Molecular Phylogenetics and Evolution 191 (2024) 107977

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Out of the desert: Paleoclimatic changes drove the diversification of arid-adapted *Ocymyrmex* ants in southern Africa

Nokuthula Mbanyana [a,b,*], Bonnie B. Blaimer [c], Johannes J. Le Roux [d], Simon van Noort [a,e], Seán G. Brady [f], Theresa C. Wossler [b]

[a] Research and Exhibitions Department, South African Museum, Iziko Museums of South Africa, P.O. Box 61, Cape Town, 8000, South Africa
[b] Department of Botany and Zoology, Stellenbosch University, Stellenbosch, 7602, South Africa
[c] Museum für Naturkunde, Leibniz-Institute for Evolution and Biodiversity Science, Center for Integrative Biodiversity Discovery, Invalidenstr. 43, Berlin, 10115, Germany
[d] School of Natural Sciences, Macquarie University, Sydney, 2113, New South Wales, Australia
[e] Department of Biological Sciences, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa
[f] Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA

ARTICLE INFO

Keywords:
Afrotropical region
Biogeography
Endemism
Miocene
Pliocene
Ultraconserved elements (UCEs)

ABSTRACT

A highly endemic ant fauna is found in the arid regions of southern Africa, including species in the genus *Ocymyrmex*. This genus of ants has higher species richness in the western arid regions of southern Africa compared to tropical and subtropical parts of the continent. The processes that have produced these patterns of diversity and distribution of arid adapted ants in southern Africa have never been investigated. The diversification of many other taxa in the region has been associated with past climate fluctuations that occurred during the Miocene epoch. In this study, the nature and timing of historical processes that may have led to the diversification within *Ocymyrmex* were assessed. We hypothesized that past climate oscillations, characterized by long periods of aridification, have driven the current distribution of *Ocymyrmex* species that resulted in the highest species richness of the genus in the Deserts & xeric shrublands biome in southern Africa.

Ninety-four *Ocymyrmex* worker specimens from Botswana, Kenya, Namibia, South Africa, Tanzania and Zimbabwe, representing 21 currently described species and six morphospecies, were included in a phylogenomic analysis. Phylogenies for the genus, based on next generation sequencing data from ultraconserved elements, were inferred using Maximum Likelihood, and a dating analysis was performed using secondary age estimates as calibration points. A distribution database of *Ocymyrmex* records was used to assign species ranges, which were then coded according to major biomes in southern Africa and used as input for biogeographical analysis. We explored the phylogenomic relationships of *Ocymyrmex* and analysed these within a biogeographical and paleoclimatic framework to disentangle the potential processes responsible for diversification in this group. Dating analyses estimated that the crown age of *Ocymyrmex* dates to the Oligocene, around 32 Ma. Diversification within this group occurred between the mid-Miocene (~12.5 Ma) and Pleistocene (~2 Ma). Our biogeographic analyses suggest that *Ocymyrmex* species originated in the south-western region of southern Africa, which is now part of the Deserts & xeric shrublands biome and diversified into eastern subtropical areas during the Pliocene. Paleoclimatic changes resulting in increased aridity during the Miocene likely drove the diversification of the genus *Ocymyrmex*. It is most likely that the diversification of grasslands, because of historical climate change, facilitated the diversification of these ants to the eastern parts of southern Africa when open grasslands replaced forests during the early Miocene.

1. Introduction

Major environmental fluctuations associated with changes in historical climate conditions have shaped ecosystems and influenced the distribution of many species within them (Linder, 2003,2005). These paleoclimatic changes are considered to be the major drivers of many

* Corresponding author at: Research and Exhibitions Department, South African Museum, Iziko Museums of South Africa, P.O. Box 61, Cape Town, 8000, South Africa.
E-mail address: nnhleko@iziko.org.za (N. Mbanyana).

https://doi.org/10.1016/j.ympev.2023.107977
Received 14 June 2023; Received in revised form 6 November 2023; Accepted 23 November 2023
Available online 24 November 2023

speciation and regional patterns of biodiversity (Linder, 2003,2005; Hewitt, 2011; Svenning et al., 2015). Past climate conditions have often led to contractions and expansions of many species' ranges that, in turn, facilitated reproductive isolation and speciation (Hewitt, 2011). For example, in Africa a shift from tropical to arid conditions in the Sahara led to the divergence of the elephant shrews in the genus *Elephantulus* during mid to late Miocene (11.6 Ma) (Douady et al., 2003). Similar diversification in several other taxa was influenced by these events, such as rodents (McDonough et al., 2015), southern African tortoises (Daniels et al., 2007), southern rock agamas (Swart et al., 2009), southern African *Pedioplenis* sand lizards (Makokha et al., 2007), and the African ground squirrel, *Xerus inauris* (Herron et al., 2005).

It is often the indirect impacts of historical climate change, such as associated changes in vegetation, that influence changes in patterns of biodiversity. For example, grasslands became a major component of African ecosystems during the mid-Miocene, replacing forests with the progression of aridity (Udeze and Oboh-Ikuenobe, 2005; Bobe, 2006; Kürschner et al., 2008) and many animal taxa responded accordingly to these changes (e.g., dwarf chameleons (Tolley et al., 2008) and dung beetles (Davis and Scholtz, 2020)). The western part of southern Africa became more arid during the Miocene due to upwelling of cold subsurface water in the Atlantic Ocean (Tankard and Rogers, 1978; Senut et al., 2009; Pickford et al., 2014). The subtropical anticyclones over the Atlantic and Indian Oceans further contributed to aridification in the western parts of southern Africa (Schulze, 1965).

The ant fauna in the arid regions of southern Africa is highly endemic, including members of the genus *Ocymyrmex* Emery. While most *Ocymyrmex* species occur in the arid regions of southern Africa (Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe), a few species do occur beyond southern Africa (Ethiopia, Kenya, Somalia, Sudan and Uganda) (Fig. 1 and Appendix A: Fig. A1 & A4). The highest diversity of these ants lies in the arid regions of Namibia (23 endemic) and South Africa (15 endemic) (AntWeb. Version 8.91.2, 2023; Janicki, et al., 2016 (antmaps)). There is only one species-group that occurs in the eastern side of the Afrotropical region (cf. *weitzeckeri*-group), which is also widespread in other parts of southern Africa (Bolton and Marsh, 1989). Despite an increase in studies reporting how the distribution of fauna and flora responded to historical climate and topographical shifts in southern Africa (Prendini, 2005; Makokha et al., 2007; Voelker et al., 2012; Engelbrecht et al., 2013; Engelbrecht et al., 2021; Raphalo et al., 2021), such information on the region's ant fauna remains scarce.

The paleoclimatic changes and topographic heterogeneity which led to habitat changes during the Miocene and shaped the biomes of southern Africa (Cowling et al., 2009) may be the main drivers of diversification in *Ocymyrmex* ants. This has been shown for other taxa in biogeographical studies from the region. For example, the distributions of endemic dung beetles in Botswana, Namibia and South Africa are thought to have been influenced by past climatic, geomorphic and vegetation changes (Davis and Scholtz, 2020). Similar changes are thought to have spurred inter- and intra-specific diversification in various taxonomic groups, including *Gittenedouardia* snails (Raphalo et al., 2021), the Cape legless skink *Acontias meleagris* (Engelbrecht et al., 2021), *Pedioplanis* lizards (Makokha et al., 2007) and scorpions (Prendini, 2005).

The topographical changes associated with tectonic plate uplifts during the Miocene may have also created opportunities for the formation of new species due to vicariance (e.g., tent tortoise (*Psammobates tentorius*; Zhao et al., 2020) and southern African geckos (*Pachydactylus* group; Bauer and Lamb, 2005)). Thus, the phylogeographic structure and diversification of *Ocymyrmex* could also be associated with these geomorphic events (Sepulchre et al., 2006) and subsequent habitat heterogeneity (Cowling et al., 2009).

Molecular genetic studies unravelling phylogenetic relationships have been instrumental in understanding the historical processes that have shaped current biodiversity (Daniels et al., 2007; Tolley et al., 2008; Branstetter et al., 2021). In this study we used next generation sequencing data from ultraconserved elements (UCEs) to assess the

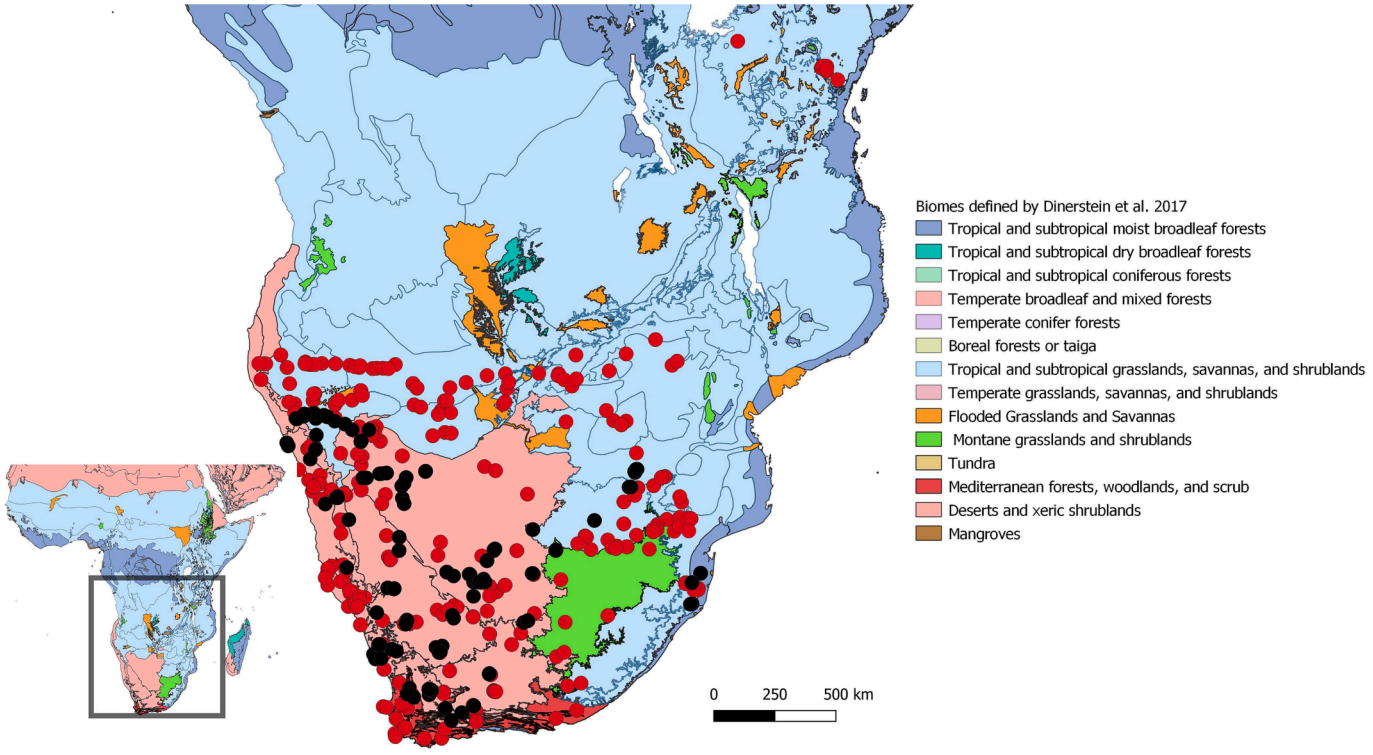

Fig. 1. Map showing the distribution records of ants in the genus *Ocymyrmex* across different biomes in southern Africa. Red dots represent occurrence records that were extracted from the Iziko Museums of South Africa Specify6 database, and black dots represent *Ocymyrmex* specimens sampled for this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phylogenomic relationships among *Ocymyrmex* species. Coupled with dating and biogeographical analyses, we then investigated the timing and nature of historical processes that may have led to the diversification and the current distribution of *Ocymyrmex* species in southern Africa. We hypothesized that past climate oscillations, characterized by long periods of aridification, have driven the current distribution patterns of *Ocymyrmex* species that resulted in the highest species richness of the genus in the Deserts & xeric shrublands biome in southern Africa.

2. Materials and methods

2.1. Sampling

Sampling was conducted throughout the known distribution of *Ocymyrmex* in the Northern Cape province, North-West province, KwaZulu-Natal province, Limpopo province and Western Cape province in South Africa, and in Namibia (Fig. 1). Fieldwork was conducted between 2011 and 2019. As *Ocymyrmex* ants are most active during the hottest seasons, fieldwork was conducted between October and April. Ants were collected using pitfall traps, yellow pan traps, Malaise traps, UV light traps or by digging up ant nests. At least 20 individuals from each nest were sampled to allow an assessment of morphological variation within and between species. Samples were preserved in ethanol (96 %) prior to DNA extraction. We identified all the material collected from this study to species level, using Bolton and Marsh's (1989) classification and verified identifications by comparing all specimens to material that is currently housed at Iziko Museums of South Africa. Putative new species included in this study (denoted by *Ocymyrmex* sp. 1, *Ocymyrmex* sp. 2, *Ocymyrmex* sp. 3, *Ocymyrmex* sp. 4, *Ocymyrmex* sp. 5, and *Ocymyrmex* sp. 6) will be formally described in a separate paper. Representatives for each species were mounted on a card point to examine morphological characters under a microscope and labelled and deposited in the Entomology dry collection of the South African Museum (SAMC) (Iziko Museums of South Africa). For long-term storage, all remaining material was preserved in 96 % ethanol. Specimen data for all the curated species were digitized in the Iziko Specify6 database (Specify version 6.7.01; Specify Collections Consortium | Software for Biological Collections and Samples). All necessary collecting permits were obtained from the relevant conservation bodies (Cape Nature: 0056-AAA041-00160, Cape Nature: CN44-87-19112, Northern Cape: FAUNA 0207/3/2017, Northern Cape: FAUNA 0426/3/2018, South African National Parks: CRC/2019-2020/012-2012/V1, North-West: NW 13478/08/2019. Northern Cape: FAUNA 099/2015, Northern Cape: FAUNA 0085/2016).

A total of 94 *Ocymyrmex* worker specimens representing 21 currently described species of *Ocymyrmex* and six morphospecies were included in the phylogenomic analyses (Appendix B: Table B1). We could not include all 34 valid species of *Ocymyrmex* in this study as we could not obtain specimen representatives of 12 species (recorded from Angola, Botswana, Kenya, Lesotho, Namibia, Somalia, South Africa, Zambia and Zimbabwe), housed in various international institutions. We believe that inclusion of these species would not change the biogeographic results from this study, as the species in our study covered all biome regions where these 12 absent species were collected and include likely close relatives of all missing species based on morphological work (Bolton, 1981; Bolton and Marsh, 1989).

Representative *Ocymyrmex* specimens included in phylogenomic analyses were selected to cover a full range of morphological variation and a wide geographical distribution. The following taxa with existing UCE data were included as outgroups: *Cardiocondyla mauritanica* Forel, *Cardiocondyla thoracica* Smith, *Nesomyrmex madecassus* Forel and *Tetramorium severini* Emery (Blaimer et al., 2018).

2.2. DNA extraction

DNA was extracted destructively (either using the whole specimen or two legs, depending on the size of the specimen) or non-destructively (by soaking the whole specimen in proteinase-K and then rinsing in ethanol and remounting) from mounted museum specimens collected between 1980 and 2015, using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). The standard protocol described by the manufacturer was followed except for the final elution step; 130 μL of AE buffer was added to ensure that there was enough DNA for quality checks and UCE library preparation. We ran 10 μL of each DNA extract on a 2 % agarose gel to assess any degradation of DNA. The quantity of extracted genomic DNA was quantified using a Qubit 2.0 fluorometer high sensitivity kit (Life Technologies). DNA concentrations ranged between 0,06 to 20,9 ng/μL (Appendix B: Table B1). All UCE laboratory work was conducted in the Laboratories of Analytical Biology (L.A.B.) facilities of the National Museum of Natural History, Smithsonian Institution (Washington, U.S.A.).

2.3. UCE library preparation, enrichment and sequencing

DNA was sheared to a target size of approximately 250–600 bp by sonication using a Qsonica Q800 sonicator (Qsonica LLC, Newton, CT, U.S.A.). Sheared DNA fragments were used for genomic library preparations following a modified protocol described in Faircloth et al. (2015), using a Kapa Hyper Prep Library Kit (Kapa Biosystems, Wilmington, MA, U.S.A.) and a generic SPRI substitute (Fisher et al., 2011; "speedbeads" (Faircloth et al., 2015)) for bead-based clean-up steps. We ligated dual-indexing Illumina TruSeq-style adapters (iTru i5 and i7 primers) (Faircloth and Glenn, 2012) to 15 μL DNA template during a PCR reaction consisting of 25 μL HiFi HotStart polymerase (Kapa Biosystems, Wilmington, MA, U.S.A.), 2.5 μL each of iTru i5 and i7 primers (5 nM each) and 5 μL ddH20. The following thermal protocol was used: 98 °C for 45 s, 13 cycles of 98 °C for 15 s, 65 °C for 30 s, 72 °C for 60 s and final extension at 72 °C for 5 min. PCR products were purified using 1.0X speedbeads and eluted with 23 mL of pH 8 elution buffer (EB; 10 mM Tris-Cl, pH 8.5; ddH_2O). DNA concentration was measured using a Qubit 2.0 fluorometer. In addition, we ran 2 μL of library products on an agarose gel. We pooled libraries together at equimolar concentrations into enrichment pools, and pool concentrations were adjusted accordingly using a vacuum centrifuge.

Enrichment was performed using the 'Hymenoptera-v2-ANT-SPECIFIC' bait set, which includes 9446 unique baits targeting 2590 UCE loci specific to the order Hymenoptera (Branstetter et al., 2017). The library enrichment procedures for the MYcroarray (now ArborBiosciences) MYBaits kit protocol v3 (Blumenstiel et al., 2010) were followed. We added 0.7 μL of 500 μM custom blocking oligos designed for the sequence tags. Enrichment incubation was performed at 65 °C for 24 h. After this step, all pools were bound to streptavidin beads (MyOne C1; Life Technologies) and the enriched pools were purified. We used the "with-bead" approach for PCR amplification of the enriched libraries using the approach described in Faircloth et al. (2015). We combined 15 μL of enriched pool with 25 μL of HiFi HotStart Taq (Kapa Biosystems), 5 μL of Illumina TruSeq primer mix (5 nM each), and 5 μL of ddH20, and ran the reaction at 98 °C for 45 s; 18 cycles of 98 °C for 15 s; 60 °C for 30 s; 72 °C for 60 s; and a final extension of 72 °C for 5 min. We purified the resulting reactions using 1.0X speedbeads and rehydrated the enriched pools in 22 μL EB. We quantified 2 μL of each enriched pool using a Qubit fluorometer.

We quantified the DNA concentration of each library pool by performing qPCR using a SYBR® FAST qPCR kit (Kapa Biosystems) with a ViiA™ 7 Real-Time PCR System (Life Technologies). We used the measured concentrations to pool libraries at equimolar concentrations. This final pool was then size-selected to a fragment range of 250–800 bp using a BluePippin (SageScience, Beverly, MA, U.S.A.). The pooled libraries were sequenced using two partial lanes of 125 bp paired-end Illumina HiSeq 2500 sequencing runs at the University of Utah's Huntsman Cancer Institute. Raw sequencing reads are deposited at the NCBI Sequencing Read Archive (Bioproject accession PRJNA1043559).

2.4. Bioinformatic analyses

The demultiplexed FASTQ data were cleaned and trimmed of adapters using Illumiprocessor v.2.0 (Faircloth, 2013), based on the package Trimmomatic (Bolger et al., 2014). Data processing was done through a series of scripts available in the PHYLUCE package v.1.7.1 (Faircloth, 2015). Phyluce commands have been deposited in the figshare repository under accession doi.10.6084/m9.figshare.24585888. Trimmed reads were assembled into contigs using a wrapper script (phyluce_assembly_assemblo_trinity.py) and the program TRINITY (version trinityrnaseq_r20140717) (Grabherr et al., 2011). Assembled contig files have been deposited in the figshare repository under accession doi.10.6084/m9.figshare.24585849. We used the PHYLUCE pipeline to identify and extract contigs containing UCE loci. Species-specific contig assemblies were aligned to a FASTA file of all enrichment baits using phyluce_assembly_match_contigs_to_probes.py (min_coverage = 50, min_identity = 80). A list of UCE loci shared across all taxa was generated by using phyluce_assembly_get_match_counts.py. This list was then used to create FASTA files for each UCE locus using phyluce_get_fastas_from_match_counts.py. All sequence data in these FASTA files were aligned using MAFFT (Katoh and Standley, 2013) through phyluce_seqcap_align.py (min. length = 100, no trim) and trimmed using a wrapper script (get_gblocks_trimmed_alignment_from_untrimmed.py) for Gblocks (Castresana, 2000) with the following settings: b1 = 0.5, b2 = 0.5, b3 = 12, b4 = 7. After trimming, multiple subsets based on filtering UCE loci for different levels of taxon occupancy (70 %, 80 % and 90 % taxon completeness) were created using phyluce_get_only_loci_with_min_taxa.py, and we generated statistics across all subsets using get_align_summary_data.py. Individual alignments of UCE loci for each subset were then concatenated into one nexus alignment file with phyluce_align_format_nexus_files_for_raxml.py script for subsequent phylogenetic analyses. SPRUCEUP v2020.2.19 (Borowiec, 2019) was used to remove poorly aligned sequences or sequence fragments. The matrices were trimmed based on the following cut-off values: 95 %, 97 %, 98 % and 99 %. For this study, all the analyses here are based on 97 % and 98 % cut-off values, as a 95 % cut-off was too stringent, and a 99 % cut-off did not trim outlier sequences sufficiently.

2.5. Phylogenomic analyses

Data partitioning was performed using the Sliding-Window Site Characteristics (SWSC-EN) algorithm described by Tagliacollo and Lanfear (2018), using entropy to split each UCE locus into three parts: two flanking regions, which are more variable, and a core region which is more conserved. PartitionFinder2 (Lanfear et al., 2017) was used to combine data blocks with similar properties and to search for the best-fitting scheme of partitions and models of evolution for our dataset.

To assess phylogenetic relationships among *Ocymyrmex* species, we analysed both partitioned and unpartitioned datasets using Maximum Likelihood (ML) in IQ-TREE version 2.1.2 (Minh et al., 2020) or IQ-TREE version 1.6.12 (Nguyen et al., 2015), using best tree analyses with maximum number of iterations set to 5000 and 1000 ultrafast bootstraps replicates. Models GTR+F+R3, GTR+F+R4 and GTR+F+R5 were selected by ModelFinder in IQ-TREE version 2.1.2 (Minh et al., 2020) for 70 %, 80 % and 90 % levels of taxon completeness as the best-fitting models of evolution for the unpartitioned data sets, respectively; GTR+G was used as a model of evolution for the partitioned data sets. Both partitioned and unpartitioned analyses were rooted using the outgroup taxon *Tetramorium severini*. To confirm repeatability of our phylogenetic results, different levels of taxon completeness (70 %, 80 % and 90 %) for both partitioned and unpartitioned datasets were analysed. Input files for concatenated analyses have been deposited in the figshare repository under accession doi.10.6084/m9.figshare.24585954 (unpartitioned analyses) and doi.10.6084/m9.figshare.24585897 for the partitioned analyses.

The potential effects of independent lineage sorting on our dataset were explored by performing coalescent analyses with ASTRAL-III v. 5.7.7 (Zhang et al., 2018). ASTRAL-III is a java program for estimating a species tree given a set of unrooted gene trees, each inferred from a different part of a genome. This approach aims to explain the genealogical discordances between gene trees and species trees which might be caused by incomplete lineage sorting (Zhang et al., 2018). Gene trees were reconstructed from 2055 UCE loci retained in the 70 % dataset, 1235 UCE loci retained by 80 % dataset and 88 UCE loci retained by the 90 % dataset. We estimated gene trees using the following command in IQ-TREE version 2.1.2 (Minh et al., 2020): iqtree2 -S ALN_DIR –prefix loci -T AUTO. Input files for coalescent analyses have been deposited in the figshare repository under accession doi.10.6084/m9.figshare.24586005.

2.6. Divergence time estimation

To assess the timing of historical processes that may have led to diversification in *Ocymyrmex*, a dated phylogeny for the genus was inferred using MCMCTREE v4.9j in the PAML package (Phylogenetic Analysis by Maximum Likelihood; (Yang, 2007)). Divergence ages were reconstructed using the 80 % taxon completeness matrix, trimmed with 0.97 cut-off in SPRUCEUP v2020.2.19 (Borowiec, 2019), and the tree resulting from partitioned analysis of this data set as input topology (Appendix C). This tree was pruned to include only one representative of each *Ocymyrmex* species, and two outgroup taxa (*Nesomyrmex madecassus* and *Cardiocondyla thoracica*) were also removed prior to this analysis. Given the unknown fossil record of *Ocymyrmex*, secondary ages estimated by Blaimer et al. (2018) were used to calibrate two nodes in our tree. The split between *Ocymyrmex* and *Cardiocondyla* was calibrated with 46.5–71.9 Ma and the split between *Tetramorium* and the *Cataulacus* genus-group of which both *Ocymyrmex* and *Cardiocondyla* are part of (=root node) was calibrated with 60.1–84.9 Ma. Both calibration ranges were derived from 95 % highest posterior density (HPD) intervals across four analyses from Blaimer et al. (2018) to account for uncertainty.

We first estimated the Hessian gradient in MCMCTREE (set usedata = 3), then the estimates from the Hessian gradient were used to perform approximate likelihood estimation (usedata = 2). We performed four independent runs, with the following parameters: burnin was set to 500,000; sampling was performed every 100 iterations until 1,000,000 samples were gathered; the model was set to 7 (GTR); an independent rates model was applied (clock = 2); and we used a single data partition. Convergence and effective sample size (ESS) were visualized in TRACER v1.7 (Rambaut et al., 2018). Input files for MCMCTREE analyses have been deposited in the figshare repository under accession doi.10.6084/m9.figshare.24586032.

2.7. Ancestral area reconstruction

To investigate the factors that may have influenced the distribution of *Ocymyrmex* species in southern Africa, we inferred their biogeographic histories using the R package BioGeoBEARS (BioGeography with Bayesian (and likelihood) Evolutionary Analysis in R Scripts) (Matzke, 2018). This likelihood-based model is designed to perform inferences of biogeographical histories on phylogenies and to compare different models of range evolution (Matzke, 2013a). The following biogeographical models were tested against our data to estimate the ancestral area of *Ocymyrmex* species: a Dispersal-Extinction-Cladogenesis (DEC) model, which allows the geographic range to change across a phylogeny through several events (Ree, 2005; Ree and Smith, 2008); a likelihood version of the Dispersal-Vicariance model (DIVALIKE) (Ronquist, 1997); and a likelihood version of the Bayesian Analysis of Biogeography (BAYAREA-LIKE), which is a Bayesian model that samples geographical history along phylogenetic branches, together with sampling parameter values (Landis et al., 2013). These models have free parameters specifying the rate of dispersal (d = range expansion)

and extinction (e = range of contraction) along the phylogeny branches. At cladogenesis events, DEC assumes that one daughter lineage will always inherit the ancestral range if the ancestor lives in a single area; if the ancestor is widespread, one daughter lineage will live in a subset of that area, or one area will split off by vicariance. By contrast, DIVA only allows vicariance. BayArea assumes that no range evolution occurs at cladogenesis, and therefore the ancestral range is copied by both daughters (Matzke, 2013b). A founder event speciation model (+j) was also tested in combination with each base model.

BioGeoBears requires a time calibrated tree and geographic distribution units as input files. *Ocymyrmex* distribution records were extracted from Iziko Museum's Specify6 database (Specify version 6.7.01; Specify Collections Consortium | Software for Biological Collections and Samples). Given the tight habitat affinity of *Ocymyrmex*, species occurrences were assigned and coded according to the biomes defined by Dinerstein et al. (2017) (Appendix B: Table B2). The following biome regions were designated and used as biogeographical units: Tropical & subtropical moist broadleaf forests; Tropical & subtropical grasslands, savannas & shrublands; Flooded grasslands & savannas; Montane grasslands & shrublands; Mediterranean forests, woodlands & scrub; and Deserts & xeric shrublands. The dated phylogeny produced by MCMCTREE was pruned to exclude outgroups and was used as an input file. The Akaike information criterion (AIC) was used to select the best-fitting model. The maximum number of areas any species may occupy was set to five, as the *Ocymyrmex* species with the largest distribution range, *O. flaviventris*, can be found in five biomes. We followed guidelines in http://phylo.wikidot.com/biogeobears. Input files and code for biogeographic analyses have been deposited in the figshare repository under accession doi.10.6084/m9.figshare.24586143.

3. Results

We recovered well-supported phylogenies with congruent topologies for both our partitioned and unpartitioned datasets and across three different levels of taxon completeness (70 %, 80 % and 90 %), with most nodes having 100 % ultrafast bootstrap (ufBS) support (Appendix C: C1 – C12). The coalescent species trees estimated by ASTRAL-III v. 5.7.7 were mostly congruent with the trees recovered by the concatenated analyses, with clade E and F showing some discordance in terms of species relationships (Appendix C: C14 – C16). The phylogenies recovered by both partitioned and unpartitioned analyses of the 80 % level of completeness and 97 % cut-off had 100 % ufBS at almost all nodes (Appendix C: C5 & C7) and hereafter we discuss our findings based on the partitioned version of this tree (Fig. 2).

The molecular results were mostly congruent with the *Ocymyrmex* species groups delimited by Bolton (1981) and Bolton and Marsh (1989) that were based on morphological assessments only. Our phylogeny identified 26 lineages, and 24 of them (92 %) were supported by a value of 100 % ufBS and two lineages were supported by a value of >85 % ufBS (Fig. 2). Clades and subclades were identified as those recovered as reciprocally monophyletic and distinguishable based on several morphological characters, and also based on the species groups identified from previous *Ocymyrmex* revisions (Bolton, 1981; Bolton and Marsh, 1989). The species in clade D include *O. afradu*, *O. engytachys*, *O. kahas*, *O. turneri*, *O. velox*, and *O. zekhem*; with *O. turneri* recovered as a sister species to *O. engytachys*; and *O. kahas* a sister species to *O. afradu*. Clade E consists of *O. barbiger*, *O.* sp. 1, *O. dekerus*, *O. robustior*, and *O.* sp. 6, with *O. barbiger* recovered as a sister species to *O. robustior*. The only disagreement was regarding the species designations of *O. foreli*, *O. fortior*, *O. flaviventris* and *O. resekhes*. In clade F, both *O. fortior* and *O. foreli*, as well as *O. resekhes* and *O. flaviventris* specimens are intermingled and not recovered as reciprocally monophyletic lineages (Appendix C). The results from our study suggest that *O. foreli* and *O. fortior* are likely a single species. In the taxonomic revision of Bolton and Marsh (1989) these two species were separated based on the sculpture of the

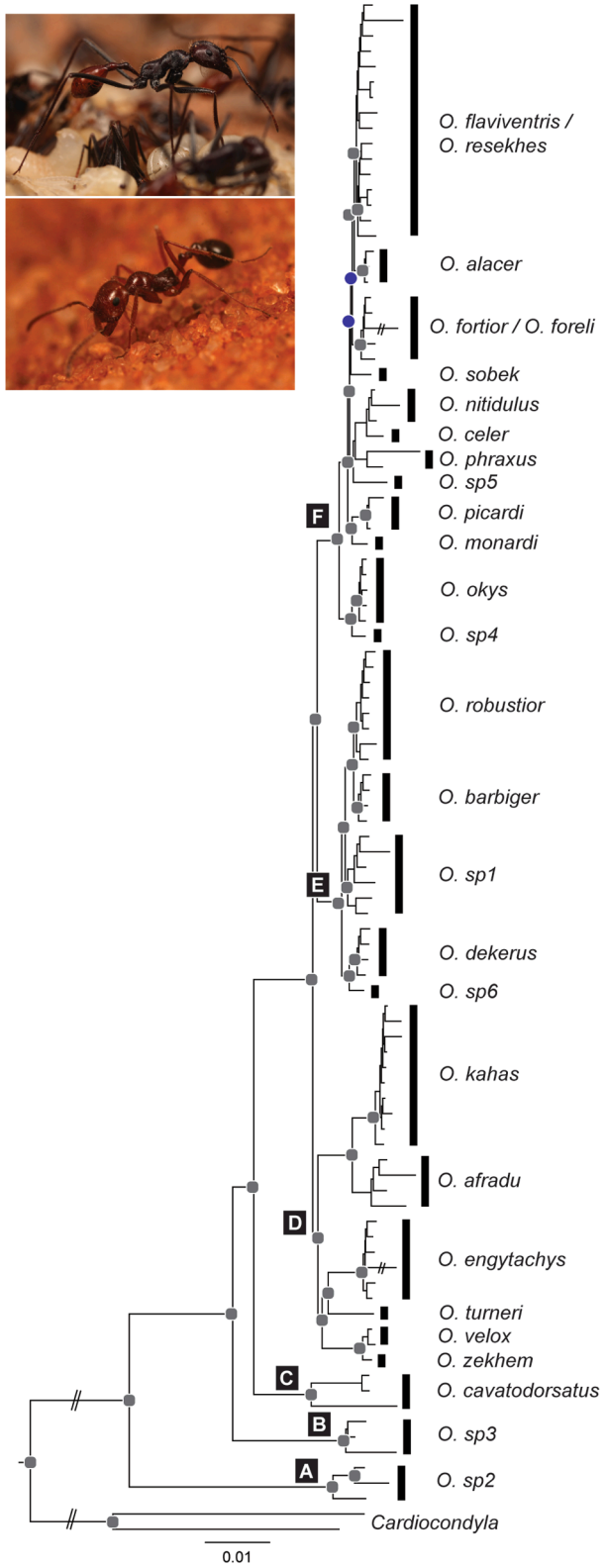

Fig. 2. Maximum likelihood phylogeny of *Ocymyrmex* ants based on 1235 UCE loci. The outermost outgroups (*Nesomyrmex madecassus* and *Tetramorium severini*) have been pruned for better visualization. Grey squares indicate ultrafast bootstrap (ufBS) support values of 100; blue circles indicate ufBS values between 88 and 92. Double slashes indicate where branches have been manually shortened for display purposes. Labels A–F denote lineages referred to in the text. Scale bar represents substitutions per site.

petiole. The entire petiolar node is covered with sharply defined regular sculpture in the specimens identified as *O. foreli*, as opposed to the weakly or unsculptured petiole in specimens identified as *O. fortior*. The workers of *O. fortior* have sharply defined longitudinal costulae on the dorsum of the head, which are parallel and running from the front to the back of the head, whereas in *O. foreli*, the cephalic sculpture is only longitudinal on the mid-section of the head, and more divergent on the sides. The distribution of these two species overlaps. Based on the interpretation of our overall results, these minor differences in sculpture represent intra-specific variation; we therefore intend to synonymise these two species in a forthcoming publication. We observed a similar situation with respect to *O. flaviventris* and *O. resekhes*. The morphological differences between these two species are superficial. They are separated based on the length of the peduncle, which is relatively short and stout in profile view in *O. flaviventris*, whereas in *O. resekhes* the peduncle is relatively long and narrow in profile (Bolton and Marsh, 1989). Specimens of these two species were retrieved nested in a single well-supported monophyletic clade, supporting a single species. This intra-specific variation in terms of colour, size, striation, and emargination of clypeus has been observed in other species within this group, including *O. barbiger* and *O. velox*. Based on molecular data and morphological evaluation of these species, we also plan to synonymise *O. flaviventris* and *O. resekhes*.

The BioGeoBEARS model comparisons showed that the BayArea-Like model with the +j founder-speciation and the DEC model were favoured as both received the lowest AIC scores compared to other models tested (Table 2). BayArea-Like +j model was the best fit model to the data as it performed slightly better than DEC (AICs: 169.1 vs 169.5). Here, we present results produced by the DEC model as the difference in model scores was not sufficiently significant to justify using the founder event (+j) parameter. Ree and Sanmartín (2018) indicated that the +j parameter was a poor model of founder event speciation, and its statistical comparisons were inappropriate. Irrespective, results from biogeographical analyses using different models showed relatively consistent patterns, as they all inferred the same ancestral ranges.

Our dating analyses estimated the crown age of *Ocymyrmex* to date back to the Oligocene, around 32 Ma (million years ago; all ages quoted in the following are median ages) (node 1, Fig. 3, Table 1) and Appendix C: Fig. C13). The ancestral ranges estimated by the DEC model mapped onto the time-calibrated phylogeny suggested that *Ocymyrmex* originated in the south-western dry parts of southern Africa during the Oligocene, a region that is now part of the Deserts & xeric shrublands biome in southern Africa, and subsequently diversified to the eastern subtropical parts of this region during the Pliocene (~5 Ma) (node 7, Fig. 3, Table 1). The divergence of the remaining *Ocymyrmex* species from a most recent common ancestor with *O.* sp. 3 (node 2, Fig. 3,

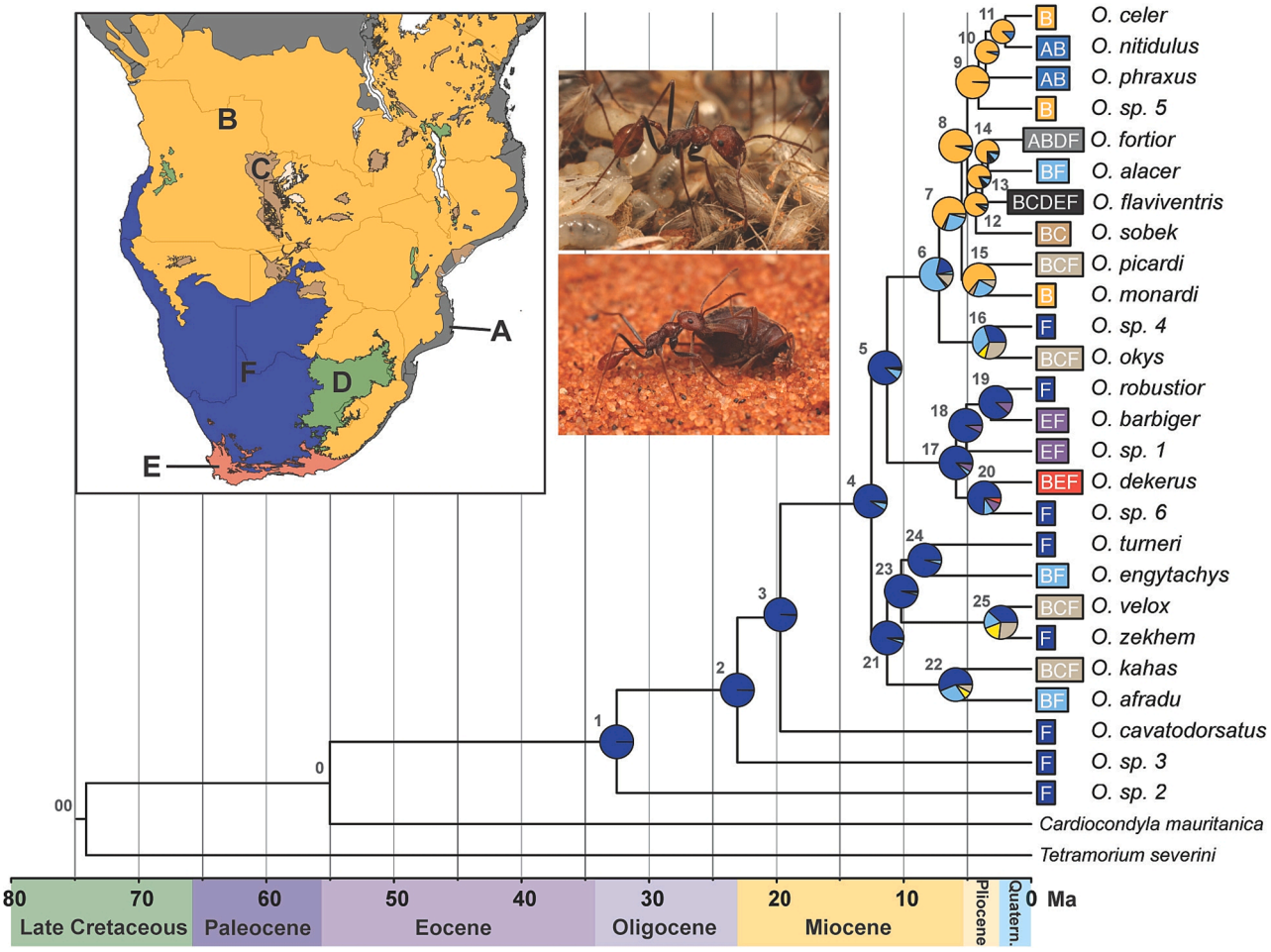

Fig. 3. Time-calibrated phylogeny of ants in the genus *Ocymyrmex*. The dataset was pruned prior to analysis to include only one exemplar per species. Ages are displayed in million years (Ma). Node numbers refer to results of dating and biogeographic analyses in Table 1 and Appendix B: Table B3. The biogeographical history of *Ocymyrmex* species, inferred using BIOGEOBEARS and the best-fitting Dispersal-Extinction-Cladogenesis (DEC) model, is mapped onto the time-calibrated phylogeny in form of piecharts. Distributions of extant taxa are indicated by letters at terminal nodes. Colours of the piecharts and boxes correspond to the geographical areas on the map (on the left). Geographic areas are: A = Tropical & subtropical moist broadleaf forests; B = Tropical & subtropical grasslands, savannas & shrublands; C = Flooded grasslands & savannas; D = Montane grasslands & shrublands; E = Mediterranean forests, woodlands & scrub; F = Deserts & xeric shrublands. Images are of *O. resekhes* © H.G. Robertson.

Table 1
Summary of divergence estimates and ancestral ranges. Divergence ages estimated with MCMCTREE from the concatenated 80 % taxon filtered matrix, trimmed with a 0.97 cutoff. Given are median ages and 95 % highest posterior densities (HPD) in Ma. Ancestral range reconstructions are shown as highest probabilities for each node estimated under the DEC model. Node numbers refer to Fig. 3. B = Tropical & subtropical grasslands, savannas & shrublands; F = Deserts & xeric shrublands.

Node number	Median age (Ma)	95 % HPD	Range	Probability
00	74.13	61.05,85.36		
0	55.03	45.35,68.85		
1	32.54	19.98,46.1	F	1.00
2	23,08	12.42,35.06	F	1.00
3	19.71	10.35,30.35	F	0.99
4	12.53	5.77,20.96	F	0.91
5	11.29	5.17,18.87	F	0.87
6	7.22	3.07,12.71	BF	0.64
7	5.46	2.35,9.62	B	0.68
8	5.01	2.17,8.79	B	0.95
9	4.13	1.77,7.27	B	0.99
10	3.57	1.52,6.3	B	0.95
11	2.02	0.73,3.79	B	0.88
12	4.33	1.86,7.59	B	0.90
13	3.85	1.63,6.77	B	0.81
14	3.4	2.17,8.79	B	0.76
15	4.09	1.57,7.43	B	0.64
16	3.29	0.92,6.7	BF	0.33
17	5.89	2.3,10.76	F	0.87
18	5.08	1.95,9.33	F	0.92
19	3.17	1.02,6.14	F	0.89
20	3.68	1.16,7.16	F	0.74
21	11.29	5.17,18.82	F	0.94
22	5.92	2.09,11.0	F	0.55
23	10.18	4.68,16.98	F	0.96
24	8.34	3.68,14.17	F	0.96
25	2.38	0.63,5.05	F	0.38

Table 1) occurred during the early Miocene. Based on our inferences, diversification of *Ocymyrmex* species occurred between Miocene (23 Ma) (node 2, Fig. 3, Table 1) and Pleistocene (~2 Ma) (node 11, Fig. 3, Table 1).

Ocymyrmex species within clade F extended their distribution ranges to the eastern part of southern Africa (Tropical & subtropical grasslands, savannas & shrublands biome) around 7 Ma, and spread to the Tropical & subtropical moist broadleaf forests biome around 5 Ma. The MRCA of *O. afradu*, *O. engytachys*, *O. kahas*, *O. turneri*, *O. velox* and *O. zekhem* (node 21, Table 1, Fig. 3) originated around 11 Ma in the Deserts & xeric shrublands biome. *Ocymyrmex turneri* and *O. zekhem* are still restricted to this region, whereas other extant species in this clade expanded their ranges to the Tropical & subtropical grasslands, savannas and shrublands biome. Only *O. kahas* and *O. velox* have extended their habitat range to the Flooded grasslands & savannas biome. Other *Ocymyrmex* lineages, including species such as *O. barbiger*, *O.* sp. 1, *O. dekerus*, *O. robustior*, and *O.* sp. 6, originated slightly later in the Deserts & xeric shrublands biome around 5.8 Ma (node 17, Table 1, Fig. 3), where they are still found today. *Ocymyrmex robustior* and *O.* sp. 6 are still restricted to this habitat today, while *O. barbiger*, *O.* sp. 1 and *O. dekerus* have extended their habitat ranges to the Mediterranean forests, woodlands and scrub biome. *Ocymyrmex* sp. 1 is the only species in this clade that has been recorded in the Montane grasslands & shrublands biome, while *O. dekerus* is the only species found in the Tropical & subtropical grasslands, savannas & shrublands biome.

The MRCA of Clade F, comprised of *O. alacer*, *O. celer*, *Ocymyrmex* sp. 5, *O. flaviventris*, *O. fortior*, *O. monardi*, *O. nitidulus*, *O. okys*, *O. phraxus*, *O. picardi*, *Ocymyrmex* sp. 4, and *O. sobek*, emerged around 7 Ma (node 6, Table 1; Fig. 3) and diversified into the Tropical & subtropical grasslands, savannas & shrublands and Tropical & subtropical moist broadleaf forests biomes between the late Miocene (ca. 7 Ma) and Pleistocene (ca. 2 Ma, node 11, Table 1; Fig. 3). Species within this group are also widespread in other parts of southern Africa. By contrast, *O. celer*, *O.* sp. 5, *O. monardi*, *O. nitidulus*, *O. phraxus*, and *O. sobek* have remained restricted to these Tropical & subtropical grasslands, savannas and shrublands, and Tropical & subtropical moist broadleaf forests biomes (Fig. 3).

The highest species richness of *Ocymyrmex* is found in the Deserts & xeric shrublands biome, with 20 of the 28 recognized species included in this study being recorded from there, nine of which are endemic (Table 1). Sixteen *Ocymyrmex* species are present in the Tropical & subtropical grasslands, savannas & shrublands biome which experiences summer rainfall (Nicholson, 2000) and three of these species are endemic to these regions. Only three species were recorded in the Tropical & subtropical moist broadleaf forests and are endemic to this region. Six species were recorded from Flooded grasslands & savannas; furthermore, three species were recorded from Montane grasslands & shrublands and four from Mediterranean forests, woodlands & scrub (Table 1).

4. Discussion

Southern Africa is home to a variety of diverse biomes, spanning the winter rainfall regions in the south-west to the summer rainfall region in the east. The vegetation changes driven by past climate and geomorphic changes, especially during the Neogene period (Cowling et al., 2009), are thought to have shaped the current biomes in the region (Neumann and Bamford, 2015). The development of the cold Benguela Current (Tankard and Rogers, 1978) and the tectonic plate upliftment resulted in significant topographical changes (Sepulchre et al., 2006), which contributed to greater rainfall seasonality in the region. These climatic shifts have had a significant influence on the distribution, endemism, and richness of species in the region (Burgess et al., 2004). The Deserts & xeric shrublands biome in south-western Africa is the continent's driest biome that experiences winter rainfall (Nicholson, 2000). This biome consists of Kaokoveld desert, Namibian savanna woodlands, Namib desert, Kalahari xeric savanna, Succulent Karoo and Nama Karoo ecoregions (Dinerstein et al., 2017). A highly endemic fauna has been recorded in this biome, including dung beetles (Davids & Scholtz, 2020), pollen wasps (Masarinae, Vespidae; Gess, 1992), pycnostigmine wasps (Figitidae) (Buffington and van Noort, 2007), ants in the *Tetramorium solidum*-group (Formicidae) (Mbanyana et al., 2018), and scorpions (Prendini, 2005).

Contemporary species richness, abundance and distributions often reflect historical processes that influenced the evolutionary trajectories of regional biotas. The highest species richness of *Ocymyrmex* lies in the Deserts & xeric shrublands biome in southern Africa. We investigated the underlying evolutionary processes that may have led to this diversity

Table 2
Comparison of biogeographic models (in BioGeoBEARS) based on Akaike (AIC) scores.

Model	LnL	numparams	D	E	J	AIC	AICwt
DEC	−82,741	2	0,020	1,000000e-12	0,0000e+00	169,5	0,73
DEC+J	−82,742	3	0,020	3,995504e-09	1,0000e-05	171,5	0,27
DIVALIKE	−85,715	3	0,021	1,000000e-12	1,0002e-05	175,4	0,73
DIVALIKE+J	−85,715	3	0,021	7,321697e-09	1,0000e-05	177,4	0,27
BAYAREALIKE	−82,531	2	0,013	4,707896e-02	0,0000e+00	171,1	0,27
BAYAREALIKE+J	−82,533	3	0,013	4,716592e-02	1,0000e-05	169,1	0,73

and that resulted in the current distribution patterns of the genus. Our biogeographical analyses and dated phylogeny supported our hypothesis that *Ocymyrmex* likely originated and evolved in the arid regions of south-western Africa. According to our results, the genus appeared during the Oligocene (around 32 Ma) and subsequently transitioned and diversified into the eastern subtropical parts of the region during the late Miocene and Pleistocene (7–2 Ma). The genus emerged when arid conditions were prevalent in southern Africa, which were associated with the development of the cold Benguela Current after the appearance of the first Antarctic ice sheet between 35 and 26 Ma and intensified during the mid-Miocene (Feakins and Demenocal, 2010). The early diversification of these ants also coincides with aridification in the early Oligocene. The divergence of the remaining *Ocymyrmex* species from a most recent common ancestor (MRCA) with *O.* sp. 2 occurred during the Oligocene (ca 32 Ma) in the Deserts & xeric shrublands biome. Much of the diversification of the genus occurred within this biome, and some species, namely *O.* sp. 2, *O.* sp. 3 and *O. cavatodorsatus,* have remained restricted to its dry sandy regions. The results from this study suggest that the evolution of these ants has also been shaped by historical geological and associated environmental factors that led to habitat fragmentation. During the Eocene (56–33 Ma) Africa was dominated by tropical rainforests; then a shift from tropical to arid conditions occurred during the Miocene when the western part of southern Africa became more arid as a result of upwelling of cold subsurface water in the Atlantic Ocean (Tankard and Rogers, 1978; Udeze and Oboh-Ikuenobe, 2005; Bobe, 2006; Kürschner et al., 2008, Senut et al., 2009). The west coast region of southern Africa experienced hyper-arid conditions during the early Miocene (Pickford et al., 2014). The diversification of some *Ocymyrmex* lineages (e.g., Clades D–F in Fig. 2) began towards the end of the mid-Miocene (around 12 Ma), which coincides with the replacement of C3 grasses (and other vegetation components such as trees and shrubs) by C4 grasslands in south-western Africa (Rossouw et al., 2009). These changes in vegetation led to a major change in biomes as grasslands became a dominant component of ecosystems alongside the progression of aridity (Bobe, 2006). These open habitats likely contributed to the diversification of *Ocymyrmex* when these ants expanded their habitat ranges into other biomes, including those in the eastern region of southern Africa, as these ants prefer to nest in the soil. C4 plants are still dominant in the subtropical and tropical grasslands today. Alternatively, the divergence in some taxa may not have been triggered by vegetation changes but rather by vicariance events. For example, the Cape Fold Mountains are thought to be a physical barrier responsible for diversification between the western and the southern clades of the angulate tortoise, *Chersina angulata* (Daniels et al., 2007). It is therefore evident that not all taxa in southern Africa responded similarly to all historical processes. For example, some *Ocymyrmex* species (*O. cavatodorsatus*, *O.* sp. 2 and *O.* sp. 3) are restricted to sandy regions in Namibia, Kalahari and isolated parts of the Northern Cape (South Africa). These species demonstrate habitat specificity (i.e., niche specialization), which could explain the observed centres of endemism in these sandy regions. Other species (*O. celer, O. nitidulus, O. phraxus* and *O. robechii*) only occur in the eastern parts of the Afrotropical region (Ethiopia, Kenya, Mozambique, Somalia, Sudan, Tanzania and Uganda), and this fragmentation of the overall distribution of *Ocymymex* could have been triggered by vicariance events or driven by development of vicariant barriers such as provided by the Orange and Zambezi rivers (Matthee et al., 2002; Moore et al., 2007). Only a couple of species have a wide distribution (*O. fortior, O. flaviventris, O. resekhes* and *O. weitzeckeri*) that appears not to have been influenced by any of these factors.

The distribution of *Ocymyrmex* ants suggests that past climate oscillations, associated with aridity in the western parts of southern Africa (Schulze, 1965), may have influenced the current biogeographic patterns of this group in southern Africa. However, in addition to climate change and associated changes in vegetation, the Miocene was also characterized by tectonic plate uplifts that resulted in topographical change, in turn resulting in rainfall seasonality in the western part of southern Africa (Sepulchre et al., 2006). Seasonal rainfall, in particular, may have been an important driver of the high endemicity that is symbolic of the arid regions of southern Africa and has been hypothesized to underlie the disparate distribution of arid adapted taxa such as the Masarinae (Gess, 1992), Pycnostigminae (Figitidae) (Buffington and van Noort, 2007), *Spathioplites* (Braconidae, Doryctinae) (van Noort et al., 2021), and dung beetles (Scarabaeinae) (Davis and Scholtz, 2020).

In summary, our study delivers additional evidence to help understand historical factors that could have shaped distribution patterns of insect taxa present in the arid regions of southern Africa. For example, the distribution of some ant taxa here closely reflects borders between particular biomes, e.g., the ponerine ant, *Ophthalmopone hottentota*, and the sugar ant, *Camponotus storeatus*, which follow the borders of the Nama and Succulent Karoo biomes respectively (Robertson, 2000) and ants within the *Tetramorium solidum*-group, which are mainly restricted to dry semi-desert areas in southern Africa (Bolton, 1981; Mbanyana et al., 2018). Our study is timely, as no attempt has been made to date to unravel the history of arid-adapted ant diversification in southern Africa or to estimate the evolutionary relationships of these ants. In addition, this study provides a useful model to make conservation management recommendations for other ant species endemic to these dry regions (e.g., the *Tetramorium solidum* species-group). The predicted future climate change to increasing aridity in the region might favour future range expansion of some *Ocymyrmex* species to the subtropical regions.

5. Contribution statement

N.M., B.B.B., J.J.L.R., S.v.N., and T.C.W. conceptualized the study. N.M. and S.v.N. conducted fieldwork. N.M. and B.B.B. conducted bioinformatic analyses, phylogenomic analyses, dating analyses and biogeographic analyses. N.M. wrote the first draft and edited the final draft of the manuscript. B.B.B., J.J.L.R., S.v.N. and T.C.W. contributed substantially to the writing of the final draft. S.G.B. funded UCE extractions and contributed resources for UCE laboratory work and commented on the final draft. All authors approved submission of the final version of the manuscript.

6. Funding information

National Research Foundation – Thuthuka Grant: UID109916.
Peter Buck Fellowship - Smithsonian Institution.
National Science Foundation (DEB-1555905) awarded to SGB.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank Hamish Robertson, Phumezo Ndzunga and Tiffany Wynford for assisting with fieldwork; Zikhona Njeza, Mujahid Hector, Abusisiwe Ndaba, Lindiwe Khoza and Aisha Mayekiso for assisting with curation and databasing of samples; Caswell Munyai, Peter Hawkes and Francisco Hita Garcia for donating *Ocymyrmex* material for this study, and Michael Lloyd for assisting with DNA extractions and ultraconserved elements Library preparations. All the UCE laboratory work was conducted in and with the support of the Laboratories of Analytical Biology (L.A.B.) facilities of the National Museum of Natural History, Smithsonian Institution, Washington, United States. This research was supported by Iziko Museums of South Africa, South Africa; National Research Foundation, South Africa (Thuthuka grant (UID109916) awarded to NM); Stellenbosch University, South Africa; the National Science Foundation (DEB-1555905 awarded to SGB), and Peter Buck Fellowship at the Smithsonian Institution. We would also like

to thank the Editor and the two anonymous reviewers for their vaulable comments on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2023.107977.

References

AntWeb. Version 8.91.2. California Academy of Science, online at https://www.antweb.org. Accessed 29 May 2023.

Bauer, A.M., Lamb, T., 2005. Phylogenetic relationships of Southern African geckos in the *Pachydactylus* group (Squamata: Gekkonidae). Afr. J. Herpetol. 54, 105–129. https://doi.org/10.1080/21564574.2005.9635525.

Blaimer, B.B., Ward, P.S., Schultz, T.R., Fisher, B.L., Brady, S.G., 2018. Paleotropical diversification dominates the evolution of the hyper diverse ant tribe Crematogastrini (Hymenoptera: Formicidae). Insect Syst. Divers. 2 (5), 1–14. https://doi.org/10.1093/isd/ixy013.

Blumenstiel, B., Cibulskis, K., Fisher, S., DeFelice, M., Barry, A., Fennell, T., Abreu, J., Minie, B., Costello, M., Young, G., 2010. Target exon sequencing by in-solution hybrid selection. Curr. Protoc. Hum. 1–8 (Unit 18.4) https://doi.org/10.1002/0471142905.hg1804s66.

Bobe, R., 2006. The evolution of arid environments in eastern Africa. J. Arid Environ. 66, 564–584. https://doi.org/10.1016/j.jaridenv.2006.01.010.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. J. Bioinform. 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bolton, B., 1981. A revision of six minor genera of Myrmicinae (Hymenoptera: Formicidae) in the Ethiopian zoogeographical region. Bull. Nat. Hist. Mus. Entomol. Ser. 43, 245–307.

Bolton, B., Marsh, A., 1989. The Afrotropical thermophilic ant genus *Ocymyrmex* (Hymenoptera: Formicidae). J. Nat. Hist. 23, 1267–1308. https://doi.org/10.1080/00222938900770681.

Borowiec, M.L., 2019. Spruceup: fast and flexible identification, visualization, and removal of outliers from large multiple sequence alignments. J. Open Source Softw. 4, 1635. https://doi.org/10.21105/joss.01635.

Branstetter, M.G., Longino, J.T., Ward, P.S., Faircloth, B.C., 2017. Enriching the ant tree of life: Enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. Methods Ecol. Evol. 8 (6), 768–776. https://doi.org/10.1111/2041-210X.12742.

Branstetter, M.G., Müller, A., Griswold, T.L., Orr, M.C., Zhu, C.D., 2021. Ultraconserved element phylogenomics and biogeography of the agriculturally important mason bee subgenus *Osmia (Osmia*). Syst. Entomol. 46 (2), 453–472. https://doi.org/10.1111/syen.12470.

Buffington M.L., van Noort, S., 2007. A world revision of the Pycnostigminae (Cynipoidea: Figitidae) with descriptions of seven new species. Zootaxa 1392, 1–30. 10.11646/zootaxa.1392.1.1.

Burgess, N.D., Hales, J., Underwood, E., Dinerstein, E., Olson, D., Itoua, I., Schipper, J., Rickketts, T., Newman, K., 2004. Terrestrial eco-regions of africa and Madagascar: A conservation assessment. World Wildlife Fund (united States) 23.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Cowling, R.M., Proches, S., Partridge, T.C., 2009. Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. Mol. Phylogenet. Evol. 51, 64–74. https://doi.org/10.1016/j.ympev.2008.05.034.

Daniels, S.R., Hofmeyr, M.D., Henen, B.T., Crandall, K.A., 2007. Living with the genetic signature of Miocene induced change: Evidence from the phylogeographic structure of the endemic angulate tortoise *Chersina angulata*. Mol. Phylogenet. Evol. 45, 915–926. https://doi.org/10.1016/j.ympev.2007.08.010.

Davis, A.L.V., Scholtz, C.H., 2020. Dung beetle conservation biogeography in southern Africa: current challenges and potential effects of climatic change. Biodivers. Conserv. 29, 667–693. https://doi.org/10.1007/s10531-019-01904-7.

Dinerstein, E., Olson, D., Joshi, A., Vynne, C., Burgess, N.D., Wikramanayake, E., Hahn, N., Palminteri, S., Hedao, P., Noss, R., Hansen, M., Locke, H., Ellis, E.C., Jones, B., Barber, C.V., Hayes, R., Kormos, C., Martin, V., Crist, E., Sechrest, W., Price, L., Baillie, J.E.M., Weeden, D., Suckling, K., Davis, C., Sizer, N., Moore, R., Thau, D., Birch, T., Potapov, P., Turubanova, S., Tyukavina, A., de Souza, N., Pintea, L., Brito, J.C., Llewellyn, O.A., Miller, A.G., Patzelt, A., Ghazanfar, S.A., Timberlake, J., Klöser, H., Shennan-Farpón, Y., Kindt, R., Lillesø, J.P.B., van Breugel, P., Graudal, L., Voge, M., Al-Shammari, K.F., Saleem, M., 2017. An ecoregion-based approach to protecting half the terrestrial realm. J. Biosci. 67 (6), 534–545. https://doi.org/10.1093/biosci/bix014.

Douady, C.J., Catzeflis, F., Raman, J., Springer, M.S., Stanhope, M.J., 2003. The Sahara as a vicariant agent, and the role of Miocene climatic events, in the diversification of the mammalian order Macroscelidea (elephant shrews). PNAS 100 (14), 8325–8330. https://doi.org/10.1073/pnas.0832467100.

Engelbrecht, H.M., van Niekerk, A., Heideman, N.J.L., Daniels, S.R., 2013. Tracking the impact of Pliocene/Pleistocene Sea level and climatic oscillations on the cladogenesis of the Cape legless skink, Acontias meleagris species complex, in South Africa. J. Biogeogr. 40, 492–506. https://doi.org/10.1111/jbi.12024.

Engelbrecht, H.M., Branch, W.R., Tolley, K.A., 2021. Snakes on an African plain: the radiation of *Crotaphopeltis* and *Philothamnus* into open habitat (Serpentes: *Colubridae*). PeerJ 9, e11728.

Faircloth, B.C., Bransletter, M.G., White, N.D., Brady, S.G., 2015. Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. Mol. Ecol. Resour. 15, 489–501. https://doi.org/10.1111/1755-0998.12328.

Faircloth, B.C., Glenn, T.C., 2012. Not all sequence tags are created equal: designing and validating sequence identification tags robust to indels. PLoS One 7, e42543.

Faircloth, B.C., 2013. illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming. Available at: https://github.com/faircloth-lab/illumi processor.

Feakins, S.J., Demenocal, P.B., 2010. Global and African regional climate during the Cenozoic. Cenozoic Mammals of Africa 45–55.

Fisher, S., Barry, A., Abreu, J., Minie, B., Nolan, J., Delorey, T.M., Young, G., Fennell, T. J., Allen, A., Ambrogio, L., Berlin, A.M., Blumenstiel, B., Cibulskis, K., Friedrich, D., Johnson, R., Juhn, F., Reilly, B., Shammas, R., Stalker, J., Sykes, S.M., Thompson, J., Walsh, J., Zimmer, A., Zwirko, Z., Gabriel, S., Nicol, R., Nusbaum, C., 2011. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. Genome Biol. 12, R1. https://doi.org/10.1186/gb-2011-12-1-r1.

Gess, S.K., 1992. Biogeography of the masarine wasps (Hymenoptera: Vespidae: Masarinae), with particular emphasis on the southern African taxa and on correlations between masarine and forage plant distributions. J. Biogeogr. 19, 491–503. 10.2307/2845768.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652. https://doi.org/10.1038/nbt.1883.

Herron, M.D., Waterman, J.M., Parkinson, C.L., 2005. Phylogeny and historical biogeography of African ground squirrels: the role of climate change in the evolution of *Xerus*. Mol. Ecol. 14 (9), 2773–2788. https://doi.org/10.1111/j.1365-294X.2005.02630.x.

Hewitt, G.M., 2011. Quaternary phylogeography: the roots of hybrid zones. Genetica 135, 617–638. https://doi.org/10.1007/s10709-011-9547-3.

Janicki, J., Narula, N., Ziegler, M., Guénard, B., Economo, E.P., 2016. Visualizing and interacting with large-volume biodiversity data using client-server web-mapping applications: The design and implementation of antmaps.org. Ecol. Inform. 32, 185–193.

Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. Mol. Biol. Evol. 30 (4), 772–780. https://doi.org/10.1093/molbev/mst010.

Kürschner, W.M., Kvacek, Z., Dilcher, D.L., 2008. The impact of Miocene atmospheric carbon dioxide fluctuations on climate and the evolution of terrestrial ecosystems. PNAS 105, 449–453. https://doi.org/10.1073/pnas.0708588105.

Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. Syst. Biol. 62, 789–804. https://doi.org/10.1093/sysbio/syt040.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773. https://doi.org/10.1093/molbev/msw260.

Linder, H.P., 2003. The radiation of Cape flora, southern Africa. Biol. Rev. 78, 597–638. https://doi.org/10.1017/S1464793103006171.

Linder, H.P., 2005. Evolution of diversity: the Cape flora. Trends Plant Sci. 10, 536–541. https://doi.org/10.1016/j.tplants.2005.09.006.

Makokha, J.S., Bauer, A.M., Mayer, W., Matthee, C.A., 2007. Nuclear and mtDNA phylogenetic inferences among southern African sand lizards, *Pedioplanis* (Sauria: Lacertidae). Mol. Phylogenet. Evol. 44 (2), 622–633. https://doi.org/10.1016/j.ympev.2007.04.021.

Matthee, C.A., Flemming, A.F., 2002. Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in Southern Africa. Mol. Ecol. 11, 465–471. https://doi.org/10.1046/j.0962-1083.2001.01458.x.

Matzke, N.J., 2013. BioGeoBEARS: biogeography with Bayesian (and likelihood) evolutionary analysis in R scripts. University of California, Berkeley, CA.

Matzke, N.J., 2013. Probabilistic historical biogeography: New models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 5 (4), 242–248. https://doi.org/10.5811/westjem.2011.5.6700.

Matzke, N.J., 2018. BioGeoBEARS: BioGeography with Bayesian (and likelihood) Evolutionary Analysis with R Scripts. version 1.1.1, published on GitHub on November 6, 2018. 10.5281/zenodo.1478250.

Mbanyana, N., Hita, G.F., Robertson, H.G., Le Roux, J.J., 2018. A taxonomic revision of seed harvester ants of the *Tetramorium solidum*-group (Hymenoptera: Formicidae) in southern Africa. Eur. J. Taxon. 454, 1–59. https://doi.org/10.5852/ejt.2018.454.

McDonough, M.M., Šumbera, R., Mazoch, V., Ferguson, A.W., Phillips, C.D., Bryja, J., 2015. Multilocus phylogeography of a widespread savanna–woodland-adapted rodent reveals the influence of Pleistocene geomorphology and climate change in Africa's Zambezi region. Mol. Ecol. 24 (20), 5248–5266.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol. 37, 1530–1534. 10.1093/molbev/msaa015.

Moore, A.E., Cotterill, F., Main, M.P.L., Williams, H.B., 2007. The Zambezi River. In: Gupta, A. (Ed.), 1428 Large Rivers: Geomorphology and Management. Wiley, Chichester, pp. 311–332.

Neumann, F.H., Bamford, M.K., 2015. Shaping of modern southern African biomes: Neogene vegetation and climate changes. Trans. r. Soc. South Africa 70, 195–212. https://doi.org/10.1080/0035919X.2015.1072859.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300.

Nicholson, S., 2000. The nature of rainfall variability over African on time scales of decades to millennia. Glob. Planet. Change 26, 137–158.

Pickford, M., Senut, B., Mocke, H., Mourer-Chauviré, C., Rage, J.C., Mein, P., 2014. Eocene aridity in southwestern Africa: timing of onset and biological consequences. *T*Rans. r. Soc. South Africa 26 (3), 139–144. https://doi.org/10.1080/0035919X.2014.933452.

Prendini, L., 2005. Scorpion Diversity and Distribution in Southern Africa: Pattern and Process. In: African Biodiversity. *Springer, Boston, MA*. 10.1007/0-387-24320-8_2.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. syy032 https://doi.org/10.1093/sysbio/syy032.

Raphalo, E.M., Cole, M.L., Daniels, S.R., 2021. Climatic oscillations during the Mio/Pliocene epochs induced cladogenesis in the terrestrial snail genus *Gittenedouardia* (Mollusca: Gastropoda: Cerastidae) from South Africa. Mol. Phylogenet. Evol. 155, 107000 https://doi.org/10.1016/j.ympev.2020.107000.

Ree, R.H., 2005. Detecting the historical signature of key innovations using stochastic models of character evolution and cladogenesis. Evolution 59 (2), 257–265. https://doi.org/10.1111/j.0014-3820.2005.tb00986.x.

Ree, R.H., Sanmartín, I., 2018. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. J. Biogeogr. 45 (4), 741–749. https://doi.org/10.1111/jbi.13173.

Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14. https://doi.org/10.1080/10635150701883881.

Robertson, H.G., 2000. Afrotropical ants (Hymenoptera: Formicidae): taxonomic progress and estimation of species richness. J. Hymenopt. Res. 9, 71–84.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203. https://doi.org/10.1093/sysbio/46.1.195.

Rossouw, L., Stynder, D.D., Haarhof, P., 2009. Evidence for opal phytolith preservation in the Langebaanweg 'E' Quarry Varswater Formation and its potential for palaeohabitat reconstruction. S. Afr. J. Sci. 105, 223–227. https://doi.org/10.4102/sajs.v105i5/6.95.

Schulze, B.R., 1965. Climate of South Africa. Part 8, General survey = Klimaat van Suid-Afrika. Deel 8, Algemene oorsig. Weather Bureau, [Pretoria].

Senut, B., Pickford, M., Ségalen, L., 2009. Neogene Desertification of Africa. CR GEOSCI 346 (8–9), 591–602. https://doi.org/10.1016/j.crte.2009.03.008.

Sepulchre, P., Ramstein, G., Fluteau, F., Schuster, M., Tiercelin, J.J., Brunet, M., 2006. Tectonic Uplift and Eastern Africa Aridification. Science 313 (5792), 1419–1423. https://doi.org/10.1126/science.1129158.

Specify Collections Consortium | Software for Biological Collections and Samples. http://specifysoftware.org/.

Svenning, J.C., Eiserhardt, W.L., Normand, S., Ordonez, A., Sandel, B., 2015. The Influence of Paleoclimate on Present-Day Patterns in Biodiversity and Ecosystems. Annu. Rev. Ecol. Evol. Syst. 46, 551–572. https://doi.org/10.1146/annurev-ecolsys-112414-054314.

Swart, B.L., Tolley, K.A., Matthee, C.A., 2009. Climate change drives speciation in the southern rock agama (*Agama atra*) in the Cape Floristic Region, South Africa. J. Biogeogr. 36 (1), 78–87. https://doi.org/10.1111/j.1365-2699.2008.01988.x.

Tagliacollo, V.A., Lanfear, R., 2018. Estimating improved partitioning schemes for ultraconserved elements. Mol. Biol. Evol. 35, 1798–1811. https://doi.org/10.1093/molbev/msy069.

Tankard, A.J., Rogers, J., 1978. Late Cenozoic palaeoenvironments on the west coast of Southern Africa. J. Biogeogr. 5, 319–337. https://doi.org/10.2307/3038026.

Tolley, K.A., Chase, B.M., Forest, F., 2008. Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. J. Biogeogr. 35, 1402–1414. https://doi.org/10.1111/j.1365-2699.2008.01889.x.

Udeze, C., Oboh-Ikuenobe, F.E., 2005. Neogene palaeoceanographic and palaeoclimatic events inferred from palynological data: Cape Basin off South Africa, ODP Leg 175. PANGAEA, 10.1594/PANGAEA.694040, Supplement to: Udeze, C; Oboh-Ikuenobe, FE (2005): Neogene palaeoceanographic and palaeoclimatic events inferred from palynological data: Cape Basin off South Africa, ODP Leg 175. Palaeogeogr. Palaeoclimatol. Palaeoecol. 219(3–4), 199–223. 10.1016/j.palaeo.2004.12.026.

van Noort, S., Belokobylskij, S.A, Touret-Alby, A., 2021. Rediscovery of the endemic Afrotropical genus Spathioplites (Hymenoptera, Braconidae, Doryctinae) with major range extension records for *Spathioplites phreneticus*. Afr. Invertebr. 62, 497–520. 10.3897/AfrInvertebr.62.74103.

Voelker, G., Bowie, R.C.K., Wilson, B., Anderson, C., 2012. Phylogenetic relationships and speciation patterns in an African savannah dwelling bird genus (*Myrmecocichla*). Biol. J. Linn. 106, 180–190. https://doi.org/10.1111/j.1095-8312.2012.01856.x.

Yang, Z., 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol. Biol. Evolut' 24, 1586–1591. https://doi.org/10.1093/molbev/msm088.

Zhang, C., Rabiee, M., Sayyari, E., Mirarab, S., 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinform. 19, 153. https://doi.org/10.1186/s12859-018-2129-y.

Zhao, Z., Heideman, N., Bester, P., Jordaan, A., Hofmeyr, M.D., 2020. Climatic and topographic changes since the Miocene influenced the diversification and biogeography of the tent tortoise (*Psammobates tentorius*) species complex in Southern Africa. BMC Evol. Biol. 20 (153), 1471–2148. https://doi.org/10.1186/s12862-020-01717-1.