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Phylogeny and historical biogeography of the southern African lacewing genus *Afroptera* (Neuroptera: Nemopteridae: Nemopterinae)

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Abstract

The lacewing genus *Afroptera* Abdalla & Mansell (Neuroptera: Nemopteridae: Nemopterinae) is endemic to southern Africa, predominantly found in the Fynbos and Succulent Karoo biomes. The taxonomy of the genus has been recently resolved. However, the monophyly and evolutionary history of the genus has never been addressed. This study employs an integrative phylogenetic approach, by incorporating three ribosomal genes (16S, 28S and 18S) and two protein-coding genes (cytochrome oxidase subunit I and carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase), and morphological data to examine the monophyly and historical biogeography of *Afroptera*. We use Bayesian, parsimony and maximum likelihood phylogenetic methods to assess the monophyly and relatedness of *Afroptera* within the Nemopterinae. We also use ancestral range reconstruction and diversification analysis to infer the historical biogeography of the genus. Our analyses reveal the genus as a monophyletic lineage. The genus *Afroptera* originated during the Pliocene (5.24–3.13 Mya) in a desert environment, experiencing rapid speciation during the Pleistocene, primarily within the Fynbos and Succulent biomes; and secondarily dispersed into the Nama Karoo and Savannah (Kalahari) biomes. The current distribution patterns of *Afroptera* species likely stem from intensified aridification in the southwest during the Plio-Pleistocene, consistent with the dry-adapted nature of *Afroptera's* ancestors. Therefore, our findings suggest a climatically driven diversification model for the genus *Afroptera*.

KEYWORDS
Cape flora, diversification, endemism, historical biogeography, insects, molecular analysis, monophyly, Nemopterinae, Neuroptera

INTRODUCTION

Nemopteridae (thread-, spoon- and ribbon-winged lacewings) are unique by virtue of the unusual shape of their hind wings that are narrow and much longer than the forewings, and their short metathorax (Tjeder, 1967). The family includes approximately 142 species mainly confined to arid habitats. They occur in the Middle East and India, Mediterranean Europe, South America, Australia, Socotra Island and Africa, with the greatest concentration in southern Africa (Sole et al., 2013). The family does not occur in North America, except for two fossil records of the genus *Marquettia* Navás (Carpenter, 1959). The family comprises two subfamilies: Crocinae (thread-winged lacewings), which have filiform hind wings that act as tactile sensors enabling the insect to interact with its surroundings, and in mate

recognition (Mansell, 1996). This subfamily comprises about 43 species, primarily distributed in the arid and desert zones on the southern fringes of the Palaearctic and Oriental Regions and the Neotropical, Afrotropical and Australian Regions (Mansell, 1996; Monserrat, 1996). The adult and larval biology and morphology of Crocinae have been documented by Tjeder (1967), Mansell (1976, 1977, 1980, 1981a, 1981b, 1983a, 1983b, 1986, 1996) and Monserrat (1983), while the taxonomy, biogeography and phylogeny were treated by Hölzel (1975) and Monserrat (1996).

Nemopterinae (spoon- and ribbon-winged lacewings) by contrast have broader hindwings with an apically dilated region, which provides stability during flight and in mating signals and camouflage (Mansell, 1996). The subfamily has a similar distribution to that of Crocinae. The subfamily Nemopterinae was recovered as a monophyletic group by molecular analysis, including 10 of the 11 southern African genera, *Nemia* Navás, *Barbibucca* Tjeder, *Halterina* Navás, *Derhynchia* Tjeder, *Knersvlaktia* Picker, *Palmipenna* Tjeder, *Nemeura* Navás, *Sicyoptera* Navás, *Semirhynchia* Tjeder and *Nemopterella* Banks (*Nemopistha* Navás could not be sequenced). *Nemia*, *Barbibucca*, *Derhynchia*, *Halterina*, *Palmipenna* and *Knersvlaktia* were found to be monophyletic, whereas *Nemopterella* was paraphyletic (Sole et al., 2013). Divergence time of the subfamily was dated to the Late Jurassic (145.6 Mya) with most genera diversifying during the period ca. 44–11 Mya, while recent rapid speciation occurred in the Late Miocene (ca. 6–5 Mya). Among these genera, *Nemia* and *Nemopterella* were always considered to be taxonomically controversial.

The new southern African genus *Afroptera* Abdalla & Mansell (Figure 1) was recently described after a comprehensive taxonomic revision of the two genera *Nemia* and *Nemopterella*. The taxonomy of these two genera was uncertain, as there were no reliable diagnostic characters to distinguish them. The first taxonomic work on the two genera was by Navás (1910) when he proposed the genus *Eretmoptera* and designated *Nemopteryx africana* Leach, as type species of the genus. However, the name is a junior homonym of *Eretmoptera* (Diptera) (Kellogg, 1900). Banks (1910) consequently proposed *Nemopterella* as a replacement name for *Eretmoptera* Navás (Nemopteridae). A few years later, Navás (1915) split the genus *Nemopterella* into *Nemeva* Navás with type species *Nemopteryx africana*, and *Nemia* Navás with type species *Nemoptera costalis* Westwood, 1836. This classification was based on forewing venation patterns: in *Nemia*, the '*confluentiam cubitorum*', that is, veins Cu_{1a} and Cu_2 are fused before reaching the forewing margin. However, as more specimens were investigated it transpired that this character was insufficient to distinguish the two genera, as it is frequently present in many other nemopterids, even in the type species of *Nemeva* (Tjeder, 1967). Tjeder (1967) subsequently differentiated the genera based on more consistent characters. He discovered a pair of pleuritocavae in the abdomen of male *N. africana* that were absent from those of *N. costalis*, and used this character to differentiate *Nemia* and *Nemopterella*. He then synonymised *Nemeva* with *Nemopterella*. The taxonomic status of the genera, however, remained unclear owing to the discovery of new species that were morphologically very close to *Nemia* yet had pleuritocavae, which are characteristic of males of *Nemopterella*.

The recent revision by Abdalla et al. (2019) suggested the separation of the two genera into four: *Nemopterella* Banks sensu stricto with three species, *Nemia* Navás with six species, *Siccanda* Abdalla & Mansell with one species and *Afroptera* with 28 species. The division

FIGURE 1 (a–d) Representatives of *Afroptera* species in their natural environment.

was based on molecular evidence as well as morphologically diagnostic characters. These include body size, shape of the tip segment of the antennae in both males and females, presence of a pair of transverse spots along the postfrontal suture on the frons above antennae, colour of the pterostigma of the forewing, forewing anal area tinged or not tinged, number of costal cells, pubescence patterns, colour of the hind wing from the base to the apical whitish area, presence of a pair of pleuritocavae in the male abdomen and striping of the body. The most significant diagnostic character, however, for separating the genus *Afroptera* from the remaining genera is the membranous terminal segment of the antennae, which is characteristic of all the species in the genus *Afroptera*. The last segment is either entirely or partially membranous, while in the remaining genera, it terminates in a tooth-like structure. The revision of the genus increased the number of known species to 28, eight of which were recently described (Abdalla et al., 2019).

As in most southern African Nemopterinae, members of *Afroptera* are distributed mainly in the Western and Northern Cape Provinces of South Africa (Abdalla et al., 2019; Sole et al., 2013; Tjeder, 1967), with some species recorded further north in Namibia, and extending as far south as the Succulent Karoo and the Nama Karoo Biomes. The genus is considered to be one of the most complex taxonomic groups since the species are morphologically very similar and there are no significant differences between the females of the different species. The identification of the species is consequently based largely on male specimens. Little is known about the biology of the genus except that they are attracted to light in large numbers and adults feed exclusively on pollen and nectar, while the carnivorous larvae live freely in sand (Mansell Pers. Obs.).

However, the phylogeny and historical biogeography, including the dynamics and mode of diversification of *Afroptera* species, have not been studied. Given its endemism in southern Africa, the genus is a particularly interesting model system to understand the evolutionary biology of Nemopteridae in the region. Thus, the aims of this study are (1) to test the monophyly and to resolve the relationships between the genera of the Nemopterinae and (2) to use a dated phylogeny to infer the historical biogeography of the genus *Afroptera* by addressing the following research questions. (a) Where did the ancestral lineage of the genus *Afroptera* originate within the current biomes? (b) How did Plio-Pleistocene geoclimatic changes in southern Africa affect the diversification of *Afroptera* species?

MATERIALS AND METHODS

Sampling and taxon selection

a. To test the monophyly and relatedness of the Nemopterinae genera, we included as in-group taxa all known southern African Nemopterinae genera: *Barbibucca*, *Derhynchia*, *Halterina*, *Knersvlaktia*, *Nemeura*, *Nemia*, *Palmipenna*, *Semirhynchia* and *Sicyoptera*, as well as the Australian *Chasmoptera* Westwood, in addition to the newly proposed *Nemopterella* Banks sensu stricto, *Afroptera* Abdalla & Mansell, and *Siccanada* Abdalla & Mansell. The only genus not included in this study is *Nemopistha*. A representative of the subfamily Crocinae, *Laurhervasia setacea* (Klug), was chosen as the out-group taxon, following Sole et al. (2013).
b. To study the biogeography and diversification estimate time of the genus *Afroptera*, we included 14 species from the genus *Afroptera* on the basis of availability and suitability of fresh material for DNA extraction. We also include species of two genera, *Knersvlaktia* (one sp.) and *Palmipenna* (three spp.), as these taxa were regarded as phylogenetically close to *Afroptera* (Sole et al., 2013) before the problematic *Nemopteralla* was split into three genera (Abdalla et al., 2019). Additionally, we sought an outgroup entirely distinct from the problematic *Nemopterellla*, which *Knersvlaktia* and *Palmipenna* fit perfectly. Of these, 12 species of *Afroptera* were sequenced for the first time, the remainder taxa were retrieved from GenBank (see Table S1). All the sequences are deposited in Genbank (see Table S1).

DNA extraction, amplification, processing and alignment of sequences

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. Five gene regions were selected to construct the phylogenetic relationships among the species of *Afroptera*. Partial sequences of three ribosomal genes; 16S rDNA, 18S rDNA and a portion of the nuclear rRNA large subunit 28S domain 2 and two protein-coding genes, cytochrome oxidase subunit I (COI) and carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase-dihydroorotase (CAD). The oligonucleotide primers used to amplify the five gene regions are summarised in Table S2. For details on polymerase chain reaction (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 & Toh, 2008). All sequences were deposited in GenBank.

Phylogenetic reconstructions

Monophyly and relatedness of *Afroptera* among the genera of Nemopterinae

Bayesian inference (BI) was used to infer the phylogenetic relationship between the genera of the subfamily Nemopterinae using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Prior to analyses, jModelTest (Posada, 2008) was conducted to estimate the best-fitting model of nucleotide evolution based on Bayesian information criteria (Schwarz, 1978) (Table S3). Markov Chain Monte Carlo (MCMC) consisted of two independent runs with one cold and three heated chains

(0.01) for 20 million generations starting from random trees and sampling every 200 generations. The stationary probability distribution of Markov chains was verified by measuring the effective sample size (ESS) using the programme TRACER v.1.6 (Rambaut & Drummond, 2014). The first 25,000 (25%) trees were discarded as burn-in.

Genus Afroptera

To construct phylogenetic relationships between the species of *Afroptera*, two data partitions were analysed: molecular and combined morphological/concatenated molecular datasets. The concatenated molecular dataset was analysed under three molecular algorithms: maximum parsimony (MP) using the computer software PAUP*4.0b10 (Swofford, 2003), maximum likelihood (ML) using RAXML-HPC version 8.1.20 (Stamatakis, 2014) and BI. The combined morphological/concatenated molecular dataset was analysed with Parsimony and Bayesian methods.

In the parsimony analyses, a heuristic tree search protocol with 10 random addition sequences and tree bisection and reconnection was applied. All characters were unordered and equally weighted, uninformative characters were excluded from the analysis and gaps were treated as missing data. The branch support was estimated by calculating bootstrap values (Felsenstein, 1985) based on 1000 replicates.

For ML analyses, ML searches were conducted using RAXML-HPC version 8.1.20 (Stamatakis, 2014). To acquire an optimal ML tree, tree searches were performed using the option ML+rapid bootstrapping under the GTRGAMMA model with 1000 bootstrap pseudo-replicates.

For Bayesian analyses, the analyses followed the same procedure mentioned above, but with MCMC run for 30 million generations and 37,500 (25%) trees were discarded as burn-in.

Divergence estimation of *Afroptera*

The node ages for the major lineage-splitting events within *Afroptera* were estimated using the BEAST v.2.5.1 software package (Rambaut & Drummond, 2014). The programme BEAUti v 2.5.1 (Rambaut & Drummond, 2014) was used to generate xml files that were then executable by BEAST. The concatenated molecular datasets used five partitions, the substitution models were set as unlinked and the molecular clock and trees were set as linked. The favoured substitution models that were chosen by the jModelTest were assigned to each gene. The tree prior was set to Yule speciation. A fossil record for the genus *Marquettia* from the Eocene–Oligocene boundary (33.9 million years ago [Mya]) (Carpenter, 1959) was used to constrain the minimum age of *Afroptera*, where we used the midpoint of (34.00 Mya) as a hard-minimum age constraint in a lognormal prior distribution, other parameters were used in default settings. Two independent runs were performed for 10 million generations. The log files of the two runs were combined using the programme LOG COMBINER v 2.5.1 (Rambaut & Drummond, 2014). The results were then checked for convergence and ESS >200 using TRACER v 1.6. (Rambaut & Drummond, 2014). The first 20% of the logs were discarded as burn-in. The resulting trees were also combined, and then interpreted in TREE ANNOTATOR v 1.8.4, and viewed in FigTree v 1.4.3 (Rambaut, 2009).

Biogeographical analysis

We conducted ancestral range reconstruction for *Afroptera* in southern Africa using the R package 'BioGeoBEARS' (Matzke, 2013). Three models of biogeographic inferences were considered: dispersal-extinction-cladogenesis (Ree & Smith, 2008), a ML version of dispersal-vicariance analysis (DIVALIKE) (Ronquist, 1997), and a likelihood 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 model of biogeographic inference, we assessed the fit of six model combinations (Table 1) based on the Akaike information criterion (AIC) scores and Akaike weights. For all models, the analyses were conducted using the following South African biomes (Mucina et al., 2006; Figure 5): (A) Desert; (B) Fynbos; (C) Savannah; (D) Succulent Karoo and (E) Nama Karoo. The distribution data for *Afroptera* species was sourced from a recent taxonomic revision on the group (Abdalla et al., 2019).

TABLE 1 Summary statistics of biogeographic model testing in 'BioGeoBEARS', in order to infer the most likely biome occupied by the ancestors of *Afroptera*.

Models	LnL	Numparams	d	e	j	AIC	AIC_weight
DEC	−61.25	2	0.049	0.06	0	127.3	0.0001
DEC+J	−52.08	3	0.014	1.00E−12	0.11	111.8	0.29
DIVALIKE	−62.79	2	0.082	0.092	0	130.3	2.70E−05
DIVALIKE+J	−54.63	3	0.028	0.032	0.11	116.9	0.023
BAYAREALIKE	−68.99	2	0.092	0.22	0	142.7	5.50E−08
BAYAREALIKE+J	**−51.24**	**3**	**0.021**	**0.053**	**0.11**	**110.1**	**0.68**

Note: The best-fitting model is highlighted in bold. Values of parameters of dispersal (d), extinction (e), founder effect (j), likelihood scores (lnL) and Akaike information criterion (AIC) are detailed.
Abbreviation: DEC, dispersal-extinction-cladogenesis.

Diversification analyses

The diversification analyses were performed on an ultrametric tree topology with branch lengths scaled to time. A plot of lineage through time (LTT) (Nee et al., 1995) was calculated using the R (R Core Team, 2019) package 'APE' (Paradis et al., 2004). We also used the compound Poisson process on mass extinction times (CoMET) as implemented in the R (R Core Team, 2019) package 'TESS' (Höhna et al., 2016; May et al., 2016) to estimate speciation and extinction rates through time. The compound Poisson process allows us to detect shifts in speciation or extinction rates that may be correlated with geoclimatic changes in southern Africa since the Plio-Pleistoceno. We ran a constrained MCMC analysis where the mean speciation rate (λ), mean extinction rate (μ) and their standard deviations are set to zero, which generated the posterior distributions of these hyper-priors (May et al., 2016). These distributions were then used as hyper-priors in a full Bayesian analysis. The prior on the number of mass extinction events was set to 1.0. The reversible jump MCMC chains were run for 1 million generations with a burn-in of 25% and sampling thinning interval of 100.

RESULTS

Monophyly and relatedness of *Afroptera* among the genera of Nemopterinae

The total alignment matrix contained 3318 bp of which 736 bp were from CO1, 863 bp from CAD, ≈336 bp from 16S, ≈791 bp from 18S and ≈592 bp from 28S. The Bayesian analysis revealed two major clades in the subfamily Nemopterinae, both clades supported with strong Bayesian posterior probability (PP): clade A with (1.00 PP) and clade B with (1.00 PP). Clade A comprises well-supported monophyletic genera: *Afroptera* (1.00 PP), *Siccanda* (1.00 PP), *Halterina* (1.00 PP), *Nemia* (1.00 PP), *Barbibucca* (1.00 PP), *Nemopterella* (sensu stricto) (1.00 PP), *Knersvlaktia* (1.00 PP) and *Palmipenna* (1.00 PP). Within clade A, the genera are clustered into three groups I, II and III: Clade I includes *Afroptera*, *which* is sister to *Siccanda*, and, in turn, they are sister to *Halterina*. Clade II includes the sister genera *Nemia* and *Barbibucca*, which are, in turn, sister to *Nemopterella* sensu stricto and *Knersvlaktia*. The genus *Palmipenna* formed clade III and was recovered as being a close relative to all genera in the clade. Clade B includes the genera: *Nemeura*, *Sicyoptera*, *Semirhynchia*, *Chasmoptera* and *Derhynchia*. The genus *Derhynchia* was recovered as the only monophyletic genus in the clade while the remainder of the genera are polyphyletic. The genus *Derhynchia* appears as sister to all genera in the clade (Figure 2).

Phylogeny of the genus *Afroptera*

Molecular data

The total alignment matrix contained 3222 bp of which 736 bp were from CO1, 863 bp from CAD, ≈329 bp from 16S, ≈679 bp from 18S and ≈615 bp from 28S. Parsimony analysis for the concatenated molecular dataset resulted in 394 parsimony-informative sites and 10 most equally parsimonious trees with a tree length of 668 steps (confidence interval [CI] = 0.7246; retention index [RI] = 0.8160). The values of PP, MP and ML bootstraps (MLB) for concatenated molecular datasets are summarised on a Bayesian consensus tree. Nodes with bootstrap support (BS) values above 70% and/or posterior probability above 0.90 are considered as strongly supported nodes (Alfaro & Holder, 2006; Hillis & Bull, 1993).

All analyses (BI, PB and ML) for concatenated molecular datasets resulted in strong support with 1.00 PP; PB 100% and MLB 100% (Figure 3). In addition, all analyses produced similar tree topologies that consisted of two major clades labelled A (1.0 PP; 86% PB; 94% MLB) and B (1.00 PP; 83% PB; 98% MLB) with *A. alba* recovered as sister taxon (1.00 PP; 100% PB; 100% MLB) to all the other species (Figure 3). Clade A consisted of four sub-clades labelled A1, A2, A3 and A4. In BI analyses (Figure S1), the phylogenetic relationships between the four sub-clades were well supported, whereas in MP and ML, the sister relationship between sub-clades A1 and A2 were poorly supported. Sub-clade A1 is strongly supported (1.0 PP; 80% PB; 86% MLB) with five species recovered representing three sister groups. The first group comprises the species *A. bitis* (Tjeder, 1967), *A. olivacea* (Tjeder, 1967) and *A. sabuleti* (Tjeder, 1967) with (0.79 PP). Within the group, *A. bitis* and *A. olivacea* clustered together with low support (0.54 PP; 58% MLB), and, in turn, they were placed sister to *A. sabuleti* with (0.79 PP). The second group comprises *A. maraisi* Abdalla (2019) that resolved separately forming a strong sister relationship with other species in the first group (1.00 PP; 75% PB; 85% MLB). The third group includes *A. aequabilis* (Tjeder, 1967), which was also recovered separately and formed a strong sister relationship with the remainder of the species in the sub-clade A1 (1.00 PP; 80% PB; 86% MLB). Phylogenetic relationships among the species in sub-clade A2 were strongly supported by Bayesian analysis and ML while PB analyses reported poor support (1.00 PP; 54% PB; 94% MLB) and two clusters were recovered. One cluster contains *A. longicornis* (Tjeder, 1967), *A. lanata* (Tjeder, 1967) and *A. peringueyi* (Tjeder, 1967) (0.99 PP; 85% PB; 83% MLB), and, in turn, they form a sister relationship with *A. pilosa* (Tjeder, 1967). The phylogenetic relationship between the species of sub-clade A1 and A2 was strongly supported by Bayesian analysis (1.00 PP) and moderately supported by ML analysis (58% MLB). The sub-clade A3 was recovered with (0.99 PP; 93% MLB) and comprises a single species *A. papio* (Tjeder, 1967), while sub-clade A4 comprises *A. munroi* (Tjeder, 1967) (1.0 PP; 73% PB; 100% MLB). Strong phylogenetic relationships were recovered in clade B between *A. obtusa* and *A. koranna* Abdalla et al. (2019) (1.00 PP; 83% PB; 98% MLB) (Figure 3).

Combined morphological and molecular data

Parsimony analysis for the combined morphological/concatenated molecular datasets resulted in 489 parsimony-informative sites of 3325 and one most parsimonious tree with a tree length of 1166

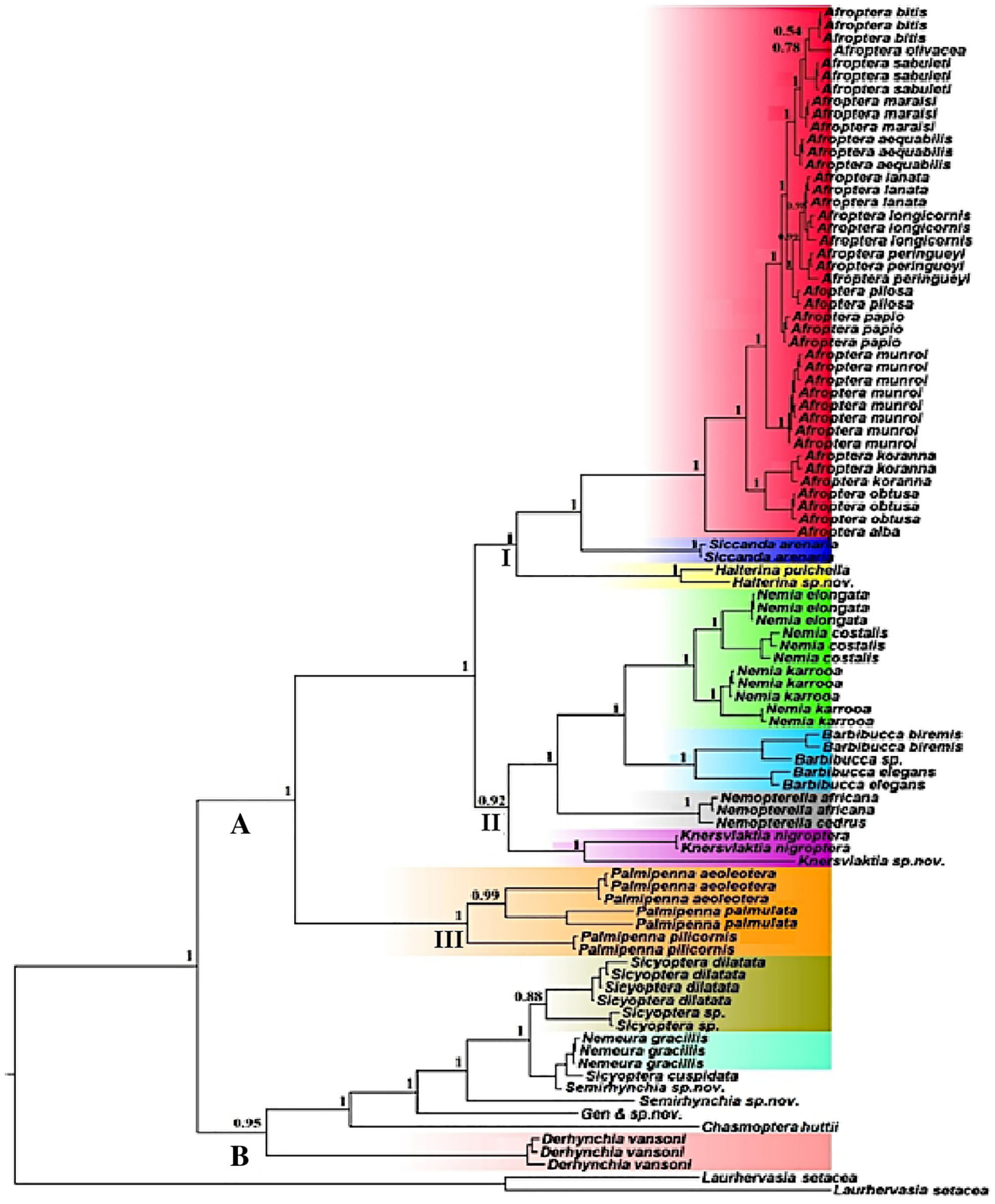

FIGURE 2 Bayesian phylogram of combined dataset analysis (COI; 16S; 18S; CAD and 28S) for Nemopterinae. CAD, carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase; COI, cytochrome oxidase subunit I; PP, Bayesian posterior probability.

steps (CI = 0.6454; RI = 0.7447). The strict consensus trees with BS values are presented (Figure S2).

Parsimony analyses for the combined morphological/concatenated molecular datasets resulted in a well-resolved monophyletic tree with high BS (100% BS). The tree's topology is strongly congruent with tree topologies resulting from Bayesian and ML analyses for molecular data. The most significant differences between the topologies of the two datasets are in the position of *A. maraisi* and in the position of *A. peringueyi* in

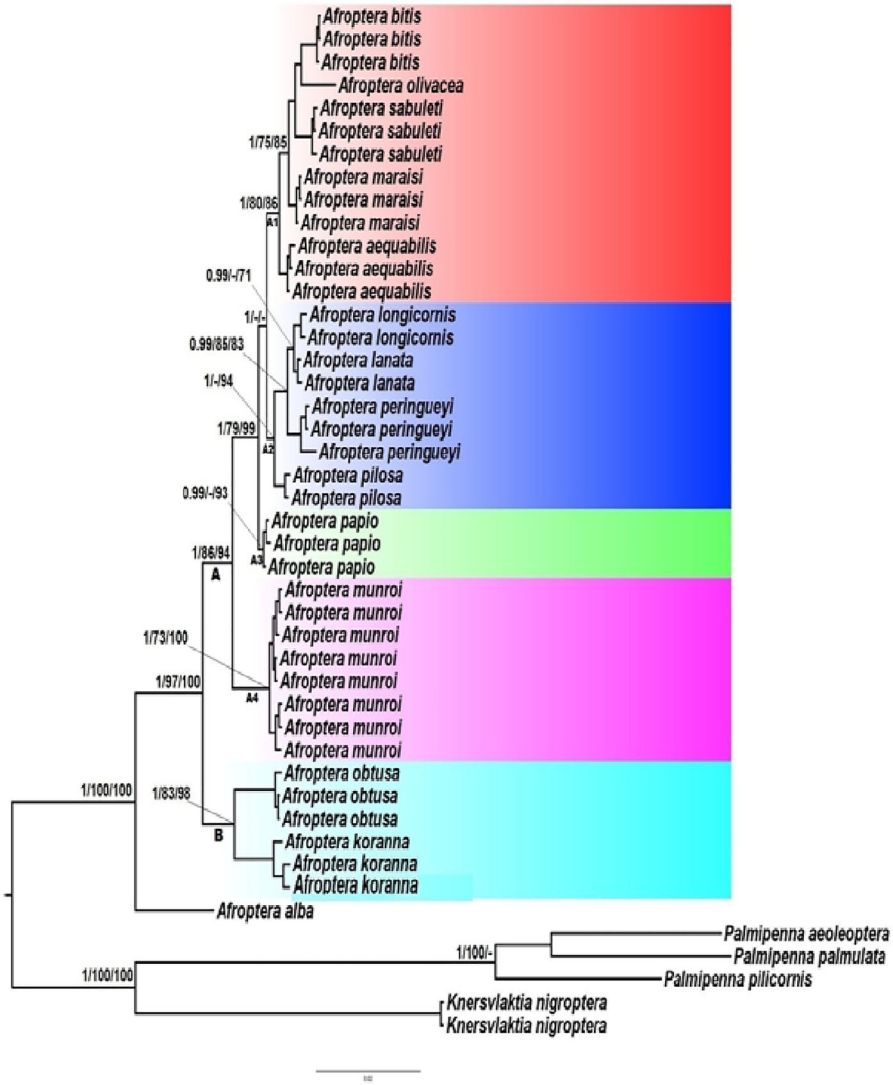

FIGURE 3 Bayesian phylogram of combined dataset analysis (COI; 16S; 18S; CAD; 28S and morphological data) for phylogenetic relationship inference in the genus *Afroptera*. Support nodes are represented by Bayesian posterior probabilities (PP), maximum likelihood bootstrap (MLB) and parsimony bootstrap (PB). CAD, carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase; COI, cytochrome oxidase subunit I.

sub-clade A1 and A2, respectively. In the trees resulting from the molecular data, *A. maraisi* (sub-clade A1) is sister to the group comprising *A. bitis*, *A. olivacea* and *A. sabuleti*, while in the tree resulting from the combined morphological/concatenated molecular dataset *A. maraisi* appears as sister to *A. bitis*. Also, *Afroptera peringueyi* (sub-clade A2) appears as sister taxon to *A. longicornis* while in the tree resulted from the combined morphological/concatenated molecular dataset *A. peringueyi* is sister to the cluster that contains *A. longicornis* and *A. lanata*, although these relationships are weakly supported.

Bayesian analysis for the combined morphological/concatenated dataset also retrieved a very strong phylogeny with high posterior probability (Figure 3; 1.00 PP, 100 BS for ML and MP) and two major clades (A and B). The only difference between this phylogeny and that of the Bayesian analysis of the molecular dataset is in the position of *A. peringueyi*, which is sister to *A. longicornis*.

Divergence time estimates

The BEAST chronogram is largely congruent with those resulting from Bayesian, MP and ML analyses for the concatenated molecular dataset. Our analysis suggested that the genus *Afroptera* originated during the Pliocene (4.12 Mya; 95% CI: 5.24–3.13 Mya) followed by a rapid diversification and speciation in the Pleistocene when *A. alba* split from the rest of the species (Figure 4).

Ancestral range reconstruction

Among six models of biogeographic inference used to reconstruct the ancestral range of the genus *Afroptera*, BAYAREALIKE+J was selected as the best-fit model by BioGeoBEARS for *Afroptera*

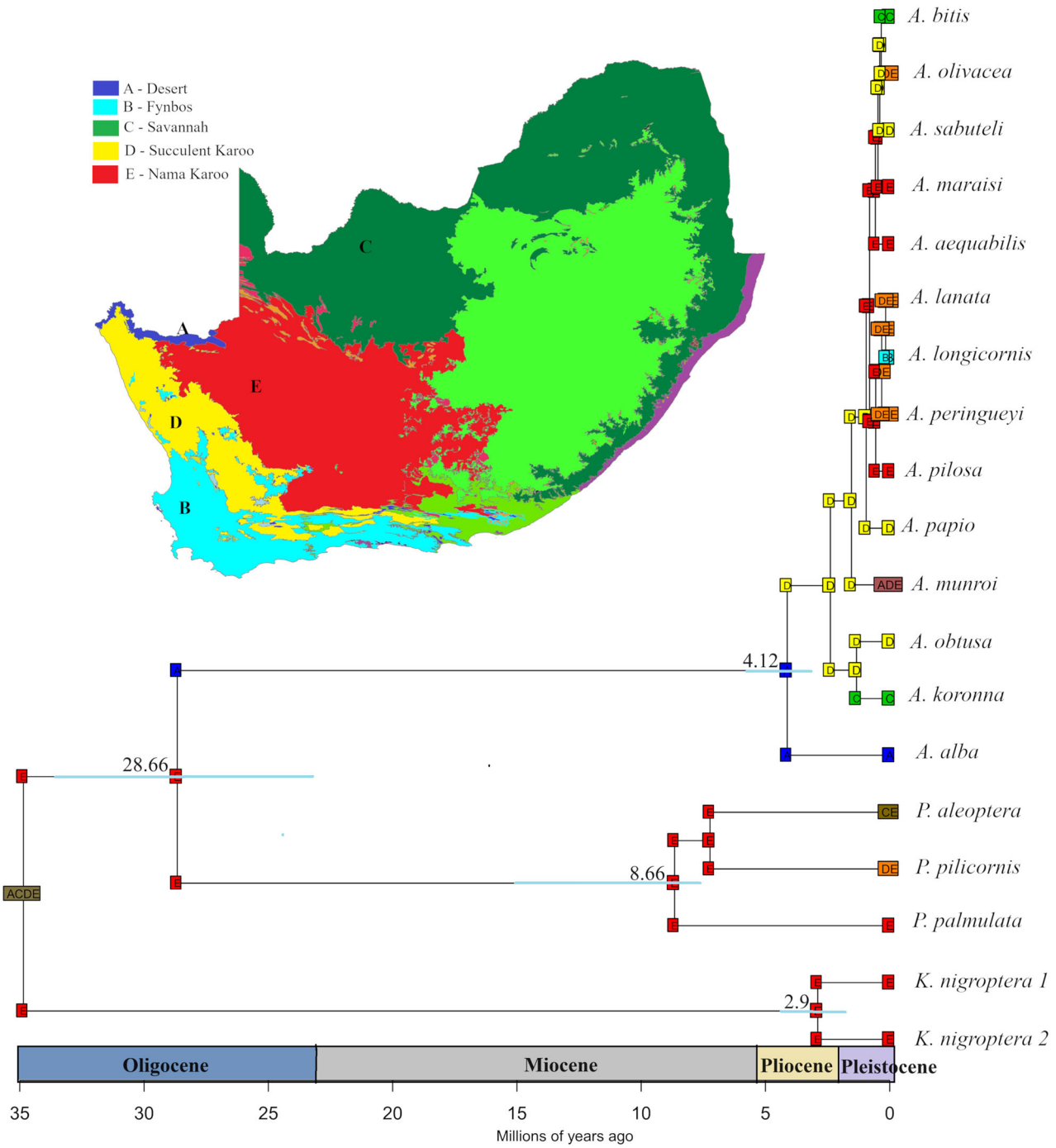

FIGURE 4 Relative estimated time of divergence for *Afroptera* species. The blue bars in the main nodes represent the time intervals for the 95% probability of actual age. Also the cladogram shows the ancestral ranges reconstruction for the genus inferred under the BAYAREALIKE+J model in 'BioGeoBEARS'. The letters A–E represent biomes in southern Africa based on Mucina et al. (2006): (A) Desert, (B) Fynbos, (C) Savannah, (D) Succulent Karoo and (E) Nama Karoo. Values on nodes represent mean estimated ages of divergence.

(lnL = −51.24, AICc = 110.1, Table 1, Figure 5). All models including founder-event speciation (J) were significantly better than models without this parameter (Table 1). The inclusion of the founder event reduced the estimates for dispersal (*d*) and extinction (*e*) parameters and increased the log-likelihood (Table 1). The BAYAREALIKE+J model suggested that the ancestor of *Afroptera* originated in the current Desert biome during the transition between Plio-Pleistocene followed by radiation into Succulent Karoo in early Pleistocene, and more recently the genus dispersed into Fynbos, Nama Karoo and Savannah (Figure 4).

Patterns of diversification

To conduct the LTT analysis, we calculate the gamma (ɣ) statistic and its significance (Pybus & Harvey, 2000) as an estimate of the change

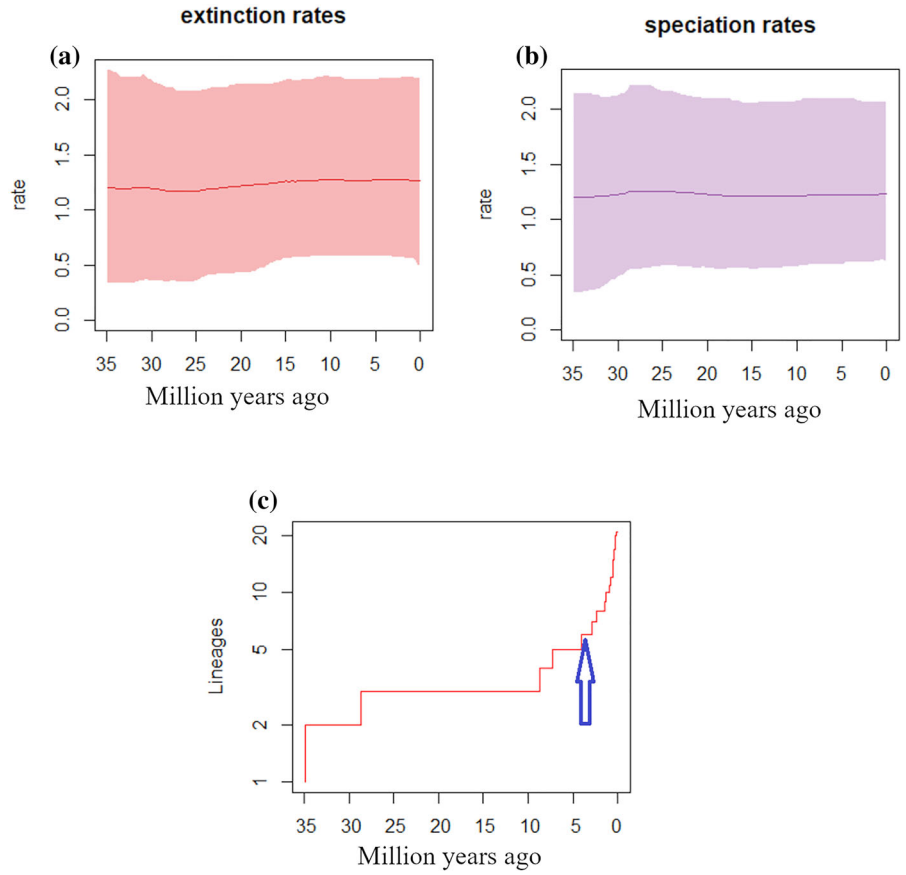

FIGURE 5 (a–c) Diversification analyses of *Afroptera* from TESS showing extinction rates (a), speciation rates (b) and *Afroptera* lineages through time (LTT).

diversification rate over the phylogeny. These values were −2.876124 ($p = 0.0017632$), respectively. The LTT suggests that the diversification of *Afroptera* lineages started in the end of Pliocene, followed by a rapid and intense lineage accumulation rate in the Pleistocene (Figure 5c). On the other hand, the CoMET analysis shows very stable extinction and speciation rates (Figure 5a,b) in the Plio-Pleistocene.

DISCUSSION

Phylogenetic relationships within Nemopterinae

Nemia and *Nemopterella* have always been taxonomically complex genera owing to the lack of conclusive characters to differentiate them (Sole et al., 2013). The classification of Tjeder (1967) was based on the presence or absence of pleuritocavae in the species of *Nemopterella* Banks and *Nemia* Navás, respectively. He accordingly assigned the name *Nemopterella* to all species that have pleuritocavae while those that lack the character were assigned to the genus *Nemia*.

Revision of the genus *Nemopterella* by Abdalla et al. (2019) indicated that the genus includes groups of species that are morphologically different and consequently suggested the division of *Nemopterella* into three genera: *Afroptera*; *Siccanda* and *Nemopterella* Banks, sensu stricto. The key morphological character to distinguish the species of *Afroptera* from *Siccanda* and *Nemopterella* sensu stricto is the presence of a membranous area on the last segment of the antennae. The last segment of the antennae in *Siccanda* and *Nemopterella* sensu stricto terminates in an acute bare tooth (Abdalla et al., 2019).

In addition, *Siccanda* is further distinguished from *Afroptera* by a faintly visible light yellowish pterostigma, with the shading over the costal veins being weakly manifest. *Afroptera* is characterised by a distinct yellowish, brown or dark brown pterostigma and the shading over the costals is very distinct. *Siccanda* is also characterised by a blackish-grey body, while the bodies of *Afroptera* species are yellowish, yellowish-brown or brown. Moreover, the frons above the antennae has a pair of yellow transverse spots along the postfrontal suture and the ventral side of the thorax is tinged dark brown in *Siccanda*, while the species of *Afroptera* lack these characters.

The second group comprising *Nemia*, *Barbibucca*, *Nemopterella* sensu *tricto*, *Knersvlaktia* and *Palmipenna* were statistically strongly supported as being monophyletic. The sister-group relationship between *Nemia* and *Barbibucca* is supported by the absence of pleuritocavae in both genera. *Barbibucca* is morphologically distinct from *Nemia* and *Nemopterella* sensu stricto by having a very robust body

and evenly broad hind wings. *Nemopterella* sensu stricto and *Nemia* are also morphologically different. According to Abdalla et al. (2019), the species comprising *Nemopterella* sensu stricto are distinguished by having the following characteristics: the presence of pleuritocavae in the fifth tergite of the male abdomen, while *Nemia* species lack this character. In addition, the species in *Nemopterella* sensu stricto are characterised by a whitish pterostigma in the forewings, while those of *Nemia* are characterised by a brown or dark brown pterostigma. Although there are distinctions between the two genera, they also manifest many common characteristics that place them into a single group. Characteristics include: terminal segment of the antennae terminates in an acute tooth; vertex of head broad with a pair of yellow or dark transverse spots along the postfrontal suture on the frons above the antennae; anal area tinged brown or dark brown; number of costal cells sometimes exceeds 30; the entire hind wing from the base to the apical whitish area bears black setae; thorax and abdomen with distinct brown to dark brown longitudinal mid and lateral-stripes; costals and area between the costals tinged brown to light brown.

Phylogenetic relationship within *Afroptera*

This is the first molecular and morphological study carried out on the phylogeny of the southern African genus *Afroptera*. All cladograms obtained from BI, MP and ML analyses recovered the genus *Afroptera* as a monophyletic lineage, revealing well-resolved trees with two major clades being recognised (labelled A and B) with *A. alba* being sister to all the species in the tree (Figures 2 and 3). The major Clade (A) split into four sub-clades: A1 with five species, *A. bitis*, *A. olivacea*, *A. sabuleti*, *A. maraisi* and *A. aequabilis*. Sub-clade A2 comprises two clusters; one cluster consisted of *A. longicornis*, *A. lanata*, *A. peringueyi*, and, in turn, they are sister to the cluster comprising *A. pilosa*. Another two sub-clades resolved each with single species: *A. papio* in sub-clade A3 and *A. munroi* in sub-clade A4. Major Clade B comprised two sister species *A. obtusa* and *A. koranna*.

The species in sub-clade A1 are very similar in their superficial appearance. They manifest many morphological characteristics that reveal them as close relatives. All the species are characterised by their large size, elongated forewings that end with sub-acute apex and the broad pterostigma in the forewings. All have the prescutum entirely covered with black hairs and the abdomen clothed in white hairs, except for *A. bitis* in which the black hairs are restricted to the anterior-lateral sides of the prescutum. The antennae of the species are long, reaching the pterostigma of the forewing and always ending with the last segment entirely membranous except for *A. aequabilis* in which the antennae are relatively short but still with the last segment completely membranous.

Sub-clade A2 contains *Afroptera longicornis*, *A. lanata*, *A. peringueyi* and *A. pilosa*. All the species share the markedly striped mesonotum and the reddish-yellow vertex of the head. The species, *A. longicornis*, *A. peringueyi* and *A. lanata* formed a well-supported sister-group. Morphologically these three species have mainly white hairs on the mesonotum and black hairs on the anterior lateral portions of the prescutum as well as white hairs on the dorsum and venter of the abdomen. Moreover, they have a brown pterostigma. *Afroptera papio* and *A. munroi* are single species that form sub-clades A3 and A4, respectively, and are sister to all species in the clade. *Afroptera papio* is remarkably different from the other species in the clade by having the whole mesonotum and abdomen pubescence white without intermingling black hairs. *Afroptera munroi* is differentiated from its congeners by having an indistinctly striped thorax and the middle and hind coxae of the legs are devoid of hairs. The close relationship between *A. obtusa* and *A. koranna* in major clade B is supported morphologically by having a reddish yellow vertex, short antennae, the same patterns of pubescence on the thorax and abdomen, length of the hind wing's dark area being the same length as the white apical area and legs with a tinged tip to the femurs.

Afroptera alba emerges as the sister to all species within clades A and B, standing out morphologically from its counterparts. This distinction is notably observed in its whitish appearance, attributed to an exceptionally pruinose body, whereas other species typically exhibit yellow or greyish-yellow hues. Environmental influences, as suggested by Rowell (1972), might contribute to the contrasting body colours between the lineages. *A. alba* thrives exclusively in desert environments characterised by extreme aridity and heat, prompting adaptations to tolerate such harsh conditions. Insects, including *A. alba*, have evolved various physiological, behavioural and morphological mechanisms to cope with heat stress (Sheikh et al., 2017). Pruinosity, a feature found in many insects such as Odonata, serves functions like reflecting ultraviolet light and dissipating heat by reflecting solar radiation (Corbet, 1999). Given the thermal challenges of desert habitats, marked by temperature fluctuations from low in winter to scorching in summer, it is plausible that *A. alba* evolved its pruinose body as an adaptation to survive such extremes.

Contrarily, according to Abdalla et al. (2019), the remaining *Afroptera* species not included in the phylogeny share morphological similarities, except for *A. alba*. Consequently, it is unlikely that they could be considered 'basal' to *A. alba*. This assertion refrains from speculation, given the absence of these species in the phylogenetic analysis. This scenario underscores a crucial aspect of evolutionary biology and phylogenetics: the interplay between morphology and genetic data. While morphological traits can offer valuable insights into evolutionary relationships, genetic data often provide a more precise depiction of evolutionary history. It is not uncommon for morphological similarities to be misleading, especially when considering factors like convergent evolution, which can yield similarities that do not reflect true genetic relatedness. This highlights the importance of integrating both morphological and genetic data in the future study for a comprehensive understanding of evolutionary relationships (Dayrat, 2005).

Biogeography and diversification of *Afroptera*

Our analyses suggest that the ancestors of *Afroptera* originated in the Pliocene (5.24–3.13 Mya) in a desert environment, which currently comprises the Desert biome in southern Africa (Figure 4). This was

followed by a recent diversification and radiation into the Succulent Karoo and Fynbos biomes, where a high diversity of species is recorded. However, more recently, a few representatives of the genus *Afroptera* colonised the Nama Karoo and Savannah (Kalahari) biomes. The timing of the evolutionary history of the genus *Afroptera* coincides with the second uplift during the Pliocene in the southeast, resulting in the isolation of the interior plateau (Dauteuil et al., 2015; Partridge & Maud, 1987). Another significant event during the Pliocene was the inception of the winter rainfall regime and summer drought in the southwest region (Deacon, 1983; Sciscio et al., 2016). These two geoclimatic events are thought to have greatly contributed to the aridification of the western part of the subcontinent (Deacon, 1983; Diester-Haass et al., 2004; Petrick et al., 2015; Sepulchre et al., 2006).

Climatic and topographic changes act as primary factors that influence species diversification (Crisci et al., 2006; Liu et al., 2013; Mantooth & Riddle, 2011; Posadas et al., 2006). On the one hand, topographic heterogeneity may influence species diversification by increasing habitat diversity and by limiting gene flow between populations inhabiting different ecological niches (Verboom et al., 2015). On the other hand, climatic fluctuations and, in particular, those associated with glacial periods are the primary factors that enhance species diversification shaping their distributions (Demenocal, 2004; Potts, 1996). So how did Plio-Pleistocene geoclimatic changes in southern Africa affect the diversification of *Afroptera* species?

Species of the southern African genus *Afroptera* exhibit wide but disjunctive distributions, primarily in the Fynbos and Succulent Karoo biomes, with recent dispersion into the Nama Karoo and Savannah (Kalahari) biomes; and a few Plio-Pleistocene relict species in the Desert biome. Our analysis suggests that the eco-climatic fluctuations during the Plio-Pleistocene did not result in an environmental crisis leading to a mass extinction of *Afroptera* species. The current high diversity of *Afroptera* species in xeric environments could have been favoured by the intensification of aridification in the southwest during the Plio-Pleistocene, which aligns with the dry-adapted nature of the ancestors of the genus *Afroptera*. In this scenario, a climatically driven diversification model for *Afroptera* would explain our observed constant speciation rates (Figure 5b) throughout the Plio-Pleistocene. The role of climate as a driver of insect speciation has been reported in several groups in southern Africa (for details, see Daniel et al., 2020, 2021; Hemp et al., 2020; Matenaar et al., 2016; Price et al., 2007; Sole et al., 2013; Strümpher et al., 2016; Switala et al., 2014).

The subsequent radiation in *Afroptera* occurred during the late Pliocene (2.39 Mya) when clade A split from clade B (Figure 4). The driving force behind this radiation is presumably the general climatic changes at that time, including the inception of arid and cooler conditions in the southwestern region of the subcontinent. The increasing aridity, with sporadic hyper-arid habitats in the Cape region, acts as a centre for species radiation (Linder, 2003; Sepulchre et al., 2006).

Our LTT analysis indicates a marked increase in lineage accumulation and rapid diversification of *Afroptera* during the transition between the Pliocene and Pleistocene (Figure 5c). This may have been facilitated by the prevalent Mediterranean climate in the region and the emergence of new arid and semi-arid habitats, as observed in plants (Cowling et al., 2005, 2009) and reptiles (Swart et al., 2009; Tolley et al., 2009). Moreover, a pattern of rapid recent origin of species (Figure 5c), resulting from extensive habitat changes due to climatic shifts as reported in our study, has been documented for many Cape faunal and floral elements. For example, the most recent diversification of many Cape clades (e.g., Restionaceae) is attributed to the effect of the Antarctic (Benguela) current around 5.2 Mya (Axelrod & Raven, 1978; Demenocal, 1995, 2004; Goldblatt, 1997; Linder, 2003; Linder & Hardy, 2004; Richardson et al., 2001). Similarly, many evolutionary studies of fauna confined to the Cape region have correlated recent speciation with climatic shifts in the post-late Miocene era (Linder et al., 2010; McDonald & Daniels, 2012; Sole et al., 2013; Swart et al., 2009; Tolley et al., 2006). Interestingly, the close proximity of recent rapid radiation events during the transition between the Pliocene and Pleistocene for most current *Afroptera* species may explain their close morphological similarity, as there has been inadequate time for significant differentiation, making their distinction challenging in most cases—as represented by short terminal branches in the trees (Figures 2–4).

Have the *Afroptera* species co-evolved with the Cape Flora? The Cape flora consists of many plant families including Asteraceae, Fabaceae, Iridaceae, Ericaceae, Scrophulariaceae, Proteaceae, Restionaceae, Cyperaceae, Orobanchaceae, Rutaceae, Diosmeae, Polygalaceae, Rhamnaceae, Thymelaeaceae and Poaceae (Goldblatt, 1978). Among these, Ruchioideae (Aizoaceae) is the second largest family in the southern African flora and the largest family in Namaqualand and in southwestern Namibia (Goldblatt, 1978). Molecular dating of the Aizoaceae suggested that the family originated about 8.7–3.7 Mya (Klak et al., 2004). As the southern African Nemopterinae and Aizoaceae occur in the same areas of distribution, Sole et al. (2013) hypothesised that Nemopterinae may have a diet that relies on Aizoaceae and that they may have co-evolved at the same time. The results of Sole et al. (2013) of divergence time of Nemopterinae have shown that most genera including *Nemia* and *Nemopterella* (currently *Afroptera*, *Nemopterella* sensu stricto and *Siccanda*) underwent recent rapid adaptive radiations during the Late Miocene (ca. 6–4.5 Mya), which accords with our results. Although our results strongly support the Sole et al. (2013) hypothesis; a recent unpublished study by Chirango (2014) on the gut contents of southern African nemopterids provided different insights on the co-evolution of nemopterids and Cape flora. One of the aims of that study was to test the hypothesis of Sole et al. (2013) as to whether the speciation of southern African Nemopteridae follows Aizoaceae radiation as an adaptive radiation; thereby hypothesising that Aizoaceae would be the main diet of this group. By collecting specimens from different types of vegetation and biomes, Chirango's (2014) study included two Crocinae genera (*Laurhervasia* Navás and *Concroce* Tjeder) and nine Nemopterinae genera (*Palmipenna*, *Nemopterella*, *Nemia*, *Knersvlaktia*, *Nemeura*, *Sicyoptera*, *Semirhynchia*, *Derhynchia* and *Nemopistha*).

An analysis of gut contents in different genera showed that of the 11 genera examined, *Nemopterella* contained a variety of pollens that included about 24 pollen types, *Nemeura* 13 and *Palmipenna* and *Nemia* had 12 each, *Derhynchia* and *Concroce* did not contain any

pollen and the other genera contained 2–4 pollen types (Chirango, 2014). The study also indicated that the plant family Asteraceae is the most abundant pollen type in nemopterid's gut contents. It was present in 7 of the 11 genera examined and constituted about 100% of *Knersvlaktia* diet and 99% of *Palmipenna aeoleoptera* Picker, 1987, 90% of *Palmipenna pilicornis* Tjeder, 1967 and 57% of *Nemopterella* (Chirango, 2014). *Nemia* was found to be the most reliant on Aizoaceae pollens, which reached 75%–94% of the diet. Aizoaceae pollen was less in *Sicyoptera* (15%) and *Nemopterella* (10%), and in *Palmipenna*, it was less than 10% (Chirango, 2014). The study concluded that *Nemia* is the only Nemopteridae genus that may have co-evolved with Aizoaceae, while the remaining genera, including the genus *Afroptera* evolved independently, and their recent diversification was presumably to accommodate the high variety of food resources available in the Cape region (Chirango, 2014).

CONCLUDING REMARKS

We demonstrate, for the first time, the monophyletic nature of the genus *Afroptera* and elucidate the phylogenetic relationships among its species. Our findings indicate that *Afroptera* originated during the Pliocene in the Desert biome, followed by rapid diversification primarily in the Fynbos and Succulent Karoo biomes during the Pleistocene. This recent origin aligns with previous suggestions by Sole et al. (2013). Furthermore, our analysis suggests that climatic changes, such as the intensification of arid conditions and the inception of a winter rainfall regime in the southwest region of southern Africa, have been driving forces behind the diversification of the genus *Afroptera*. This study presents an opportunity for future studies to explore further the co-evolution and radiation of nemopterids or herbivorous insects with the Cape Flora. Consequently, our results serve as a crucial benchmark for future studies investigating the diversification and evolution of eco-climatic ranges of invertebrates in southern Africa and beyond.

AUTHOR CONTRIBUTIONS

Ishtiag H. Abdalla: Conceptualization; writing – original draft; methodology; visualization; writing – review and editing; formal analysis. **Mervyn W. Mansell:** Conceptualization; writing – original draft; visualization; writing – review and editing; supervision. **Catherine L. Sole:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing; supervision; visualization. **Gimo M. Daniel:** Formal analysis; writing – review and editing; methodology.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All molecular sequence data is available on GenBank. All molecular sequence data is available on GenBank. Datasets, R scripts and trees are available on Dryad (https://doi.org/10.5061/dryad.tmpg4f56k).

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SUPPORTING INFORMATION

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

Appendix S1. Morphological characters list and their corresponding states used in this study.

Figure S1. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the combined (COI, CAD, 16S, 28S and 18S) with PP, MP and MLB given, respectively. Dashes (-) on nodes indicate weak/no support. Letters below branches indicate the clade's name.

Figure S2. The single most parsimonious tree of the combined morphological/molecular concatenated dataset with bootstrap support values on each branch. Dashes (-) on branches indicate weak/no support.

Table S1. Species included in this study, with collection locality and GenBank accession numbers.

Table S2. Primers used for PCR amplification.

Table S3. Estimated model parameters for COI, 16S, 28S 18S and CAD for *Afroptera* using JMODELTEST.

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