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Editors' Choice

Phylogenomics of *Messor* harvester ants (Hymenoptera: Formicidae: Stenammini) unravels their biogeographical origin and diversification patterns

La phylogénomique des fourmis moissonneuses *Messor* (Hymenoptera: Formicidae: Stenammini) clarifie leur origine biogéographique et leurs patrons de diversification

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Abstract

As a major abiotic factor, climate change is expected to profoundly alter biological communities. On this basis, identifying how past temperature variations affected species diversification and distribution can help to predict the effects of the ongoing climate change. In this study, we focused on the harvester ant genus *Messor* Forel, which is adapted to dry environments by specializing in a granivorous diet. The phylogenomic analyses of 2524 ultraconserved-element loci obtained from 58 *Messor* species and subspecies support their monophyly. Phylogenetic relationships uncovered in this study enabled us to redefine historical taxonomic groups, providing a solid basis for future revisions that encompass the entire genus diversity. Molecular dating and biogeographical analyses indicate an emergence in the Irano-Indian area approximately 20 million years ago. Our results suggest a rapid geographical dispersal from their ancestral range towards the Western Palaearctic, reaching Northeastern Africa during the early Miocene. We found a major diversification event during the mid-Miocene climatic optimum, from which we inferred the emergence of the common ancestor of all *Messor* groups.

Their diversification appears to be affected by temperature, suggesting a higher speciation rate during warmer periods. This confirms that the ecological specialization of *Messor* makes them strongly dependent on thermal conditions. Our results highlight the importance of abiotic factors on diversification processes, especially for highly specialized species that may exhibit predictable evolutionary responses to climate changes.

KEYWORDS

global warming, historical biogeography, Palaearctic, social hybridogenesis, ultraconserved elements

INTRODUCTION

Climate change is expected to influence species distributions in complex ways by influencing both the biotic and abiotic components of ecosystems (Bellard et al., 2012). This interplay challenges our ability to predict distribution shifts or local extinctions of species (Parmesan et al., 1999; Urban et al., 2016). However, not all species are equal in this regard, and some species can be expected to respond in more predictable ways than others to climate change. This is the case for biome specialists who should be strongly impacted (Vrba, 1980, 1987), while biome generalists should be less dependent on abiotic factors (Gamboa et al., 2022).

Based on climate and vegetation zonation, biome distribution is heterogeneous at the global scale with, for instance, the Palearctic region being composed of at least six types of biome (Donoghue & Edwards, 2014; Walter, 1970). However, some of these biomes expanded or disappeared over time (Pound et al., 2012; Pound & Salzmann, 2017). During the Cenozoic, when the climate cooled down (Westerhold et al., 2020), the tropical biomes vanished or shifted southward (Pound et al., 2012; Ziegler et al., 2003). These changes impacted diversity and diversification at global (Meseguer & Condamine, 2020; Quintero et al., 2023) and regional (Graham, 2011; Jaramillo, 2023) scales. During the Neogene, and more specifically during the last 11 million years (Westerhold et al., 2020), global climate cooling induced substantial aridification over the Northern Hemisphere (Eronen et al., 2012). One of the main biome shifts over the late Cenozoic consisted of the expansion of grasslands (Edwards et al., 2010; Pound et al., 2012; Spriggs et al., 2014; Strömberg, 2011). Most grassland ecosystems are dominated by grasses (family Poaceae) that employ C_4 photosynthetic pathways, which are better adapted to dry and low CO_2 conditions (Strömberg, 2011). Although their origin remains debated, fossil and phylogenetic data indicate an early Miocene diversification and a Pliocene dominance of grasses linked with the drop in atmospheric CO_2 (Palazzesi et al., 2022).

The combination of climate and biome changes is thought to have played a key role in the evolution of species communities, notably by promoting the diversification of phytophagous lineages associated with grasslands (e.g., Kergoat et al., 2018; Liu et al., 2024). There is growing evidence that past climate change has influenced the pace at which groups of organisms diversified (Benton, 2009; Erwin, 2009). Analyses of the fossil record and/or dated phylogenies suggest that past temperature fluctuations could likely be a driver of speciation rates in many clades (e.g., Condamine, Romieu, & Guinot, 2019). For tetrapods, a meta-analysis on families has proposed that speciation rates were generally higher when the climate was warmer, which led to a slowdown of speciation towards the present (Condamine, Rolland, & Morlon, 2019). The support for a temperature-dependent speciation pattern across tetrapods has been recovered with a different machine-learning approach (Skeels et al., 2023). However, there is no such a consensus on invertebrates and insects because we still lack macroevolutionary studies performed at the species-level phylogenetic scale (but see Baird et al., 2021; Condamine et al., 2015, 2018; Kergoat et al., 2018). Indeed, some clades show a positive dependence on temperature like stemborer moths (Kergoat et al., 2018), but others like Ectemnorhinini weevils (Baird et al., 2021) show a negative correlation with temperatures. To our knowledge, there is no study linking ant speciation to past climate changes, but a fossil-based study of ant diversification did not recover any significant association between speciation and temperature variations at the family scale (Jouault et al., 2024).

Within insects, the harvester ant genus *Messor* Forel, could represent a relevant biological model to better assess the role of climate change in insect diversification. These ants are highly specialized for granivory (Hahn & Maschwitz, 1985; Lévieux & Diomande, 1978) and mainly found in open areas of semi-arid to arid habitats of the Old World. Their interaction with seed-producing plants makes them abundant around the Mediterranean basin, but also extending to South Africa and Japan (Plowes et al., 2013). Within the subfamily Myrmicinae, *Messor* currently includes 134 species (based on the AntCat database, antcat.org, accessed on 13 November 2024), making it the second most diverse genus in the tribe Stenammini (7 genera, 458 species). Based on our current knowledge, two distant regions (Western North Africa and Middle-East) comprise a large part of the species diversity. According to AntMaps (Guenard et al., 2017), 30 species of *Messor* are recorded from Morocco to Iran. The emergence of the genus has been previously inferred to be within the Palearctic, between 12.6 and 16.13 Mya (Branstetter et al., 2022), however with only a few species included in the analysis. Whether the biogeographic origin of *Messor* corresponds to one of the current diversity hotspots, or whether this diversity pattern is not representative of their ancestral area, remains an open question. It is also unknown if the highly specialized ecology of harvester ants conditioned their diversification. Because aridification and habitat opening promoted the dominance of easily harvestable plants such as grasses and forbs (Hui et al., 2021), one can wonder whether such environmental transitions also shaped the diversification of the granivorous *Messor* ants. As a genus mostly associated with warm and arid biomes, we hypothesize that it experienced higher diversification rates during warmer periods of past climatic changes.

Several challenges may hamper our ability to unravel the origin and diversification of the genus *Messor*. Because of the continuous worker polymorphism (Schlick-Steiner et al., 2006), identification depends on specimen size (Salata & Borowiec, 2019). Large individuals with large heads (majors) are preferred as they present more marked morphological features (Bernard, 1981), but without proper sampling or colony maturity, species identification can be difficult. Examination of sexual castes may help to better distinguish species, but as in most ant genera, their observation is mostly seasonal dependent (Bernard, 1953) and many of them are still unknown or undescribed (following the AntCat database). Species of *Messor* can also exhibit strong variations between populations of what is presumably the same species (Bernard, 1955; Salata, Lapeva-Gjonova, et al., 2023). On the contrary, entities that are supposed to represent distinct species are morphologically extremely close, not to say cryptic (Bernard, 1985; Steiner et al., 2018). Some studies have been conducted on broad geographic scales and taxonomic ranges, such as in the Maghreb (Barech et al., 2020) or the Afrotropics (Bolton, 1982), but none has yet tried to investigate and link most of the known diversity in a global taxonomic assessment. Finally, some species appear to hybridize (*Messor minor* × *Messor wasmanni*, Steiner et al., 2011; *Messor mcarthuri* × *Messor hellenius*, Lapeva-Gjonova & Borowiec, 2022) and others are known to possess complex reproductive systems called social hybridogenesis (in *Messor barbarus*, *Messor ebeninus* and *Messor ibericus*; Romiguier et al., 2017; Steiner et al., 2018). If these phenomena frequently occur across the genus, they could be one of the main factors explaining morphological variability and thus the difficulty in identifying consistent species boundaries.

Facing these difficulties, it appears important to complement morphology-based systematics with genomics to clarify the higher-level systematics and species-group taxonomy of the genus *Messor*. Here, we aimed to infer a dated species-level phylogeny of *Messor* using genomic data to lay the systematic foundation of the genus and study their evolutionary history by estimating their most likely ancestral origin and dispersal routes to explain the current diversity pattern. We also explored the deep-time environmental factors tied to their diversification. A first attempt has been conducted in a phylogenetic study of the tribe Stenammini, but this study contained only six sequenced *Messor* species with limited geographic representation (Branstetter et al., 2022). To fill this gap, we sequenced 58 *Messor* species/subspecies from 23 countries across the genus geographic range and reconstructed their evolutionary history through a phylogenomic approach including molecular dating and macroevolutionary analyses. We paid attention to identify potential cases of hybridization, which could create discordant gene topologies and low branch supports.

MATERIALS AND METHODS

Taxon sampling and sequencing

Among the investigated specimens, 62 samples were obtained from the ant collection hosted at the London British Museum of Natural History (BMNH) in 2019, and 31 were sampled between 2005 and 2023 from alcohol then stored in the University of Montpellier freezers (Table S1). Non-destructive extraction was achieved using the Qiagen (Valencia, CA) DNeasy Blood and Tissue kit, following the manufacturer's protocol with a few modifications, as detailed in (Cruaud et al., 2019) and summarized hereafter. To maximize the recovery of DNA and preserve the integrity of the specimens, small holes have been punctured with minutens in the three tagmata, mostly through integument membranes. Specimens were incubated overnight in an Eppendorf thermomixer (temperature = 56°C, mixing frequency = 300 rpm). Following incubation, specimens were removed, dried and re-mounted using standard water-soluble and non-toxic glue. The remaining buffer was treated with ethanol to precipitate DNA and filtered using binding columns. To increase DNA yield, two successive elutions (50 μL each) were performed with a heated AE buffer (56°C) and an incubation step of 15 min followed by centrifugation (8000 rpm for 1 min at room temperature). Eppendorf microtubes LoBind 1.5 mL were used for elution and stored at −20°C until library preparation. DNA was quantified with a Qubit 2.0 Fluorometer (Invitrogen).

To avoid sequencing contaminants inherent to conservation issues on dry specimens (mould/environmental DNA), we sequenced museum specimens after hybrid capture of 2524 ant-specific ultra-conserved elements (UCE probes designed by Branstetter et al., 2017). UCE sequences are indeed adequate for fine taxonomical studies at species level, and still effective with low-quality samples from museums (Blaimer et al., 2016; Harvey et al., 2016) while being good markers for generating phylogenies, investigating complex species boundaries and estimating divergence times (Blair et al., 2019). Sequencing libraries were prepared according to the protocol outlined by Faircloth et al. (2012, 2015) for UCE capture, with modifications detailed in Cruaud et al. (2019). DNA was fragmented to a size of 400 bp using the Bioruptor Pico (Diagenode). Subsequent steps included end repair, 3′-end adenylation, adapter ligation and polymerase chain reaction (PCR) enrichment, all performed using the NEBNext Ultra II DNA Library prep kit for Illumina (NEB). Barcoded adapter pairs were utilized, containing amplification and Illumina sequencing primer sites, along with a 5 or 6 bp nucleotide barcode for sample identification. Pools of 16 samples were created at an equimolar ratio. UCE capture was accomplished by enriching each pool using the 'Insect Hymenoptera 2.5K version 2, Ant-Specific' probe set (Branstetter et al., 2017) and MYbaits kits (MYcroarray, Inc.). The manufacturer's protocol (MYbaits, user manual version 3) was followed. The hybridization reaction was conducted for 24 h at 65°C. Post-enrichment amplification was performed on beads using the KAPA Hifi HotStart ReadyMix. Enriched libraries were quantified with Qubit, an Agilent Bioanalyzer and qPCR using the Library Quantification Kit Illumina/Universal from KAPA (KK4824). The libraries were then pooled at an equimolar ratio. Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq platform at UMR AGAP (Montpellier, France).

For well-conserved specimens stored in alcohol, we sequenced whole genomes (WGS) as it allows more complete genomic analyses. DNA extractions have been done on the whole body of each sample using the Macherey-Nagel NucleoMag Tissue kit. Individuals of the same nest series have been preserved in alcohol, with corresponding voucher IDs listed in Table S1. Library preparation for WGS has been performed using a custom Illumina protocol modified from Meyer and Kircher (2010) and detailed in Tilak et al. (2015).

To complement the taxonomic range of the sequenced specimens, especially for other Stenammini genera, UCE capture data from Branstetter et al. (2022) has been added (see Table S1 for details). In total, the dataset of this study contains sequences of 192 specimens from 143 taxa investigated: 51 sp. and 7 ssp. of *Messor*; 29 sp. of *Aphaenogaster* Mayr; 37 sp. of *Stenamma* Westwood; 5 sp. of *Goniomma* Emery; 2 sp. of *Oxyopomyrmex* André; 9 sp. of *Veromessor* Forel; 3 sp. of *Novomessor* Emery.

Phylogenomic inference

We used phyluce v.1.7.1 (Faircloth, 2016) to identify UCE loci among 2524 Formicidae UCEs references (probes designed by Branstetter et al., 2017). Each UCE locus was aligned using MAFFT v.7.490 (Katoh et al., 2019). We used trimaAl v1.2 (Capella-Gutiérrez et al., 2009) to only keep sites present in at least 50% of the sequences analysed, then concatenated in a single alignment the trimmed sequences using catsequences v.1.5 (Creevey et al., 2023). Phylogenetic analyses on the concatenated alignment obtained have been performed using IQ-TREE v.2.0.7 (Minh et al., 2020), setting up the substitution model GTR + F + I + G4 and 1000 ultrafast bootstrap replicates (Hoang et al., 2018).

To produce gene trees, individual alignments for each locus have been trimmed with the heuristic method '-automated1' of trimAl v.1.2, and gene trees inferred with IQ-TREE (following the same options as above). These gene trees have been used to assess the gene (gCF) and site concordance factors (sCF) of the concatenated phylogeny (Minh et al., 2020). We then compared this concatenated phylogeny with a coalescent tree-reconciliation approach using wASTRAL-hybrid (Zhang & Mirarab, 2022) to weigh the inference depending on the branch lengths and branch supports of every gene tree.

Detecting hybrids

Before investigating phylogenetics, genome heterozygosity has been estimated using a pipeline designed to detect first-generation hybrids (F1) in single individuals (Weyna et al., 2022). For this, the approach estimates the parameters of a coalescent-based model of divergence which partitions observed individual heterozygosity into two components: the component γ due to putative divergence between the two parental lineages, and the component θ due to polymorphism in the ancestral population of these parental lineages. The signal captured when estimating these parameters is primarily based on the mean and variance of heterozygosity across loci, as the divergence across two parental lineages results in an increase of polymorphism which is uniformly distributed along the genome. Because both parameters depend on observed heterozygosity, it is expected that γ and θ are both positively correlated. To ensure independence from sample heterozygosity, we focused on the γ/θ ratio, which is expected to be close to zero in nonhybrids. Following the strictest threshold suggested by Weyna et al. (2022), we only considered ratios above 1.0 as F1 hybrids. Outliers of the distribution of γ/θ ratios (points beyond 1.5 times the interquartile range) were mentioned as potential hybrid candidates that might explain unexpected positioning in the tree. Values of θ and $\gamma < 0.0005$ were considered as too low to be considered, regardless of the corresponding ratio value.

Both UCEs and Benchmarking Universal Single-Copy Orthologs (BUSCOs) can be used in estimating the heterozygosity, but only WGS specimens could be tested with the two genetic markers. Contigs of interest have been detected using phyluce v.1.7.1 with a 2524 Formicidae UCE loci probe set (same as for phylogeny); and BUSCO v.5.3.1 with a 5991 BUSCO dataset (Manni et al., 2021, dataset hymenoptera_odb10 from orthodb.org) and with *Camponotus floridanus* as the reference species for the integrated gene finder tool Augustus (Stanke et al., 2008). Per-contig counts of heterozygous sites have been performed with the snp-calling tool freebayes v.1.3.2 (Garrison & Marth, 2012), with an additional contamination filter. This process uses REF (allele of reference) and ALT (any other allele at REF locus) allelic depth to compute and compare genotype probabilities for each site. If the likelihood that the site is truly heterozygous is lower than the probability that the site is truly homozygous, then the SNP is rejected (see https://github.com/arthurWeyna/hybrid_scan for details). Because museomics are highly prone to contamination, we used a stringent value of 0.2 for the parameter *e*, which defines the expected frequency of ALT at true homozygous sites.

Estimating divergence times

To infer the divergence times of *Messor*, we relied on Bayesian relaxed clock inferences as implemented in MCMCtree v.4.10.7 (Yang, 2007) using the species tree obtained from the concatenated analysis of the Stenammini. All *Messor* individuals have been included, but only one representative species for the other genera was retained (i.e., the one with the highest number of UCE loci). We did not use fossil calibration for divergence time estimation; rather, we used secondary calibrations, with 95% height posterior densities (HPD) inferred by Branstetter et al. (2022). We retrieved the minimum and maximum ages of the 95% HPD for setting up a uniform prior to calibrate five nodes (see Table S4) on a pruned Stenammini phylogeny, which, besides *Messor*, was represented by the best-covered specimen for other genera. To date the Stenammini, Branstetter et al. (2022) used the oldest known Stenammini fossil, †*Aphaenogaster dlusskyana* Radchenko and Perkovsky, and a secondary calibration for the crown of Myrmicinae based on the phylogenetic study of Ward et al. (2015). We did not take into account the only known *Messor* species fossil, †*Messor sculpturatus* Carpenter, which has been attributed to the genus based on the presence of two closed cubital cells on the forewing—a feature that has since been identified as unreliable (Bolton, 1982). Furthermore, the fossil †*M. sculpturatus* was described from the Nearctic region (Florissant shales), further reducing the likelihood of it being a true *Messor*. The molecular dataset was generated with the SortaDate package (Smith et al., 2018) to extract the 10 most clock-like UCEs. We set up the HKY substitution model for the dataset. We ran two Markov Chain Monte-Carlo (MCMC) with correlated clock rates (Thorne & Kishino, 2002) and 2000 (burn-in) + 20 (sample frequency) × 20,000 (number of samples) for a total of 402,000 iterations. The convergence of the MCMC has been verified with Tracer v1.7.1 (Rambaut et al., 2018) checking for the values of effective sample sizes (ESS), considering ESS > 200 as good convergence for a given parameter. The dated tree has then been used for *Messor* biogeographic inference, but only one specimen of each taxon was kept.

Inferring ancestral geographic ranges

To estimate the biogeographic origin and diversification of *Messor*, we divided the Palearctic and Afrotropics into 14 areas as follows: 1—Southern Africa; 2—Sahelian and Eastern Africa; 3—Northeast Africa; 4—Northwest Africa; 5—Southwest Europe; 6—Northwest Europe; 7—Italian Peninsula; 8—Northeast Europe; 9—Balkans; 10—Türkiye and Caucasus; 11—Arabian Peninsula; 12—Irano-Indian Asia; 13—Central Asia; and 14—Eastern Asia (Figure S2).

For the inference, we used DECX (Beeravolu & Condamine, 2016), an extension of the Dispersal-Extinction-Cladogenesis model that can easily handle computational constraints with such many defined areas. The distribution matrix of the investigated *Messor* species (Table S2) has been mainly constructed from the AntMaps distribution data (Guenard et al., 2017). We coded the presence and absence of the studied species for each region by considering native species in a country but discarding introduced species. The following minor modifications have been made to better suit current knowledge. The distribution of *Messor asmaae* (Sharaf), which is still regarded as an *Aphaenogaster* on AntMaps despite its recent new combination (Schifani et al., 2022), has been completed with the origin of our sample, making it the first mention for Pakistan and the first mention outside the Arabian Peninsula (NHMUK010363418, BMNH). *Messor meridionalis* (André) was not considered to occur in the Italian peninsula and Southwest Europe because there is a lack of evidence for the presence of this dubious species in these well-studied areas. Despite *Messor bouvieri* Bondroit being present in France, its distribution is restricted to areas near the Mediterranean coasts (Lebas et al., 2016), which is why we assigned it to Southwest Europe only in addition to the Italian Peninsula. We discarded mention of *Messor sanctus* Emery from Palestine and Syria (Bernard, 1967), as the species has not been mentioned in recent checklists (Vonshak & Ionescu-Hirsch, 2010 for Israel and Tohmé & Tohmé, 1981 for Syria). Despite presence on the two circum-Sicilian islands Pantelleria and Linosa (Mei, 1995), *M. sanctus* was not included as part of the Italian peninsula, as these two islands are geographically closer to Africa than to mainland Europe. We discarded *Messor aegyptiacus* (Emery) from the Sahelian and Eastern Africa areas as there is only one mention of it in Mauritania (Cagniant & Espadaler, 1998; found in Zouérat, Cagniant personal communication). We assigned the repartition of *M. minor maurus* Barquín according to Santschi (1927b), as this subspecies is absent from AntMaps.

A time-stratified geographic model was constructed using connectivity matrices that account for palaeogeographical changes through time, with time slices indicating whether or not a species was able to colonise a new area. Based on paleogeographic reconstructions (e.g., Kocsis & Scotese, 2021), we defined three time periods of 10 million years between the present and 30 Mya, which correspond to the cooling phase since the late Miocene (0–10 Mya), the late to early Miocene (10–20 Mya) and the early Miocene to mid-Oligocene (20–30 Mya). The adjacency matrix of the areas has been binarily coded, with 0 whenever two areas are not directly connected at the time considered, and 1 when areas are geographically linked (Table S3). We created one connectivity matrix per period to represent major changes in tectonic conditions that may have affected species distributions. We imposed constraints on patch connectivity by coding 0 if any two areas were not connected, or 1 if they were connected at a given time. Biogeographic ranges larger than four areas were not allowed as valid biogeographic states if they were not subsets of the terminal species' ranges so that extensive ranges that included areas that were never geographically connected were also removed. We estimated the most likely ancestral states at each node, using both the species distribution matrix and the connectivity matrices. For comparison, we also ran a null DECX model without any connectivity matrices, allowing for any area composed of four areas, even if they are not geographically adjacent.

Estimating diversification rates

We investigated the diversification rates of *Messor* (58 taxa considered). We first tested whether rates of diversification (speciation minus extinction) remained constant or varied through time, and then tested the impact of palaeoenvironmental variables on diversification rates using a birth-death likelihood method in which rates may change continuously with an environmental variable, itself varying through time (Condamine et al., 2013; Condamine, Rolland, & Morlon, 2019). As implemented in RPANDA 2.1 (Morlon et al., 2016), we tested four models: (1) a constant-rate birth-death model in which diversification is not associated with the environmental variable; (2) speciation rate varies according to the environment and extinction rate does not vary; (3) speciation rate does not vary and extinction rate varies according to the environment; and (4) both speciation and extinction rates vary according to the environment. We also tested the corresponding models in which speciation and/or extinction are allowed to vary with time, but independently from the environmental variable (time-dependent birth-death models, Morlon et al., 2011). When rates varied with the environment (E), we assumed exponential variation, such that $\lambda(E) = \lambda_0 \times e^{\alpha E}$ and $\mu(E) = \mu_0 \times e^{\beta E}$, in which λ_0 and μ_0 are the speciation and extinction rates for a given environmental variable for

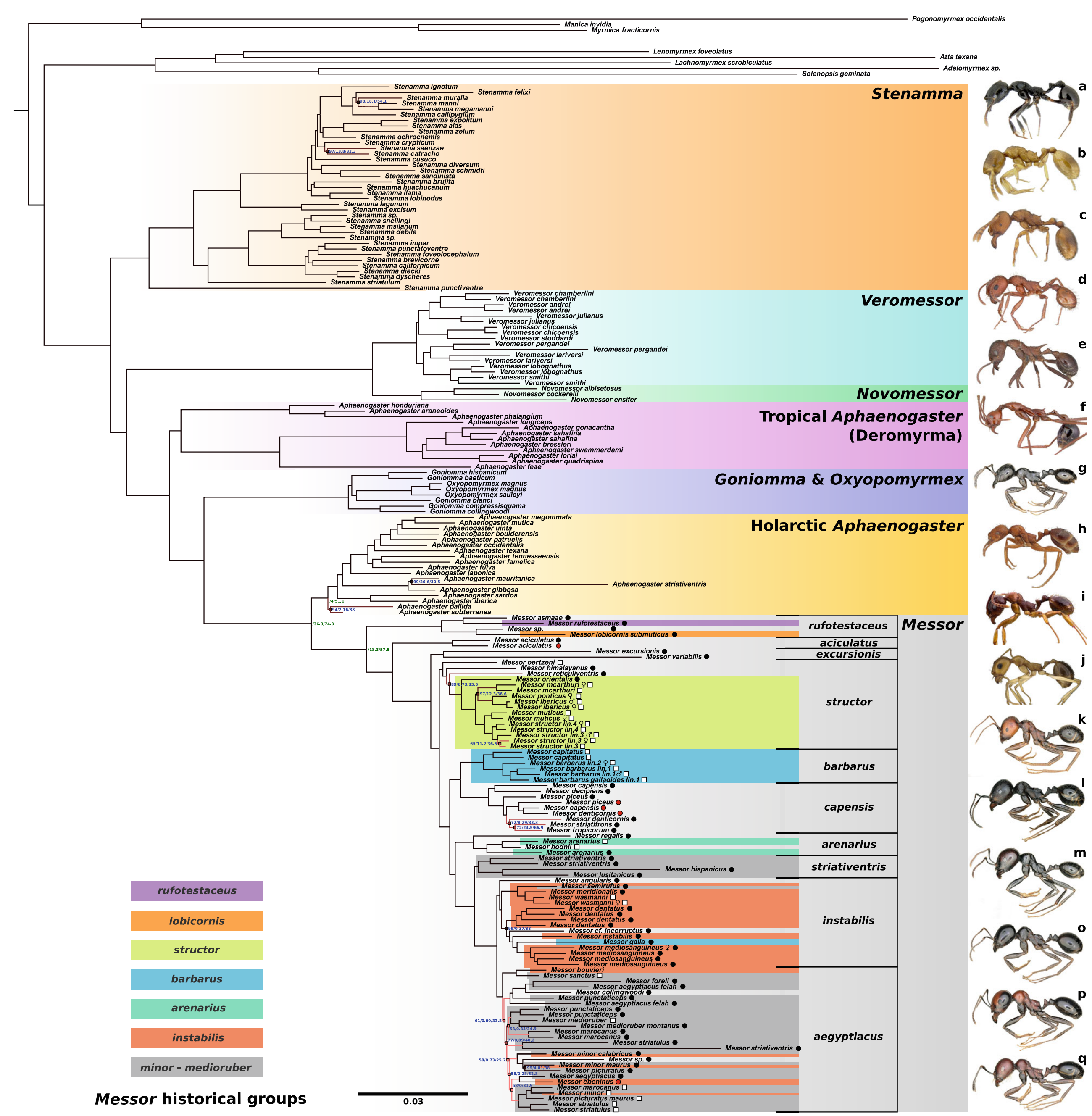

FIGURE 1 Maximum-likelihood phylogeny of the Stenammini tribe. Topology obtained with IQ-TREE on a concatenated alignment of 2524 ultraconserved elements (UCEs). Only ultrafast bootstrap values inferior to 100 are written in blue at nodes, followed by gene (gCF) and site concordance factors (sCF). Concordance factors of important nodes between Holarctic *Aphaenogaster* and *Messor* are written in green. For the *Messor* genus, circles mean that the sample has been sequenced after capture of UCEs (black circle for new data, red circle for Branstetter et al., 2022 data), and white squares mean that the whole genome of the sample has been sequenced. Images of worker specimens (with a code if from antweb.org): (a) *Stenamma callipygium* (CASENT0606207, credit to M. Branstetter); (b) *Stenamma lagunum* (CASENT0622371, holotype, credit to M. Branstetter); (c) *Stenamma californicum* (CASENT0221920, credit to X. Yang); (d) *Veromessor lobognathus* (CASENT0104781, credit to A. Nobile); (e) *Novomessor albisetosus* (CASENT0102824, credit to A. Nobile); (f) *Aphaenogaster swammerdami* (CASENT0421554, credit to A. Nobile); (g) *Goniomma blanci* (personal coll., credit to L. Soldati—CBGP); (h) *Aphaenogaster uinta* (FMNHINS0000062702, paratype, credit to G. Brilmyer); (i) *Aphaenogaster subterranea* (ANTWEB1041832, credit to E. Collins-Sussman); (j) *Messor asmaae* (CASENT0922290, credit to M. Esposito); (k) *Messor oertzeni* (personal coll., credit to L. Soldati—CBGP); (l) *Messor ibericus* (personal coll., credit to L. Soldati—CBGP); (m) *Messor barbarus* (personal coll., credit to L. Soldati—CBGP); (o) *Messor wasmanni* (personal coll., credit to L. Soldati—CBGP); (p) *Messor picturatus* (personal coll., credit to L. Soldati—CBGP); (q) *Messor aegyptiacus* (personal coll., credit to L. Soldati—CBGP).

which the value is 0, and α and β are the rates of change according to the environment. Positive values for α or β mean a positive effect of the environment on speciation or extinction (and conversely). As environmental variables, we used paleotemperature (data retrieved from Westerhold et al., 2020, converted to sea-surface temperature by Boschman & Condamine, 2022) and proportion of C_4 grasses, a proxy for grassland expansion (data retrieved from Kergoat et al., 2018). The R package pspline was used to build environmental vectors from the data as input for the birth-death models. In total, we fitted 14 different models to the dated phylogeny of *Messor*. For the list of the tested models and their results, see Table S5. The best-fitting model was selected based on corrected Akaike information criterion (AIC_c) scores and Akaike weight (AIC_ω).

RESULTS

Phylogeny of the genus *Messor* within the tribe Stenammini

In general, ultrafast bootstrap values of the concatenated (4,255,038 bp) inferred phylogeny suggest strong support for the main subdivisions of Stenammini. Most genera are monophyletic with maximal bootstrap support (Figure 1). As already inferred in previous work (Branstetter et al., 2022), *Oxyopomyrmex* is phylogenetically embedded within *Goniomma*, while *Aphaenogaster* species are divided into a tropical clade (named *Deromyrma*, see Branstetter et al., 2022) and a Holarctic one (simply named *Aphaenogaster*). In our analysis, *Aphaenogaster* is recovered monophyletic and is the closest relative of *Messor*, a result that contrasts with Branstetter et al. (2022) in which *Messor* was nested within *Aphaenogaster*. The monophyly of both genera is supported by maximal bootstrap values. However, the coalescent analysis retrieves *Aphaenogaster* as paraphyletic, with *Aphaenogaster subterranea* (Latreille) being the closest relative of *Messor*. gCF confirms phylogenetic conflict among genes, with only 4% of UCEs supporting *Aphaenogaster* as monophyletic. Nevertheless, those 4% of loci appear to carry most of the phylogenetic signal of the dataset, as more than half of the global phylogenetic signal supports *Aphaenogaster* monophyly instead of alternative topologies (sCF of 51.1%).

Within *Messor*, the historical *structor* species group (hereafter group) appears monophyletic and is supported on both concatenated and coalescent phylogenies (Figure 1, Figure S1). However, we included three species, *Messor oertzeni* Forel, *Messor himalayanus* (Forel) and *Messor reticuliventris* Karavaiev, which to our knowledge were never mentioned as belonging to this group in recent work (Barech et al., 2020; Salata, Demetriou, et al., 2023; Steiner et al., 2018). In addition to their phylogenetic placement, these three species share the general morphology of the *structor* group (dense pilosity, marked sculpture on head and mesosoma, lack of psammophore, see Bernard, 1955). Other congruently supported clades are observed, but without having been specifically defined until now. Based on phylogenetic evidence, we propose to name the *capensis* group for the Southern Afrotropical *Messor* clade, and the *excursionis* group for *Messor excursionis* (Ruzsky) and *Messor variabilis* Kuznetsov-Ugamsky clade. The *aciculatus* group is only composed of one species considering our sampling, as its phylogenetic position is clearly apart from the rest of the tree. We extend the *rufotestaceus* group with *M. asmaae*. This result confirms the recent morphological revision and transfer of *M. asmaae* (Sharaf) from *Aphaenogaster* to *Messor* (Schifani et al., 2022). *Messor lobicornis submuticus* also appears in our extended *rufotestaceus* group despite being morphologically divergent. The same occurs in the *arenarius* group with *Messor hodnii* Barech et al., which is surprisingly nested between two *Messor arenarius* (Fabricius) specimens. We also included *Messor regalis* (Emery) in the group to form a slightly anterior clade. In our sampling, the historical *barbarus* group was only represented by three species, but *Messor galla* (Mayr) does not group with the two others (*M. barbarus* and *Messor capitatus*) and is thus excluded from the *barbarus* group.

Groups *minor-medioruber* and *instabilis*, used in Barech et al. (2020) and Salata, Lapeva-Gjonova, et al. (2023) respectively, do not constitute clades. This result was expected as these two groups have overlapping species composition in the aforementioned references. The *minor-medioruber* group is divided into a clade containing species from the Iberian peninsula and Northwest Africa (*Messor hispanicus* Santschi, *Messor lusitanicus* Tinaut and *Messor striativentris* Emery); and a large clade of mostly Northwest African species. We renamed these two clades the *striativentris* and *aegyptiacus* group, respectively. More particularly, the *aegyptiacus* group is a large clade of closely related species/subspecies with diverse morphologies and poorly supported relationships. Further work might help to subdivide it into several species complexes. The historical *instabilis* group is one of the most anciently used and species-rich groups if considered to its greater extent (Santschi, 1927b). It appears that the group supported by our phylogeny contains almost all the species considered by Salata, Lapeva-Gjonova, et al. (2023) for which we had material, with the exception of *M. bouvieri*, *M. ebeninus* Santschi and *M. minor* (André) + ssp. that instead belong to the *aegyptiacus* group.

In our phylogeny, the following species appear to be non-monophyletic: *Messor capensis* (Mayr), *Messor piceus* Stitz, *Messor denticornis* Forel, *Messor striatulus* (Dalla Torre), *Messor strativentris* Emery, *Messor punctaticeps* Barech et al., *Messor arenarius*, *Messor aegyptiacus felah* Santschi, *Messor marocanus* Santschi, *Messor minor* + ssp. and *Messor picturatus* Santschi + ssp. While this result might be due to technical artefacts or identification inconsistencies between taxonomists, we tested whether it might be due to the presence of hybrid individuals in our dataset.

F1 hybrid detection within the tribe Stenammini

To check whether some of the sequenced individuals are hybrids, we computed the γ/θ ratio of each individual following an approach designed to detect F1 hybrids from single individual genetic data (Weyna et al., 2021). As suggested in Weyna et al.

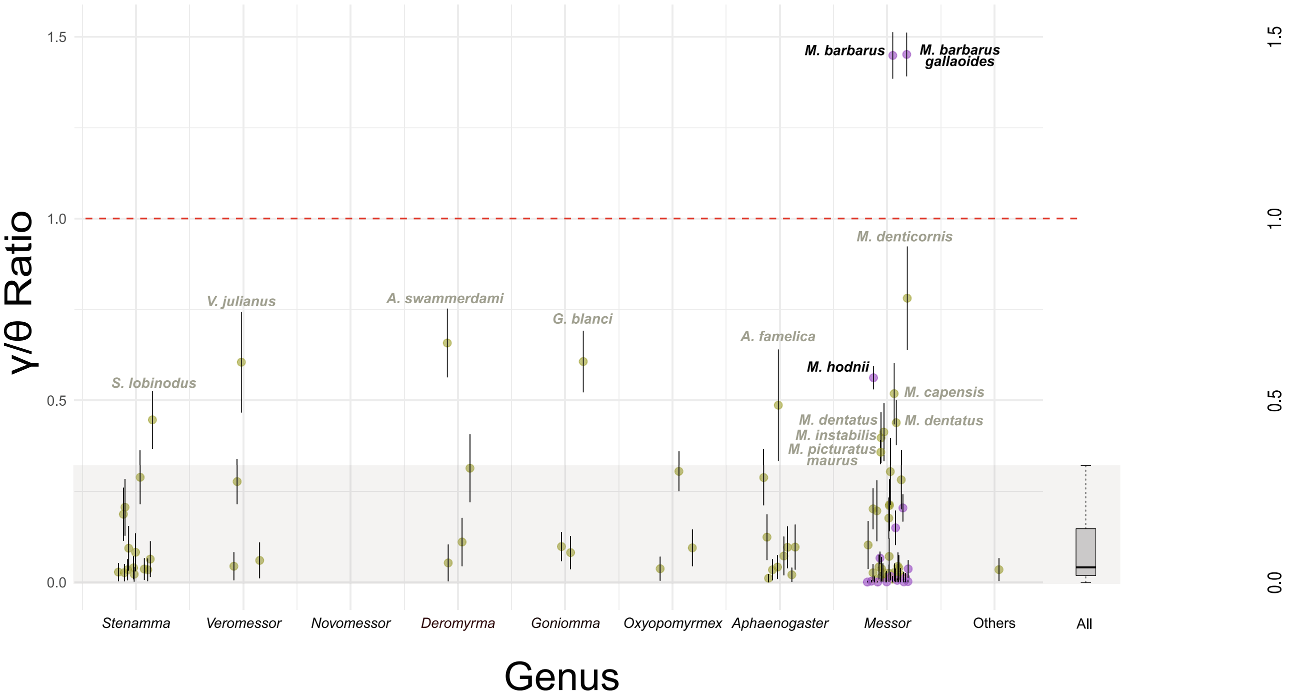

FIGURE 2 Hybrid scan of investigated specimens with a contamination filter. Hybrid status is estimated by the ratio between the divergence parameter γ and the ancestral population mutation rate θ. Purple points are ratios estimated with BUSCO genes on whole genome sequencing (WGS) specimens, whereas yellow points come from ultraconserved elements (UCE) capture. Points above 1.0 are considered as hybrids. A boxplot displays the distribution of all data samples, with the box representing interquartile range and whiskers extending to 1.5 times the interquartile range. Points beyond the whiskers are outliers that may indicate potential hybrid candidates. Points with mean theta values inferior to 0.0005 have been discarded from the plot.

(2022), individuals with γ/θ ratio superior to 1.0 were considered hybrids. Results on our dataset retrieved 2 samples as hybrids (see Figure 2): *M. barbarus* (Linnaeus), which is known for practising social hybridogenesis, a reproductive system involving workers that are exclusively hybrids (Romiguier et al., 2017). Interestingly, this also includes a worker from the subspecies *Messor barbarus gallaoides* Santschi, a morphological variant from North Africa that also appears unambiguously hybrid. Besides these two clear hybrids, we also identified 12 outliers of the γ/θ ratio distribution (Figure 2), which may be indicative of past or ongoing hybridization that may bias phylogenetic inferences. Notably, this includes *M. denticornis* and *M. capensis*, which may explain some inconsistencies observed in the phylogeny, where two putative hybrid individuals do not group with their respective non-hybrid conspecifics. As their potential hybridism may be responsible for this incongruent clustering, we decided to remove these individuals from the dating and diversification analysis. Another potential hybrid retrieved within the genus is *M. hodnii*, which has been inferred from WGS data. Again, this could explain why our sequenced specimen appears misplaced, nested within *M. arenarius* individuals. Outside of *Messor* candidates, the other hybrids indicated are in the genera *Veromessor*, *Goniomma*, *Aphaenogaster* and the clade *Deromyrma*. *Veromessor julianus* (Pergande) is the only one of these represented by more than one specimen; however, these do cluster together in the phylogeny.

Age and historical biogeography of *Messor*

The Bayesian inferences of divergence times with MCMCtree converged well, with most parameters having ESS values above 200. The divergence time between the genus *Aphaenogaster* and the genus *Messor* was estimated at 21.68 Mya (95% HPD: 22.97–20.39 Mya). The initial diversification of crown *Messor* is inferred at 19.67 Mya (95% HPD: 21.13–18.22 Mya; Figure 3).

The biogeographic analyses estimated an Irano-Indian range as the ancestral area for *Messor* (Figure 3). Our results suggested a rapid geographic dispersion from their origin area towards the Western Palearctic, reaching Northeastern Africa during the early Miocene. We inferred a major diversification event during the middle Miocene climatic optimum (17–14 Mya; Steinthorsdottir et al., 2021). During this climatic event, we inferred the emergence of the common ancestors of all *Messor* groups except for the *rufotestaceus* and *aciculatus* groups (which diverged earlier), around the latitudinal axis from Northwest Africa to the Irano-Indian region. After the middle Miocene climatic transition (14 Mya), when temperatures started to decrease, the *arenarius* group emerged from Northwest Africa. The end of the middle Miocene saw then the emergence of *capensis* and *barbarus* groups from the same ancestral clade, as well as the *striativentris* group, in the northern half of Africa. Finally, the two last groups, *instabilis* and *aegyptiacus*, differentiated during the middle Miocene from a common ancestor in Northwest Africa,

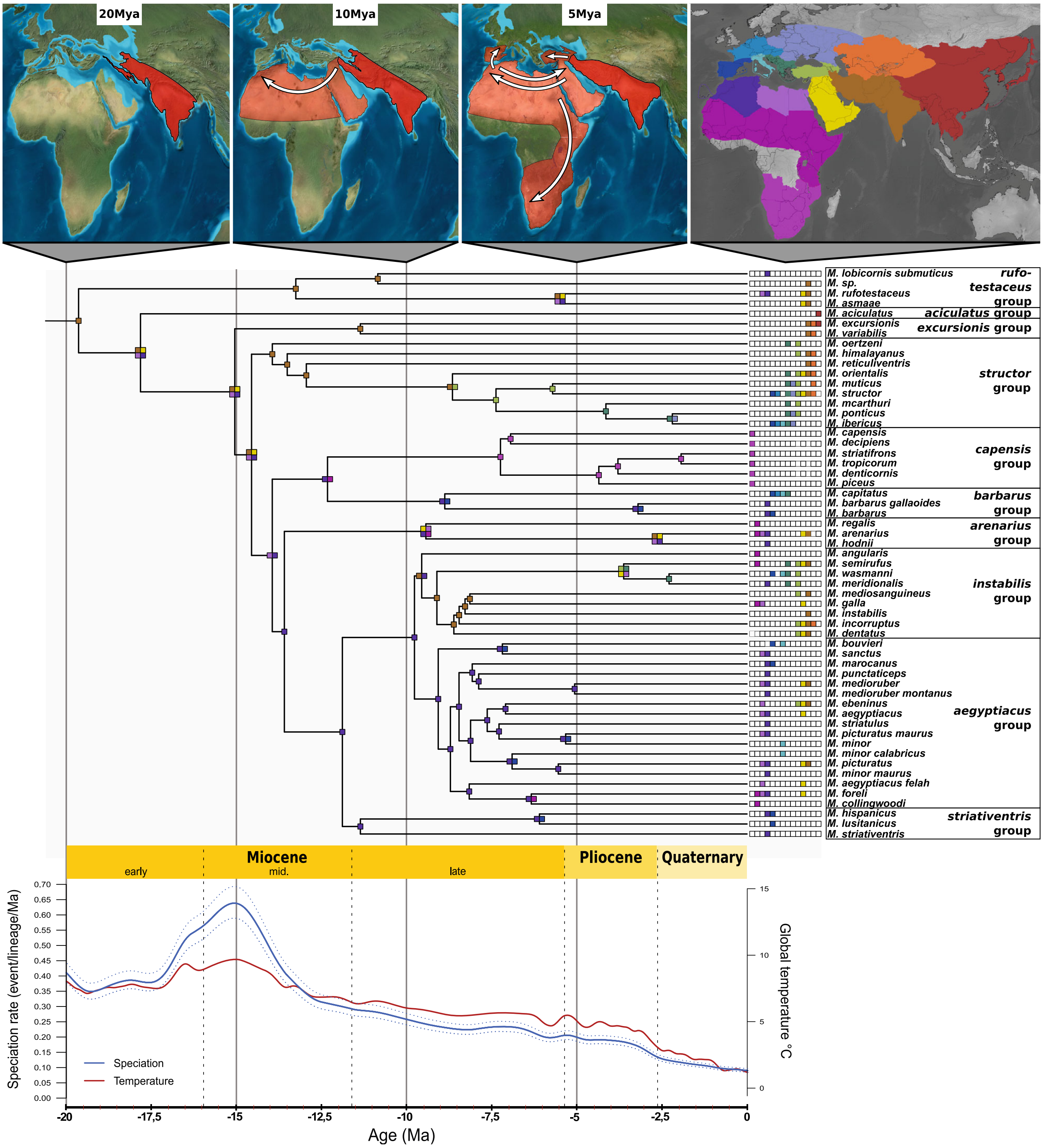

FIGURE 3 Time-calibrated phylogeny, historical biogeography and speciation rates of the ant genus *Messor*. The taxonomic groups are shown on the right side of the phylogeny along with the current distribution range of *Messor* species. The distribution range of the unidentified specimen corresponds to the locality of its sampling. Selection of only one representative for each taxon has been made depending on the best loci-covered individual. The dated tree shows median ages as estimated with MCMCtree and at each node the most likely ancestral range as inferred with DECX is shown (colours follow the top right map). At the bottom, the plot shows the best-fit diversification model with a temperature-correlated speciation rate, suggesting that speciation was higher during periods of global warming and thus decreased towards the present.

approximately 12 Mya (Figure 3). We found that Europe was reached a few million years later in the late Miocene, from Northeast Africa to Southwest Europe with the origin of the *barbarus*, *aegyptiacus* and *striativentris* groups; and later during the Pliocene, from Türkiye to the Balkans with the diversification of *structor* and *instabilis* groups.

Compared to the time-stratified DECX model, the unconstrained DECX model estimated a broader ancestral range composed of Irano-Indian Asia, Eastern Asia, Central Asia and Northwest Africa (see Figure S3). Although such a range is biologically unlikely because Northwest Africa has never been connected to any of the three regions, this unconstrained analysis indicates the importance of Central Asian regions for the origin of *Messor* ants. In particular, the Irano-Indian Asia region is recovered in 8 of 10 most likely ancestral ranges for the root of *Messor*. The fact that Northwest Africa is part of the most likely ancestral range can be explained by the high species richness of *Messor* in this region today and sampled in this study.

Diversification pattern of *Messor*

Fitting a series of birth-death models to the phylogeny of *Messor* indicated that the null hypothesis of constant diversification rates can be rejected, suggesting that rates have varied through time (Table S5). The rate variation was better explained by temperature fluctuations rather than time, with a model assuming temperature-dependent speciation and no extinction receiving the lowest AIC_c and highest AIC_ω ($AIC_\omega = 0.522$, $\Delta AIC_c > 2$ with the second-best model; Table S5). Maximum-likelihood parameter estimates of the best model indicated a positive correlation ($\alpha = 0.3$) with speciation rate for the clade, suggesting higher speciation rates during warm periods. This translates into faster rates of speciation during the middle Miocene, for instance, and lower speciation rates in the Pleistocene (Figure 3). Note that the second-best model was a model with temperature-dependent speciation and constant extinction estimated to be close to zero.

DISCUSSION

Phylogenomics

Previous work retrieved the *Messor* genus as potentially nested within a Holarctic *Aphaenogaster* clade (hereafter, abbreviated as *Aphaenogaster* in contrast to the *Aphaenogaster* tropical clade renamed *Deromyrma*, Branstetter et al., 2022). Here, our phylogeny separates the *Messor* and *Aphaenogaster* genera with 100% ultrafast bootstrap support. This difference likely stems from the high number of *Messor* taxa in our dataset, an important improvement compared to previous work (6 vs. 51 species here), representing approximately a third of the currently known diversity. However, a major limitation of our dataset is the low number of *Aphaenogaster* species, especially in groups originating from the Palearctic bioregion. Further studies with a higher number of *Aphaenogaster* species will be necessary to confirm that both genera are consistently separated.

Greater taxon depth and redundancy could have been useful to clarify poorly supported relationships or to better recognize potential wrong identifications causing inconsistent species clustering. Nevertheless, the main species groups are not expected to change drastically, as most historical taxonomic groups are already represented. The majority of ambiguous relationships within *Messor* stem from the *instabilis* and *aegyptiacus* groups, whose origin seems to be in Northwest Africa. This is not surprising as it is a major diversity hotspot of the genus. This species-rich region might have promoted rapid and numerous speciation events, leading to short branches difficult to resolve through phylogenetic inference. Resolving these relationships might be additionally hampered by the volume of data from a UCE dataset, as separating closely related species that diversified through rapid and successive speciation events may require a quantity of data more akin to that provided by WGS sequencing.

Contrary to expectations, some individuals thought to belong to the same species do not always cluster together. This could be due to wrong identifications, which are not uncommon for this genus, or due to few informative substitutions, as suggested by low node support values. However, some cases are interesting, like *M. hodnii*, which renders *M. arenarius* paraphyletic. Those two species cannot be morphologically confused as they are strongly unalike. Little is known about *M. hodnii*, as its discovery has been made recently (Barech et al., 2020). Another interesting case is observed with incongruent taxa clustering for Southern African *Messor* species. This specifically includes *M. capensis*, *Messor decipiens* Santschi and *M. piceus*, which do not seem to be monophyletic. Although this could be explained by wrong identifications, it was mentioned before that these extremely similar species could in fact be the same species (Bolton, 1982). If this is indeed the case, *M. piceus* and *M. decipiens* need to be synonymized with *M. capensis*. *M. denticornis* also appears polyphyletic; however, in this case, incorrect identification would be surprising, as this species should be reliably distinguishable from its relatives through eye size (Bolton, 1982). The most likely explanation for such inconsistencies is a methodological artefact due to heterogeneous data origin. Indeed, we note that *Messor* individuals with genetic data retrieved from the literature (Branstetter et al., 2022) cluster together in a separate group than individuals of the same species with genetic data produced in this study. This could be explained because the earlier study has targeted the capture of 1510 UCE loci, whereas we have targeted 2524 UCE loci, which is likely to cause a non-random distribution of missing data and artifactual clustering among closely related specimens. Incidentally, a similar artefact might affect the monophyly of Holarctic *Aphaenogaster*, as all *Aphaenogaster* in our phylogeny are from the 1510 loci dataset.

We suggest that the investigated *M. meridionalis* specimen, because of its morphological inspection, its dubious status (*incertae sedis* taxon; Borowiec, 2014; Kiran & Karaman, 2021; Salata & Borowiec, 2019; Salata, Lapeva-Gjonova, et al., 2023), its sampling origin (Yougoslavia, 1986, NHMUK010363380, BMNH) and its phylogenetic position, should correspond to *M. wasmanni* Krausse.

Messor lobicornis submuticus appears in our extended *rufotestaceus* group, but its morphological divergence and low UCE coverage (118/2524) make this result uncertain. Moreover, the existence and historical use of the morphological *lobicornis* group would need to be genetically investigated, with a better sampling to infer its systematics properly.

Regarding unidentified *Messor* species, the *M.* sp. (from Israel) clustering in the *aegyptiacus* group could not be properly identified due to the small size of the specimen. However, the one clustering in the *rufotestaceus* group, which comes from a pin series of three workers sampled from Iraq (NMUK010363417, BMNH), corresponds the most to *Messor thoracica* (Mayr, 1862), a junior synonym of *Messor rufotestaceus* (Foerster 1850). We believe this taxon should be restored under the correct spelling *Messor thoracicus* as its synonymization seems arbitrary, but further work will be needed to prove it.

Hybrid scan results confirm the social hybridogenesis of *M. barbarus* (Romiguier et al., 2017) in extra-European samples (investigated specimens come from Morocco and Algeria), as for the subspecies *M. barbarus gallaoides*, which is newly reported as hybrid. It is therefore still unclear whether *M. barbarus gallaoides* hybridizes with lineages of *M. barbarus* already investigated in Europe (Romiguier et al., 2017). Among other samples, 12 appear as outliers of the γ/θ ratio distribution, which may indicate potential hybridization and call for further studies in these species to explore a potentially unusual reproductive system such as social hybridogenesis. It is to note that the sequenced *M. ebeninus* does not appear as hybrid, as it would be expected in a worker of this species (Romiguier et al., 2017). This might be due to a misidentification, or if the specimen was an alate, as queens or males are expected to not be hybrid in case of social hybridogenesis.

Origin, biogeographic history and diversification

The inferred ancestral area of the *Messor* genus corresponds to Irano-Indian Asia in the early Miocene, approximately 20 Mya. This origin suggests that the evolution of this genus might be linked to climatic and environmental changes induced by the Himalayan orogenic process that was still ongoing at this time (Ji et al., 2020) and may have contributed to the isolation and emergence of the genus (see Tardif et al., 2023). Moreover, this major geological event is suspected to have promoted C4 grasses spread due to the progressive aridification of the region (Wu et al., 2014), which are an important source of food for *Messor* ants due to their granivorous diet.

Regarding our results, the *Messor* genus likely reached North Africa during its early history. This first and rapid dispersal route out of their ancestral area could help understand the current observed diversity hotspots. Indeed, Northwest Africa and especially the Middle East are geographic crosspoints that could have allowed repeated migrations, which likely contributed to the high diversity of these regions. Furthermore, the arid climate and mountainous topography—Atlas range in Northwest Africa and the Zagros, Alborz, Taurus and Pontic ranges in the Middle East—may have created ideal conditions for speciation and diversification of *Messor* species.

Regarding Europe, two potential reasons may explain why *Messor* species arrived in the region later than in others. The first one is the connectivity of the continent, which was not directly reachable from the time and emergence area of the *Messor* genus (see Table S2). The second one is related to its latitude with a colder climate, thus limiting the grassland dominance to Mediterranean coasts. However, two different dispersal routes can be reported towards the continent. The first to have been achieved approximately 9 Mya comes from the Gibraltar strait and involves the *barbarus* group. The other route from Türkiye towards the Balkans occurred later, nearly 4 Mya and implies most of the *structor* group. Glaciation events and the lowering of global sea levels due to ice cap formation have likely influenced European migrations. Because of the geographic bottleneck of the Gibraltar strait between Africa and Europe, and of the Bosphorus and Dardanelles straits between Asia and Europe, crossings might have been temporarily impeded or allowed depending on the sea level and land connectivity, thus contributing to successive vicariance and speciation events (Mas-Peinado et al., 2022). Examples of intermittent Gibraltar-crossing dynamics, as hypothesized for other ants (Tinaut & Ruano, 2021), could coincide with several *Messor* species pairs: (i) *M. hispanicus* Santschi/*M. lusitanicus* Tinaut, which are closely related and on both sides of Gibraltar strait (*M. lusitanicus* is considered as exotic in Morocco, Wong et al., 2023); (ii) *M. bouvieri*/*M. sanctus*, which are also sister species, but the first one is only present in Europe while the second is present in North Africa and a few nearby islands (Mei, 1995). Whereas the latter example could illustrate a typical allopatric speciation, the observed sympatry of *M. hispanicus* and *M. lusitanicus* cannot exclude other speciation types in which the Gibraltar strait may not be involved.

Other minor dispersal routes can be noticed towards Southern Africa and Central Asia. The first one is clearly depicted as it has led to the *capensis* group, probably passing through the East African 'arid corridor' during the late Miocene (Bobe, 2006). This matches with an untested hypothesis that *M. regalis* and *Messor cephalotes* (Emery 1895), two Afrotropical species, came from the Nile Valley due to their relatedness with *M. arenarius* (Santschi, 1938). The second minor route is not that obvious due to our incomplete diversity sampling for these areas but is expected to have led to most if not all of the Central and East Asian diversity, circling the Himalayan range from the West towards Northern steppes and the Gobi desert, where we suspect an undescribed diversity could still await description.

These results should nevertheless be taken with caution. A possible bias of our analysis is potential species misidentifications when selecting one representative individual per species/subspecies. This could lead to wrongly inferred areas, as well as poor taxonomic representation. Moreover, we still lack taxon depth to better define biogeographic history dynamics and their taxonomic consequences. Our sampling is biased towards the occidental diversity despite the genus originating in Asia. Also, we still lack crucial knowledge about some species delimitations and relevance, especially since hybridization can occur during obligatory or facultative interspecific interactions (Romiguier et al., 2017; Steiner et al., 2011). Another linked problem is the reliability of AntMaps (Guenard et al., 2017) data for area assignment, as in the cryptic species complex unravelled from *Messor structor* (Latreille) (Steiner et al., 2018). The latter species has many old and unrevised mentions that may often designate *Messor ponticus* Steiner et al., *Messor muticus* (Nylander), *M. mcarthuri* Steiner et al. or *M. ibericus* Santschi. However, all these issues are limited to recent species history details, but are unlikely to have affected the overall biogeographic history of the *Messor* genus.

Interestingly, this history appears consistent with a specialized ecology into arid environments, as the initial dispersal route of the genus appears to have followed progressive aridification around the Indo-Iranian region (Figure 3). This pattern is strikingly similar to what has been recently inferred for *Cataglyphis* Foerster, which is considered the other major genus of desert ants in the Palearctic. Both genera emerged approximately 20 Mya between the Middle East and South Asia and then dispersed towards Europe and Northern Africa (Lecocq De Pletincx et al., 2024). Remarkably, the parallel biogeographical histories of *Messor*, reconstructed here from molecular data, and *Cataglyphis*, were predicted over a century ago (Emery, 1912, 1920; Kugler, 1988). The similar dispersal routes of these two desert ant genera reinforce the idea that species specialized for extreme environments have evolved more stereotypically in response to past climate changes compared to generalist species. Such trends may extend to their responses to global warming, making predictions for extreme specialists more straightforward.

Besides ants, other clades with similar ecology to *Messor* have diversified in response to climate changes. This is the case of other taxa associated with grasslands such as the *Pardosa* wolf spiders, which also originated during the Miocene climatic optimum (Liu et al., 2024). In vertebrates, some taxa sharing the granivorous diet of *Messor* ants such as Heteromyidae (kangaroo rats and spiny pocket mices) or Gerbillinae have also been hypothesized to have diversified during the expansion of arid habitats that occurred in the Miocene (Cui et al., 2025; Samuels et al., 2023). This supports the view that rising temperatures may favour the diversification of taxa specialized for warm and arid environments. More generally, biome specialists appear to respond to temperature changes, as exemplified by taxa specialized for cold environments that diversified during global cooling periods rather than global warming ones (Baird et al., 2021).

While this study suggests a major impact of temperature changes on biome specialists such as *Messor* harvester ants, ants in general appear to have remained largely unaffected by past climate change (Jouault et al., 2024). Further research assessing how past and ongoing global warming differentially affects biome specialists and generalists may improve our understanding of how climate change differentially affects biodiversity.

AUTHOR CONTRIBUTIONS

Yannick Juvé: Investigation; data curation; writing—original draft. **Arthur Weyna:** Investigation; data curation; methodology; writing—review and editing. **Elodie Lauroua:** Data curation. **Sabine Nidelet:** Data curation. **Mourad Khaldi:** Data curation. **Ghania Barech:** Data curation. **Claude Lebas:** Data curation. **Jean-Yves Rasplus:** Methodology; writing—review and editing. **Astrid Cruaud:** Methodology; writing—review and editing. **Fabien L. Condamine:** Conceptualization; investigation; methodology; writing—original draft. **Jonathan Romiguier:** Conceptualization; investigation; funding acquisition; data curation; methodology; writing—original draft; project administration; supervision; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are openly available in Zenodo: https://zenodo.org/records/14768612. The raw data sequenced for this project (fastq) are deposited on NCBI (Project PRJNA1259445) with individual ID detailed in Table S1.

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SUPPORTING INFORMATION

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

Figure S1. Consensus tree of 2203 gene (UCEs) trees inferred with wASTRAL-hybrid.

Figure S2. Map of the areas defined for the historical biogeography.

Figure S3. Time-calibrated phylogeny of historical biogeography from an unconstrained model.

Table S1. Information for the taxon sampling of the study.

Table S2. Distribution matrix of *Messor* species among the defined biogeographic areas.

Table S3. Adjacency matrix on the defined areas in the last 30 Myr.

Table S4. Calibrated nodes for molecular dating inference with MCMCtree.

Table S5. Palaeoenvironment-dependent diversification for the *Messor* ants.

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