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day microallopatric speciation patterns, we performed ancestral character reconstruction analyses. These examined *Macroderes*' origins, environmental adaptations (semi-arid vs. moist habitats), and the emergence of flightlessness as an adaptive trait, specifically investigating whether the genus descended from volant or flightless ancestors. Bayesian diversification models evaluated diversification dynamics.

Results: Our findings support *Macroderes*' monophyly, with its most recent common ancestor (MRCA) originating in the late Miocene (~8.44 mya; 95% CI: 9.74–7.06 mya). The MRCA was flightless and associated with a semi-arid environment resembling the present-day Succulent Karoo biome. Subsequent diversification involved multiple southward colonisation events into Fynbos, indicating repeated environmental transitions.

Main Conclusions: The evolutionary history of *Macroderes* highlights how aridification and flightlessness have shaped its diversification. Flightlessness in *Macroderes* is closely linked to adaptation to semi-arid environments, restricting dispersal and contributing to the genus's short-range endemism. Repeated southward movement highlights the dynamic evolutionary history of *Macroderes* and the significant role of environmental transitions in shaping its lineage. Our findings highlight the role of historical climatic changes in driving speciation within flightless dung beetles.

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1 | Introduction

Microallopatric speciation is an intriguing evolutionary phenomenon in which new species originate from geographically isolated (sub)populations on a very small scale—as in classic allopatric speciation, there is no migration of individuals or gene flow between the diverging (sub) populations (Mayr 1942; Gavrillets 2003; Fitzpatrick et al. 2008). This form of speciation has been observed in various organisms, including cichlid fish in Lake Malawi, where populations separated by habitat discontinuities as small as 35 m have diverged into distinct species (Rico and Turner 2002). Another notable example involves weevils on the island of Rapa and its surrounding islets, where species have speciated microallopatrically (Paulay 1985). Additionally, a sympatrically distributed triplet of diving beetle species (*Paroster*) in Australia's Yilgarn region has likely speciated microallopatrically within a 3.5 km² area (Guzik et al. 2009).

To date, the only documented cases of microallopatric speciation in dung beetles (Scarabaeidae: Scarabaeinae) have been recorded in mountain rainforests—*Grebennikovius* in Tanzania's Eastern Arc Mountains (Montanaro et al. 2024) and *Temnoplectron* in the Wet Tropics of Australia (Bell et al. 2004, 2007). However, to the best of our knowledge, no cases of microallopatric speciation in dung beetles have been recorded from semi-arid environments. Our study on the flightless dung beetle genus *Macroderes* from southwestern South Africa offers a rare and powerful lens into the evolutionary processes driving microallopatric speciation in semi-arid environments—an ecological context where such patterns have remained largely undocumented. This research goes beyond adding another lineage to the phylogenetic tree; it challenges conventional narratives about speciation, adaptation and survival in marginal habitats. By revealing how flightlessness and environmental transitions have shaped diversification, *Macroderes* emerges as a model system for understanding evolutionary dynamics in flightless taxa. These insights not only inform conservation strategies for endemic species but also enrich our grasp of biodiversity evolution in one of the planet's most ecologically distinctive regions.

All species of the dung beetle genus *Macroderes* lack a humeral umbone, a trait associated with aptery and flightlessness, which may explain their restricted distribution (Frolov and Scholtz 2004; Abdalla et al. 2018; Davis et al. 2020). Their biology remains largely unknown; while they are presumed to exhibit tunnelling behaviour, their nesting habits have not been documented. Field observations in Grootbos Nature Reserve, Western Cape Province, South Africa, indicate that some species are attracted to cattle and human dung, as well as carrion, and have been collected using dung-baited traps set in the late afternoon and retrieved the following morning (GM Daniel, personal observation). This suggests a primarily nocturnal foraging pattern. However, diurnal activity has also been observed under particularly cold conditions, leaving their dominant diel activity pattern uncertain (Davis et al. 2020).

The genus *Macroderes* was first proposed by Westwood (1842) with *Onthophagus greeni* as its type species. Subsequent contributions by various authors, including De Borre (1880); Harold (1877); Sharp (1880); Kolbe (1908) and Péringuey (1901), expanded the genus with new species and taxonomic keys.

Janssens (1939) later described *Macroderes arrowi* and provided a comprehensive identification key to species. The first major revision was conducted by Frolov and Scholtz (2004), adding seven new species, synonymising two and designating a neotype for *Macroderes bias*. The most recent revision by Abdalla et al. (2018) covered all known species and described seven new ones, increasing the total number of known valid species to 21.

The genus *Macroderes* is particularly interesting as an evolutionary study model, as it is endemic to the Greater Cape Floristic Region (GCFR) of South Africa (Figure 1), which encompasses the Fynbos and Succulent Karoo biomes (Frolov and Scholtz 2004; Abdalla et al. 2018; Davis et al. 2020). The Fynbos biome, located at the southern tip of Africa, is bordered by the Atlantic Ocean to the west and the Indian Ocean to the southeast. Inland, the Cape Fold Mountains separate it from the Nama-Karoo Biome, while the Albany Thicket Biome lies to the east, and Namaqualand, a region of the Succulent Karoo, lies to the northwest. The Fynbos biome is renowned for its rich diversity of plants and associated fauna, the majority of which are endemic (Neumann and Bamford 2015). Similarly, the Succulent Karoo is famous for its extraordinary plant diversity, including stunning carpets of daisy flowers in spring, dwarf succulent shrubs, euphorbs, stone plants and a variety of geophytes from the iris, orchid and hyacinth families (Mucina et al. 2006; Ellis et al. 2014; Neumann and Bamford 2015). The GCFR harbours a globally unique and exceptionally diverse biota. The spectacular evolutionary radiations of multiple lineages within this relatively small region make the GCFR an ideal natural laboratory for studying the mechanisms driving biotic diversification and the processes underlying these patterns of diversification (for more details, see Linder 2003; Born et al. 2007; Ellis et al. 2014; Samways et al. 2024).

The remarkable level of endemism in the biota of the GCFR is the result of a dynamic interplay between geological events and climate changes that shaped the landscape throughout the late Cenozoic (Linder 2003, 2008; Cowling et al. 2009; Ellis et al. 2014). These environmental shifts triggered dramatic transformations in vegetation and associated fauna, ultimately contributing to the region's extraordinary biodiversity. Although the geological history of southern Africa is complex, there is a broad consensus among geologists that the region experienced two significant uplift events. The first uplift, occurring around 20 million years ago (mya), affected the eastern part of southern Africa, giving rise to the central southern African plateau, which includes prominent features such as the Drakensberg, Highveld and Upper Karoo (King 1944; Partridge and Maud 1987; Paul et al. 2014; Dauteuil et al. 2015; Rudge et al. 2015). This was followed by a second uplift during the Pliocene, between 2 and 5 mya, concentrated in the southeastern region. This latter uplift is believed to have played a crucial role in isolating the interior plateau, a process that accelerated the aridification of the subcontinent (Partridge and Maud 1987; Haddon and McCarthy 2005; Dauteuil et al. 2015).

In parallel with these geological shifts, the region experienced significant climatic transformations that profoundly influenced its ecosystems. One of the most notable trends was the transition following the Mid-Miocene Climatic Optimum (about 17–14.5 mya), during which environmental conditions

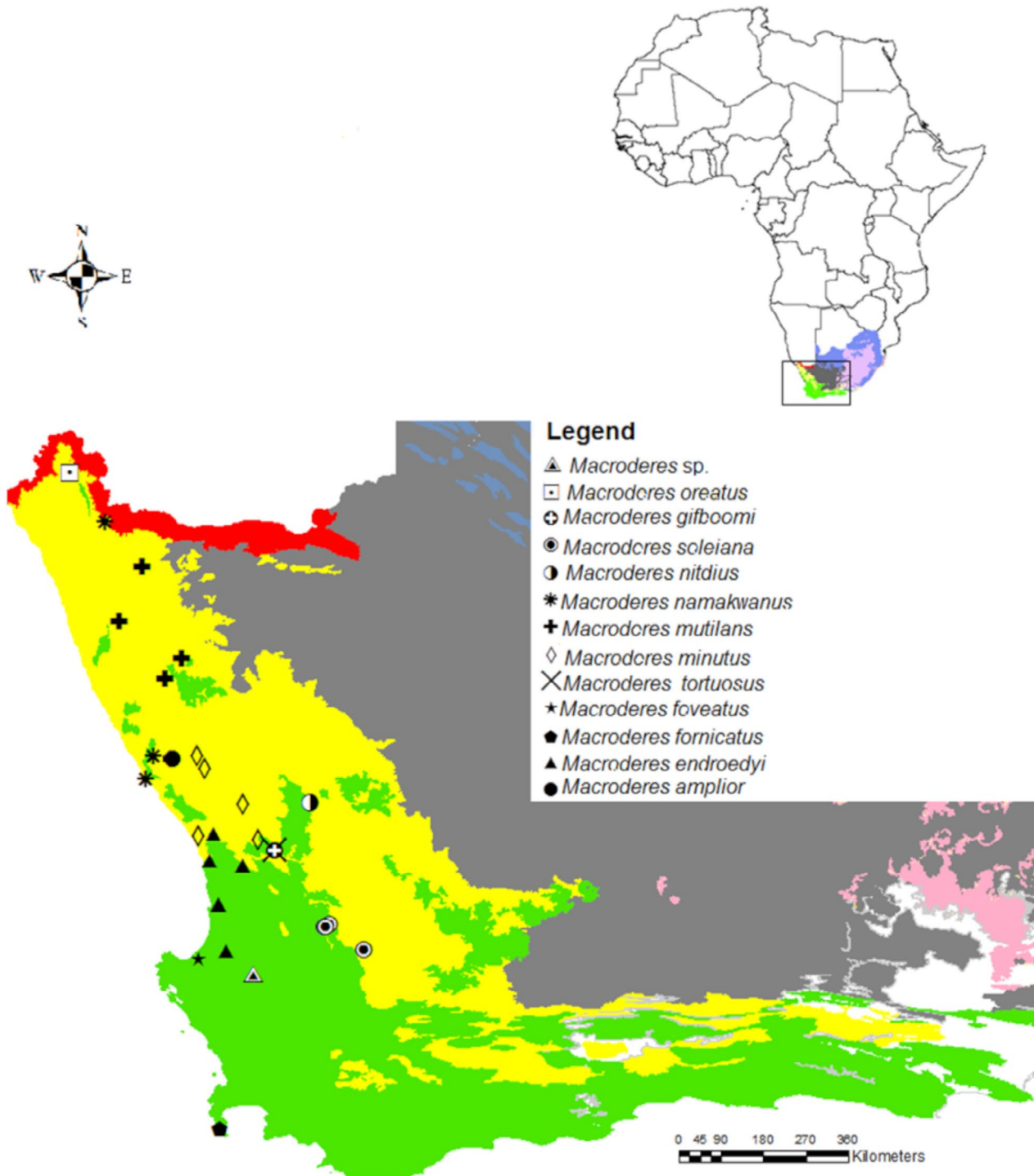


FIGURE 1 | Southwestern Africa map illustrating the distribution patterns of *Macroderes* species observed in the present study. *Macroderes* sp. (= *Macroderes* sp. pikterberg), while *Macroderes mutilans* (= *Macroderes mutilans* in the south and *Macroderes* aff. *mutilans* in the north).

shifted from warm and moist to cooler and drier (Tyson 1991; Demenocal 1995, 2004; Zachos et al. 2001; Hansen et al. 2008). By the late Miocene–Pliocene transition, the southwestern Cape had developed a winter rainfall climate (Deacon 1983; Sciscio et al. 2016), while the west coast of southern Africa became increasingly dry due to the cooling effects of the Benguela Current (Tyson 1986, 1991; Chase and Meadows 2007; Chase et al. 2015). These shifts in precipitation patterns—from a predominantly summer to a winter rainfall regime—along with increasing aridification, had a significant impact on the Fynbos

and Succulent Karoo biomes, where our study model, the dung beetle genus *Macroderes*, is endemic. Additionally, these climatic changes played a crucial role in shaping vegetation dynamics in the southwestern Cape, transitioning from earlier wooded subtropical elements to the development of modern succulents and fynbos elements respectively (Neumann and Bamford 2015).

While a few studies have examined the impact of late Cenozoic environmental changes on the diversification of dung beetles

in southern Africa—for example, *Pachysoma* (Sole et al. 2005), *Circellium* (Sole and Scholtz 2013), *Sisyphus* (Daniel, Sole, et al. 2020a; Daniel, Davis, et al. 2020b) and *Epirinus* (Daniel et al. 2021)—our broader understanding of how this region's remarkable dung beetle diversity, comprising approximately 1000 species, was shaped, remains largely unexplored. Each dung beetle genus or lineage undoubtedly has its own unique evolutionary history, yet many of these histories remain to be investigated. In this study, we address this knowledge gap by exploring the evolutionary history of the genus *Macroderes* using molecular data. Specifically, we aim to answer the following research questions: (a) Is *Macroderes* a monophyletic lineage? (b) When and where did the genus originate? (c) When did flight loss evolve within the genus? (d) How did late Cenozoic climatic changes in southwestern Africa influence the observed patterns of microallopatric speciation? To address these questions, two hypotheses are summarised as follows: (a) *Macroderes* constitutes a monophyletic lineage that originated in the late to middle Miocene within the region now comprising the Fynbos and Succulent biomes, where the genus is currently endemic. (b) We predict that the genus's ancestor was adapted to semi-arid conditions and that the intensification of aridification in the Mio-Pliocene of southwestern Africa triggered the evolution of flightlessness—this could explain the present-day pattern of short-range endemism observed in *Macroderes* species.

2 | Material and Methods

2.1 | Taxon Sampling

Fifteen species of the genus *Macroderes* were used as ingroup: *Macroderes amplior*, *M. arrowi*, *M. endroedyi*, *M. fornicatus*, *M. foveatus*, *M. gifboomi*, *M. minutus*, *M. namakwanus*, *M. nitidus*, *M. oreatus*, *M. soleiana*, *M. tortuosus*, *M. mutilans*, *M. aff. mutilans* and *Macroderes* sp. Piketberg—the latter two species are possibly undescribed. Ten species were sequenced for the first time, and the others were retrieved from GenBank (Data S1 available in the following link: <https://doi.org/10.5061/dryad.j0zpc86v1>). In order to test the hypothesis of monophyly of an African genus like *Macroderes* and also to infer its hypothesised biogeographical history, we also included several other African dung beetle taxa in the phylogeny, some of which are hypothesised to be closely related to *Macroderes* (Sole et al. 2013; Mlambo et al. 2015; Tarasov and Génier 2015; Tarasov and Dimitrov 2016) as follows: *Aliuscanthoniola similaris*, *Anonychonitis freyi*, *Aphengoecus multiserratus*, *Caccobius* sp., *Coptorhina klugii*, *Cyptochirus ambiguus*, *Delopleurus pullus*, *Dicranocara deschodti*, *Digitonthophagus gazella*, *Dwesasilvasedis medinae*, *Elassocanthon ovalis*, *Epirinus aquilus*, *Epirinus flagellatus*, *Frankenbergerius armatus*, *Hammondantus psammophilus*, *Hyalonthophagus alcyonides*, *Namakwanus davisii*, *Odontoloma pusillum*, *Onthophagus interstitialis*, *Pachysoma hippocrates*, *Phalops* sp1, *Phalops* sp2, *Proagoderus aciculatus*, *Pycnopanelus krikkeni*, *Sarophorus* sp., *Scarabaeolus* sp., *Sceliages* sp., *Silvaphilus oubosiensis* and *Xinidium dewitzi* (Data S2 available in the following link: <https://doi.org/10.5061/dryad.j0zpc86v1>).

3 | DNA Extraction, Amplification, Processing and Alignment of Sequences

Specimens were collected in the field and directly preserved in absolute ethanol and kept in the freezer (−20°C) until use. Total genomic DNA was extracted from the muscle tissue of a single hind leg of alcohol-preserved specimens and two legs from dried specimens by using the Macherey Nagel (NucleoSpin Tissue) extraction kit. Four gene regions were selected to construct the phylogenetic relationships and address our proposed research questions. Partial sequences of two ribosomal genes, 16S rDNA, and a portion of the nuclear rRNA large subunit 28S domain 2 and two protein-coding genes: the mitochondrial Cytochrome Oxidase subunit I (COI) and carbamoyl-phosphate synthetase-aspartate-transcarbamoylase-dihydroorotase (CAD). The oligonucleotide primers used to amplify the four gene regions are summarised in Table S1. For details for PCR amplification as well as extraction, amplification and sequencing protocols, see Sole et al. (2013) and Table S2. Sequence chromatograms were first visualised and edited in Chromas (Version 2.0), and then the forward and reverse sequences were assembled in CLC BIO Main Workbench version 6 (<http://www.clcbio.com>). Sequence alignment was implemented using default settings of the online MAFFT (Katoh and Toh 2008). All sequences were deposited in GenBank (Data S1, S2).

3.1 | Partitioning

We partitioned our datasets using partitionfinder v.2.1 (Lanfear et al. 2016). The appropriate model selection and partitioning (Table S2) were determined under the corrected Akaike information criterion. According to Kainer and Lanfear (2015), partitioning involves two steps: (i) defining groups of sites that are assumed to have evolved in similar ways; and (ii) choosing an appropriate model of molecular evolution for each group of sites. The first step in partitioning involves the assignment of each site in an alignment to a data block. Data blocks are user-defined sets of sites, typically encompassing distinct DNA features such as genes, introns, exons and codon positions. We identified codon positions first, second and third for the two protein-coding genes (COI and CAD). The noncoding genes (16S and 28S) are regarded as a single data block (Kainer and Lanfear 2015).

3.2 | Maximum Likelihood

Maximum likelihood (ML) analyses were performed on each gene individually and on the total concatenated dataset. All ML analyses were implemented in RAxML v8.2.4 (Stamatakis 2014). As RAxML allows only a single model of rate heterogeneity in partition analyses, we implement the general time reversible (GTR) model of nucleotide substitution under the GAMMA model of rate heterogeneity (Stamatakis 2014). Nodal support confidence of the majority-rule consensus tree topology was estimated from 1000 nonparametric bootstrap replicates of likelihood (Felsenstein 1981, 1985).

3.3 | Bayesian Inference

Individual gene and concatenated phylogenies were estimated using Bayesian inference (BI) in **MrBayes** v3.2.6 (Ronquist and Huelsenbeck 2003). To determine the best-fit partitioning schemes and models of evolution, we used **PartitionFinder** (Table S2). Flat Dirichlet priors were applied in all analyses. Bayesian analyses were performed using two independent Metropolis-coupled Markov Chain Monte Carlo (MCMC) runs for 30 million iterations, with trees sampled every 200th iteration. The first 25% of sampled trees were discarded as burn-in to ensure proper convergence, which was assessed using **Tracer** v1.6 (Rambaut et al. 2014). Clade support was evaluated based on posterior probability (PP) estimates. The resulting tree topologies from both maximum likelihood (ML) and Bayesian inference (BI) analyses were visualised in **FigTree** v1.4.3 (Rambaut 2009).

3.4 | Divergence Time Estimation

The origin and radiation of the genus *Macroderes* were estimated using BEAST v2.4.5 (Bouckaert et al. 2014). Since there are no known fossil records for *Macroderes* or any phylogenetically informative fossils within Scarabaeinae (Tarasov et al. 2016), calibrating absolute divergence times is challenging. In such cases, two common approaches can be used: (a) inferring divergence times by applying a substitution rate estimated from studies of closely related taxa (Ho 2007; Weir and Schluter 2008); and (b) using secondary calibrations based on previous molecular dating of fossil relatives or the estimated age of the earliest reliable fossil within the target group (Shaul and Graur 2002). However, it is widely recognised that the latter approach tends to yield younger age estimates (Sauquet et al. 2012; Schenk 2016).

For this study, we utilised a secondary calibration point derived from a recent and comprehensive phylogenomic study of Coleoptera (McKenna et al. 2019), as it was also employed in a recent biogeographical dung beetle study (Rossini et al. 2022). Specifically, we selected the most recent common ancestor (MRCA) of *Copris* and *Onthophagus*, estimated at 59.5 mya. The MRCA prior was modelled with a normal distribution, with a mean equal to the divergence estimate and a standard deviation of 1, ensuring that the priors remained concentrated around the inferred ages. Otherwise, we followed the default settings of BEAST v2.4.5.

TABLE 1 | Summary statistics of biogeographic model testing in 'BioGeoBEARS', in order to infer the most likely ancestral area occupied by *Macroderes*. The best-fitting model is highlighted in bold. Number of free parameters (num params); values of parameters of dispersal (d), extinction (e), founder effect (j), likelihood scores (lnL) and Akaike information criterion (AIC) are detailed.

Models	LnL	Num params	d	e	j	AICc	AICc_wt
DEC	-95.42	2	0.0054	1.00E-12	0	195.1	0.0013
DEC+J	-88.51	3	0.0037	1.00E-12	0.058	183.6	0.41
DIVALIKE	-100	2	0.007	1.00E-12	0	204.3	1.30E-05
DIVALIKE+J	-88.17	3	0.0043	1.00E-12	0.051	182.9	0.58
BAYAREALIKE	-112.8	2	0.014	0.038	0	229.9	3.80E-11
BAYAREALIKE+J	-92.94	3	0.0033	1.00E-07	0.07	192.5	0.0049

Two independent MCMC analyses were run for 30 million generations, with parameters sampled every 200th generation. The first 25% of trees sampled in each run were discarded as burn-in. The program LogCombiner (BEAST 2 package) was used to merge the log and tree output files from the two independent runs. Tracer v1.6 was employed to assess convergence between runs. TreeAnnotator (BEAST 2 package) was used to generate the consensus tree and determine the mean ages under the 95% highest posterior density (HPD). The tree topology was visualised in FigTree v1.4.3.

3.5 | Biogeographical Analysis

We conducted an ancestral range reconstruction for *Macroderes* in southern Africa using the R package **BioGeoBEARS** (Matzke 2013; R Core Team 2025). Three models of biogeographic inference were considered: dispersal-extinction-cladogenesis (DEC) (Ree and Smith 2008), a maximum likelihood (ML) version of dispersal-vicariance analysis (DIVALIKE) (Ronquist 1997) and a likelihood-based interpretation of Bayesian biogeographic inference (BAYAREALIKE) (Landis et al. 2013). For each model, we repeated the analysis with a founder-event speciation parameter (+J) (Matzke 2014). To determine the best-fitting biogeographic model, we evaluated six model combinations (Table 1) based on Akaike information criterion (AIC) scores and Akaike weights. All analyses were conducted using the following South African biomes (Mucina et al. 2006; Figure 5): (A) Succulent Karoo, (B) Fynbos, (C) Savanna, (D) Grassland and (E) Forest. Distribution data for *Macroderes* and other dung beetle species included in this study were sourced from the following taxonomic studies: Frolov and Scholtz (2004), Abdalla et al. (2018) and Davis et al. (2020).

3.6 | Diversification Analyses

Diversification analyses were performed using an ultrametric tree topology with branch lengths scaled to time, including only *Macroderes* species. A lineage-through-time (LTT) plot (Nee et al. 1995) was generated using the R package **APE** (Paradis et al. 2004; R Core Team 2025). We also used the compound Poisson process on mass extinction times (CoMET), as implemented in the R package **TESS** (Höhna et al. 2016; May et al. 2016; R Core Team 2025), to estimate speciation and extinction rates over time. The compound Poisson process enables the detection of shifts in speciation or extinction rates that may be associated with climatic changes, particularly the intensification of aridification in southwestern

Africa during the transition between the Miocene and Pliocene. A constrained MCMC analysis was conducted, in which the mean speciation rate (λ), mean extinction rate (μ) and their standard deviations were initially set to zero. This approach generated posterior distributions of these hyperpriors (May et al. 2016), which were subsequently used in a full Bayesian analysis. The prior on the number of mass extinction events was set to 1.0. Reversible-jump MCMC chains were run for 1 million generations, with a burn-in of 25% and a thinning interval of 100.

3.7 | Ancestral Character Reconstruction

To reconstruct the historical biogeography of the genus *Macroderes* and infer whether its most recent common ancestor was associated with arid or moist environments, we categorised species into two humidity preferences: moist or semi-arid. This classification was based on distribution and annual rainfall dung beetle data from Davis et al. (2020), where species inhabiting regions with annual rainfall below 200 mm were classified as semi-arid-associated, while those experiencing over 200 mm of rainfall were considered moist-associated. Generally, species associated with the Succulent Karoo biome were treated as adapted to semi-arid environments, whereas those from the Fynbos biome were regarded as moisture-associated.

Ancestral character states were reconstructed using the R packages Phytools (version 2.0.3) and Geiger, following the guidelines of Revell and Harmon (2022). The phylogenetic and trait data were imported, with the phylogenetic tree loaded as a tree file and trait data as a CSV file containing two categorical traits: moisture preference (moist or semi-arid). For ancestral state reconstruction of a discrete trait, selecting an appropriate model is essential. For a two-state character, four models are possible: an equal-rates (ER) model, an all-rates-different (ARD) model and two irreversible models—one allowing only 0–1 transitions (but not the reverse, e.g., semi-arid \rightarrow moist) and another permitting only 1–0 transitions (e.g., moist \rightarrow semi-arid).

We applied the fitDiscrete function to compare these models; once the best-fitting model was identified (ER model, see Tables S3 and S4), we performed stochastic character mapping with 1000 simulations under the ER model to visualise the posterior probability of each state across the phylogeny. The densityMap function generated density maps, with states visualised in red and blue (for details, see Revell and Harmon 2022). Additionally, we used the same approach to reconstruct ancestral character states for flightlessness, testing whether the most recent common ancestor of *Macroderes* species was flightless or volant. The only modifications applied were to the two irreversible models: one permitting only 0–1 transitions (but not the reverse, e.g., fly \rightarrow flightless) and another allowing only 1–0 transitions (e.g., flightless \rightarrow fly).

4 | Results

4.1 | Phylogenetic Relationships

The combined datasets for four gene regions comprise a total of 2441 bp (base pairs); COI = 836 bp; CAD = 871 bp; 28S rDNA domain 2 (D2) \approx 465 bp; 16S \approx 269 bp. The genus *Macroderes*

constitutes a monophyletic group with high posterior probability (PP = 1.0) and Maximum Likelihood bootstrap support (MLB = 100%). Within the subfamily Scarabaeinae, *Macroderes* is recovered as a sister group to a clade comprising members of the tribes Onthophagini and Oniticellini (*Caccobius*, *Hyalonthophagus*, *Onthophagus*, *Proagoderus*, *Cyptochirus*, *Phalops*, *Digitonthophagus*) along with Onitini (*Anonychonitis*) and *Pycnopanelus*, currently not placed in a recognised tribe (Figure 2).

Meanwhile, the phylogenetic relationships within the genus suggest that *Macroderes* is subdivided into two major clades (Clade A and Clade B; PP = 1.0; MLB = 100%). Clade A includes two species: *Macroderes minutus* and *M. oreatus* (PP = 1.0; MLB = 100%). Clade B comprises the rest of the remaining species, which are further divided into the following subclades: subclade B1 (*Macroderes* sp. *Piketberg* + *M. fornicatus*; PP = 0.8; MLB = 90%), subclade B2 (*M. nitidus* + *M. aff. mutilans* + *M. mutilans*; PP = 0.9; MLB = 79%), subclade B3 (*M. gifboomii*), subclade B4 (*M. tortuosus*), subclade B5 (*M. foveatus* + *M. arrowi* + *M. amplior* + *M. namakwanus*; PP = 1.0; MLB = 100%) and subclade B6 (*M. endroedyi* + *M. soleiana*; PP = 0.9; MLB = 79%) (Figure 2).

4.2 | Divergence Time Estimates

The BEAST chronogram is largely congruent with those resulting from Bayesian and Maximum Likelihood inferences for the concatenated molecular dataset. Our analysis suggests that the genus *Macroderes* originated at the end of the Miocene (8.44 mya; 95% confidence interval (CI): 9.74–7.06 mya), followed soon after by bifurcation into clades (A and B), and that diversification and speciation within these clades took place in the Plio-Pleistocene (Figure 3).

4.3 | Historical Biogeography

Among the six biogeographic models used to reconstruct the ancestral range of the genus *Macroderes*, BioGeoBEARS identified DIVALIKE+J as the best-fit model (lnL = -88.17, AICc = 182.9; Table 1, Figure 4). Models incorporating founder-event speciation (J) performed significantly better than those without this parameter (Table 1). The inclusion of founder-event speciation reduced estimated dispersal (d) and extinction (e) rates while increasing log-likelihood (Table 1). According to the DIVALIKE+J model, the MRCA of *Macroderes* likely originated in a region resembling the present-day Succulent Karoo biome, and later a few elements of the group dispersed into Fynbos (Figure 4). While our ancestral state reconstruction analyses suggest that the MRCA of *Macroderes* was adapted to a semi-arid environment, subsequently, during the Plio-Pleistocene, multiple independent dispersal events led various lineages southward from the Karoo into the Fynbos, where they adapted to the moist conditions characteristic of the region (Figure 7).

4.4 | Patterns of Diversification

To conduct the lineage-through-time (LTT) analysis, we calculated the gamma (γ) statistic and its significance (Pybus

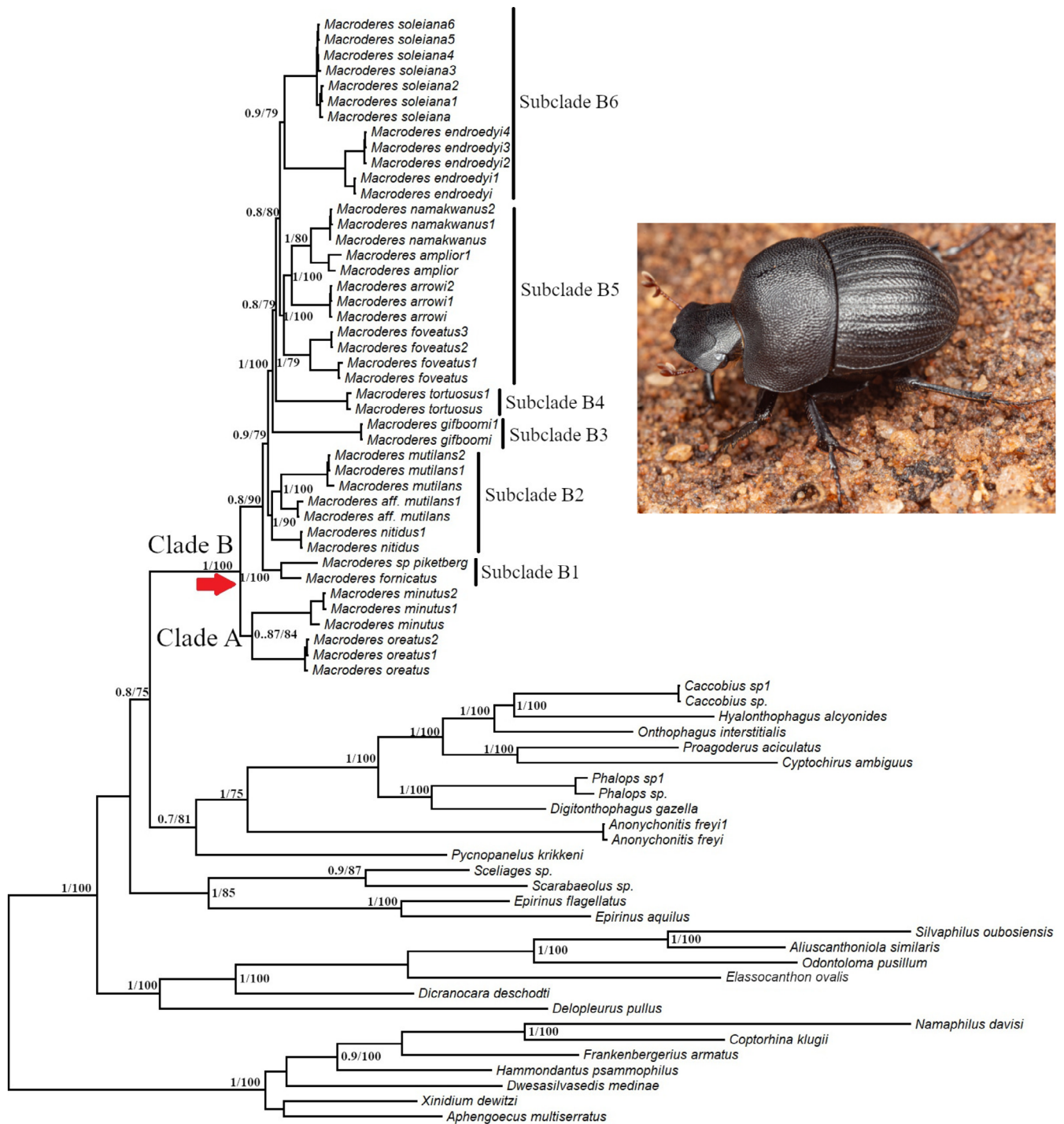


FIGURE 2 | Bayesian phylogram resulting from the combined molecular dataset analysis (COI, 16S, CAD and 28S). This analysis was conducted to infer the phylogenetic relationships within the genus *Macroderes*, with support nodes represented by Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (MLB). On the side, *Macroderes gifboomi*, representing the genus, thrives in its natural habitat. Photograph courtesy of Hennie de Klerk.

and Harvey 2000) as an estimate of the change in diversification rate over the phylogeny. These values are: -3.144589 ($p = 0.001663201$), respectively. During the late Miocene (approximately 8–5 mya), the lineage accumulation rate may have been relatively slow, reflecting limited diversification as the genus established itself in the region. A further noticeable increase in lineage accumulation occurred during the Plio-Pleistocene (5–2 mya), where the slope changes dramatically. The curve flattens

towards the present day, suggesting a recent stabilisation of diversity (Figure 5D).

We also estimated the speciation (λ) and extinction (μ) rates, as well as the net diversification rate ($\lambda - \mu$) in order to provide insights into the diversification dynamics of *Macroderes*. These were as follows: $\lambda = 0.3067$; $\mu \approx 0.0000001$; $\lambda - \mu = 0.3067$. A relatively high value of 0.3067 indicates active speciation. While

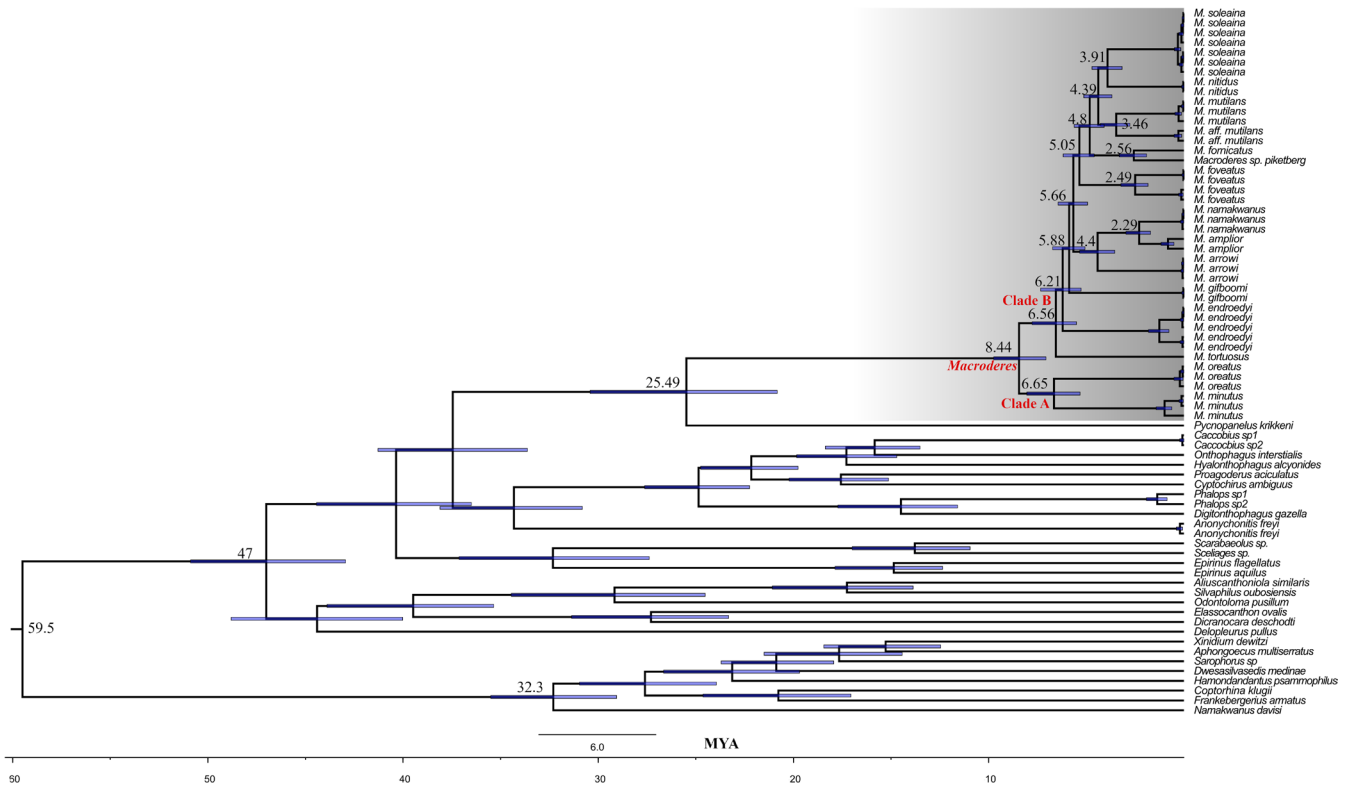


FIGURE 3 | Illustrates the maximum clade credibility cladogram derived from the BEAST analysis, depicting the relative estimated time of divergence for *Macroderes*. Blue bars at the nodes indicate the time intervals corresponding to the 95% probability of the actual age. The values at the nodes represent the mean estimated ages of divergence, with the geologic timescale shown in millions of years ago (MYA).

the extinction rate is close to zero, this suggests that extinctions have been negligible or undetectable within the genus over the timescale analysed. The net diversification rate, equivalent to the speciation rate due to the near-zero extinction, confirms that *Macroderes* is a genus with steady lineage expansion (Figure 5A–C).

4.5 | Evolution of Flightlessness in *Macroderes*

Our ancestral state reconstruction analyses suggest that the MRCA of *Macroderes* was flightless—so the flightlessness condition in the genus *Macroderes* is an ancient trait, arising shortly after its divergence from its sister group. This trait has remained present throughout the evolutionary history of *Macroderes* (Figure 6, Figure S1).

5 | Discussion

5.1 | Phylogenetic Relationships of *Macroderes* Within Scarabaeinae

The genus *Macroderes* is one of the many dung beetle genera without a confirmed tribal placement, pending a comprehensive tribal revision of the subfamily Scarabaeinae using a methodological approach different from previous studies, as suggested by Daniel and Davis (2024) and Lopes et al. (2024). In this study, we successfully tested, for the first time, the monophyletic nature of *Macroderes*, a genus characterised by its black, convex,

bulky body and absence of wings (Frolov and Scholtz 2004; Abdalla et al. 2018; Davis et al. 2020). Additionally, we corroborated previous studies on its sister-group relationship with other dung beetle taxa, such as Onthophagini, Oniticellini, Onitini and *Pycnopanelus* (Sole and Scholtz 2010; Mlambo et al. 2015; Tarasov and Génier 2015; Tarasov and Dimitrov 2016; Gunter et al. 2016, see also Figures 2 and 3).

Although the taxonomy of the genus has been established (see Frolov and Scholtz 2004; Abdalla et al. 2018), species delimitation remains somewhat challenging, since species within it are superficially similar. However, a thorough examination of the micro-sculpture on the pronotum and elytra has revealed notable variation among species, which can serve as diagnostic characters (Frolov and Scholtz 2004). From a phylogenetic perspective, the genus comprises two clades (A and B). Clade A includes two smaller species (7–8.9 mm) that share a similar shape of the internal sac of the aedeagus, which is almost straight and has a weakly developed lateral process (Frolov and Scholtz 2004; Abdalla et al. 2018). Clade B, in contrast, consists of larger species (9–15 mm) and is further divided into six subclades. Subclade B1 contains only two species: *Macroderes fornicatus* and an undescribed species from Piketberg Mountain in South Africa (*Macroderes* sp. Piketberg) (Figure 2). These two species are characterised by strongly shagreened, matte elytral intervals, punctate and crenulate lateral margins of the pronotum, and similar micro-structures in the pronotal punctures. However, the remaining subclades do not show a strong correlation between morphological and genetic data, meaning that inferred sister-species relationships do not always align with

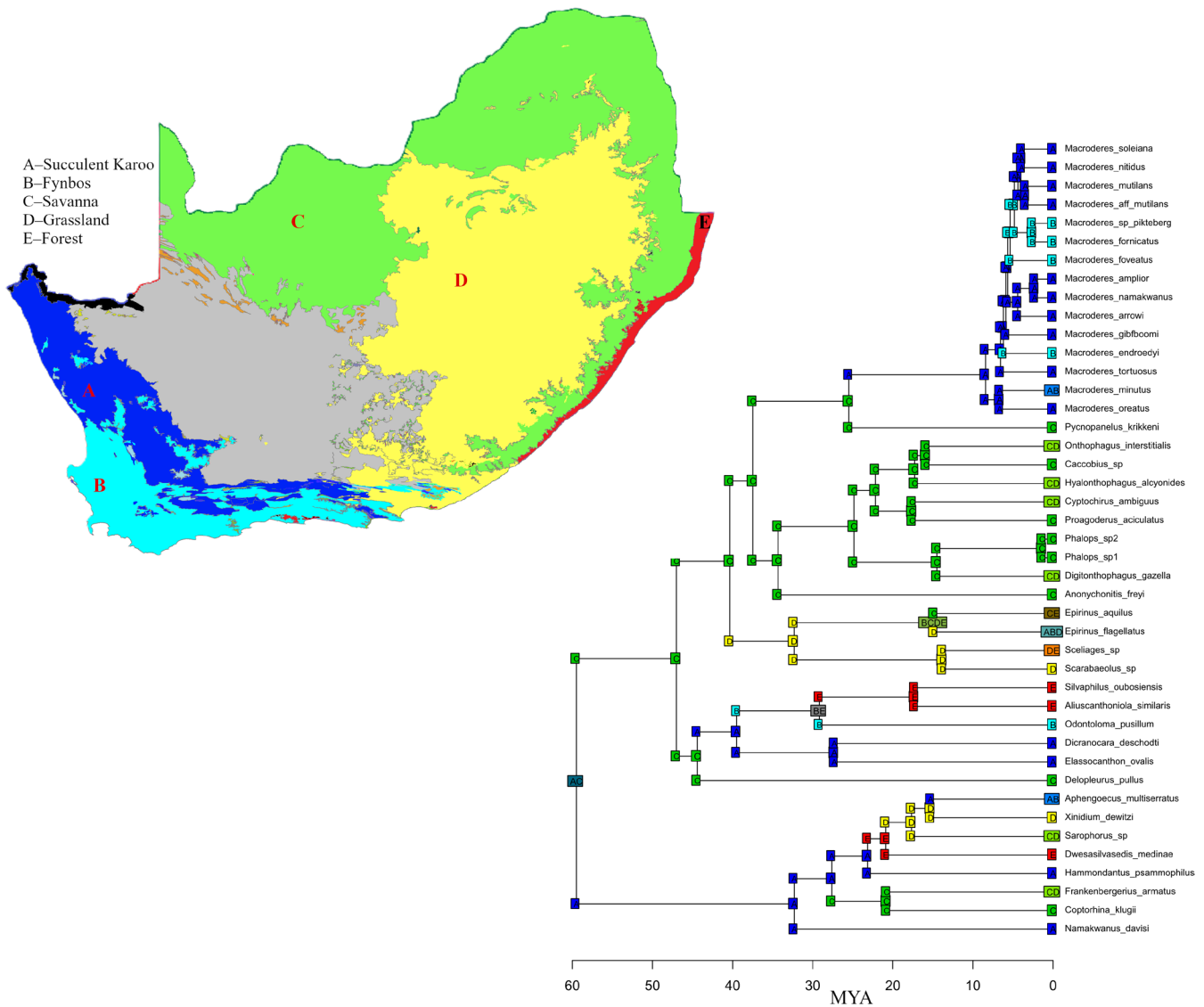


FIGURE 4 | Represents a cladogram illustrating the ancestral range reconstruction for the genus, as inferred using the DIVALIKE+J model in ‘BioGeoBEARS.’ The letters A–E correspond to southern African biomes defined by Mucina et al. (2006): (A) Succulent Karoo, (B) Fynbos, (C) Savannah, (D) Grassland and (E) Forest.

the diagnostic characters described by Abdalla et al. (2018) and Frolov and Scholtz (2004).

Our findings support the notion that, while morphological traits provide valuable insights into evolutionary relationships—particularly when examining fossil records or extinct species—genetic data often offer a different and, at times, more precise representation of evolutionary history (Dayrat 2005). This is largely because genetic data are less prone to convergent evolution or phenotypic plasticity, both of which can obscure true relationships (Giribet 2015). Nevertheless, it is crucial to integrate both morphological and molecular data, as their combined use has proven robust and has resolved numerous phylogenetic relationships (Neumann et al. 2021; Hunt et al. 2025).

We interpret the external morphological similarities observed in *Macroderes* species as potentially misleading, particularly due to convergent evolution, where distantly related species independently evolve similar traits. For instance, most *Macroderes*

species appear highly similar, except for *Macroderes cornutus* and *M. foveatus*, which are distinctly characterised by a deep triangular concavity at the base of the pronotum. However, despite this distinct morphology, *M. foveatus* was recovered as a sister species to *M. amplior*, *M. arrowi* and *M. namakwanus*. This finding further illustrates the complexity of morphological and genetic congruence within this genus and highlights the probable role of environmental stability in shaping the observed morphological similarities in *Macroderes*—a phenomenon commonly observed in other coleopterans (Maruyama and Parker 2017; Zhao et al. 2024).

5.2 | Aridification in Southwest Africa and Flightlessness as Putative Drivers of Microallopatric Speciation in the Genus *Macroderes*

The most recent common ancestor of *Macroderes* likely originated in a biome resembling the present-day Succulent Karoo

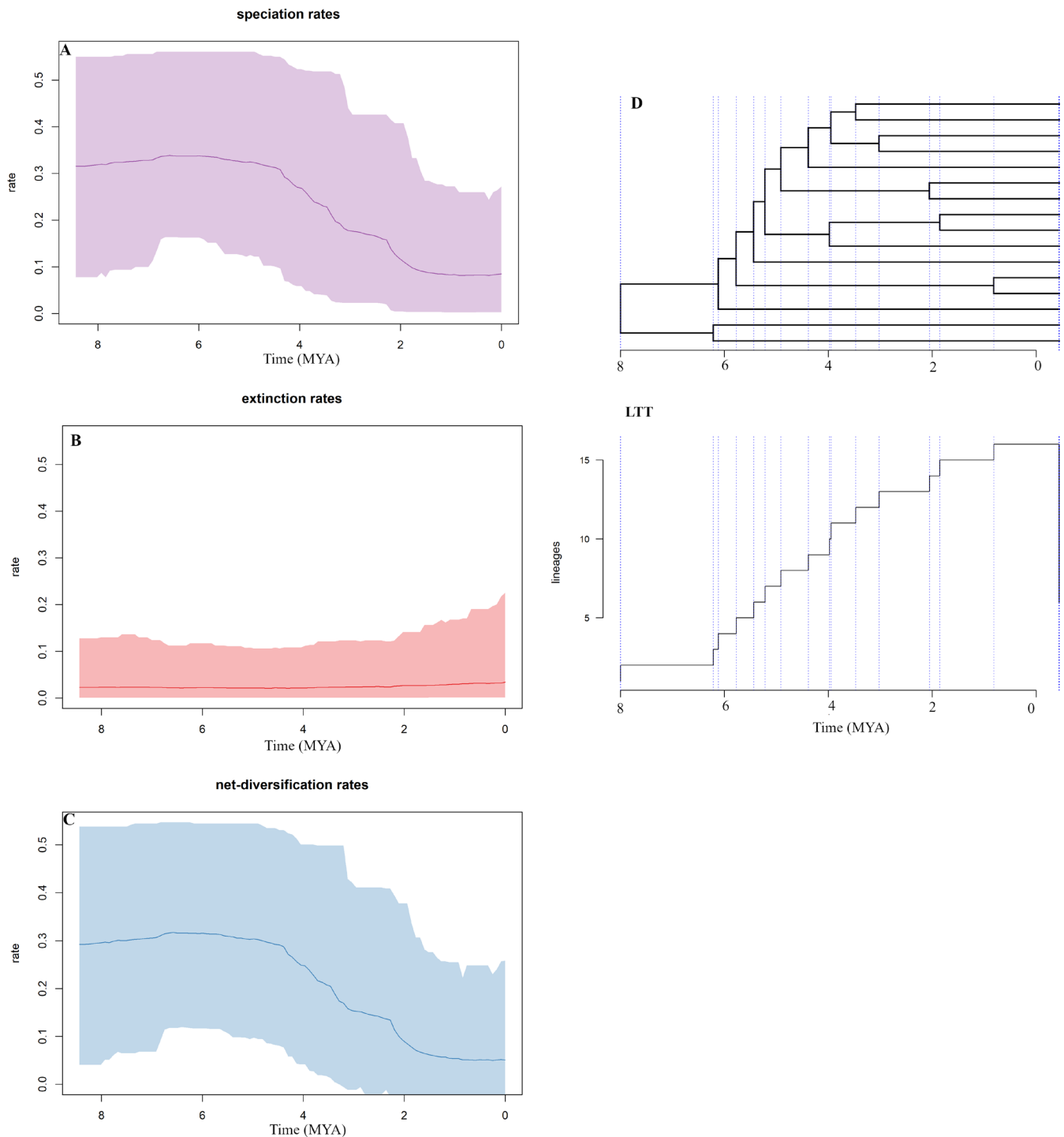


FIGURE 5 | (A–C) Illustrates the diversification analyses of *Macroderes* conducted using TESS. The figure displays speciation rates (A), extinction rates (B), net diversification (C) and the lineages-through-time (LTT) for *Macroderes* (D).

in the late Miocene (Figures 3 and 4). During the transition between Miocene and Pliocene, the ancestral area that *Macroderes* inhabited changed from a summer to a winter rainfall regime, followed by intensification of semi-arid conditions to which the flightless ancestor of the genus was adapted. How did this change in climate affect speciation in *Macroderes*?

Our analyses suggest that the evolutionary history of *Macroderes* dates back to the late Miocene, coinciding with the onset of the winter rainfall regime and the intensification of semi-arid

climatic conditions in southwestern Africa. These environmental changes, along with various geomorphological events, played a significant role in fragmenting the habitats of the most recent common ancestors of *Macroderes*. During the late Miocene (approximately 8–5 mya), lineage accumulation may have been relatively slow, reflecting limited diversification as the genus became established in the region (Figure 4D). Ancestral populations were likely adapting to a transition from warmer, wetter conditions to cooler, drier climates. This may explain why only two species within subclade A diverged significantly around 6.65 mya.

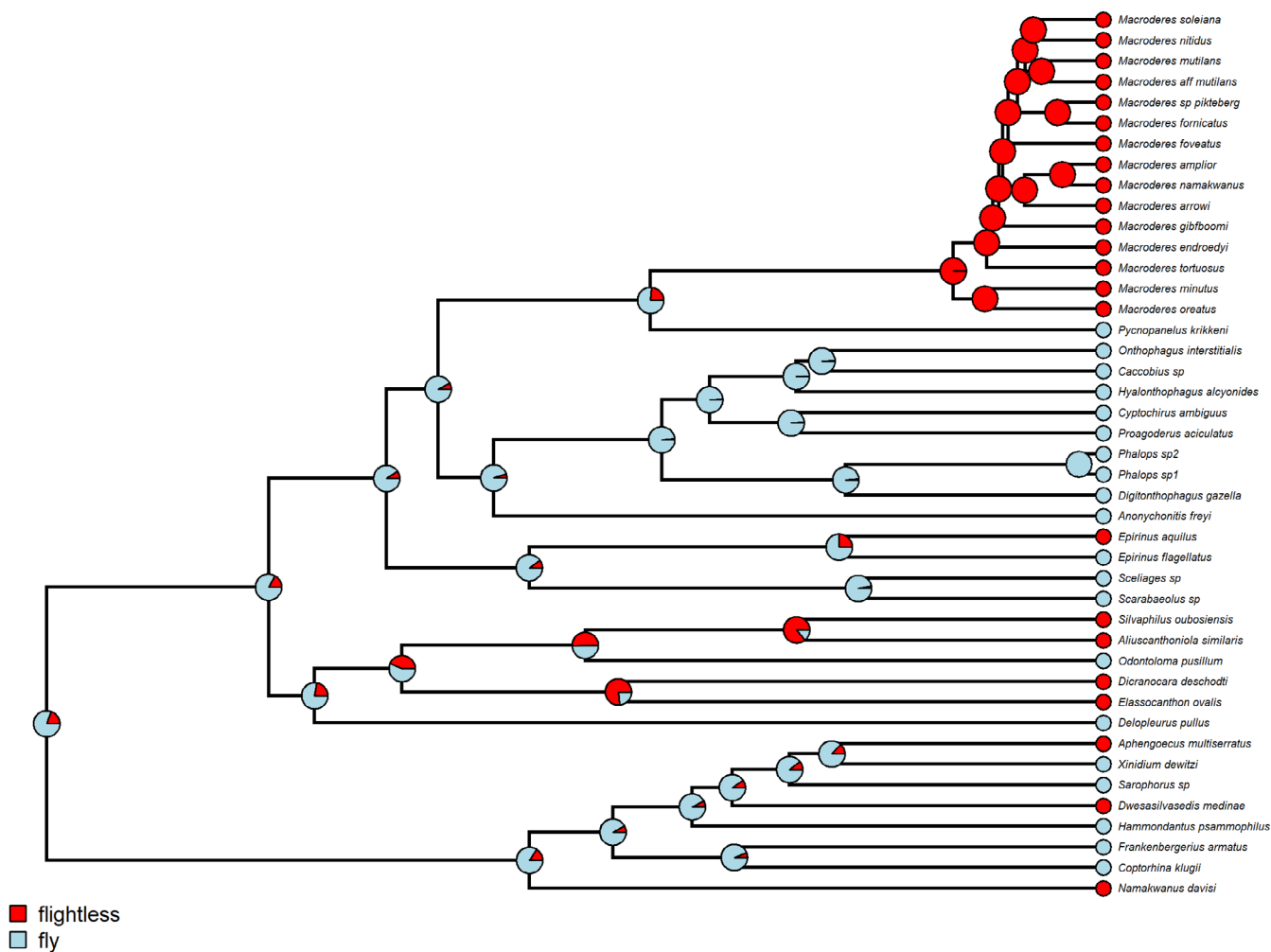


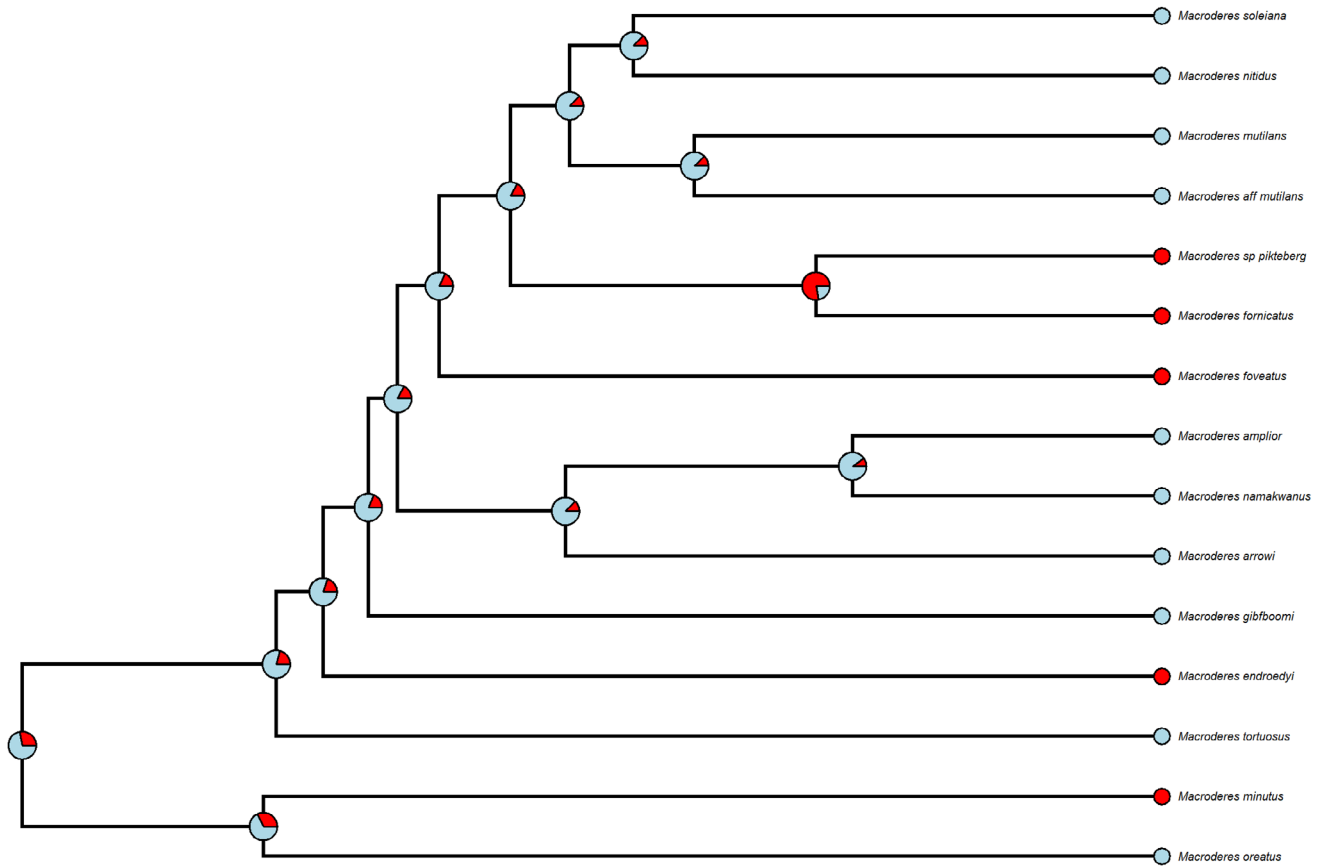
FIGURE 6 | Shows the ancestral character reconstruction of the flightlessness condition within the genus *Macroderes*.

In contrast, lineage accumulation increased during the Plio-Pleistocene (5–2 mya), particularly in subclade B (Figure 4D). The highest period of lineage accumulation in *Macroderes* coincides with Miocene aridification in southwestern Africa and global Pleistocene glacial cycles (Demenocal 1995, 2004). Regional climatic shifts are commonly associated with the fragmentation of wooded vegetation into more arid shrubland or open habitats in the GCFR (Cowling et al. 2009; Neumann and Bamford 2015). Such fragmentation likely isolated populations and reduced gene flow, thereby promoting divergence and microallopatric speciation in *Macroderes*. As the ancestor of this group was likely semi-arid adapted, it can be inferred that the climatic conditions of the time favoured the diversification of *Macroderes*.

This interpretation is supported by the relatively high speciation rate estimate (0.3067) and negligible extinction rates, which together indicate active diversification within the genus (Figure 5A–B). This pattern is likely driven by the intensification of aridity in the region—a plausible scenario for a semi-arid-adapted and flightless genus such as *Macroderes*. However, caution is warranted in interpreting these evolutionary patterns, as extinction rate estimates can be sensitive to incomplete taxon sampling or model assumptions (Helmstetter et al. 2022). Low extinction values may also reflect artefacts resulting from the

lack of fossil evidence, which could be the case for *Macroderes*. This highlights an important opportunity for future studies to test our diversification models on other dung beetle lineages, particularly those with more complete extant and fossil records. Nevertheless, several studies have shown that climatic processes have played a major role in driving speciation across southern Africa, including within the GCFR. Supporting examples come from both vertebrate (Tolley et al. 2008; Swart et al. 2009; Barlow et al. 2013; Maswanganye et al. 2017) and invertebrate taxa (Price et al. 2007; Sole et al. 2005, 2013; Matenaar et al. 2016; Strümpher et al. 2016; Daniel, Sole, et al. 2020; Daniel, Davis, et al. 2020; Daniel et al. 2021; Abdalla et al. 2025). Furthermore, comparable mechanisms have been implicated in the evolution of microendemic species beyond Africa, for example, in leaf beetles (Platania and Gómez-Zurita 2023) and crickets (Anso et al. 2016) from New Caledonia—where climatic fluctuations similarly promoted speciation through habitat fragmentation and isolation.

If our ancestral trait reconstruction analyses reflect a plausible evolutionary history, it is reasonable to argue that a combination of historical climatic events in southwestern Africa and the condition of flightlessness has microallopatrically driven speciation within the genus *Macroderes*. This pattern resembles the speciation observed in the flightless southwestern Cape



■ moist
■ semi-arid

FIGURE 7 | Presents the ancestral character reconstruction of habitat preferences within the genus *Macroderes*, contrasting semi-arid conditions with moist environments.

Fold Mountains endemic stag beetle *Colophon*, where the evolutionary history of the genus is thought to have originated in the Great Karoo and southern coastal plain. With the gradual increase in aridity and temperature during the Miocene, the group was forced into more hospitable mountainous habitats (Switala et al. 2014; Scholtz and de Klerk 2024). As previously mentioned, flightlessness in *Macroderes* is an ancient trait (Figure 6), much like in *Colophon*. This is in contrast to the vast majority (approximately 99%) of extant flightless insects, which evolved from winged, more recent common ancestors (Scholtz 2000, 2009b; Scholtz et al. 2021; Scholtz and de Klerk 2024).

So, how has aridification in the southwestern region shaped the current flightless condition observed in *Macroderes* species? Our results suggest that by the end of the Miocene and into the early Pliocene, the ancestral range of *Macroderes* experienced increasingly semi-arid conditions (Figure 7). In such environments, the principal selective pressures would include water acquisition and the reduction of desiccation risk. Accordingly, *Macroderes* exhibits sealed elytra, which function in reducing water loss: moisture lost during respiration is trapped beneath the elytra, creating a subelytral cavity that quickly becomes saturated with water vapour. In flightless species like *Macroderes*, this cavity is often enlarged and maintained in a permanently saturated

state, allowing for efficient water conservation—an adaptive trait especially suited to dry environments (Slobodchikoff and Wismann 1981; Zachariassen 1991; Draney 1993; Chown et al. 1998; Scholtz 2000, 2009a), such as the Succulent Karoo biome, which is the ancestral area of *Macroderes*.

Conversely, if *Macroderes* species were volant, the saturated air would dissipate during flight, resulting in significant water loss through exhaled vapour (Slobodchikoff and Wismann 1981; Zachariassen 1991). However, recent findings by Duncan (2002), using another flightless dung beetle species, *Circellium bacchus*, cast doubt on the water-saving function of the subelytral cavity. She suggests that this species uses the mesothoracic spiracle—rather than abdominal spiracles—as the main structure for gas exchange. If this is true for other flightless beetles as well, including *Macroderes*, then the long-standing water conservation hypothesis may need to be re-evaluated. This indicates that further studies are required to fully understand the evolutionary mechanisms underlying the high incidence of flightlessness among beetles in arid and semi-arid ecosystems.

Yet, a key question remains: how did *Macroderes* maintain its flightless condition despite the climatic fluctuations of the Pleistocene, a period marked by alternating glacial and

interglacial cycles that profoundly influenced both global and regional species distributions (deMenocal 2004; Barlow et al. 2013)? Again, the lack of fossil records complicates this inquiry. Nevertheless, Potts et al. (2013) propose that the Fynbos and Succulent Karoo biomes—likely refugial zones for proto-*Macroderes*—remained relatively stable throughout the Pleistocene and were largely decoupled from broader climatic changes. This long-term environmental stability may have provided a consistent habitat and reliable food sources over millennia, enabling *Macroderes* to retain flightlessness as a beneficial trait. As argued by Roff (1990, 1994) and Scholtz (2000), stable environments often favour the evolutionary loss of flight in insects.

How did the condition of flightlessness contribute to microallopatric speciation in *Macroderes*? Flightlessness likely played a crucial role in the microallopatric speciation of *Macroderes* by limiting dispersal and increasing population isolation. In fragmented landscapes, reduced mobility can promote genetic divergence among populations, particularly when combined with ecological specialisation—as observed in another flightless, dry-adapted dung beetle genus, *Pachysoma* (Sole et al. 2005). The heterogeneous environments of the Fynbos and Succulent Karoo biomes, characterised by features such as sand dunes, heuweltjies and mountain ranges, support a rich diversity of mammals and therefore provide a wide range of dung types essential for the feeding and breeding of dung beetles, including *Macroderes* (Davis and Scholtz 2001). These unique landscapes likely acted as refugia for isolated populations of *Macroderes*, allowing them to adapt to localised ecological conditions, which may have driven microallopatric speciation. For example, in Namaqualand (Succulent Karoo biome), some *Macroderes* species are separated by distances of less than 10 km², although a few cases of overlap are observed (e.g., *M. gifboomi* and *M. soleiana*, which do exhibit some overlap). This indicates that fine-scale geographic isolation, likely facilitated by their flightlessness, has played a role in their distribution. A similar pattern of microallopatric speciation has been documented in other flightless dung beetle taxa, such as *Grebennikovius* from the rainforests of the Eastern Arc Mountains (Montanaro et al. 2024), and *Temnoplectron* from Australia (Bell et al. 2004, 2007).

Furthermore, the vast majority of *Macroderes* species are highly localised or have restricted distribution ranges, typically associated with shrublands and open vegetation types. For instance, the Succulent Karoo biome supports 13 species, followed by the Fynbos biome with six. Only one species, *Macroderes cornutus*, displays a disjunct distribution, occurring in both the Fynbos and Succulent Karoo biomes along the coastline. In contrast, the only truly widespread species is *Macroderes bias*, which occurs across four biomes: Albany Thicket, Fynbos, Grassland and Savanna (Frolov and Scholtz 2004; Abdalla et al. 2018; Davis et al. 2020), most likely representing a recent colonisation. Overall, our study corroborates the distribution patterns outlined by Davis et al. (2020), identifying four main trends in *Macroderes* biogeography: (1) an east–west or north–south gradient; (2) a westward coastal dune distribution; (3) a range extending from the western to south-eastern Cederberg Mountains and (4) a disjunct pattern in cooler upland regions, with centres of endemism in Namaqualand, the Cape Peninsula and the southern Cape.

6 | Concluding Remarks

Although the clade analysed is relatively small, and we acknowledge the limited statistical power inherent in diversification analyses of such groups, we believe these preliminary insights remain valuable. They highlight the potential for further exploration of diversification dynamics in microendemic taxa, particularly when integrated into broader phylogenetic frameworks.

Notably, this study is the first to investigate microallopatric speciation in flightless dung beetles inhabiting semi-arid environments, providing a novel perspective on evolutionary processes within these ecosystems. Climatic shifts during the late Cenozoic, which led to habitat fragmentation, alongside long-term habitat stability in the ancestral range, likely contributed to the evolution of flightlessness observed in extant *Macroderes* species. Our findings suggest that both environmental factors (e.g., aridification) and adaptive traits (e.g., flightlessness) have jointly shaped the current pattern of microallopatric speciation within the genus. As the first study to address this phenomenon in xeric-adapted dung beetles, we hope it serves as a benchmark and comparative model for future cladogenetic studies on faunal diversification in semi-arid ecosystems globally. Moreover, given that flightlessness in *Macroderes* is an ancient trait, our research offers a valuable opportunity to explore broader evolutionary mechanisms underpinning the loss of flight across other insect lineages.

Author Contributions

G.M.D., C.H.S. and C.L.S.: designed the study; I.H.A.: planned and conducted the field collection of specimens and curation; I.H.A.: generated molecular data in the laboratory of C.L.S. and C.H.S.; G.M.D.: analysed the data; G.M.D.: wrote the first draft and all authors contributed revising the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All molecular sequence data are publicly available on GenBank. The raw datasets and other relevant information used in this study are archived in Dryad and can be accessed via the following link: <https://doi.org/10.5061/dryad.j0zpc86v1>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Ancestral character reconstruction shows the evolution of flightlessness. **Table S1:** Primers used for PCR amplification. **Table S2:** Data from PartitionFinder v.2.1: subset partitions and best model used for phylogenetic inference analyses. **Table S3:** Model selection for the ancestral character reconstruction of habitat preferences within the genus *Macroderes*, contrasting semi-arid conditions with moist environments. This analysis does not show a huge difference between models, except for the model that permits only transitions from semi-arid conditions to moist environments, but not the reverse, which explains our data very poorly. However, it does not indicate any real justification for using a model more complex than the simple equal-rates (ER) model. Consequently, we will use this model for the rest of our analyses, as highlighted in bold. **Table S4:** Model Selection for the ancestral character reconstruction of the flightlessness condition within the genus *Macroderes*. This analysis does not show a huge difference between models, except for the model that permits only transitions from volant to flightless, but not the reverse, which explains our data very poorly. However, it does not indicate any real justification for using a model more complex than the simple equal-rates (ER) model. Consequently, we will use this model for the rest of our analyses, as highlighted in bold.