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Paleogene forest fragmentation and out-of-Africa dispersal explain radiation of the Paleotropical dung beetle tribe Epactoidini *trib. nov.* (Coleoptera: Scarabaeinae)

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Abstract

Paleotropical clades with largely disjunct distributions are ideal models for biogeographic reconstructions. The dung beetle genera *Grebennikovius* Mlambo, Scholtz & Deschodt, *Epactoides* Olsouffief and *Ochicanthon* Vaz-de-Mello are distributed in Tanzania, Madagascar and Réunion, and the Oriental region, respectively. We combine morphology and molecular dataset to reconstruct the phylogenetic relationships between these taxa. Our analyses corroborate previous hypotheses of monophyly of the group, which is here described as new tribe Epactoidini **trib. nov.** *Grebennikovius* is recovered as sister to *Epactoides*, while *Ochicanthon* emerges as sister to them both. The disjunct distribution of our focal clade is unusual within the subfamily Scarabaeinae. Bayesian divergence time estimates and ancestral range reconstructions indicate an African origin of the crown group of the tribe Epactoidini **trib. nov.** in the early mid Eocene, ca. 46 Ma. The divergence between *Epactoides* and its sister is dated to 32.3 Ma, while the crown age for the genus *Ochicanthon* is dated to 27 Ma. We investigate the factors that may have shaped the current distribution of the tribe Epactoidini **trib. nov**. The formation of the *Gomphotherium* landbridge, along with favourable environmental conditions would have allowed dry-intolerant organisms, such as *Ochicanthon*, to disperse out of Africa. Remarkable climatic stability of the Eastern Arc Mountains was critical for the retention of the monotypic genus *Grebennikovius*. We suggest two subsequent overwater dispersal events: the migration of the most recent common ancestor (MRCA) of *Epactoides* from Africa to Madagascar (32.3–29.5 Ma); the lately dispersal of the MRCA of the today's extinct *Epactoides giganteus* Rossini, Vaz-de-Mello & Montreuil to Réunion island from Madagascar (3.4 Ma). We suggest that the high potential of dispersal of Epactoidini **trib. nov.** dung beetles and the strict association to forest habitat might have triggered two major radiations, one in Madagascar and one in the Oriental Region.

KEYWORDS
biogeography, evolution, phylogenetics

INTRODUCTION

Major geological events and paleoclimatic fluctuations are among the main drivers that shape biotas. Disentangling their interplay helps explaining biogeographic history of organisms (Lomolino et al., 2010). Many lineages display Paleotropical distribution, with a disjunction between Afrotropical and Oriental Regions. Recent studies have elucidated numerous biogeographic factors that have shaped such disjunct ranges in Paleotropical clades (Biju & Bossuyt, 2003; Gustafson et al., 2017; Joshi & Edgecombe, 2019; Lecompte et al., 2008; Raxworthy et al., 2002; Sparks & Smith, 2004; Tamar et al., 2016; Toussaint et al., 2016, 2019, 2021; Yoder & Nowak, 2006; Yuan et al., 2005; Zhou et al., 2012). All these factors fall into four major scenarios. The '*biotic ferry*' scenario (Datta-Roy & Karanth, 2009) suggests that the Paleotropical disjunction is associated with the fragmentation of Gondwana when biotas could disperse from Africa to Asia by crossing Indian Ocean on drifting tectonics plates. This scenario implies a correspondence between divergence times in Paleotropical clades and chronology of Gondwana break-up (Rutschmann et al., 2004; Sparks & Smith, 2004; Wilkinson et al., 2002). The *boreotropical* dispersal scenario (Zachos et al., 2001) proposes that forests distributed throughout southern Eurasia during Late Palaeocene – Early Eocene could serve as a bridge to facilitate biotic exchange between Africa and Asia (Couvreur et al., 2011; Wolfe, 1975). The *Gomphotherium* corridor scenario (Rögl, 1999) suggests that the migrations between Africa and Asia in both directions were caused by the closure of the Tethys sea and the existence of pockets of tropical forests distributed at low to mid-latitudes during Late Oligocene – Mid Miocene (Morley, 2000). Lastly, the *routine transoceanic* dispersal scenario proposes various and constantly occurring transport mechanisms, such as floating vegetation and oceanic currents, to drive intercontinental migration and disjunction (Gillespie, 2012; Nathan, 2006).

The biotic ferry scenario implies that lineage divergence time should be older than break-up of eastern Gondwana, and these examples are quite rare in the literature (Pyron, 2014; Toussaint et al., 2016). On the other hand, the boreotropical and *Gomphotherium* dispersal scenarios assume younger lineage age and suggest the pivotal role of forest corridors in driving biotic exchange between Africa and Asia. For example, out-of-Africa migrations via the *Gomphotherium* corridor have been well documented for tetrapods (Harzhauser et al., 2007) and snakes (Pook et al., 2009), while Asia-to-Africa dispersals through the same corridor have been proposed for termites (Bourguignon et al., 2017), butterflies (Kaliszewska et al., 2015; Toussaint et al., 2019, 2021), amphibians (Yuan et al., 2018) and lizards (Tamar et al., 2016, 2018). However, it is still unclear whether these four scenarios can sufficiently explain all cases of disjunct Paleotropical distributions.

In this study, we aim at reconstructing the biogeographic history of a clade of forest-dwelling dung beetles (Coleoptera: Scarabaeinae) with remarkable Paleotropical range that is disconnected among Eastern Arc Mountains (EAMs) in Africa, Madagascar, Réunion island and Asia (Figure 1). This disjunct distribution is uncommon in animals, which makes it particularly interesting for studying Paleotropical fragmentations and assessing the impact of the four aforementioned scenarios in shaping Paleotropical biotas.

Our focal dung beetle clade comprises 92 valid species classified in three genera: *Grebennikovius* Mlambo, Scholtz & Deschodt, *Epactoides* Olsoufieff and *Ochicanthon* Vaz-de-Mello. The monotypic *Grebennikovius* is endemic to the Uluguru mountains (EAMs) which are known to have served as refugia for ancient and peculiar biotas (Lovett et al., 2004; Lovett & Wasser, 2008). The genus *Epactoides* comprises ~40 species, many of which are microendemics in Madagascar (Fairmaire, 1898; Montreuil, 2003, 2005, 2017; Paulian, 1935, 1975, 1976, 1991, 1992; Wirta & Montreuil, 2008), and one recently extinct species from Réunion island (Rossini et al., 2021). Finally, the Oriental genus *Ochicanthon* includes over 50 species distributed from southern India to southern Philippines (Krikken & Huijbregts, 2007; Latha et al., 2011).

The monophyly of our focal clade is supported by earlier morphological and molecular phylogenetic studies (Mlambo et al., 2014; Monaghan et al., 2007; Tarasov & Dimitrov, 2016; Tarasov & Génier, 2015; Wirta et al., 2010; Wirta & Montreuil, 2008). There is a wide agreement that the divergence age of this clade is younger than the break-up of Madagascar from India (88–85 Ma) (Mlambo et al., 2014; Wirta et al., 2010). However, the relationships among the three genera and their divergence times still remain unclear or controversial (Mlambo et al., 2014; Tarasov & Dimitrov, 2016; Wirta et al., 2010), which hinders biogeographic analysis.

Here, we use an increased species sample for the three genera to infer their dated phylogeny using a total evidence approach and reconstruct their biogeographic history. Our combined dataset comprises eight gene loci and 103 morphological characters, which were assembled for 84 Operational Taxonomic Units (OTUs) of Scarabaeidae of which 33 OTUs belong to our focal clade. We estimate the divergence dates using Bayesian framework in BEAST (Drummond et al., 2012) by selecting among alternative clock models. Our combined phylogenetic analyses corroborate previous studies and support the isolated position of the three focal genera with respect to other dung beetle lineages. Based on this evidence and distinct shared morphology, we place them in the new tribe Epactoidini **trib. nov.** We reconstruct ancestral ranges of Epactoidini **trib. nov.** members by comparing alternative biogeographic models in BioGeoBEARS (Matzke, 2018). Finally, we assess and discuss the contribution of various biogeographic mechanisms, as well as geologic and paleoclimatic events in shaping the disjunct distribution of the new tribe.

MATERIAL AND METHODS

Combined dataset

The combined dataset of morphological and molecular characters included 84 OTUs (Table S1): (i) 33 ingroup OTUs from the genera *Epactoides* (25 OTUs), *Grebennikovius* (1 OTU) and *Ochicanthon* (7 OTUs); (ii) 41 outgroup OTUs belonging to various other genera of

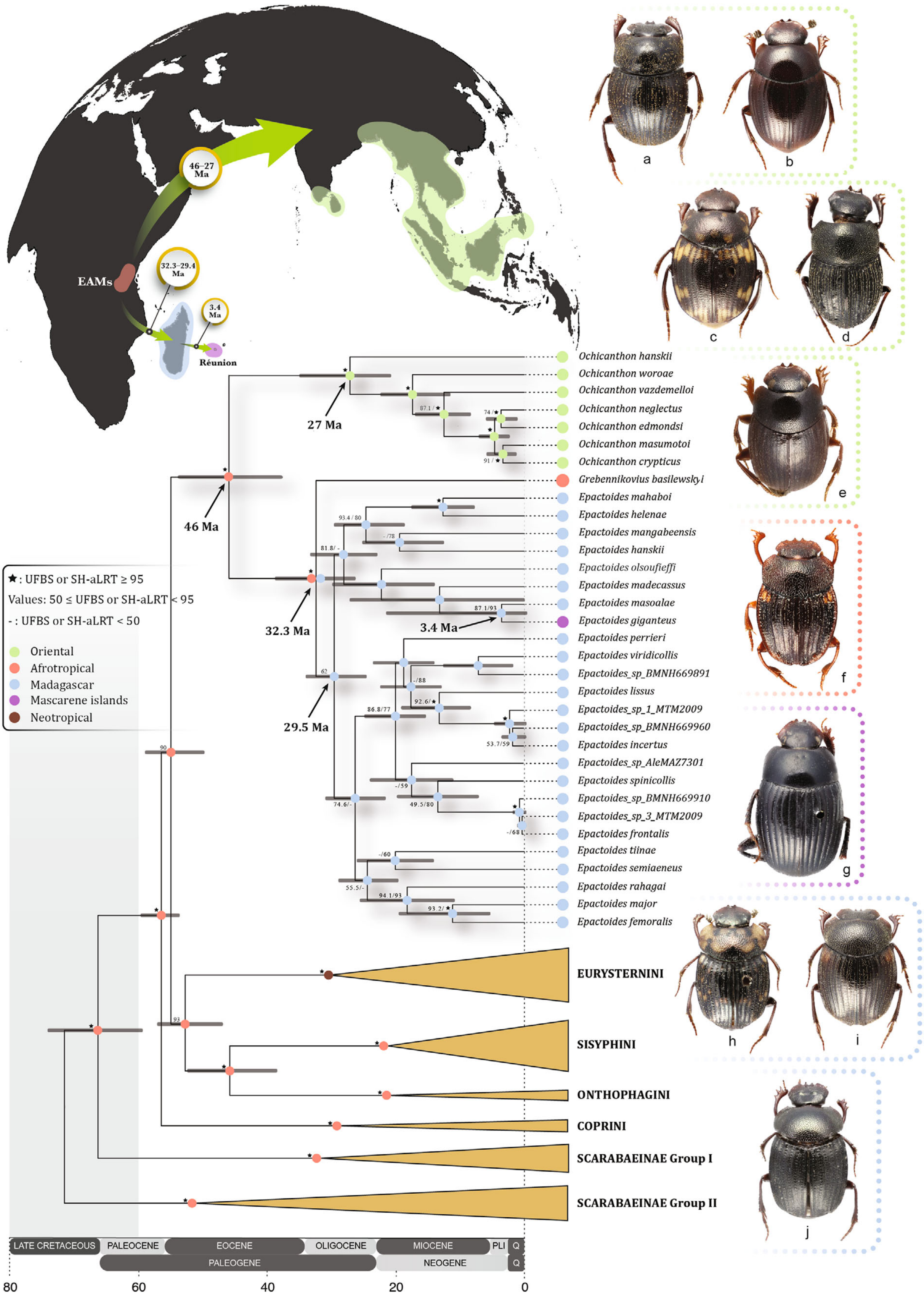


FIGURE 1 Legend on next page.

Scarabaeinae and (iii) 10 outgroup OTUs belonging to the subfamilies Hybosorinae Erichson, and Aphodiinae Arrow, which have been previously identified as close relatives of Scarabaeinae (Ahrens et al., 2014; Lawrence et al., 2011; Philips, 2011; Smith et al., 2006). Our taxonomic sample covers 35% of the ingroup species diversity and a representative set of its phenotypes. In the combined dataset only one species (*Epactoides giganteus* Rossini, Vaz-de-Mello & Montreuil) was missing molecular data, as it is only known by the holotype, an old museum specimen. Eight OTUs from outgroup and two from ingroup (*Epactoides femoralis* [Montreuil] and *E. lissus* Lebis) were missing morphological data due to their unavailability. The details on morphological and molecular data alone are given below.

Morphological data

In assembling morphology, we identified 11 characters (Appendices S1–S2) that were considered to be putatively informative for resolving the relationships within the ingroup: *Epactoides* + *Grebennikovius* + *Ochicanthon*. Next, we combined them with the global morphological matrix (205 characters) from Tarasov and Génier (2015) and scored the combined character matrix for 74 OTUs from ingroup and outgroup. We removed constant characters and the final matrix contained 103 characters that were used in all downstream analyses.

The examined material is deposited in the Coleoptera collection of the Finnish Museum of Natural History (FMNH), Musee des Sciences Naturelle of Genéve (MNHG) and Musée royal de l'Afrique centrále (RMCA).

In examining morphology, head, prothorax, abdomen and associated sclerites were disarticulated and genitalia of both sexes were dissected for detailed investigation. Body parts were firstly cleaned in a 10% KOH solution, and then bleached in a 30% hydrogen peroxide solution for some minutes to facilitate the comprehensive study of the phenotypic traits of the body parts. After dissection, genitalia were cleaned in a KOH solution and then stored in bioassay plates with glycerol, along with disarticulated body parts of the same specimen.

Morphological terminology follows Tarasov and Solodovnikov (2011), Tarasov and Génier (2015) and Génier (2019).

Molecular data

Molecular sequences for the analysed taxa were retrieved from GenBank. Additionally, seven *Ochicanthon* species were sequenced in this study to enrich representation of this genus in molecular dataset. The final dataset included three protein-coding loci (CAD, TOP1, COI), one mitochondrial (16S) and three nuclear rRNA gene fragments (18S, 28Sd1, 28Sd2). The proportion of missing loci was 58%. GenBank accession numbers are summarized in Table S1 (supporting information). Gene fragments were individually aligned using MAFFT with the L-INS-i algorithm (Katoh et al., 2002).

Maximum likelihood (ML) analyses

The combined initial dataset was concatenated into eight prior partition subsets: three codon partitions, 16S, 18S, 28sd1, 28sd2 and morphology. The best partition and substitution models were selected using ModelFinder (Kalyaanamoorthy et al., 2017) and implemented in IQ-TREE (Nguyen et al., 2015) under Bayesian Information Criterion. The best partition was inferred to be the same as the prior one except that 18S and 28Sd2 were placed in the same subset. For the morphological subset, MK $+ \Gamma$ model (Lewis, 2001) and ascertainment bias correction were selected, which is the usual model for this type of data. ModelFinder results were used in the subsequent IQ-TREE search to infer the ML tree. We ran 20 individual runs to bypass potential local optima using the following settings: *iqtree2 -spp myfile -m TESTMERGE -ninit 200 -nstop 1000 -pers 0.2 -ntop 100 -nbest 20 -rcluster 30 -alrt 1000 -bb 1000*. Branch support was calculated using ultrafast bootstrap (UFBS) approximation (Minh et al., 2013) and the Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT) (Anisimova et al., 2011), with 1000 replicates. Additionally, we analysed molecular and morphological data separately using the same settings but 10 individual runs to assess the impact of different data sources.

Combined analysis and divergence time estimation

BEAST was used to generate a dated phylogenetic tree using Bayesian inference on the fixed topology obtained from the combined ML analysis. We ran analyses under strict, and uncorrelated exponential relaxed clock models and selected the best one using Bayes factor (BF). In both models, a Yule process was used to generate the timetree with an exponentially distributed birth rate prior (*mean* $= 0.1$). In the relaxed clock model, the prior for mean branch rate (ucedMean) was set to *Normal* (0.008, 0.001). For other model parameters, we used default BEAST's priors. The data partition was the same as that inferred in the ML analysis, except that HKY $+ \Gamma$ model was assigned

FIGURE 1 Ancestral range reconstruction of Epactoidini **trib. nov.** BioGeoBEAR tree and map showing suggested dispersal routes of *Epactoides* and *Ochicanthon* from Afrotropical ancestors, including date estimation. Nodal support values are expressed as UFBS and SH-aLRT. Bars at nodes indicate 95% CI of estimated ages. Distribution areas of Epactoidini members: Eastern Arc Mountains (EAMs) (red); Madagascar (light blue); Réunion (purple); Oriental (green). Species illustrated: (a) *Ochicanthon hanskii* Krikken & Huijbregts, (b) *O. woroae* Ochi, Ueda & Kon, (c) *O. crypticus* Krikken & Huijbregts, (d) *O. devagiriensis* Sabu & Latha, (e) *O. ceylonicus* Cuccodoro, (f) *Grebennikovius basilewskyi* (Balthasar), (g) *Epactoides giganteus*, (h) *E. madecassus* (Paulian), (i) *E. major* (Paulian), (j) *E. frontalis* (Montreuil).

to the molecular subsets to achieve sufficient mixing and convergence in the Markov Chain Monte Carlo (MCMC) sampling. For each clock model, we ran four MCMC analyses: two for tree dating and two for marginal likelihood calculation with 50 Million and 2 Million generations respectively. Marginal likelihood was calculated using path sampling (Lartillot & Philippe, 2006) with $alpha = 0.3$ and 100 steps.

Calibration points for divergence time estimation

Scarabaeinae are lacking sufficient and phylogenetically informative fossils to use as direct calibration points for tree dating (Tarasov et al., 2016). Therefore, we used secondary calibration points obtained from a recent and comprehensive phylogenomic study of Coleoptera (McKenna et al., 2019). Three relevant divergence estimates were selected for constructing most recent common ancestor (MRCA) priors in BEAST: Hybosoridae Erichson + Scarabaeidae Latreille (147 Ma), Aphodiinae Arrow + Scarabaeinae (93 Ma) and *Copris* Geoffroy + *Onthophagus* Latreille (59.5 Ma). The MRCA priors were normally distributed with mean equal to the divergence estimate and standard deviation 1. This standard deviation was chosen to make the MRCA priors concentrated around mean values of the inferred ages.

Historical biogeographic reconstructions

Ancestral range reconstruction was performed in the R package BioGeoBEARS (Matzke, 2018). The input dataset of our analyses included the dated phylogenetic tree obtained in BEAST, and an area distance matrix, which incorporates geographic distance (Appendix S6). Since our species sample is biased with respect to biogeography, we analyse four different subsets of the dated tree to test the sensitivity of the results. Specifically, the following trees were analysed: (SUB1) full time-tree obtained in BEAST, which includes all terminals; (SUB2) tree that excludes remote outgroup (i.e., *Aphodius* Hellwig, *Phaeochrous* Castelnau, *Rhyparus* and *Hybosorus* MacLeay) to avoid potential bias that might be associated with significantly older biogeographic events; (SUB3) the same tree as in SUB2 but where non-ingroup terminals belonging to the same genus were collapsed into single OTU (i.e., *Eurysternus* Dalman, *Copris*, *Onthophagus*, *Sisyphus* Latreille, *Odontoloma* Boheman, *Byrrhidium* Harold, *Coptorhina* Hope and *Sarophorus* Erichson); (SUB4) tree that included only ingroup terminals (i.e., *Grebennikovius*, *Epactoides* and *Ochicanthon*). We performed maximum-likelihood inference with six biogeographic models: (i) Dispersal-Extinction-Cladogenesis (DEC), (ii) DIVALIKE, (iii) BAYAREALIKE, (iv) DEC + J, (v) DIVALIKE + J and (vi) BAYAREALIKE + J; where ‘J’ refers to the founder event parameter that is automatically estimated from data. The analysed models differ in the types of geographic range evolution that follow cladogenetic events (Matzke, 2013, 2014). Relative model fit was estimated using Akaike Information Criterion (AIC) (Matzke, 2014; Ree & Sanmartín, 2018) and ΔAIC that was calculated as $\Delta AIC = AIC(model_i) - AIC(model_{best})$. Distribution ranges were coded as follows: A (Afrotropical); M (Madagascar); R (Mascarene Islands); O (Oriental); N (Nearctic); P (Palearctic); T (Neotropical).

RESULTS

ML phylogenetic analyses

Twenty ML runs of the combined dataset yielded log likelihood (Ln) scores ranging from −36,068.755 to −36,081.147, which indicates the presence of multiple local maxima. The best topology was recovered in four analyses with the top Ln scores (from −36,068.755 to −36,071.826), which indicates that our ML search has repetitively converged to the same result. This ML tree was selected for further discussion and downstream analysis using BEAST and BioGeoBEARS (Appendix S5–S6).

The combined analysis recovers the monophyly of the genera *Epactoides* (UFBS: 83.8; SH-aLRT: 49) and *Ochicanthon* (UFBS: 100; SH-aLRT: 100) and the clade *Epactoides* + *Grebennikovius* (UFBS: 96.8; SH-aLRT: 99). The monophyly of the focal clade *Epactoides* + *Grebennikovius* + *Ochicanthon* is well supported too (UFBS: 100; SH-aLRT: 100). The same results are inferred using molecular and morphological data alone, except for *Grebennikovius* that was found nested within *Epactoides* (Appendix S4). In both molecular and morphological analyses, early splits within *Epactoides* are poorly supported indicating that the position of *Grebennikovius* is not confidently resolved. This means that separate analyses do not have enough information to correctly recover *Epactoides* and *Grebennikovius* relationships. The relationships retrieved by the molecular dataset were very similar to those recovered by the combined analysis (Appendix S4). At the same time, the morphological tree was substantially less resolved; the relationships within Scarabaeinae differed from those inferred in the global dung beetle morphological phylogeny (Tarasov & Génier, 2015) due to reduced taxon and character sampling. The clade *Epactoides* + *Grebennikovius* + *Ochicanthon* was found to be sister group to the clade Sisyphini + Onthophagini + Eurysternini in the combined analysis (UFBS: 67.3, SH-aLRT: 88) and sister to the tribe Eurysternini in the molecular analysis (UFBS: 66.6; SH-aLRT: 81) (Appendix S4).

Model comparison and divergence time estimation

BEAST analyses converged on the same posterior distribution for all parameters. The marginal likelihoods of two runs for the strict clock model (M_s) was (−38,595.67, −38,595.85), while for the exponential relaxed clock model (M_r) was (−38,912.92, −38,913.07). Given that the strict clock model has substantially outperformed the relaxed one, $BF(M_s,M_r) = 317.23$, we selected it for ancestral range reconstruction and further discussion.

The time-tree inference recovered the mean crown age for the focal clade at 46 Ma, with 95% credibility interval (CI) equal to

TABLE 1 Results of BioGeoBEARS analyses

Model	LnL	# param	*d*	*e*	*j*	AIC	Δ*AIC*
DEC + J	**−78.63**	**3**	**0.0003**	**1.0e−12**	**0.0085**	**163.3**	**0**
BAYAREALIKE + J	−80.83	3	0.0002	0.0006	0.0100	167.7	4.4
DIVALIKE	−98.45	2	0.0009	0.0040	0.0000	200.9	37.6
DIVALIKE + J	−98.36	3	0.0009	0.0040	0.0001	202.7	39.4
DEC	−178.90	2	0.0100	0.0100	0.0000	361.8	198.5
BAYAREALIKE	−194.90	2	0.0100	0.0100	0.0000	393.8	230.5

Note: Models are presented in rows. Statistics presented in the column are from left to right: log-likelihood of the model (LnL); number of parameters estimated by the model (# param); mean estimated dispersal ratrhyparuse (d); mean estimated extinction rate (e); mean estimated rate of cladogenetic events (j); Akaike Information Criterion (AIC); and AIC weights (Δ*AIC*).

50–42 Ma. The clade formed by Sisyphini, Onthophagini and the Neotropical Eurysternini was recovered as sister to Epactoidini **trib. nov.**, and they were estimated to split at 58.5 Ma (CI: 61–56). The inferred age for the separation between *Epactoides* and *Grebennikovius* was 32.3 Ma (CI: 36–29). The crown age for *Ochicanthon* was estimated at 27 Ma (CI: 31–23), and the divergence between *Epactoides giganteus* from Réunion island and its sister species from Madagascar was estimated at 3.4 Ma (CI: 10–0.001).

Biogeographic reconstructions

Given AIC scores, the best biogeographic models recovered for the subset trees were: DEC + J (SUB1 and SUB2), BAYAREALIKE+J (SUB3), DIVALIKE (SUB4) (Table 1, Appendix S6: Tables S3–S6). In trees SUB1 and SUB2, the DEC + J model suggests an Afrotropical origin for the focal clade, Oriental origin for *Ochicanthon*, and African origin for *Grebennikovius* + *Epactoides* (Figure 1; Appendix S6). Different biogeographic scenarios are estimated with SUB3 and SUB4, where the best models suggest an Afro-Oriental and Afro-Oriental-Madagascan origin for the focal clade, respectively; the same analyses suggest an Afro-Oriental (SUB3) and Afro-Madagascan (SUB4) origin for the clade *Grebennikovius* + *Epactoides* (Appendix S6).

DISCUSSION

Systematics: Tribe Epactoidini trib. nov.

Morphological and molecular evidence

Our combined ML analyses yield high support for main branching events which are consistent with the previous global molecular phylogeny of Scarabaeinae (Tarasov & Dimitrov, 2016). The backbone topology is also consistent with the Coleoptera phylogenomic tree (McKenna et al., 2019), except for the remote outgroup relationships where *Rhyparus* Agassiz comes out as a sister to the subfamily Scarabaeinae instead of *Aphodius*.

The combined, morphological and molecular analyses strongly support the monophyly of the clade formed by the genera *Grebennikovius*, *Epactoides* and *Ochicanthon* (UFBS and SH-aLRT > 95); this result corroborates earlier morphological (Tarasov & Génier, 2015) and molecular phylogenies (Mlambo et al., 2014; Tarasov & Dimitrov, 2016). The genus *Ochicanthon* emerges as sister to *Epactoides* + *Grebennikovius*, which is consistent with earlier studies as well (Mlambo et al., 2014; Monaghan et al., 2007; Tarasov & Dimitrov, 2016; Wirta et al., 2010). Interestingly, the study of Tarasov and Dimitrov (2016), as well as the present independent molecular and morphological analyses alone recover the nested position of *Grebennikovius* in *Epactoides*. We consider this to be an artefact of data deficiency as our combined analyses reveals high support toward sister relationships between these two genera (UFBS: 96.8; SH-aLRT: 99).

Our morphological survey has identified 11 diagnostic characters (Appendix S2; Figure 2), which can be used to separate the focal genera from other dung beetles. This evidence indicate that the phylogenetic position of *Epactoides*, *Grebennikovius*, and *Ochicanthon* and their morphology are quite distinct from any of the known tribes in Scarabaeinae. Therefore, we elevate the clade formed by these three genera to the rank of the tribe Epactoidini **trib. nov.** and provide its description and diagnosis below.

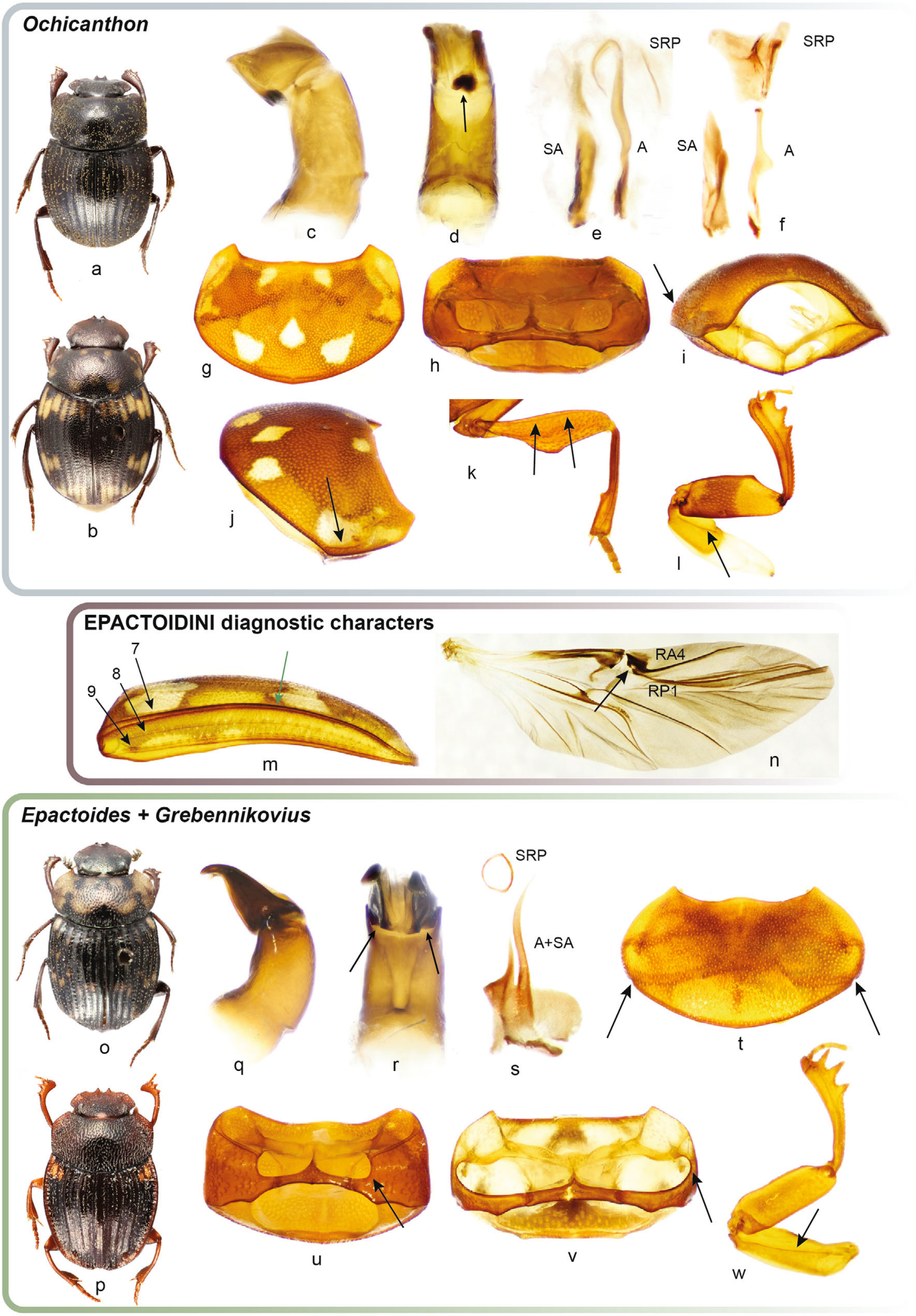
We found relatively few diagnostic characters to separate the three genera in Epactoidini (Figure 2). Specifically, no clear diagnostic characters were identified to distinguish *Grebennikovius* from *Epactoides*. The diagnostic characters for *Grebennikovius* provided in Mlambo et al. (2019) were found to be present in *Epactoides* as well. However, we follow Mlambo et al. (2019) and treat *Grebennikovius* as a separate genus. We consider that the identity of *Grebennikovius* is supported by its isolated distribution and phylogenetic position.

Diagnostic characters for the genera

The genera *Grebennikovius* and *Epactoides* share the following diagnostic characters: (i) in ventral view, proximal region of the parameres depressed (Figure 2r); (ii) A and SA endophallites fused (Figure 2s); (iii) SRP endophallite ring-shaped (Figure 2s); (iv) procoxal ventral ridge as long as procoxa (Figure 2w). The genus *Ochicanthon* differs from other Epactoidini **trib. nov.** members by: (i) proximal and ventral side of the parameres simple (Figure 2c,d); (ii) A and SA endophallites separated (Figure 2e,f); (iii) SRP differently shaped, either thin and elongate or thicker and wide (Figure 2e,f). (iv) procoxal ventral ridge as long as half procoxa (Figure 2l).

Epactoidini trib. nov. (Figure 2)

Type genus: *Epactoides* Olsouffief, 1947



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Genera

Epactoides Olsouffief, 1947

Grebennikovius Mlambo et al., 2019

Ochicanthon Vaz-de-Mello, 2003.

Distribution

Tanzania (Uluguru Mountains); Madagascar; Réunion Island; Oriental Region: from southern India to southern Philippines (Figure 1).

Diagnosis

The tribe Epactoidini is supported by the following diagnostic characters (Figure 2): (i) elytra with 9–10 striae; (ii) besides epipleural carina, elytra with one or two additional lateral carinas; (iii) endophallus without frontolateral peripheral (FLP) endophallite; (iv) radial posterior (RP_1) sclerite of hindwings (when present) approaches the posterior side the radial anterior (RA_4) sclerite.

Description

Small to medium-sized dung beetles (2–9 mm); body oval, dorsoventrally flattened to weakly convex; head, pronotal and elytral surface with superficial punctures, simple or coarse and annulate. Completely black, dark brown to reddish, tegument shiny or dull, some species dorsally with yellow spots.

Head. Edges partly (only genae) or completely (clypeus + genae) margined; without distinct cephalic processes; clypeal edge with two to four medial teeth; eyes in dorsal view either narrow (*Grebennikovius* and *Epactoides*, except for *E. giganteus*) or wide (*Ochicanthon*);

Thorax. Pronotum simple, lateral edges widely curved to straight and parallels; posterior angles simple or notched (several *Epactoides*, Figure 2t); posterolateral region of pronotum simple, with short ridge or furrow running from posterior angles to pronotal midline (several *Ochicanthon*, Figure 2i,j); procoxal cavities short laterally (Figure 2h,u) or approaching lateral margin of pronotum (some *Epactoides* and *Grebennikovius*; Figure 2v); elytra with nine (Figure 2m) to ten striae (only *E. giganteus*); interstriae flat or weakly convex, either smooth and bare or with fine to coarse punctures and rows of dense pilosity; some species with elytral interstriae tuberculate along their entire length, or (Figure 1d) on elytral apex (some *Epactoides*);

Legs. Protibiae of male simple or slender and apically widened, distal margin normal or with tooth directed forward; profemurs and procoxae simple or with anteromedial spur (Figure 2l); ventral ridge of procoxae half the length of procoxa (Figure 2l) or as long as procoxa (Figure 2w); ventrosuperior side of metafemurs simple or with thin ridge (Figure 2k);

Male genitalia. Parameres, in lateral view, elongate; ventral and proximal side simple or with strong depression (Figure 2d,r); ventral membranes of parameres asymmetric and differently sclerotized (left membrane more sclerotized; Figure 2d), or symmetric and equally sclerotized (Figure 2r); axial (A) and subaxial (SA) endophallites separated (Figure 2e,f) or fused (Figure 2s); superior right peripheral (SRP) endophallite ring shaped (Figure 2s) or differently shaped (Figure 2e,f); frontolateral peripheral (FRP) endophallite absent.

Paleotropical disjunctions and biogeography of Epactoidini trib. nov.

The BioGeoBEAR analyses revealed quite different biogeographic scenarios for the tribe Epactoidini depending on the input tree. The full time-tree that includes all analysed OTUs (SUB1) and the subtree that exclude non-Scarabaeinae OTUs (SUB2) support an Afrotropical origin of the tribe Epactoidini. The subtree where the outgroup is represented by genera rather than species (collapsed terminals) (SUB3) recovers an Afro-Oriental origin. Alternatively, the subtree that includes only Epactoidini members (SUB4) results in an uncertain ancestral reconstruction with Afro-Oriental-Madagascan range inferred for the MRCA of Epactoidini.

Evidently, the data deficiency due to the lack of outgroups in SUB4 prohibits a confident estimate of the ancestral range. We also consider the overall results from SUB3 and SUB2 as less reliable. They both support an Afro-Oriental origin for Scarabaeine and its sister lineages (i.e., Scarabaeinae Group I and II in Figure 1), which contradicts the widely accepted scenario of their African ancestry (Sole & Scholtz, 2010). This contradiction can be explained by the lack of non-Scarabaeinae outgroups in SUB2, and by the use of generic-level terminals together with species-level terminals in tree SUB3. These topological simplifications are known to mislead comparative phylogenetic analyses (Lemmon & Lemmon, 2008; Matzke, 2014). Therefore, we select the biogeographic estimates suggested by the analysis with

FIGURE 2 Morphological characters in Epactoidini **trib. nov.** *Ochicanthon* (a–l): *O. hanskii*, (a); *O. crypticus* (b); lateral and ventral view of parameres, arrow indicates sclerotization of left, ventral membrane (c, d); axial (a), subaxial (SA) endophallites separated, and superior right peripheral (SRP) endophallite (e, f); Pronotum in dorsal view (g); procoxal cavities (h); posterolateral pronotal furrow (i) and ridge (j); ventrosuperior ridge of left metafemur indicated by arrows (k); short procoxal ridge indicated by arrow (l); diagnostic characters of Epactoidini **trib. nov.** (m–n): Left elytron of *O. masumotoi* (Ochi & Araya), black arrows indicate striae, 7, 8 and 9; green arrow indicates elytral carina beside 7th stria (m); right hindwing of *Epactoides masoalae* Paulian, arrow indicates proximity, but not connection, between radial posterior (RP_1) and radial anterior (RA_4) sclerites (n). *Epactoides* and *Grebennikovius* (o–w): *E. madecassus* (o); *G. basilewskyi* (p); lateral and ventral view of parameres, arrows indicate basal depression (q, r); A and SA endophallites fused, SRP ring-shaped (s); Pronotum in dorsal view, arrows indicate notches on posterior angles (t); Procoxal cavities, arrows indicate small (u) and large (v) cavities; long procoxal ridge indicated by arrow (w).

all OTUs (SUB1) for further discussion (Figure 1; Appendix S6: Figures S1 and S2). These estimates are congruent with earlier biogeographic studies in dung beetles (Breeschoten et al., 2016; Emlen et al., 2005; Sole & Scholtz, 2010). Additionally, it is noteworthy that the biogeographic reconstructions obtained for Epactoidini based on SUB1 and SUB2 are identical, differing only in their estimates for other Scarabaeinae lineages. The selected scenario (SUB1) suggests the origin of Epactoidini in the Afrotropical Region around 46 Ma (CI: 50–42 Ma). Early to mid-Eocene (55–40 Ma) was marked by a drop of temperature that culminated with a climatic optimum in the Mid Eocene (40 Ma) (Couvreur et al., 2021; Zachos et al., 2001). In this period, climate was wetter throughout continental Africa, and today's floristic affinities between the West-Central Guineo-Congolian region and the Eastern Africa testify the existence of a Pan-African humid forest (Couvreur et al., 2021). Since Epactoidini are exclusively forest-dwellers, it is logical to speculate that the MRCA of this tribe was distributed throughout Eastern African forests during this period. In the Oligocene-Miocene (25–20 Ma), the Pan-African tropical forest experienced periodic fragmentation due to cyclic aridification events (Couvreur et al., 2008; Hamilton & Taylor, 1991; Kirk-Spriggs & Muller, 2017; Morley, 2000; Pokorny et al., 2015). Such perturbations might have triggered local extinctions in ancestral Epactoidini across Africa. This would explain present distribution of *Grebennikovius* confined solely to the Uluguru Mountains (EAMs), which would have served as a refugium for this genus.

The migration of the MRCA of *Epactoides* to Madagascar from Africa is estimated to occur around 32.3–29.5 Ma. This overwater dispersal event might have been facilitated by Africa-to-Madagascar oceanic currents (see Ali & Huber, 2010) or recurring subaerial platforms that emerged across the Mozambique Channel during Late Eocene – Early Oligocene (36–30 Ma) (Masters et al., 2021; McCall, 1997). Our ages for this event are consistent with the earlier study that estimates the colonization of Madagascar by *Epactoides* at 30–19 Ma (Wirta et al., 2010). In the same study, it is suggested that this migration initiated a successful radiation of *Epactoides* in Madagascar, where it is presently represented by ~40 species. This remarkable migration was followed by another overwater dispersal event of *Epactoides* to Réunion island from Madagascar. *Epactoides giganteus* is the only member of the genus recorded on Réunion island. Our analyses estimates that this species diverged from its closest Madagascan relative at 3.4 Ma (CI: 10–0.001 Ma), which might be taken as putative maximal age for the overwater migration. This age is consistent with the geological origin and habitable conditions on Réunion island, which are estimated at 2–3 Ma (McDougall, 1971; Thébaud et al., 2009). The species *E. giganteus* is nowadays considered to be extinct due to the lack of contemporary records and habitat loss on the island (Rossini et al., 2021), which stresses the vulnerability of insular forest-dwelling organisms to the destruction of original habitats.

The dispersal of Epactoidini to Oriental Region from Africa is estimated to occur at 46–27 Ma. This likely triggered another successful radiation of the tribe that is represented by the genus *Ochicanthon*, which currently includes ~50 living species. The age of the MRCA of the genus is inferred to 27 Ma (CI: 31–23), which is considerably older than the 4.5–7 Ma age previously proposed by Mlambo et al. (2014). Our estimates for the dispersal of the MRCAs of *Ochicanthon* to Asia significantly postdate the break-up of Gondwana (120 Ma) and the existence of Afro-Asian forest corridors (a.k.a., Boreotropical forest) during the Late Palaeocene – Early Eocene (60–55 Ma). Therefore, we can reject the 'biotic ferry' and Boreotropical dispersal models as dispersal routes for *Ochicanthon*. The most likely scenario that fits our divergence-time estimates is the existence of the *Gomphotherium* landbridge, and fragments of subtropical rainforest scattered between southern Arabian Peninsula and northwestern India that could serve as dispersal route between Africa and Asia (Morley, 2007; Pound et al., 2012). The existence of this corridor is used to explain the migrations of tropical biotas between Africa and Asia in both directions (Bourguignon et al., 2017; Cruaud et al., 2011; Toussaint et al., 2019, 2021; Zhou et al., 2012). The *Gomphotherium* corridor likely existed between 27 and 14 Ma but these dates are still debated (Hamon et al., 2013; Pirouz et al., 2017; Rögl, 1999). Our estimates for out-of-Africa dispersal events in Epactoidini (46–27 Ma) may indicate that the existence of the *Gomphotherium* landbridge should be reassessed and possibly pushed back in time. Alternatively, it is logical to propose the existence of multiple corridors that might have been separated in time due to oscillating aridification events.

Summing up, our study suggests that that the current distribution of Epactoidini was predominantly shaped by the interplay of two mechanisms. One is the routine transoceanic dispersal events: two such events have mediated the overwater migration of Epactoidini ancestors from Africa to Madagascar and further to Réunion island. Another mechanism is the *Gomphotherium* forest corridor that mediated the dispersal of Epactoidini to Oriental Region from Africa. Transoceanic and transcontinental dispersals shaped the current distribution of various Scarabaeinae clades (Cupello et al., 2020; Gillett & Toussaint, 2020; Gunter et al., 2019; Sole et al., 2011; Sole & Scholtz, 2010). The exclusively Paleotropical disjunct distribution of the tribe Epactoidini is remarkable within the subfamily Scarabaeinae. The use of the *Gomphotherium* corridor as dispersal route has never been previously proposed for any other dung beetle clade.

Members of the tribe Epactoidini are obligate forest-dwellers, which makes them habitat specialists. Nonetheless, they demonstrate a surprisingly high dispersal potential that helped them populate new geographic areas and radiate successfully. The tribe Epactoidini is estimated to have originated in Africa, where it is nowadays represented by the genus *Grebennikovius* endemic to the EAMs. This provides evidence toward a local extinction of Epactoidini in Africa. Therefore, it is conceivable to assume that the high potential of dispersal into new areas have prevented the tribe Epactoidini from global extinction by increasing the cumulative chance of lineage survival.

CONCLUSION

In this study, we have demonstrated that combined analyses of morphological and molecular data help to increase data sampling in phylogenetic reconstructions, outperforming individual molecular and morphological analyses. The combination of morphological and molecular dataset allowed us to classify the three focal genera (*Grebennikovius*, *Epactoides* and *Ochicanthon*) in the new tribe Epactoidini, and to reconstruct their biogeographic history. Our biogeographic reconstructions suggest that the disjunct Paleotropical distribution of Epactoidini, which consists of Tanzania, Madagascar, Réunion island and Asia, was shaped by different biogeographic processes, such as overwater dispersal events and the existence of forest corridors during the Late Paleogene that facilitated out-of-Africa migrations (*Gomphotherium* landbridge). Forest fragmentation in Africa due to cyclic climatic fluctuations would have led to local extinction of Epactoidini members. The Uluguru Mountains in Tanzania might have played as retention area for the survival of the monotypic genus *Grebennikovius*. We suggest that the high potential of dispersal of Epactoidini might have triggered two major radiations, one in Madagascar and one in the Oriental Region. Thus, the high dispersal ability of Epactoidini, along with their strict association to forest habitats might have facilitated the formation of discontinuous ranges during forest contraction events.

AUTHOR CONTRIBUTIONS

Michele Rossini: Conceptualization (equal); data curation (equal); formal analysis (supporting); investigation (lead); methodology (equal); project administration (equal); resources (supporting); validation (supporting); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Vasily Grebennikov:** Conceptualization (equal); data curation (supporting); investigation (supporting); methodology (supporting); resources (equal); validation (equal); writing – review and editing (equal). **Thomas Merrien:** Conceptualization (supporting); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); validation (equal); writing – original draft (supporting); writing – review and editing (supporting). **Andreia Miraldo:** Data curation (equal); investigation (equal); methodology (supporting); resources (equal); writing – review and editing (supporting). **Heidi Viljanen:** Data curation (supporting); investigation (supporting); resources (supporting); writing – review and editing (supporting). **Sergei Tarasov:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (lead); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Molecular sequences are available on GenBank and additional supporting data used in the phylogenetic and biogeographic analyses are publicly available on Zenodo (https://doi.org/10.5281/zenodo.6675412).

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SUPPORTING INFORMATION

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

Table S1. Taxon sampling, including GenBank accession numbers.

Appendix S1. Morphological character matrix used in the phylogenetic analyses.

Appendix S2. Morphological character report.

Appendix S3. Concatenated molecular dataset.

Appendix S4. Maximum Likelihood trees based on morphological, molecular and combined datasets. Node support is indicated, from left to right, by Ultra Fast Bootstrap (UFBS) and Shimodaira–Hasegawa approximate likelihood (SH-aLRT) values.

Appendix S5. BEAST divergence time tree estimated using strict clock model.

Appendix S6. BioGeoBEARS analyses. Settings of the analyses, and model selection based on input phylogenetic trees and statistics (Figures S5–S14; Tables S3–S6).

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