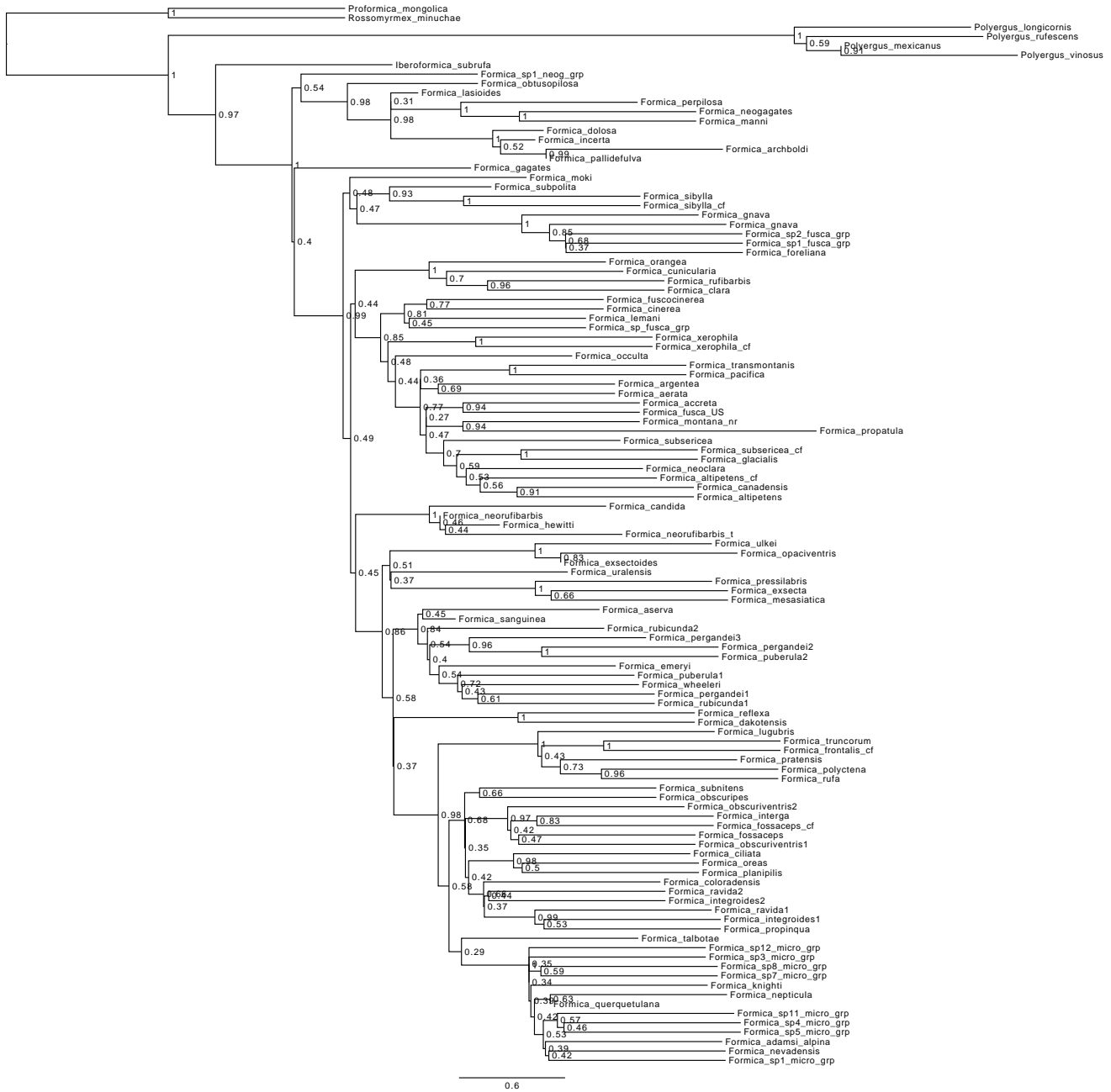


Fig. S8. Species tree inferred under coalescent using ASTRAL-III and 67 individual UCE locus trees for which at least 50 % of sequence length was available for *Formica talbotae*. Support is expressed in local posterior probability and branch lengths are in coalescent units.

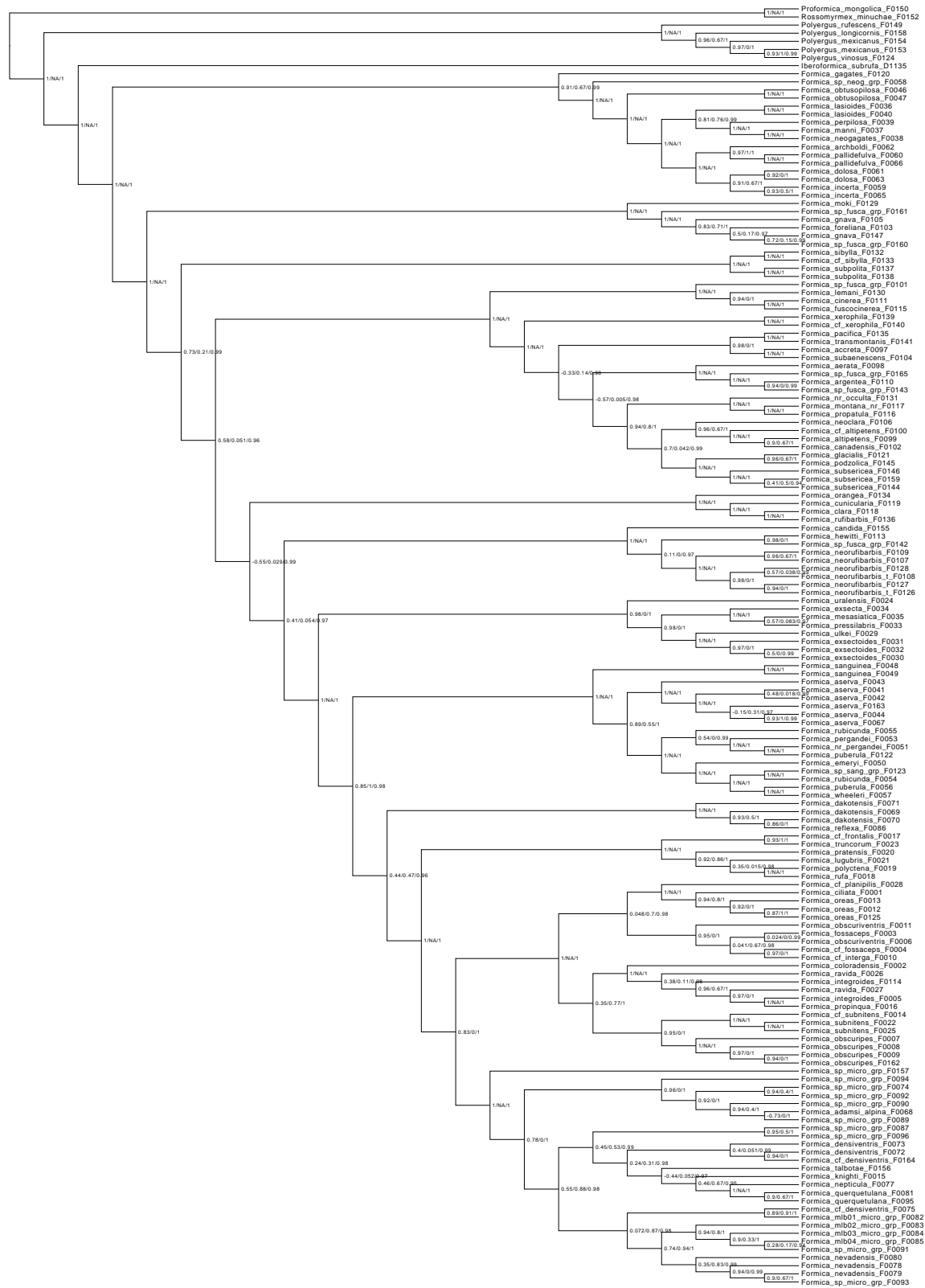


Fig. S9. Support from quartet sampling analyses. The values are: quartet concordance score (QC) / quartet differential score (QD) / quartet informativeness score (QI). Briefly, QC measures how often the concordant quartet topologies are inferred over discordant quartets in different replicates, with a value equal 1 when all replicates result in concordant topology, QD measures whether frequencies of two discordant topologies are equal (=1) or skewed (<1), and QI measures what proportion of replicates were informative. Contrast values for clade of Palearctic species related to *Formica rufibarbis* (-0.55/0.029/0.99) indicating that discordant topologies were inferred more often than those concordant with the input tree, that quartet topologies were highly skewed, but most replicates were informative. The former indicates maximal support, while the latter shows that the position of this clade within the "fusca grade" is highly uncertain, perhaps due to incomplete lineage sorting.

Table S1. Voucher specimens used in this study. Detailed collection data can be accessed at Zenodo (DOI: [10.5281/zenodo.4341310](https://doi.org/10.5281/zenodo.4341310)).

Extraction ID	Taxon	Specimen ID	Country	Latitude	Longitude
D1135	Iberoformica subrufa	ASU-SIBR2148	ES	37.185	-3.485
F0001	Formica ciliata	ASU-SIBR2001	US	38.26117	-112.51433
F0002	Formica coloradensis	ASU-SIBR2002	US	38.42233	-109.18017
F0003	Formica fossiceps	ASU-SIBR2003	US	38.39467	-109.165
F0004	Formica cf fossiceps	ASU-SIBR2004	CA	44.53050	-64.31912
F0005	Formica integroides	ASU-SIBR2005	US	38.67525	-119.99428
F0006	Formica obscuriventris	ASU-SIBR2006	US	39.12017	-107.44767
F0007	Formica obscuripes	ASU-SIBR2007	US	48.1728	-122.6714
F0008	Formica obscuripes	ASU-SIBR2008	US	38.23367	-112.44917
F0009	Formica obscuripes	ASU-SIBR2009	US	42.44788	-84.01688
F0010	Formica cf interga	ASU-SIBR2010	CA	44.68839	-63.66808
F0011	Formica obscuriventris	ASU-SIBR2011	US	42.45103	-84.01878
F0012	Formica oreas	ASU-SIBR2012	US	38.67577	-119.99431
F0013	Formica oreas	ASU-SIBR2013	US	38.84233	-106.53817
F0014	Formica cf subnitens	ASU-SIBR2014	US	38.67757	-119.98343
F0015	Formica knighti	ASU-SIBR2015	US	41.8652	-70.64777
F0016	Formica propinqua	ASU-SIBR2016	US	39.44008	-120.32349
F0017	Formica cf frontalis	ASU-SIBR2017	KG	42.8	77.47
F0018	Formica rufa	ASU-SIBR2018	PL	51.155	16.985
F0019	Formica polycтена	ASU-SIBR2019	PL	51.39	17.5
F0020	Formica pratensis	ASU-SIBR2020	PL	51.3867	16.805
F0021	Formica lugubris	ASU-SIBR2021	IT	46.586	12.105
F0022	Formica subnitens	ASU-SIBR2022	US	39.82308	-120.13748
F0023	Formica truncorum	ASU-SIBR2023	PL	50.33	16.55
F0024	Formica uralensis	ASU-SIBR2024	PL	51.38	23.55
F0025	Formica subnitens	ASU-SIBR2025	US	39.591	-108.818
F0026	Formica ravida	ASU-SIBR2026	US	39.1695	-107.94817
F0027	Formica ravida	ASU-SIBR2027	US	39.43221	-120.24265
F0028	Formica cf planipilis	ASU-SIBR2028	US	40.72538	-120.20708
F0029	Formica ulkei	ASU-SIBR2029	CA	44.49808	-63.92692
F0030	Formica exsectoides	ASU-SIBR2030	US	41.87383	-70.65183
F0031	Formica exsectoides	ASU-SIBR2031	US	38.73367	-104.896
F0032	Formica exsectoides	ASU-SIBR2032	US	43.01548	-77.57365
F0033	Formica pressilabris	ASU-SIBR2033	SE	68.3381	18.7639
F0034	Formica exsecta	ASU-SIBR2034	PL	50.577	22.984
F0035	Formica mesasiatica	ASU-SIBR2035	KG	42.33	78.24
F0036	Formica lasioides	ASU-SIBR2036	US	33.18522	-116.28849
F0037	Formica manni	ASU-SIBR2037	US	41.63632	-119.84183
F0038	Formica neogagates	ASU-SIBR2038	US	39.42998	-120.24087
F0039	Formica perpilosa	ASU-SIBR2039	US	31.83945	-109.03615
F0040	Formica lasioides	ASU-SIBR2040	US	39.43163	-120.24059
F0041	Formica aserva	ASU-SIBR2041	US	37.68319	-119.17078
F0042	Formica aserva	ASU-SIBR2042	US	48.66648	-122.96887
F0043	Formica aserva	ASU-SIBR2043	CA	44.46380	-63.58094
F0044	Formica aserva	ASU-SIBR2044	US	38.81400	-106.28217
F0046	Formica obtusopilosa	ASU-SIBR2046	US	38.71217	-111.94833
F0047	Formica obtusopilosa	ASU-SIBR2047	US	39.50633	-108.76217
F0048	Formica sanguinea	ASU-SIBR2048	PL	50.448	19.995
F0049	Formica sanguinea	ASU-SIBR2049	KG	42.67	77.18
F0050	Formica emeryi	ASU-SIBR2050	US	39.03167	-104.79633
F0051	Formica nr pergandei	ASU-SIBR2051	US	39.21529	-121.04322
F0053	Formica pergandei	ASU-SIBR2053	US	42.45278	-84.01939
F0054	Formica rubicunda	ASU-SIBR2054	US	39.03033	-105.44217
F0055	Formica rubicunda	ASU-SIBR2055	US	42.45919	-84.01429
F0056	Formica puberula	ASU-SIBR2056	US	37.88800	-109.45433
F0057	Formica wheeleri	ASU-SIBR2057	US	31.91433	-109.271
F0058	Formica sp neog grp	ASU-SIBR2058	US	37.84367	-109.36967
F0059	Formica incerta	ASU-SIBR2059	US	42.45151	-84.01901
F0060	Formica pallidefulva	ASU-SIBR2060	US	42.45948	-84.0256
F0061	Formica dolosa	ASU-SIBR2061	US	30.35995	-84.41848
F0062	Formica archboldi	ASU-SIBR2062	US	30.35995	-84.41848
F0063	Formica dolosa	ASU-SIBR2063	US	41.81444	-70.66315
F0065	Formica incerta	ASU-SIBR2065	US	41.8652	-70.64777

Table S1. Voucher specimens used in this study, continued.

Extraction ID	Taxon	Specimen ID	Country	Latitude	Longitude
F0066	<i>Formica pallidefulva</i>	ASU-SIBR2066	US	30.2275	-98.1788
F0067	<i>Formica aserva</i>	ASU-SIBR2067	US	38.66283	-111.94117
F0068	<i>Formica adamsi alpina</i>	ASU-SIBR2068	US	38.39317	-107.19683
F0069	<i>Formica dakotensis</i>	ASU-SIBR2069	US	34.02867	-109.1855
F0070	<i>Formica dakotensis</i>	ASU-SIBR2070	US	38.63500	-111.94817
F0071	<i>Formica dakotensis</i>	ASU-SIBR2071	US	39.41344	-120.32162
F0072	<i>Formica densiventris</i>	ASU-SIBR2072	US	32.65729	-109.85841
F0073	<i>Formica densiventris</i>	ASU-SIBR2073	US	39.75867	-108.78783
F0074	<i>Formica sp micro grp</i>	ASU-SIBR2074	US	38.64883	-111.94983
F0075	<i>Formica cf densiventris</i>	ASU-SIBR2075	US	38.21541	-119.74609
F0077	<i>Formica nepticula</i>	ASU-SIBR2077	US	41.87367	-70.65233
F0078	<i>Formica nevadensis</i>	ASU-SIBR2078	US	39.35788	-122.74716
F0079	<i>Formica nevadensis</i>	ASU-SIBR2079	US	41.56204	-123.21177
F0080	<i>Formica nevadensis</i>	ASU-SIBR2080	US	39.41318	-120.32038
F0081	<i>Formica querquetulana</i>	ASU-SIBR2081	US	41.87383	-70.65183
F0082	<i>Formica mlb01 micro grp</i>	ASU-SIBR2082	US	37.21317	-118.64671
F0083	<i>Formica mlb02 micro grp</i>	ASU-SIBR2083	US	40.94628	-123.0427
F0084	<i>Formica mlb03 micro grp</i>	ASU-SIBR2084	US	39.29111	-120.67918
F0085	<i>Formica mlb04 micro grp</i>	ASU-SIBR2085	US	39.40022	-120.55818
F0086	<i>Formica reflexa</i>	ASU-SIBR2086	CA	44.53050	-64.31912
F0087	<i>Formica sp micro grp</i>	ASU-SIBR2087	US	38.42033	-107.62783
F0089	<i>Formica sp micro grp</i>	ASU-SIBR2089	US	38.18833	-107.62067
F0090	<i>Formica sp micro grp</i>	ASU-SIBR2090	US	38.83800	-106.56167
F0091	<i>Formica sp micro grp</i>	ASU-SIBR2091	US	38.83800	-106.56167
F0092	<i>Formica sp micro grp</i>	ASU-SIBR2092	US	38.64983	-111.95083
F0093	<i>Formica sp micro grp</i>	ASU-SIBR2093	US	41.56204	-123.21177
F0094	<i>Formica sp micro grp</i>	ASU-SIBR2094	US	38.838	-106.56167
F0095	<i>Formica querquetulana</i>	ASU-SIBR2095	US	41.87167	-70.651
F0096	<i>Formica sp micro grp</i>	ASU-SIBR2096	US	39.04167	-104.6615
F0097	<i>Formica accreta</i>	ASU-SIBR2097	US	39.30805	-120.66831
F0098	<i>Formica aerata</i>	ASU-SIBR2098	US	37.80145	-118.5299
F0099	<i>Formica altipetens</i>	ASU-SIBR2099	US	33.90683	-109.1245
F0100	<i>Formica cf altipetens</i>	ASU-SIBR2100	US	38.6739	-119.99411
F0101	<i>Formica sp fusca grp</i>	ASU-SIBR2101	US	37.38932	-118.76612
F0102	<i>Formica canadensis</i>	ASU-SIBR2102	US	39.12017	-107.44
F0103	<i>Formica foreliana</i>	ASU-SIBR2103	US	31.43100	-111.16967
F0104	<i>Formica subaenescens</i>	ASU-SIBR2104	US	37.92051	-122.57471
F0105	<i>Formica gnava</i>	ASU-SIBR2105	US	32.64983	-109.81717
F0106	<i>Formica neoclara</i>	ASU-SIBR2106	US	39.50332	-120.24157
F0107	<i>Formica neurufibarbis</i>	ASU-SIBR2107	US	38.71467	-106.223
F0108	<i>Formica neurufibarbis t</i>	ASU-SIBR2108	US	36.67239	-118.34463
F0109	<i>Formica neurufibarbis</i>	ASU-SIBR2109	US	39.42505	-120.24285
F0110	<i>Formica argentea</i>	ASU-SIBR2110	MX	31.00261	-115.5497
F0111	<i>Formica cinerea</i>	ASU-SIBR2111	PL	51.39	17.5
F0113	<i>Formica hewitti</i>	ASU-SIBR2113	US	38.67984	-119.98373
F0114	<i>Formica integroides</i>	ASU-SIBR2114	US	40.11490	-120.32086
F0115	<i>Formica fuscocinerea</i>	ASU-SIBR2115	DE	48.16452	11.50075
F0116	<i>Formica propatula</i>	ASU-SIBR2116	MX	17.1873	-96.62085
F0117	<i>Formica montana nr</i>	ASU-SIBR2117	MX	17.61752	-96.36799
F0118	<i>Formica clara</i>	ASU-SIBR2118	KG	42.66	77.47
F0119	<i>Formica cunicularia</i>	ASU-SIBR2119	PL	51.175	17.068
F0120	<i>Formica gagates</i>	ASU-SIBR2120	CZ	48.869	16.652
F0121	<i>Formica glacialis</i>	ASU-SIBR2121	CA	44.98735	-64.06002
F0122	<i>Formica puberula</i>	ASU-SIBR2122	US	45.0446	-110.68078
F0123	<i>Formica sp sang grp</i>	ASU-SIBR2123	US	45.13217	-111.06283
F0124	<i>Polyergus vinosus</i>	ASU-SIBR2124	US	32.85323	-116.43738
F0125	<i>Formica oreas</i>	ASU-SIBR2125	US	38.05273	-119.32138
F0126	<i>Formica neurufibarbis t</i>	ASU-SIBR2126	US	38.06165	-119.34966
F0127	<i>Formica neurufibarbis</i>	ASU-SIBR2127	US	38.06207	-119.34888
F0128	<i>Formica neurufibarbis</i>	ASU-SIBR2128	US	38.04167	-119.29781
F0129	<i>Formica moki</i>	ASU-SIBR2129	US	38.9	-121.02
F0130	<i>Formica lemani</i>	ASU-SIBR2130	PL	50.33	16.55

Table S1. Voucher specimens used in this study, continued.

Extraction ID	Taxon	Specimen ID	Country	Latitude	Longitude
F0131	<i>Formica nr occulta</i>	ASU-SIBR2131	US	31.91375	-109.26757
F0132	<i>Formica sibylla</i>	ASU-SIBR2132	US	39.43221	-120.24258
F0133	<i>Formica cf sibylla</i>	ASU-SIBR2133	US	39.431	-120.331
F0134	<i>Formica orangea</i>	ASU-SIBR2134	KG	42.6	75.85
F0135	<i>Formica pacifica</i>	ASU-SIBR2135	US	48.1728	-122.6714
F0136	<i>Formica rufibarbis</i>	ASU-SIBR2136	PL	52.76917	14.30778
F0137	<i>Formica subpolita</i>	ASU-SIBR2137	US	39.50444	-120.23817
F0138	<i>Formica subpolita</i>	ASU-SIBR2138	US	38.72450	-112.20717
F0139	<i>Formica xerophila</i>	ASU-SIBR2139	US	35.80350	-112.11767
F0140	<i>Formica cf xerophila</i>	ASU-SIBR2140	US	38.85876	-122.41834
F0141	<i>Formica transmontanis</i>	ASU-SIBR2141	US	39.188	-123.757
F0142	<i>Formica sp fusca grp</i>	ASU-SIBR2142	US	38.83767	-106.55933
F0143	<i>Formica sp fusca grp</i>	ASU-SIBR2143	US	39.21533	-121.04019
F0144	<i>Formica subsericea</i>	ASU-SIBR2144	US	35.695	-78.69688
F0145	<i>Formica podzolica</i>	ASU-SIBR2145	US	43.01616	-77.57632
F0146	<i>Formica subsericea</i>	ASU-SIBR2146	US	42.45103	-84.01885
F0147	<i>Formica gnavia</i>	ASU-SIBR2147	US	31.93200	-109.17783
F0149	<i>Polyergus rufescens</i>	ASU-SIBR2149	KG	42.67	77.18
F0150	<i>Proformica mongolica</i>	ASU-SIBR2150	KG	42.77	74.66
F0152	<i>Rossomyrmex minuchae</i>	ASU-SIBR2152	ES	36.89	-2.78
F0153	<i>Polyergus mexicanus</i>	ASU-SIBR2153	US	39.41591	-120.31704
F0154	<i>Polyergus mexicanus</i>	ASU-SIBR2154	US	31.90882	-109.25211
F0155	<i>Formica candida</i>	ASU-SIBR2155	KG	42.08	76.73
F0156	<i>Formica talbotae</i>	ASU-SIBR2156	US	43.38	-95.185
F0157	<i>Formica sp micro grp</i>	ASU-SIBR2157	US	42.45947	-84.02559
F0158	<i>Polyergus longicornis</i>	ASU-SIBR2158	US	30.35995	-84.41848
F0159	<i>Formica subsericea</i>	ASU-SIBR2159	US	38.9879	-77.2495
F0160	<i>Formica sp fusca grp</i>	ASU-SIBR2160	US	31.8729	-109.23504
F0161	<i>Formica sp fusca grp</i>	ASU-SIBR2161	US	32.59105	-107.97398
F0162	<i>Formica obscuripes</i>	ASU-SIBR2162	US	48.03381	-110.22798
F0163	<i>Formica aserva</i>	ASU-SIBR2163	US	46.7059	-114.53617
F0164	<i>Formica cf densiventris</i>	ASU-SIBR2164	US	38.10233	-111.33653
F0165	<i>Formica sp fusca grp</i>	ASU-SIBR2165	US	48.52302	-113.3808

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