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Four new species, DNA barcode library and pre-Pliocene speciation of the euedaphic Afromontane Clivinini genera *Trilophidius* and *Antireicheia* (Coleoptera: Carabidae, Scaritinae)

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Abstract
We describe and extensively illustrate four new species of euedaphic (= dwelling in the soil) Clivinini ground beetles: *Trilophidius acastus* **sp. nov.** and *T. argus* **sp. nov.** (both from Bioko, Equatorial Guinea), as well as *Antireicheia calais* **sp. nov.** and *A. zetes* **sp. nov.** (both from the South Pare Mountains, Tanzania). We generate and report all currently available DNA barcode (= cytochrome oxidase subunit I) data for euedaphic Afromontane Clivinini of the genera *Trilophidius* (2 species, four records) and *Antireicheia* (13 species, 43 records). We infer a phylogeny for these beetles using a Maximum Likelihood approach based upon a matrix of 53 sequenced specimens (dx.doi.org/10.5883/DS-ANTIREI) with 658 aligned positions. All nominative species represented by two or more sequences are recovered as monophyletic. Both new species of *Trilophidius* form a weakly supported clade, while all seven species of South African *Antireicheia* form a moderately supported clade. The genus *Antireicheia* and the geographical assemblage of its six Tanzanian species are not monophyletic. We perform divergence time estimation in Afrotropical *Antireicheia*, and our analysis indicates that these lineages diverged predominantly in the middle or late Miocene. We highlight the notable lack of phylogenetic hypothesis linked with the vaguely and variably defined taxon "subfamily Scaritinae" and its subordinated taxa.

Key words: South Africa, Tanzania, Eastern Arc Mountains, forest litter, COI, DNA barcode, phylogeography.

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Introduction

Relatively low dispersing and habitat-specific animals are often used for bio- and phylogeographic analyses seeking to understand past evolutionary events leading to the presently observed diversity and distribution (Avise 2000). Of particular interest are clades whose members occur in a number of relatively small habitable spots that are widely scattered in otherwise acutely hostile areas. Such an archipelago-type of distribution might be formed by terrestrial organisms on oceanic islands (Tänzler et al. 2016), or by freshwater organisms in different drainages (Daniels et al. 2016), or by high-altitude biota of "sky-island" (Grebennikov 2016), or by subterraneous organisms (Gómez et al. 2016). Each of the aforementioned settings facilitates an intriguing comparison of a clade's phylogenetic and geographical patterns. Even more intellectually rewarding are situations when the dates of the geographically significant events, such as continental drift, or well dated climate cycles, or volcanic activity, can be brought into the analysis to shed light on their possible evolutionary significance.

Soil constitutes a multifaceted and diverse environment with little or no light, high moisture and relatively low temperature fluctuations. Meso- and microfauna inhabiting the deep layers of the soil contains a number of lineages highly suitable for such bio- and phylogeographic analyses (Andújar et al. 2016). Such euedaphic organisms (sensu Eisenbeis & Wichard 1987) or "cryptofauna" (sensu Lawrence 1953; Leleup 1965) often remain underutilized for their evolutionary value due to inadequate taxonomic knowledge.

In this work we attempt to detect and interpret the phylogeographic signal from one of such neglected euedaphic groups: the Afrotropical Clivinini ground beetles (Carabidae). Like the majority of the soil dwellers, these are small organisms with body length varying between 1.5 and 4.5 mm. Exceedingly little is known about them, with all available information consisting of traditional taxonomic descriptions based on adult morphology. Never before have these beetles been a subject of a phylogenetic analysis, and therefore, their current taxonomic attribution to the subtribe Reicheiina is an untested hypothesis.

This subtribe is variously defined, *i.e.* with or without six genera mentioned by Casale & Marcia (2011): *Italodytes* Müller, 1938, *Leleuporella* Basilewsky, 1956, *Psilidius* Jeannel, 1957, *Syleter* Andrewes, 1941, *Trilophidius* Jeannel, 1957 and *Trilophus* Andrewes, 1927. Adults of many Afrotropical Reicheiina are flightless and have eyes variably reduced in size. These beetles are rarely seen outside of their preferred euedaphic habitat, appear to be intolerant to desiccation, have not been sequenced for any genes, and their immature stages are unknown. Similar to other beetles committed to the euedaphic lifestyle (such as Carabidae: Anillini by Andújar et al. 2016; Staphylinidae: Leptotyphlinae by Fancello et al. 2009; Leiodidae: Leptodirini by Fresneda et al. 2011; Curculionoidea: Raymondionyminae by Grebennikov 2010), those of Reicheiina are thought to have severely restricted dispersal capabilities. This hypothesis agrees with the observation that all Afrotropical Reicheiina species are known from either a single collecting event or from a relatively small locality, even though this observation might also suggest the lack of adequate sampling.

Three Reicheiina genera are known from the sub-Saharan Africa. One of them is the monotypic *Kenyoreicheia* Bulirsch & Magrini, 2007 from the Aberdare Mountains in Kenya, and is not considered here due to the lack of DNA-grade specimens. The total of 19 nominal *Trilophidius* Jeannel, 1957 species are split between Afrotropical (13) and Oriental (6) Regions, forming the *congoanus*- and *impunctatus*- species groups, respectively (Balkenohl 2001). The winged type species (*T. impunctatus* (Putzeys, 1868)) is somewhat aberrant by being widely distributed in Indonesia, Laos, Malaysia, Philippines, Thailand and Vietnam (Balkenohl 2001). Hind wings and adult eyes of *Trilophidius* are either fully developed, or variously reduced in size. While the Oriental species have been recently revised and their number increased from one to six (Balkenohl 2001), all information on the Afrotropical species consists of the original descriptions of various authors (listed in Balkenohl 2001; last key to species by Jeannel 1957; last species described by Basilewsky 1962). Biological information on *Trilophidius* is restricted to the adult collecting circumstances, which are either at light (for some Oriental species), or by litter sifting.

The genus *Antireicheia* Basilewsky, 1951 is more diverse and seemingly more committed to the euedaphic lifestyle. It comprises slightly over 50 named species (Bulirsch & Magrini 2006, 2011, 2012a, 2012b, 2016; Grebennikov et al. 2009) with adult eyes either entirely lacking, or indicated by a small unfacetted remnant. The genus displays the classical Afromontane distribution with species predominantly reported from the forested highlands of Madagascar and from those of East Africa, South Africa and Cameroon. Two *Antireicheia* described in this genus from Vietnam and from the mainland China (see Bulirsch et al. 2013), plus two more new species from Vietnam, have recently been transferred to a newly erected *Asioreicheia* Bulirsch & Magrini, 2014, thus making *Antireicheia* proper exclusively Afrotropical. The latter taxonomic action was made in the absence of a phylogenetic analysis and, therefore, its validity needs to be assessed.

The present paper reports the first DNA barcode library (http://www.boldsystems.org/) for euedaphic Afrotropical Reicheiina and seeks to utilize these data for inferring phylogeographic patterns. More specifically, we will test monophyly of all included species and both analysed genera, as well as that of the two regional faunas of the genus *Antireicheia*: in Tanzania and in South Africa. We shall attempt to date the detected evolutionary events using molecular clock approach and to compare the estimated age with the time of the regional climatic events (such as drying climate corresponding to shrinking forest cover) potently significant in beetle evolution. We describe four new species, two species in both *Trilophidius* and *Antireicheia* and provide a key to the Tanzanian species of *Antireicheia*. We also call attention to the lack of the phylogenetic hypothesis in Clivinini and in all Scaritinae beetles, which might potentially mean that both taxa are not monophyletic, as implied by their historical taxonomic recognition.

Material and Methods

All herein reported specimens of *Trilophidius* and *Antireicheia* were collected by sifting litter in the primary African forests (Fig. 1A-D) with subsequent specimen extraction using funnels (Fig. 1E). To fully document presence/absence data for Tanzanian euedaphic Clivinini, 130 litter samples from 14 discrete forested blocks of different genesis (Fig. 1A) were taken. Nine of these localities are those of the ancient mountain forests of the Eastern Arc Mountains [= EAM], three from geologically recent volcanic forests and two from lowland forests (Fig. 1A). Individual samples from all 14 Tanzanian forested blocks are coded two letter and digit codes (for example "SP08" refers to sample #8 in South Pare), which appear on both trees (Figs 3, 4) and are explained elsewhere (Grebennikov 2017). Specimens were preserved in 96% ethanol and processed for downstream DNA extraction and sequencing (Hebert et al. 2003; Ratnasingham & Hebert 2007). Genomic DNA was extracted from either a single leg, or (considering the small size of specimens) following the whole-body non-destructive protocol developed for Collembola (Porco et al. 2010). DNA extraction, purification, amplification and sequencing was performed in a commercial laboratory "Canadian Center for DNA Barcode" (CCDB, http://www.ccdb.ca/) at the University of Guelph, Ontario, Canada following standard protocol (Ivanova et al. 2006). Resulting sequences and additional relevant information such as gel images and trace files were uploaded to the "Barcode of Life Database" (=BOLD, http://www.boldsystems.org/). All 53 specimens used for DNA

Fig 1 – **A**, Map of sampled African localities, those in Tanzania were most systematically sampled and are of three markedly different types (map generated with the online SimpleMappr tool by Shorthouse 2010), Tanzanian localities supporting *Antireicheia* are circled in red; **B**, forest floor in East Usambara, habitat of *A. grebennikovi*; **C**, sifter in operational position and with litter in the bag resting on the ground; **D**, typical sample with collapsed sifter and finer mesh insert seen on the right; **E**, Winkler funnel with suspended bags in operational position.

analysis (including all six outgroup terminals, Fig. 3) can be traced through a unique identifier label with the code CNCCOLVG0000XXXX (the last four X's correspond to a unique number referred to on our trees, Figs. 3 and 4) linked to a GenBank accession (Figs 3, 4).

For morphological studies, the specimens were dry-mounted and some of them dissected. Male and female genitalia were slide-mounted in Euparal. Label locality data of holotypes are quoted verbatim. Type specimens of the newly described species are deposited in the National Museum, Prague, Czech Republic (NMPC) and in the collection of the second co-author (PBPC). The following abbreviations were used: HT: Holotype; PT: Paratype(s); BSP: basal (prescutellar) setiferous puncture(s); DSP: dorsal setiferous puncture(s); SP: setiferous puncture(s). Single (/) and double (//) slash in locality labels indicate end of line and end of an individual label, respectively.

Phylogenetic analyses

Two analyses were designed and implemented.

Analysis 1 (**A1, phylogenetic and phylogeographic**) was designed to test monophyly of both *Antireicheia* (18 sequenced specimens representing seven species from South Africa and 25 specimens representing six species from Tanzania, of them two newly named) and *Trilophidius* (four specimens representing two newly named species from Equatorial Guinea), as well as monophyly of all analysed nominal species and that of both regional faunas of *Antireicheia*. The outgroup was formed by five terminals representing three species of the Mediterranean genera *Reicheia* Saulcy, 1862 and *Typhloreicheia* Holdhaus, 1924. All the aforementioned 52 sequences are newly generated. The trees were rooted on the branch leading to *Clivina fossor fossor* (Linnaeus, 1758) (GBOL_Col_FK_2830, AAH0274; sequence data from Hendrich et al. 2015). The resulting matrix consisted of 53 terminals and 658 trivially aligned positions of COI-5' mitochondrial DNA containing no indels. Analysis was conducted in MEGA7 (Kumar et al. 2016) using Maximum Likelihood (ML) methods and a GTR + G nucleotide substitution model (chosen for being best one-for-all model in simulations; D. Posada, personal communication). Clade support values were obtained with 1000 bootstrap replicates and interpreted as follows: strong if 75% or higher, moderate when between 40% and 75% and weak when below 40%. GenBank accession numbers for 53 terminals are seen on the topology linked to the last four unique digits of the CNCCOLVG0000XXXX codes (Fig. 3), while individual specimen images, locality data, gel images, electropherograms and sequences can be found online in a public BOLD dataset DS-ANTIREI 53 (dx.doi.org/10.5883/DS-ANTIREI).

Analysis 2 (**A2, temporal**) was focused on the history of genus *Antireicheia* and was informed by the results of analysis **A1** (recovery of the monophyly of all nominal species of *Antireicheia*, supported monophyly of the South African fauna, presence of two separate clades in Tanzania). It was aimed at estimating relative and absolute time of the evolutionary events in mtDNA leading to the present day diversity and distribution of *Antireicheia*. For this purpose the **A1** matrix was reduced in size to contain only 25 sequences best representing the early divergence events in the evolution of *Antireicheia*, as detected in **A1** (Fig. 3). Bayesian phylogenetic analysis in BEAST 1.8 (Drummond et al. 2012) was used to simultaneously estimate an ultrametric phylogenetic tree and ages of diversification. Lacking fossils or unambiguous biogeographical events to calibrate the phylogeny, a uniform a priori substitution rate was implemented. It was based on the rate of 0.0113 nucleotide substitutions per site per million years per lineage (subs/s/Myr/l), in agreement with results ob-

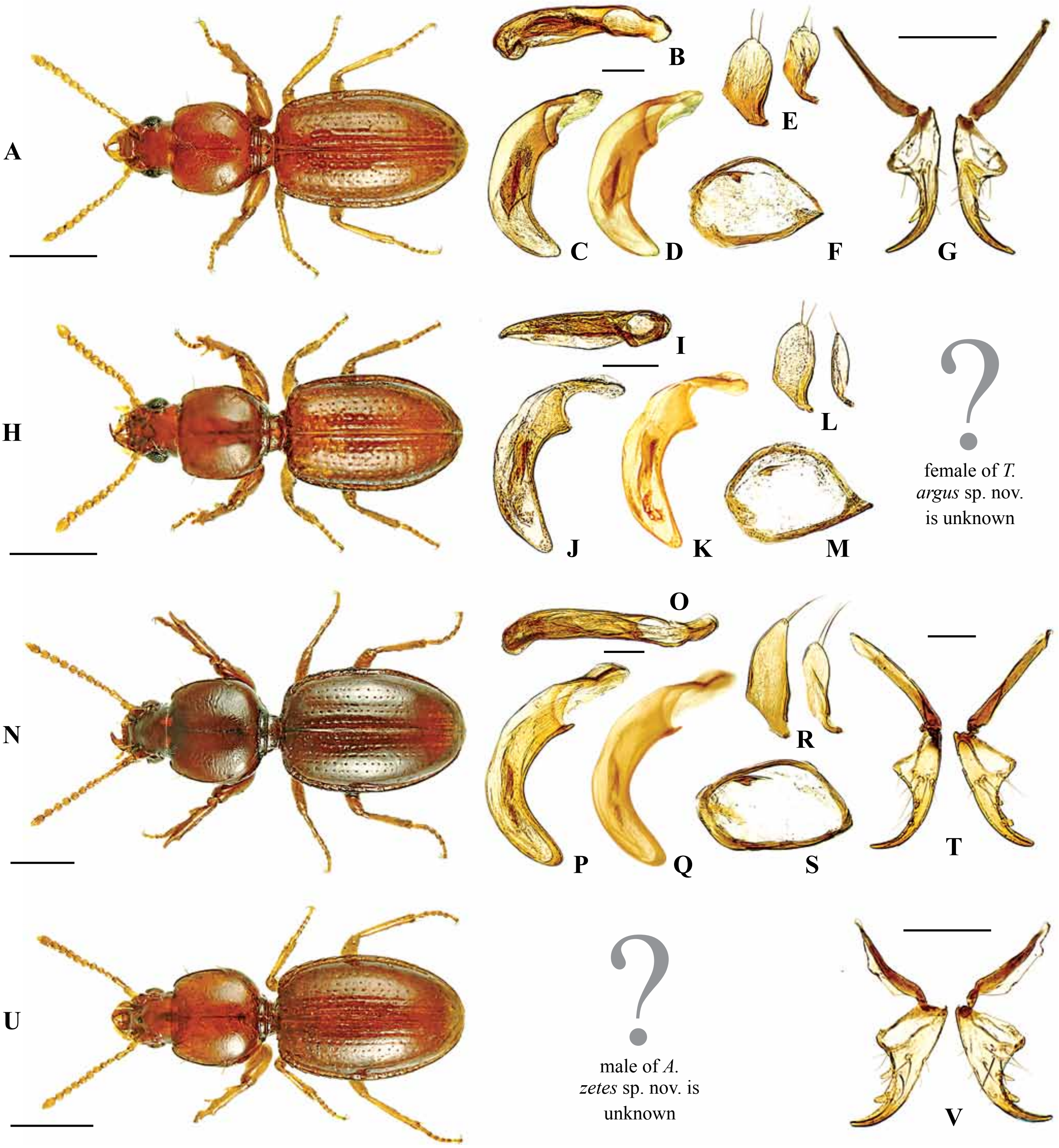

Fig 2 – Adult Afrotropical Clivinini. **A-G**, *Trilophidius acastus* sp. nov.; **H-M**, *T. argus* sp. nov.; **N-T**, *Antireicheia calais* sp. nov.; **U-V**, *A. zetes* sp. nov. **A-F**, **H-M**, **N-S**, **U**, **V**, holotypes. **A, H, N, U**: habitus; **B-D**, **I-K**, **O-Q**: aedeagi; **E**, **I**, **R**: parameres; **F**, **M**, **S:** urites (= abdominal ventrites 9), **G**, **T**, **V**: stylomeres. Scale bars: 0.5mm for habitus; 0.1mm for genitalia.

tained for COI-5’ in Carabidae (Andújar et al. 2012) and similar to the rates in other beetles (Papadopoulou et al. 2010; but see on the unusually high rate of 0.0793 subs/s/Myr/l estimated for *Trigonopterus* Fauvel, 1862 weevils inhabiting forest litter of the Oriental region, analysis 2 in Tänzler et al. 2016, not implemented herein). No monophyly enforcement was implemented prior to the analysis. GTR+G evolutionary model was used, 10 million generations were run, and a tree was sampled every 1000 generations. Consensus trees were estimated with TreeAnnotator (Drummond et al. 2012) after discarding the 25% initial trees as a burn-in, checking the ESS of likelihood, evolutionary rates and root age values, and ensuring that the tree likelihood values had reached a plateau. Posterior probabilities were considered as a measure of node support. Topologies from both analyses were visualized in FigTree1.4 (Rambaut 2014).

Analytical disclaimer. Our oversimplified assumption that the gene tree of the DNA barcoding region adequately represents the species phylogenies is vulnerable to criticism as having all of the well-known uncertainties, including such phenomena as data limitation, incomplete lineage sorting, pseudogenes and horizontal gene transfer (Mallo & Posada 2016). Acknowledging all of them, we still find it beneficial to move forward and base our hypotheses on the hard-won available data, limited as they are, pending the moment when larger and more diversified data become available.

Trilophidius Jeannel, 1957

Attribution of two of four herein newly described species to *Trilophidius* is not based on an explicit phylogeny, but

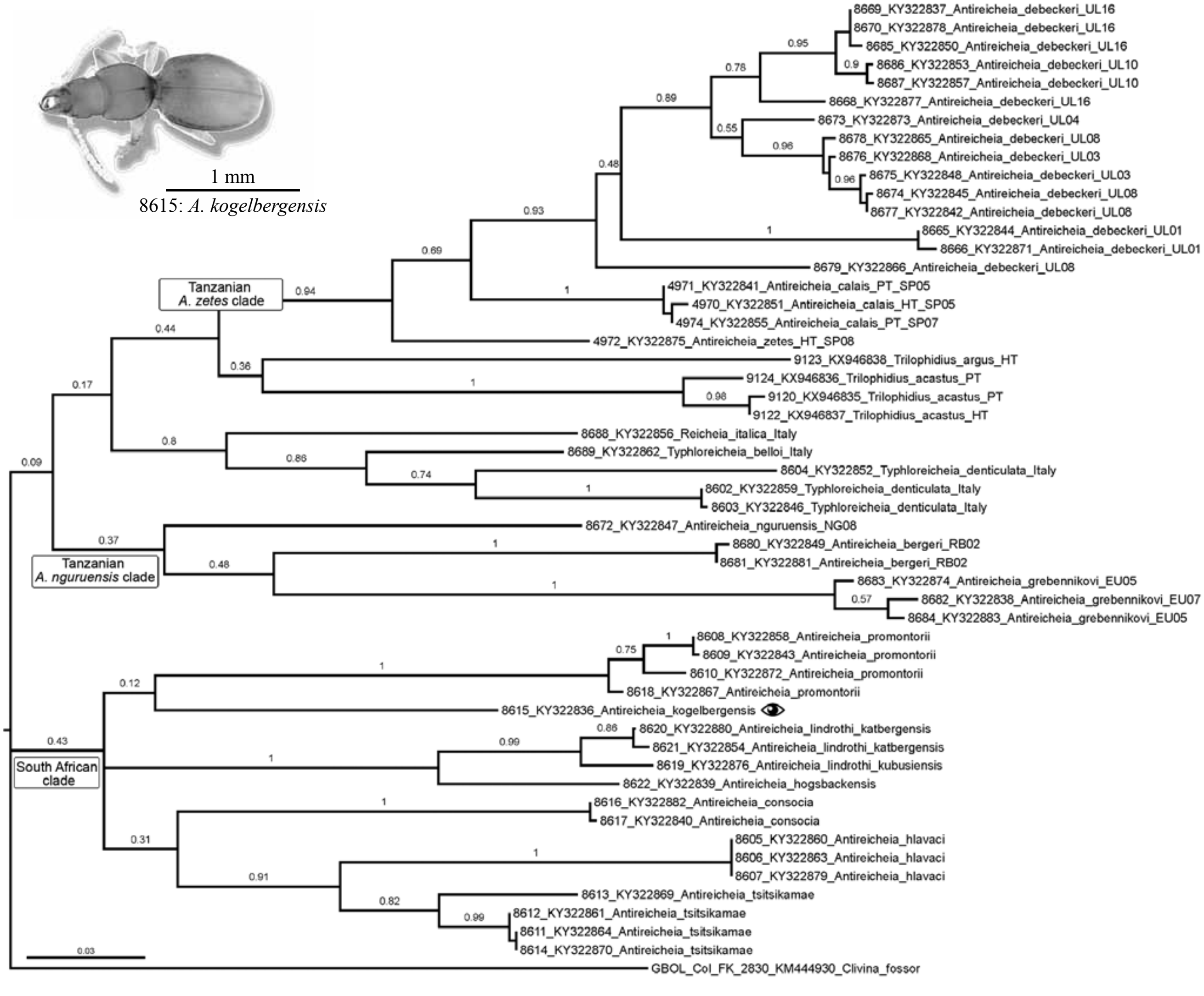

Fig 3 – Phylogeny of Afromontane euedaphic Clivinini, as obtained with MEGA7 (analysis **A1**). Numbers on nodes are bootstrap support values. Four digit voucher numbers in terminal names precede GenBank accessions; HT/PT denote the holotypes/paratypes, respectively; Tanzanian specimens have also sample numbers. Eye symbol denotes imaged specimen 8615.

on morphological similarity. All herein reported specimens share with the rest of the Afrotropical *Trilophidius* (the *congoensis* species group sensu Balkenohl 2001) the following nine characters distinguishing them from the congeners inhabiting the Oriental region (the *impunctatus* species group sensu Balkenohl 2001): (1.) body ferruginous, (2.) body not exceeding 3.3 mm in length, (3.) eyes multifaceted, gently flattened, varying in size from not to slightly reduced; genae inconspicuous to moderately developed, barely to distinctly shorter than eye length, (4.) clypeus prolonged posteriorly into a short keel, (5.) mandibles short and evenly convex, (6.) maxillary palpi securiform, (7.) proepisterna swollen and projecting laterally to form broadly rounded posterior angles distinctly visible in dorsal view, (8.) elytra with 3–5 DSP in interval 3 only, (9.) elytral intervals relatively flat.

Trilophidius acastus **sp. nov.**

(Figs 2A-G, 3)

urn:lsid:zoobank.org:act:83E380CB-7915-4804-B765-268B86250673

Material examined. Holotype (NMPC), male, **Equatorial Guinea**: “Eq. GUINEA, Bioko, / 03.3001, 008.6482 / 938 m, 23.xii.2015, sift. / for. lit., V. Grebennikov // CNCCOLVG00009122”. Paratypes (PBPC): 2 males and 13 females, same locality labels as HT, each of three females additionally labelled CNCCOLVG00009120, CNCCOL VG00009121 and CNCCOLVG00009124, respectively.

Diagnostic description. GenBank accession of DNA barcode: Fig. 3. Body length 2.16 mm (2.00–2.30 mm, HT 2.10 mm, n=16). Pronotum 0.94x (0.92–0.96, HT 0.94) as long as wide, 1.52x (1.46–1.59, HT 1.51) as wide as head width (n=16). Elytra 1.66x (1.63–1.71, HT 1.65) as long as wide, 1.25x (1.22–1.29, HT 1.26) as wide as pronotal width, 2.19x (2.15–2.24, HT 2.20) as long as pronotal length (n=16). This species is characterised as follows: relatively large body, head with reticulated vertex, eyes relatively large and slightly flattened, distinctly longer than antennomere 2, genae inconspicuous, elytra with distinct humeri and with deep inner striae and vaulted inner intervals. The distinct and regular reticulation of the vertex as seen in this species is unique within the Afrotropical *Trilophidius*. It can further be distinguished from the most similar *T. devroeyi* Jeannel, 1957 and *T. decorsei* Jeannel, 1957 by these species having different shape of the median lobe of the aedeagus (Figs. 16–17 in Jeannel 1957). The new species differs from *T. congoanus* (Burgeon, 1935) by having smaller body, by the head without reticulation and by distinctly shorter elytra. Poorly known *T. alluaudi* Jeannel, 1957 from Ivory Coast has not been seen by us and supposedly has smaller body and different shape of the median lobe (Fig. 14 in Jeannel 1957). Another poorly known species, *T. basilewskyi* Jeannel, 1957, from the Democratic Republic of the Congo (= DRC) is only known from the female HT, which has large and strongly vaulted eyes. *Trilophidius rudebecki* (Basilewsky, 1946) from West Africa (Senegal, Gambia and Ivory Coast) and *T. pallidus* (Basilewsky, 1950) from DRC differ from the new species by having lighter body and by non-protruding anterior angles of pronotum. The two remaining species, *T. bayoni* Jeannel, 1957 from Kenya and *T. ellenbergeri* Jeannel, 1957 from Gabon, could be distinguished from the new species by the elytra having shallow striae and flat intervals. For differences with another newly described sympatric congeneric species see below.

Etymology. The species epithet is a Latinized Greek mythical name of Acastus, an Argonaut, son of the wicked Thessalian king Pelias, Jason’s taskmaster; noun in apposition.

Trilophidius argus **sp. nov.**

(Figs 2H-M)

urn:lsid:zoobank.org:act:277FB78F-BFD6-4F42-818E-301D972C0EC7

Material examined. Holotype (NMPC), male, **Equatorial Guinea**: “Eq. GUINEA, Bioko, / 03.3001, 008.6482 / 938 m, 23.xii.2015, sift. / for. lit., V. Grebennikov // CNCCOLVG00009123”.

Diagnostic description. GenBank accession of DNA barcode: Fig. 3. Body length 1.95 mm. Pronotum 0.95x as long as wide, 1.47x as wide as head width. Elytra 1.57x as long as wide, 1.21x as wide as pronotal width, 1.98x as long as pronotal length. This new species is similar to the sympatric *T. acastus* sp. nov., from which it can be distinguished by the following characters: different shape of the median lobe of the aedeagus; body slightly smaller (1.95 mm versus 2.0–2.3 mm); eyes slightly larger; antennae slightly shorter; vertex surface with finer microreticulation. Additionally, analysis of COI-5’ mtDNA of both species, although grouping them in a clade (Fig. 3), shows deep divergence comparable or exceeding those found in other clades of two sister species (see Discussion). Female is unknown.

Etymology. The species epithet is a Latinized Greek mythical name of Argus, an Argonaut, co-builder (with the goddess Athena) of Argo; noun in apposition.

Antireicheia **Basilewsky, 1951**

All six *Antireicheia* species previously known from Tanzania were recently taxonomically revised (Bulirsch & Magrini 2011). Attribution of both herein described species to this genus is supported by numerous similarities considered diagnostic to the genus (Grebennikov et al. 2009), including eyes either entirely absent or each eye indicated as a small, strongly protruded, unfacetted field, as well as the presence of 0 to 4 DSP on the third elytral interval. Moreover, analysis of COI-5’ mtDNA of both new species grouped them in a clade with another *Antireicheia* species (Figs 3, 4), even though the genus has not been recovered as monophyletic (see Discussion).

Antireicheia calais sp. nov.

(Figs 2N-T)

urn:lsid:zoobank.org:act:86C9613A-6602-4EA6-82BB-444F1EA7FF0C

Material examined. Holotype (NMPC), male, **Tanzania**: "Tanzania, South Pare / Mts., Chome For., / S 4.27064° E 37.92595° / 2159 m, 3.i.2013, sift. 38 / V. Grebennikov leg. // CNCCOLVG / 00004970". Paratypes (PBPC): female, same locality label as HT and "CNCCOLVG00004971"; male: "Tanzania, South Pare / Mts., Chome For., / S 4.27145° E37.92347° / 2072 m, 4.i.2013, sift. 40 / V. Grebennikov leg. // CNCCOLVG / 00004974 // CNCCOLVG / 00004975".

Diagnostic description. GenBank accession of DNA barcode: Fig. 3. Body length 3.05 mm (3.05–3.10 mm, HT 3.05 mm, n=3). Pronotum 0.95x (0.94–0.96, HT 0.96) as long as wide, 1.64x (1.64–1.67, HT 1.64) as wide as head width (n=3). Elytra 1.54x (1.54–1.58, HT 1.54) as long as wide, 1.16x (1.15–1.19, HT 1.16) as wide as pronotal width, 1.89x (1.89–1.97, HT 1.89) as long as pronotal length (n=3). *Antireicheia calais* sp. nov. has strongly protruded eye remnants, large body, distinctly micro-reticulated head and pronotum, as well as rather broad elytra with several humeral spines and fine striae. It differs from its sister species, *A. debeckeri* (Basilewsky, 1962), by the longer and broader body, by the dorsal reticulation especially distinct on pronotum, by the numerous humero-lateral spines and by the shape of the median lobe of the aedeagus.

Etymology. The species epithet is a Latinized Greek mythical name of Calais, an Argonaut, twin brother of Zetes, with whom he chased the Harpies; noun in apposition.

Antireicheia zetes sp. nov.

(Figs 2U-V)

urn:lsid:zoobank.org:act:2A56141D-6A85-4C82-A030-3A9B8E59148F

Material examined. Holotype (NMPC), female, **Tanzania**: 'Tanzania, South Pare / Mts., Chome For., / S 4.30624° E 37.97156° / 1648 m, 6.i.2013, sift. 41 / V. Grebennikov leg. // CNCCOLVG / 00004972 // CNCCOLVG / 00004973'.

Differential diagnosis. GenBank accession of DNA barcode: Fig. 3. Body length 2.15 mm. Pronotum 0.97x as long as wide, 1.63x as wide as head width. Elytra 1.57x as long as wide, 1.23x as wide as pronotal width, 2.02x as long as pronotal length. *Antireicheia zetes* sp. nov. is characterised by the moderately strongly protruded eye remnants, the small body and the elytra with several humeral spines and fine striae. It can be distinguished from the most similar species, *A. calais* sp. nov. by the smaller body, by the less rounded pronotum outline between lateral SP; by the elytral striae shallower latero-apically, and by the different shape of stylomeres. It differs from *A. debeckeri* by the dorsal body reticulation especially distinct on pronotum, by numerous humero-lateral spines and by the differently shaped stylomeres. Male is unknown.

Etymology. The species epithet is a Latinized Greek mythical name of Zetes, an Argonaut, son of the wing god Boreas by Oreithyia, who had wings at his ankles and temples; noun in apposition.

Key to Tanzanian *Antireicheia*

1. (4) Elytral lateral margin without humeral teeth; elytra without DSP in interval 3. Uluguru Mts.
2. (3) Smaller species with body length 2.25–2.35 mm; pronotum with indistinct reflexed lateral margin; elytra 1.55–1.60 times as long as wide; first elytral striae deep; apex of median lobe of aedeagus in lateral view broader, in ventral view broader, turned left .... *A. ulugurana* (Basilewsky, 1962)
3. (2) Larger species with body length 2.30–2.70 mm; pronotum with distinct reflexed lateral margin; elytra 1.59–1.80 times as long as wide; first elytral striae shallow; apex of median lobe of aedeagus in lateral view narrower, in ventral view narrow, not turned left .... *A. debeckeri* (Basilewsky, 1976)
4. (1) Elytral lateral margin with at least four distinct humeral teeth; elytra without or with three DSP in interval 3. Uluguru, Nguru, South Pare, Rubeho or East Usambara Mts.
5. (8) Elytral interval 3 with three DSP.
6. (7) Elytra 1.50–1.59 times as long as wide; elytral striae deep on disk, body length 2.15–2.55 mm. East Usambara Mts. .... *A. grebennikovi* Bulirsch & Magrini, 2007
7. (6) Elytra 1.74–1.75 times as long as wide; elytral striae shallow on disk. Body length 2.20–2.30 mm. Uluguru Mts. .... *A. alesi* Bulirsch & Magrini, 2011
8. (5) Elytral interval 3 without DSP.
9. (15) Punctures on proepisterna absent.
11. (12) Body length 3.05–3.10 mm. South Pare Mts. .... *A. calais* sp. nov.
12. (11) Body length 2.15–2.50 mm. Uluguru, Rubeho or South Pare Mts.
13. (14) Body length 2.25–2.70 mm; elytral striae deep; intervals on elytral disk vaulted; apex of median lobe of aedeagus hooked. Uluguru or Rubeho Mts. .... *A. bergeri* Basilewsky, 1976
14. (13) Body length 2.15 mm; elytral striae shallow; intervals on elytral disk almost flat; males unknown. South Pare Mts. .... *A. zetes* sp. nov.
15. (9) Punctures on proepisterna present. Nguru Mts. .... *A. nguruensis* Bulirsch & Magrini, 2011.

Results

The Maximum Likelihood tree found in analysis **A1** is shown in Fig. 3. All nominal species were reconstructed as monophyletic. The genus *Trilophidius* was recov-

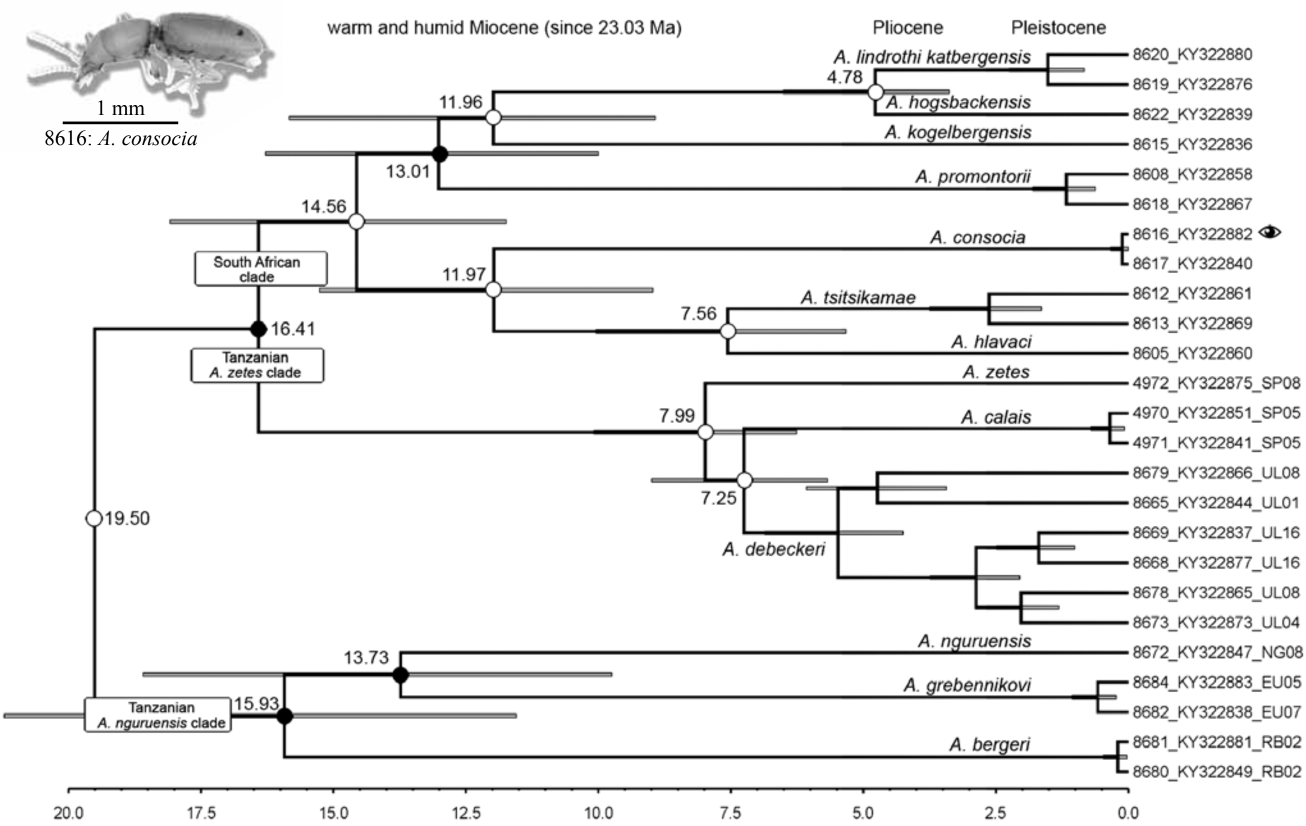

Fig 4 – Ultrametric time tree of 25 select *Antireicheia*, as obtained with BEAST using 0.013 subs/s/Myr/l rate for COI-5' (analysis **A2**). Numbers on nodes and on scale below are million years before present. Dichotomies marked with black circle are those not found in the analysis **A1**. Node bars represent 95% confidence interval of the age estimate (not shown for two basalmost dichotomies). Four digit voucher numbers in terminal names precede GenBank accessions; Tanzanian specimens have also sample numbers. Eye symbol denotes imaged specimen 8616.

ered as monophyletic but is weakly supported (bootstrap 36%). The genus *Antireicheia* is not monophyletic and instead consists of three not separate clades: the moderately supported South African clade (bootstrap 43%), the weakly supported Tanzanian *nguruensis* clade (bootstrap 37%) and the strongly supported Tanzanian *zetes* clade (bootstrap 94%). All five terminals of European Reicheiina formed a strongly supported clade (bootstrap 80%) weakly (bootstrap 17%) linked to clade consisting of the *Trilophidius* clade moderately (bootstrap 44%) linked with the Tanzanian *zetes* clade.

Analysis **A2** resulted in a similar although not identical topology (Fig. 4) with the same three *Antireicheia* clades, as found in **A1**, but with four different dichotomies (marked by black circles in Fig. 4). The root and the basalmost split leading to these three clades are dated at 19.5 Ma and 16.41 Ma, respectively. The crown-group divergence time estimates of these three clades are dated at 15.93Ma, 14.56 Ma and 7.99 Ma, respectively. With the single exception of *A. lindrothi katbergensis* Bulirsch & Magrini, 2016 and *A. hogsbackensis* Bulirsch & Magrini, 2016 diverging at 4.78 Ma, all other divergences between nominative species from their sister-groups took place between 15.93 Ma and 7.25 Ma, *i.e.* in the middle and late Miocene.

Discussion

Dated phylogeny of *Antireicheia*

The main "positive" result of our analysis is that all taxonomically delimited species of *Antireicheia* have been found reciprocally monophyletic. This, however, is not surprising, since the nominal species are normally narrowly localized, morphologically distinct and in many cases widely allopatric. Monophyly of all South Africa *Antireicheia*, the second "positive" result, if indeed true, would suggest that the region has been colonized only once from, however, an unknown source region.

Our perhaps most significant "negative" results are that neither *Antireicheia* as a whole, nor the group of six sampled Tanzanian species are monophyletic. Both observations might not necessarily be true, since the herein presented phylogeny of the relatively fast-evolving and maternally inhered mitochondrial DNA fragment might be variously mismatching the species tree (Funk & Omland 2003). On the other hand, no convincing data are available to dismiss the presented tree as untrue, since monophyly of both these groups have never been adequately tested.

Relatively little can be said about the presence/absence pattern of *Antireicheia* in 14 sampled Tanzanian localities, nine of which are exceptionally biodiverse blocks of the EAM (Fig. 1A; Lovett & Wasser 1993). Absence of *Antireicheia* records from four extensively sampled EAM blocks (Northern Pare, West Usambara, Kaguru and Udzungwa, Fig. 1A) might well be a sampling artefact. It is, however, tempting to consider consistent lack of *Antireicheia* records from all three extensively sampled volcanoes (Mts. Hanang, Kilimanjaro and Meru, Fig. 1A) as their true absence. Such a hypothesis agrees with the geologically young age of these highlands (2–3 Myr, Nonnotte et al. 2008) and, therefore, that of their newly developed altitudinal forests supported by precipitating aerial moisture. The time of the origin of these new forests on volcanic highlands significantly post-date the last hypothesised opportunity when they might have been colonized by low-dispersing *Antireicheia* inhabiting the pan-African wet forest having its territorial maximum in the middle Cenozoic and not later than the late Miocene some 6 Ma (Hamilton & Taylor 1991). If correct, that hypothesis would predict that the aforementioned volcanic forests should be similarly species-poor in other low-dispersing euedaphic invertebrates (and perhaps have the gradient of their species richness negatively correlating with their distance from the nearest EAM forested block serving as a possible colonizing source).

Three EAM blocks are each known to support a single *Antireicheia* species (*A. grebennikovi* in East Usambara, *A. nguruensis* in Nguru and *A. bergeri* in Rubeho, Fig. 1A). South Pare supports two newly descried species. Ulugurus have four nominal *Antireicheia* species (*A. alesi*, *A. bergeri*, *A. debeckeri* and *A. ulugurana*) and appear, therefore, exceptionally diverse. The latter taxon is an enigma species known only from the type series. All other specimens assigned to it in Bulirsch & Magrini (2011) are re-identified herein as those of *A. debeckeri*. The exact type locality of *A. ulugurana* in the relatively large Uluguru mountains given as "sommet du Kidunda, 1800–1950 m" is unknown and might perhaps be an isolated and presently deforested highland outside the main Uluguru forest. All Tanzanian *Antireicheia* species, as currently defined, are endemic to a single EAM forest block, except for *A. bergeri*. This species was named from Uluguru and although not represented in our analysis from the type locality, we used this name for the externally similar specimens from Rubeho with undistinguishable male genitalia. Like any taxonomic concept, this decision is a temporary practical arrangement pending further analysis. Both pairs of sympatric Tanzanian *Antireicheia* species represented in the analysis (from South Pare and Uluguru, respectively) were not recovered as sister species, which agrees with a classical scenario that speciation does not normally occur in sympatry.

Estimated timing of the hypothesised evolutionary events leading to the present day diversity and distribution of *Antireicheia* are illustrated in Fig. 4. Even if based on an oversimplified assumptions (see Methods) and having large 95% confidence intervals, the topology consistently suggests that little or no speciation of *Antireicheia* occurred since the onset of Pliocene 5.33 Ma, when the pan-African wet forest was thought to be in its last maximum (Bobe 2006) and offering the last opportunity for the ecological dispersal (Heads 2014). Similar to the divergence time estimates obtained for other low-dispersing insect clades (*i.e.* Weirauch et al. 2017), the present analysis offers no evidence that any of the sampled and presently widely separated wet Tanzanian forests have been connected during the Plio- and Pleistocene climatic fluctuations, potentially facilitating *Antireicheia* normal ecological dispersal and subsequent vicariance.

Weakly supported monophyly of *Trilophidius*

Herein reported results shed little light on "the genus *Trilophidius*", a taxonomic unit of questionable phylogenetic validity. Detection of the weakly supported clade formed by both Bioko species does not necessarily suggest monophyly of all Afrotropical congeners, let alone that of the entire genus (*i.e.* including the Oriental species). Furthermore, occurrence of the genus on the island of Bioko (formerly Fernando-Poo) should not be considered as evidence of its dispersal over at least 30 km of shallow (<70 m) salt water separating it from the African mainland. Unlike three other truly oceanic principal islands of the Cameroonian line of volcanoes (Príncipe, São Tomé and Annobón; none of them is known to support *Trilophidius*), Bioko is a continental island repeatedly connected with the mainland during all main glacial periods of the Plio- and Pleistocene climatic fluctuations, when the water level regularly reseeded and forests likely re-connected. The only notable topological feature of *Trilophidius* is the relatively deep split between both Bioko species. Such results (and observations on non-sister relations of sympatric species in Tanzanian *Antireicheia*, see above) are again consistent with the hypothesis that speciation does not normally occur in sympatry. In other words, the depicted sister-group arrangement between both analysed *Trilophidius* species is more likely an artefact of sparse sampling, rather than the reality.

Reicheiina, Clivinini and Scaritinae: are they monophyletic?

Three aforementioned taxonomic names have herein been repeatedly used without the benefit of adequate knowledge whether they have any phylogenetic meaning. The phylogeny, however, is indispensable for any accretion pertaining to a biological object (Felsenstein 1985). Neither monophyly nor internal relationships of the subtribe Reicheiina, the tribe Clivinini, and the subfamily Scaritine have ever been adequately addressed using phylogenetic analysis. As a result, the subfamily and its subordinate family- and genus-group taxa are conflictingly defined (Lorenz 2005; Bouchard et al. 2011). Furthermore, the sister-groups of all family- and genus-group Scaritinae taxa, if they are indeed monophyletic, are entirely unknown, including that of the subfamily itself. Until an adequate phylogenetic hypothesis becomes available, all highly intriguing questions posed by various Scaritinae, such as evolution of their predominantly fossorial lifestyle and burrowing behaviour, biogeography of the highly unique and disproportionally diverse faunas of Australia (Moore et al. 1987) and Madagascar (Basilewsky 1973), parental and seed-gathering complexity of the Malagasy species (Peyrieras & Basilewsky 1976), or evolution of the bizarre larval features of Australian Carenini (Moore & Lawrence 1994) will remain unanswerable.

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