PENSOFT.

SENCKENBERG
world of biodiversity

Leibniz
Leibniz
Association

Sky islands of the Cameroon Volcanic Line support the westernmost clade of five new *Typoderus* weevils (Coleoptera: Curculionidae: Molytinae)

Vasily V. Grebennikov[1]

1 *Canadian Food Inspection Agency, 960 Carling Ave., Ottawa, ON, K1A 0Y9, Canada*

http://zoobank.org/A42504B7-FFAF-4856-884A-7B74F3E03AA3

Corresponding author: Vasily V. Grebennikov (vasily.grebennikov@canada.ca)

Citation: Grebennikov VV (2021) Sky islands of the Cameroon Volcanic Line support the westernmost clade of five new *Typoderus* weevils (Coleoptera: Curculionidae: Molytinae). Arthropod Systematics & Phylogeny 79: 57–74. https://doi.org/10.3897/asp.79.e66021

Abstract

The weevil genus *Typoderus* is for the first time reported west of the Congo basin. Analysis of 2,136 aligned positions from one mitochondrial and two nuclear fragments revealed a moderately supported clade of five new Cameroonian species: *T. amphion* **sp. nov.** (Mt. Oku), *T. canthus* **sp. nov**. (Mt. Oku), *T. clytius* **sp. nov.** (Mt. Cameroon), *T. iphitus* **sp. nov.** (Mt. Kupe) and *T. telamon* **sp. nov.** (Mt. Kupe). Molecular clock analysis of 20 DNA barcode fragments using a fixed substitution rate estimated divergences within this clade to be during the Middle to Late Miocene (10.5–5.4 million years ago, MYA), which pre-dates the onset of the Pliocene-Pleistocene global climatic fluctuations and corresponding cycles of African forest size fluctuation. Such relatively old dates are unexpected and might reflect four unavoidable shortcomings of the temporal analysis: 1. undersampled ingroup, 2. scarcity of comparative temporal data for other animal clades from the Cameroon Volcanic Line, 3. oversimplification of a fixed-rate molecular clock approach using a single maternally-inherited protein-coding marker and 4. possible overestimation of comparatively old ages when using largely saturated mitochondrial sequences. Two obscure weevil species from the Republic of the Congo are hypothesized to belong to the genus *Typoderus*: *T. distinctus* (Hoffmann, 1968) **comb. nov.** (from *Anchonidium* subgenus *Neoanchonidium*) and *T. baloghi* (Hoffmann, 1968) **comb. nov.** (from *Anchonidium* subgenus *Subanchonidium*). Three genus-group names are newly synonymized under *Typoderus*: *Entypoderus* Voss, 1965 **syn. nov.** (the only non-nominative subgenus of *Typoderus*), *Neoanchonidium* Hoffmann, 1968 **syn. nov.** (subgenus of *Anchonidium*) and *Subanchonidium* Hoffmann, 1968 **syn. nov.** (subgenus of *Anchonidium*). Habitus images and other supplementary information of all sequenced specimens are available online at dx.doi.org/10.5883/DS-VGDS005 and dx.doi.org/10.5883/DS-VGDS006.

Keywords

1. Introduction

This paper was triggered by a discovery of unexpected weevils in three Cameroonian localities: Mt. Oku, Mt. Cameroon and Mt. Kupe. When first seen, the specimens appeared to belong to five species of the flightless forest-dwelling weevil genus *Typoderus* Marshall, 1953. A peculiarity of these finds was that the genus had never
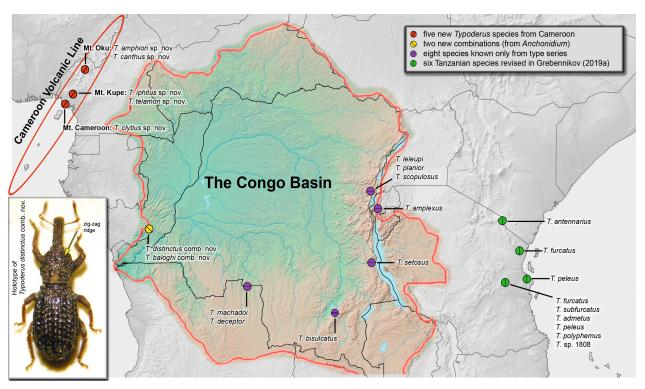
Figure 1. Distribution of the genus *Typoderus*. Map designed using the online SimpleMappr tool (Shorthouse 2010).

been recorded west of the Congo basin. For most of its history *Typoderus* contained 11 nominal Afrotropical species each known only from the type series. Grebennikov (2017) demonstrated monophyly of the genus and hypothesized *Lupangus* Grebennikov, 2017 as its sister group. Presently the genus *Typoderus* consists of 14 flightless species restricted to wet Afrotropical forests (Fig. 1). Eight *Typoderus* species each known only from the type series were described from Rwanda, the Democratic Republic of the Congo and Angola. The remaining and better known six species inhabit the Tanzanian forest archipelago (sensu Grebennikov and Heiss 2018, i.e. isolated sky island forests of different age separated by vast stretches of hot and dry savannah) and were recently studied using DNA data and phylogenetic methodology (Grebennikov 2019b).

Remarkably, the genus *Typoderus* has never been reported throughout most of the Congo basin and along its western rim. Such an askew distribution of this genus (and of other forest-dependent weevils such as *Allocycloteres* Voss, 1965, *Paocryptorrhinus* Voss, 1965, *Aparopionella* Hustache, 1939, *Prothrombosternus* Voss, 1965, or *Tazarcus* Grebennikov 2020; see Voss 1965, Grebennikov 2015, 2016, 2018, 2020 respectively) might be an artifact of insufficient sampling and/or inadequate and misleading taxonomy. This was suggested by the fact that representatives of many genera that were originally reported from one side of the Congo basin, were eventually detected on the other side. Recent examples include *Xenocaucus* China and Usinger, 1949 assassin bugs (Weirauch et al. 2017), *Coelocorynus* Kolbe, 1895 Trichiini scarab beetles (Šípek et al. 2009), soft-bodied *Dexoris* Waterhouse, 1878 net-winged beetles (Bocak et al. 2013) and a clade of two likely synonymous genera of

pill scarabs (*Afrocloetus* Petrovitz, 1968 and *Congomostes* Paulian, 1968: Grebennikov 2019c).

The first hint that *Typoderus* might be present on the western side of the Congo basin came when attempting to elucidate the identity of two obscure species, each of them the type species of an equally obscure monotypic subgenus: *Anchonidium* (*Neoanchonidium*) *distinctum* Hoffmann, 1968 and *A*. (*Subanchonidium*) *baloghi* Hoffmann, 1968 from the Republic of the Congo (Fig. 1). The only available evidence was a dorsal image of one of the holotypes (Fig. 1) exhibiting all morphological synapomorphies of *Typoderus*. This hypothesis could not be adequately tested, however, since the type series remain unavailable for study (see below), and both species have never been re-sampled.

The second and more convincing indication of *Typoderus* west of the Congo basin was the aforementioned detection of *Typoderus*–like weevils in the Cameroonian highland forests. Adults of these beetles displayed the diagnostic feature uniquely distinguishing the *Typoderus* + *Lupangus* clade among all Afrotropical weevils: pronotum on each side with two longitudinal ridges, of which the inner ridge is twice bent to form a zig-zag (Fig. 1, fig. 2G in Grebennikov 2018). Additionally, all Cameroonian specimens were distinguishable from those of *Lupangus* by two more *Typoderus*-only characters: (2) eyes not more than 2.5x as high as wide and (3) lack of a deep transverse groove on head immediately behind the posterior margin of eye (both characters illustrated for *Lupangus* in fig. 4, Grebennikov 2017). Notably, two morphologically distinct groups of such specimens were collected on both Mt. Oku and Mt. Kupe suggesting two cases of sympatry, while only one morphospecies was seen on Mt. Cameroon. These Cameroonian weevils were

distinct from those living in Tanzania (Fig. 3) and likely belonged to unnamed species.

The three forested Cameroonian highlands supporting the suspect *Typoderus* beetles are remarkable in their own right. They are parts of the Cameroon Volcanic Line (CVL, Déruelle et al. 2012) formed by a chain of (mainly extinct) volcanoes extending for about 1,700 kilometres between the island of Annobón in the southwest and Lake Chad in the north-east. Besides the prominent continental part including all three aforementioned highlands, the oceanic part of CVL includes Pico de São Tomé (2,024 metres), Pico do Príncipe (947 m) and Pico Basilé (3,011m). Similarly to the Albertine Rift located between Lake Albert and Lake Tanganyika and the Eastern Arc Mountains in mainly Tanzania, CVL supports exceedingly diverse life forms, many of them restricted to a single highland. The Albertine Rift has the highest vertebrate diversity in Africa (Voelker et al. 2010), while both Cameroon and Tanzania have the highest per-country chameleon diversity on the continent (Tilbury 2018). This extraordinary high biodiversity is normally linked with the availability of the elevational landscape gradient formed by these archipelago-type chains of highlands. Dated at about 30 million years (MY, Burke 2001; Reusch et al. 2010), the continental part of CVL is sufficiently old enough to serve as climatic refugia for moisture-dependent lowland organisms when climate change forces them to retreat into cooler and/or wetter highlands. This was particularly important during multiple repeated warm and dry interglacials of the Pliocene-Pleistocene (Plana 2004), when Afrotropical rainforest recurrently shrank to about 10% of its present size (fig. 4 in Hamilton and Taylor 1991), leaving these highlands to form sky islands of forest surrounded by vast stretches of dry and hostile savannah. The resulting extended and repeated isolation of forest biota left behind by the retreating lowland Congo basin forest supposedly drove speciation in all three chains of highlands. When tested for endemic CVL clades, this spatio-temporal hypothesis was corroborated by post-Miocene divergence dates of two species of *Otomys* Cuvier, 1824 laminate-toothed rats (Taylor et al. 2014), although in *Phrynobatrachus* Günther, 1862 puddle frogs, the timing of diversification predates the Plio-Pleistocene (Zimkus and Gvoždík 2013, see also Discussion).

Three phylogeographic processes are commonly evoked to explain the high diversity and spotty distribution of Afrotropical sky island biota. Some of these organisms are considered species-poor paleoendemics retained from the deep (=pre-Pliocene) past and sisters to sizable radiations (Fjeldså and Lovett 1997; Grebennikov 2019a). More speciose clades with more recent diversifications are thought to be products of the Plio-Pleistocene vicariant speciation achieved through habitat fragmentation (Grebennikov 2019c) and additionally enriched via habitat re-connection and secondary sympatry of recently divergent clades (=the "species-pump" hypothesis, Papadopoulou and Knowles 2015). It was, therefore, intriguing, to test, to the extent possible, each of these three scenarios using the discovered CVL beetles and by doing this, to shed light on their taxonomy and the evolutionary past.

The purpose of this paper is to document analytical steps triggered by the discovery of suspect *Typoderus* weevils west of the Congo basin. Working within the logical framework of testing falsifiable hypotheses (Popper 1959), the following questions (=hypotheses H1 to H5) were tested by performing phylogenetic analyses of DNA sequence data:

Hypothesis 1 (H1): all five visually recognized and geographically structured morphospecies of CVL *Typoderus*-like weevils correspond to biological species (following the unified species concept, De Queiroz 2007);

Hypothesis 2 (H2): these beetles taxonomically belong to the genus *Typoderus*;

Hypothesis 3 (H3): at least one of the newly discovered CVL candidate species represents a paleoendemic;

Hypothesis 4 (H4): at least one divergence between allopatric CVL candidate species is attributable to simple vicariance via habitat isolation of CVL sky islands during the post-Miocene climatic fluctuations;

Hypothesis 5 (H5): at least one case of sympatry of CVL candidate species is attributable to a secondary meeting of recently speciated populations through temporary habitat reconnection (the "species-pump" hypothesis).

Last but not least, an attempt is made to fine-tune taxonomy pertaining to the relevant parts of the weevil Tree of Life by revising rank-based names and making them to reflect the best available phylogenetic hypothesis.

2. Material and Methods

2.1. Specimen sampling, analyses design and DNA sequencing

All newly reported specimens of CVL *Typoderus*–like beetles are adults obtained from fifteen forest litter samples (Table 1) in wet primary forests of Mt. Oku, Mt. Cameroon and Mt. Kupe in Cameroon using standard sifting methods (Grebennikov 2017, 2019c). This paper is a sequel to the recent work focused on Tanzanian *Typoderus* (Grebennikov 2019c) and the sister clade of the genus (Grebennikov 2017), therefore it re-uses already reported DNA data and re-employs the same laboratory and analytical procedures. In brief, about a hundred newly sampled CVL *Typoderus*-like weevils were preliminary sorted into five geographically- and visually-coherent morphospecies. External diagnostic morphological characters for these morphospecies are given in Table 4 and were clear-cut and diagnostic for all individual specimens examined (except for two characters with intraspe-

Table 1. Cameroonian litter sifting samples.

Sample	Locality	Latitude	Longitude	Altitude	Label
CM01	Mt. Oku	6.2216	10.506	2273	CAMEROON, Mt. Oku, 6.2216 10.5063, 2273m, 23.xi.2014, sift34, local collector
CM02	Mt. Oku	6.2273	10.52	2243	CAMEROON, Mt. Oku, 6.2273 10.5202, 2243m, 24.xi.2014, sift35, local collector
CM03	Mt. Oku	6.2337	10.498	2090	CAMEROON, Mt. Oku, 6.2337 10.4980, 2090m, 26.xi.2014, sift36, local collector
CM04	Mt. Cameroon	4.0853	9.0501	314	CAMEROON, Mt .Cameroon, 4.0853, 9.0501, 314m, 28.xii.2015, sift. CM04, V.Grebennikov
CM05	Mt. Cameroon	4.0935	9.0573	524	CAMEROON, Mt. Cameroon, 4.0935, 9.0573, 524m, 28.xii.2015, sift. CM05, V.Grebennikov
CM06	Mt. Cameroon	4.1001	9.0629	638	CAMEROON, Mt. Cameroon, 4.1001, 9.0629, 638m, 28.xii.2015, sift. CM06, V.Grebennikov
CM07	Mt. Cameroon	4.1175	9.0718	1079	CAMEROON, Mt. Cameroon, 4.1175, 9.0718, 1079m, 28.xii.2015, sift. CM07, V.Grebennikov
CM08	Mt. Cameroon	4.1019	8.9793	61	CAMEROON, Mt. Cameroon, 4.1019, 8.9793, 61m, 28.xii.2015, sift.CM08, V.Grebennikov
CM09	Mt. Cameroon	4.0681	9.0717	233	CAMEROON, Mt. Cameroon, 4.0681, 9.0717, 233m, 28.xii.2015, sift. CM09, V.Grebennikov
CM10	Mt. Kupe	4.8241	9.7023	1277	CAMEROON, Mt. Kupe, 4.8241, 9.7023, 1277m, 29.xii.2015, sift.CM10, V.Grebennikov
CM11	Mt. Kupe	4.8223	9.7047	1423	CAMEROON, Mt. Kupe, 4.8223, 9.7047, 1423m, 29.xii.2015, sift.CM11, V.Grebennikov
CM12	Mt. Kupe	4.8213	9.7064	1501	CAMEROON, Mt. Kupe, 4.8213, 9.7064, 1501m, 29.xii.2015, sift.CM12, V.Grebennikov
CM13	Mt. Kupe	4.8193	9.7075	1525	CAMEROON, Mt. Kupe, 4.8193, 9.7075, 1525m, 29.xii.2015, sift.CM13, V.Grebennikov
CM14	Mt. Kupe	4.8108	9.7057	1767	CAMEROON, Mt. Kupe, 4.8108, 9.7057, 1767m, 29.xii.2015, sift.CM14, V.Grebennikov
CM15	Mt. Kupe	4.8017	9.7015	1977	CAMEROON, Mt. Kupe, 4.8017, 9.7015, 1977m, 29.xii.2015, sift.CM15, V.Grebennikov

Table 2. DNA fragments used in phylogenetic analyses (total number of sequenced terminals, followed by minimal, maximal and aligned length of each fragment, and the first and the last position of each aligned fragment in the concatenated matrix). — *Symbols*: # number of specimens.

fragment	#	min	max	aligned	positions
CO1-5P	85	589	658	658	1 to 658
ITS2	82	214	585	878	659 to 1536
28S	83	219	571	600	1537 to 2136

Table 3. GenBank accession numbers of three DNA fragments newly sequenced for 14 Cameroonian *Typoderus* and used in the A3 phylogenetic analyses; comparable data for the remaining 71 terminals are in Grebennikov (2019b).

Voucher	Species	Locality	Sample	CO1	ITS2	28S
CNCCOLVG00008341	*T. amphion*	Mt. Oku	CM01	MH917894	MH916820	MH916834
CNCCOLVG00008342	*T. amphion*	Mt. Oku	CM01	MH917902	MH916829	MH916843
CNCCOLVG00008343	*T. canthus*	Mt. Oku	CM01	MH917890	MH916816	MH916830
CNCCOLVG00008376	*T. canthus*	Mt. Oku	CM03	MH917899	MH916826	MH916840
CNCCOLVG00008918	*T. amphion*	Mt. Oku	CM02	MH917895	MH916822	MH916836
CNCCOLVG00009375	*T. clytius*	Mt. Cameroon	CM06	MH917896	MH916823	MH916837
CNCCOLVG00009511	*T. clytius*	Mt. Cameroon	CM07	MH917898	MH916825	MH916839
CNCCOLVG00009531	*T. iphitus*	Mt. Kupe	CM13	MH917886	MH916821	MH916835
CNCCOLVG00009533	*T. telamon*	Mt. Kupe	CM13	MH917901	MH916828	MH916842
CNCCOLVG00009534	*T. telamon*	Mt. Kupe	CM13	MH917892	MH916818	MH916832
CNCCOLVG00009556	*T. iphitus*	Mt. Kupe	CM15	MH917900	MH916827	MH916841
CNCCOLVG00009557	*T. telamon*	Mt. Kupe	CM15	MH917893	MH916819	MH916833
CNCCOLVG00009722	*T. iphitus*	Mt. Kupe	CM15	MH917891	MH916817	MH916831
CNCCOLVG00009732	*T. clytius*	Mt. Cameroon	CM06	MH917897	MH916824	MH916838

cific variation). Of them 20 specimens representing all five morphospecies were DNA barcoded (=sequenced for 658 bp of the 5′ end of cytochrome c oxidase subunit I, Hebert et al. 2003a, b; implemented in the Canadian Centre for DNA Barcoding, CCDB, University of Guelph, Canada, http://www.ccdb.ca) in support of analysis A1, A2 and A4 (see below); their images and DNA barcode sequences are available online at BOLD dataset dx.doi.org/10.5883/DS-VGDS006. GenBank accession numbers of 20 new DNA barcodes are MH917885–902, MH981205–6 (Table 3). Additionally, 14 of them were sequenced for two more markers, in support of analysis A3 (see below; Table 3).

To test hypotheses H1–H5, the total of four DNA analyses was implemented.

First analysis (A1) designed to assess hypothesis H1 (five morphospecies represent five biological species) by testing whether DNA barcode clusters match morphological and geographical grouping of specimens. By doing so, consistent signal was sought from different and independent sources of evidence in an attempt to delimit independent evolutionary units herein described as new species. For this purpose, 20 newly generated DNA barcodes 531–658 bp in length were analysed using the Neighbour Joining (NJ) method, Barcode Index Number cluster identification algorithm (BIN; Ratnasingham and Hebert 2013) and Kimura 2 parameter, as implemented in the online engine of the Barcode of Life online database (=BOLD, Ratnasingham and Hebert 2007, http://www.boldsystems.org).

To estimate evolutionary divergence over sequence pairs for groups recognized in section 4.2 below as five new Cameroonian *Typoderus* species, 14 terminals representing these species were analysed separately using MEGA5 (Tamura et al. 2011). The 2136 position matrix was reduced to that of a total of 1599 positions, by removing positions containing gaps and missing data. Two pairs of values were calculated: the number of base differences per site from averaging over all sequence pairs within and between species, as well as uncorrected p-distance.

Second analysis (A2): designed to test hypothesis H2 (CVL weevils taxonomically belong to the genus *Typoderus*). Considering (1.) diagnostic morphological characteristics of CVL specimens strongly in support of this hypothesis, (2.) lack of a clearly identified sister-group of the clade formed by *Typoderus* and its species-poor sister group of the genus *Lupangus*; (3.) multiple non-monophyly of Molytinae (Shin et al. 2017) and (4.) the lack of new significant DNA data on Molytinae (as compared to those recently released, Grebennikov 2017), a full-sized phylogenetic analysis with multiple non-*Typoderus* outgroups was not deemed feasible or, indeed, necessary. Instead, all 20 newly generated DNA barcodes were identified by using phenetic methods such as the BLAST algorithm implemented in GenBank (Altschul et al. 1997) or through the Neighbour Joining (NJ) clustering method (as in Analysis A1). This comparison was done against all Curculionidae records currently in BOLD (5,117 species

with barcodes, 55,115 specimens with barcodes; as on November 25, 2020).

Third (topological) analysis (A3): designed to shed light on all five hypotheses. Fourteen CVL specimens representing all five morphospecies (as recovered on the NJ tree in analysis A1) were additionally sequenced for two nuclear ribosomal loci: internal ribosomal spacer 2 (ITS2) and 28S rDNA (Table 2). All corresponding laboratory work was done in CCDB using protocols and primers described earlier (Grebennikov 2017). The three-marker dataset was formed by 14 newly sequenced CVL terminals (the ingroup) merged with the 71 terminal dataset from Grebennikov (2019b); the latter containing 70 Tanzanian *Typoderus* (the outgroup) and a single *Lupangus* terminal to root the topology. Images of all 85 sequenced specimens and their DNA data are available online at BOLD dataset dx.doi.org/10.5883/DS-VGDS005. Alignment of the ITS2 and 28S sequences was made using the MAFFT 7 online platform (http://mafft.cbrc.jp/alignment/server) and the Q-INS-i algorithm utilising the secondary structure information (Kuraku et al. 2013; Katoh et al. 2017). To correct for visually detected alignment errors, 34 positions at the 5 end of four relatively short ITS2 sequences were manually realigned (specimens 2130: 10 positions; 2154: eight positions; 3094: three positions and 7164: 11 positions). No other modifications to the alignments were made and no parts of the alignments were excluded from the analysis. The resulting matrix of 85 terminals had 2,136 aligned positions (Table 2) and 23% missing data (mainly due to insertions and deletions in ITS2). GenBank accession numbers of new DNA sequences from 14 CVL weevils are in Table 3. The concatenated matrix was partitioned into three fragments (Table 2) and an independent CAT approximation (Stamataikis et al. 2008) of the GTR+G model was applied independently to each data partition (Abadi et al. 2019). Phylogenetic analysis was conducted on the CIPRES Science Gateway online platform (Miller et al. 2010; http://www.phylo.org) using the Maximum Likelihood (ML) method and RAxML 8 tool (Stamatakis 2014). Support values were obtained with 1000 bootstrap replicates (Stamatakis et al. 2008) and topology was visualized in FigTree v1.4.0 (Rambaut 2019).

Fourth (temporal) analysis (A4) was used to partially test hypotheses H3–H5, each of them requiring dates on species divergences. Considering monophyly of CVL *Typoderus*–like weevils (see Results) and the lack of relevant calibrating points, a fixed-rate temporal analysis was the only available option. For this purpose the matrix from the analysis A1 consisting of 20 DNA barcodes of CVL weevils was re-analysed and no outgroup used. To estimate divergence times, a fixed molecular clock rate of 0.018 nucleotide substitutions per site per million years per lineage (subs/s/MY/l) was applied. This value is consistent with those obtained in other beetles (Papadopoulou et al. 2010; Andújar et al. 2012), other insects (Brower 1994) and other arthropods (Bauzà-Ribot et al. 2012), and was used for dating evolutionary events in Tanzanian
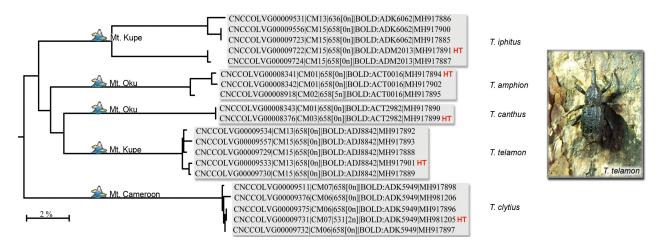
Figure 2. Neighbour Joining clustering of 20 DNA barcodes of Cameroonian *Typoderus* (analysis A1). Terminal names consist of BOLD sample ID (its last four digits correspond to specimen number), litter sifting sample number (Table 1), length of the DNA barcode (number of ambiguous nucleotides is in square brackets), BIN three-letter four-digit number and GenBank accession number. Names next to clusters are those of the new species described in section 4.2. HT denote holotypes.

Typoderus (Grebennikov 2019b). Bayesian phylogenetic analysis in BEAST 1.8 (Drummond et al. 2012) was used to simultaneously estimate an ultrametric phylogenetic tree and rates of diversification. The HKY+G evolutionary model (estimated in MEGA 7, Kumar et al. 2016) was applied and the MCMC chains ran for 10 million generations. Convergence of all parameters was checked in TRACER (Drummond et al. 2012) and consensus trees were estimated with TreeAnnotator (Drummond et al. 2012) discarding 25% initial trees as a burn-in fraction. No *a priori* topological constrains (such as rooting) were added in the analysis.

2.2.   Species diagnosis and description

Various species description workflows (Riedel et al. 2013; Meierotto et al. 2019) converged on importance of providing (1.) DNA barcode, (2.) precise locality data and (3.) image of the holotype. Here I follow the new species description workflow adopted for the *Typoderus + Lupangus* clade (Grebennikov 2017, 2019b) by presenting species-level diagnostic morphological characters in a table (Table 4). These characters consistently list all easily observed differences of five new species, including those of male genitalia. The latter were dissected only for holotype male specimens (distinguished from females by slightly depressed abdominal ventrites 1 and 2).

No attempt was made to compare male genitalia within the newly described species, nor to dissect and study female genitalia. Considering that *Typoderus* form narrow-range morphologically and genetically distinct clades in at least relatively well-sampled Tanzania (Grebennikov 2019b), no consistent attempt was made to compare morphology of five herein described new Cameroonian species with that of six *Typoderus* from Tanzania (Grebennikov 2019b). As before, the holotype body length (measured in dorsal view between anterior margin of pronotum and apex of elytra) is the only reported measurement; all other measurements and ratios

can be obtained from images (dx.doi.org/10.5883/DS-VGDS006). Overall, taxonomic procedures implemented in this paper were designed to consistently generate valid species names with the least effort, with focus on diagnosis, not descriptions (Wheeler et al. 2012; Renner 2016; Miralles et al. 2020; Vences 2020). This approach termed "turbo-taxonomy" (Butcher et al. 2012; Summers et al. 2014) or fast-track taxonomy (Riedel et al. 2013) was designed to expedite naming of new species in particularly understudied and species-rich clades, which the genus *Typoderus* appears to be. Other examples include Neotropical Gracillariidae leaf-mining moths (Leed et al. 2014) or Cecidomyiidae gall midges; the latter cosmopolitan family of some 6–7 thousand species was estimated to contain up to 1,800,000 species, a bewildering number exceeding that of the total of all scientifically named animal species (Hebert et al. 2017).

3.   Results

The first analysis (A1) clustered all 20 DNA barcodes of CVL weevils fully in agreement with their preliminary grouping in five geographical morphospecies (Fig. 2). The latter form exclusive clusters each. Four CVL morphospecies each correspond to a single BIN (ACT0016, ACT2982, ADJ8842, ADK5949), and one morphospecies was split into two BINs (ADK6062, ADM2013). The sequence data of the barcodes was nearly complete (653–658 of 658 BP) in all cases except one (the holotype of the species to be named *T. clytius* in section 4.2 below, CM07 / MH981205 only gave 531 BP, but clustering behaviour of this sample does not differ from the others). The MEGA5 analysis of the reduced dataset containing 14 Cameroonian *Typoderus* and using the 1599 position matrix identified a total of 284 variable sites (COI: 191, ITS2: 85, 28S: 8). The number of base differences per sequence from averaging over all sequence pairs between
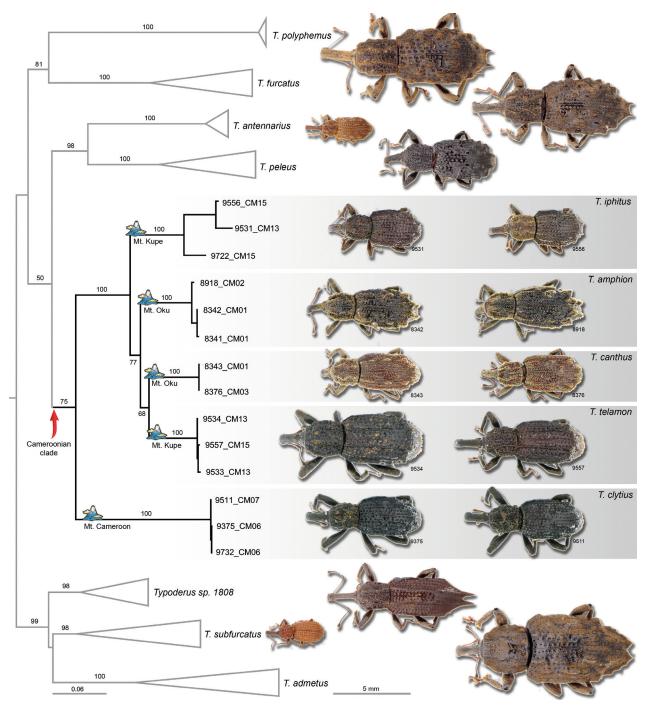
Figure 3. Maximum Likelihood inference phylogram of *Typoderus* weevils rooted on *Lupangus* (analysis A3, root is not shown). Cameroonian species form a clade. Digits at internodes are bootstrap values of 50% and above. Non-Cameroonian (=Tanzanian) terminals are collapsed in species. Terminal names consist of specimen number (Table 3) and sifting sample number (Table 1). Habitus images are to scale; their numbers indicate exact Cameroonian specimens imaged. Names next to clades are those of the new species described in section 4.2.

groups to be named as five new species (in section 4.2 below) is (followed by p-distance in parentheses): *T. amphion* vs. *T. canthus*: 91.7 (5.7%); *T. amphion* vs. *T. clytius*: 150.0 (9.4%); *T. amphion* vs. *T. iphitus*: 111.2 (7.0%); *T. amphion* vs. *T. telamon*: 89.0 (5.6%); *T. canthus* vs. *T. clytius*: 159.3 (10.0%); *T. canthus* vs. *T. iphitus*: 108.7 (6.8%); *T. canthus* vs. *T. telamon*: 85.7 (5.4%); *T. clytius* vs. *T. iphitus*: 169.0 (10.6%); *T. clytius* vs. *T. telamon*: 162.0 (10.1%); *T. iphitus* vs. *T. telamon*: 104.8 (6.6%). The same values within each species are: *T. amphion*: 7.7

(0.47%); *T. canthus*: 0.0 (0%); *T. clytius*: 1.3 (0.08%); *T. iphitus*: 47.3 (2.9%) and *T. telamon*: 4.0 (0.2%).

The second analysis (A2) had all 20 DNA barcodes of CVL weevils consistently matched (through BLAST comparison or using BOLD identification) with those of the genus *Typoderus*.

The third analysis (A3) using the ML phylogenetic method and a concatenated 2,136 bp matrix of 85 terminals produced a well-resolved tree (Fig. 3) topologically identical to the one in Grebennikov (2019b), except for
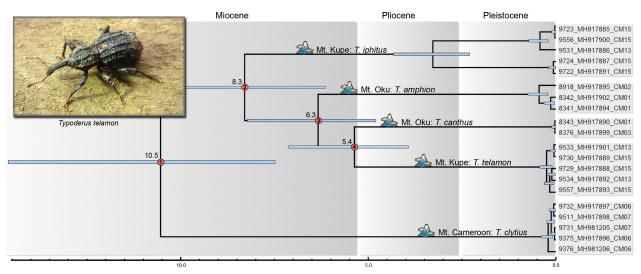
Figure 4. Maximum clade credibility Cameroonian *Typoderus* tree with median ages derived from the BEAST analysis A4. The 95% credibility interval for each node is given with a horizontal bar. Digits at nodes are million years before present. Terminal names consist of specimen number (Table 3), GenBank accession number and sifting sample number (Table 1). Names next to clades are those of the new species described in section 4.2.

the addition of the CVL terminals. All 14 Cameroonian terminals formed a moderately supported clade (bootstrap support 75%) sister to a strongly supported clade of *T. peleus* Grebennikov, 2019 and *T. antennarius* Voss, 1965 (Fig. 3). All five CVL morphospecies formed strongly supported clades (each 100%). Four CVL species (expect the one from Mt. Cameroon) formed a strongly supported clade (100%); its internal resolution was moderately supported, with a Mt. Kupe species sister to a clade (77%) consisting of a species from Mt. Oku, itself sister to two species from Mt. Oku and Mt. Kupe (68%, Fig. 3).

The fourth analysis (A4), estimating divergence dates of 20 CVL DNA barcodes, resulted in a topology (Fig. 4) identical to those recovered by analyses A1 and A3 (Figs. 2 and 3, respectively). All divergences of the Cameroonian clade were estimated to take place in the Middle to Late Miocene (Fig. 4). The most recent common ancestor of this clade lived 10.5 MYA (95% credibility interval 14.6–7.5 MYA), while the most recent species-level divergence took place at 5.4 MYA (3.9–7.1 MYA).

4.   Discussion

4.1.   Hypotheses

Interpretation of the herein presented results led to conclude that:

Hypothesis 1 (five preliminary recognized and geographically coherent morphospecies of CVL *Typoderus*-like weevils are biological species) is strongly supported, since three independent lines of evidence (morphology, geography, DNA) groups specimens in identical clusters herein assumed to be discrete biological species. The sympatric occurrence of two strongly genetically diver-

gent pairs (at Mt. Oku and at Mt. Kupe) offers an additional argument for species-level distinctness of these lineages. Moreover, the herein defined species (including both sympatric pairs) exhibit constant differences not only in COI (Fig. 2, a mitochondrial marker), but also in comparably rapidly evolving ITS2 (while having almost no differences in more conservative 28S; both are nuclear markers). Such concordance of unlinked markers (mtDNA vs. nucDNA) offers further evidence for species-level distinctness. The species to be named *T. iphitus* in section 4.2 might consist of two cryptic species due to the deep barcode divergence in the same order as that between the other species, but this needs to be investigated in more detail and is beyond the scope of this paper

Hypothesis 2 (all analysed CVL weevils belong to the genus *Typoderus*) is strongly supported, since phenetic similarities in DNA sequences (analysis A2) reinforce morphological identifications and assign all specimens to monophyletic *Typoderus*. Consequently, these CVL weevils are consistently referred to as *Typoderus* using five new species-group names introduced below.

Hypothesis 3 (at least one of the newly discovered CVL *Typoderus* is a paleoendemic) is weakly rejected, since none of CVL *Typoderus* forms a sister-group to a well-supported clade containing non-CVL species and reliably well-known and sufficiently old divergence date (although the cut-off date can hardly be precisely defined).

Hypothesis 4 (at least one divergence between allopatric CVL *Typoderus* species might be attributed to simple vicariance via habitat isolation of CVL sky islands during the post-Miocene climatic fluctuations) is weakly rejected, because by the beginning of the Pliocene at 5.3 MYA all five species of CVL *Typoderus* have already diverged (Fig. 4, but see below on the limitations of the temporal analysis).

Hypothesis 5 (at least one case of sympatry of CVL *Typoderus* might be attributed to a secondarily meeting of recently spectated populations through temporary habitat reconnection; the "species-pump" hypothesis) is weakly supported. In both cases of CVL sympatry (*T. amphion* **sp. nov.** and *T. canthus* **sp. nov.** co-occurring on Mt. Oku and *T. iphitus* **sp. nov.** and *T. telamon* **sp. nov.** co-occurring on Mt. Kupe) sister clades of each of four species occur on a different, although a relatively nearby CVL sky-island, which might have served as a source of secondary colonisation.

As a word of caution, the herein undertaken attempt to study spatial and temporal aspects of CVL *Typoderus* evolution is subject to four significant limitations. Firstly, the genus remains acutely undersampled. No DNA data on *Typoderus* are available from the >3,000 km gap separating sequenced populations in Tanzania and Cameroon, and even these two best studied countries likely have just a fraction of their evolutionary distinct *Typoderus* lineages samples and studied. It seems unlikely that *Typoderus* is truly absent from most of the Congo Basin lowland rainforest (Fig. 1), since focused litter sampling in nearby Tanzania detected this genus in all but two targeted forest blocks (Grebennikov and Heiss 2018; those two are geologically recent volcanoes Mt. Hanang and Mt. Meru likely not yet colonized by the genus). When available, new data are expected to modify, or perhaps even negate, some of the herein presented conclusions (for example, detection of non-monophyly of CVL *Typoderus* or perhaps significantly different divergence dates).

Secondly, very few other CVL animal clades were studied in sufficient detail to permit meaningful comparison. Similar to *Typoderus* weevils, all CVL laminate-toothed *Otomys* rats are monophyletic (two species diverging in the Pleistocene) and represent the western-most records of the genus, which is absent throughout most of the lowland Congo Basin (fig. 1 in Taylor et al. 2014). *Phrynobatrachus* puddle frogs widely distributed in sub-Saharan Africa are also represented in CVL by an endemic clade only and, similar to *Typoderus*, the clade's divergences took place in Middle to Late Miocene (Zimkus and Gvoždík 2013; the analysis was perhaps biased to older dates by using suboptimal priors, email correspondence with V. Gvoždík on August 12, 2019). These two papers are the only ones providing phylogeographic results comparable with other of CVL *Typoderus*.

Thirdly, and perhaps most significantly, the herein implemented temporal analysis utilizes a fixed-rate molecular clock of a single maternally-inherited protein-coding marker. This methodological oversimplification is unavoidable, since no other calibrating methods are presently available for this understudied clade. This substitution rate agrees with those of other Arthropoda (see four references in Material and Methods), however its application to CVL *Typoderus* might, or might not, be correct, because at least a four times greater rate has been detected in the same DNA fragment among similarly wingless *Trigonopterus* Fauvel, 1862 weevils from the Sunda Arc (0.0793 subs/s/MY/l, analysis 2 in Tänzler et al. 2016, versus 0.018 subs/s/MY/l implemented herein).

If the CVL *Typoderus* rate is at least twice higher than the one implemented in analysis A4, then the origin of the CVL clade's crown group and its diversification would be estimated at the beginning of the Pliocene (Fig. 4). Such a result would reverse the present rejection of hypothesis H4, and suggest a link between CVL *Typoderus* evolution and post-Miocene cyclic climatic fluctuations.

Fourthly, the herein implementer temporal analysis possibly overestimated the ages, necessarily using (largely saturated) mitochondrial sequences to estimate comparatively old ages (as demonstrated Near et al. 2017 for ray-finned fishes). Without relevant fossil evidence and improved phylogenetic resolution within the Molytinae, it is not possible to fine-tune the herein implemented temporal analysis A4 and, therefore, these results should be taken with caution.

Notwithstanding these limitations, the most significant phylogeographic result of this study is that all *Typoderus* weevils currently known west of the Congo basin form a clade (Fig. 3). This, together with the fact that the sister group of *Typoderus* is strictly Tanzanian in distribution, seemingly suggests an East African origin of *Typoderus* and single colonization of Cameroon. However, with such little DNA data available for the, likely, many dozens (if not hundreds or perhaps thousands) of other *Typoderus* species, this cannot be concluded with sufficient confidence.

4.2. Taxonomic acts

4.2.1. *Typoderus* Marshall, 1953

Marshall, 1953: 104. Type species: *Typoderus machadoi* Marshall, 1953 by original designation.

= *Entypoderus* Voss, 1965 syn. nov.

Voss 1965: 332 (as subgenus of *Typoderus* Marshall, 1953). Type species: *Typoderus deceptor* Marshall, 1953 by original designation.

Available evidence. Voss (1965: 332) established *Entypoderus* for all *Typoderus* having seven antennomeres in the funicle and contrasted it with the nominative subgenus with five antennomeres in the funicle. In this situation, use of a single diagnostic character necessitates that at least one of its two states is not synapomorphic and, therefore, the proposed classification is not phylogenetic. Moreover, no practical need is served by sustaining subgenera in *Typoderus*, a relatively small genus of only 19 species (inclusion five new one and two new combinations; see below). Perhaps the entire category of a subgenus (as well as a subspecies; see ZINK 2004) might be entirely abolished in zoological classification, since its practical advantage as a name is often compromised by the unwieldiness of non-binominal species-level nomenclature. In other words, trinomial (or even tetranomial, if both subgeneric and subspecific names are used) names are more cumbersome, than practical. An efficient naming strategy offering a practical alternative is to use infor-

mal species groups, as implemented in the jewel beetles *Agrilus* Curtis, 1825 (the larger animal genus with over 3,000 valid species, Jendek and Grebennikov 2011), or as it prevails in arranging 1,665 species of *Drosophila* Fallén, 1823 fruit flies, the classical object of biological studies (O'Grady and Desalle 2018). Only in a few cases do subgeneric names in hyperdiverse genera appear justified, and only in situations when a stable phylogeny has been first achieved (e.g. the ground beetle genus *Bembidion* Latreille, 1802 with over 1,300 species and over 50 subgenera, Maddison 2012, Maddison and Maruyama 2019; the ground beetle genus *Carabus* Linnaeus, 1858 with 940 species and 91 subgenera, Deuve et al. 2012; the fruit fly genus *Drosophila* Fallén, 1823 with 1,646 species taxonomically arranged in a number of subgenera and informal species group, O'Grady and Desalle 2018). Considering the above, both non-nominotypical *Typoderus* subgeneric names are herein synonymised and, therefore, the subgeneric classification of the genus is scrapped.

= *Subanchonidium* Hoffmann, 1968 syn. nov.

Hoffmann 1968: 23 (as subgenus of *Anchonidium* Bedel, 1884; species included: *baloghi*). Type species: *Anchonidium baloghi* Hoffmann, 1968 by monotypy.

Available evidence. The genus *Anchonidium* was recently reduced to include only five West Palaearctic species (of them two from Portugal recently described, Germann 2020), while all but two *Anchonidium* from the Afrotropical Region were transferred to a re-defined *Aparopionella* Hustache, 1939 (Grebennikov 2018). Two *Anchonidium* species described by Hoffmann (1968) from the Republic of the Congo as type species of two monotypic subgenera of *Anchonidium* were noted as belonging to neither *Anchonidium* nor *Aparopionella* (Grebennikov 2018). A decision on the taxonomic status of these four names was long delayed by the unavailability of the type series. These historical specimens were borrowed in 2007 from Hungarian Natural History Museum (=HNHM, Budapest, Hungary) by Nicolas Maughan (Aix-Marseille University, Marseille, France; loan #4240/7343) and not returned as of September 2019, despite numerous demands (personal communication, Ottó Merkl, curator of HNHM Coleoptera collection). Requests for high-resolution images of the type specimens resulted in a low-resolution dorsal view of the holotype of *A. distinctum* (Fig. 1). The image revealed a weevil consistent with *Typoderus* in its appearance (parallel-sided body, effaced elytral shoulders, two longitudinal ridges on each side of pronotum, of them the internal one zig-zag shaped). The last feature is synapomorphic for the clade of *Typoderus* plus *Lupangus*. The beetle in Fig. 1 differs from *Lupangus* by having an anteriorly directed rostrum (more ventrally directed in *Lupangus*), by lacking transverse dorsal groove behind eyes (present in *Lupangus*) and by the more rounded eyes (vertical in *Lupangus*, fig. 4 in Grebennikov 2017). These considerations, together with sympatry and great similarity of this species with *A. baloghi* ("extrêmement voisin de *baloghi*...", Hoffmann 1968: 24) are the only

Table 4. Character matrix with discrete morphological characters for diagnostics of new Cameroonian *Typoderus* weevils (see also Discussion). — *Characters*: **1.** – Body, colour of darkest specimens (Fig. 3, that is of a beetle at least a few days old, because specimens freshly emerged from the pupa are much paler): greyish: 0; brownish: 1; blackish: 2. **2.** – Elytra at middle in cross-section: triangular (Figs 5A–H, 6A–H); elytral disk flat, ridge in interstriae 6–7 forming elytral lateral contour in dorsal view: 0; cylindrical (Figs 7A–H, 8A–H, 9A–H); elytral disk evenly rounded laterally, ridge in interstriae 6–7 positioned inside lateral contour in dorsal view: 1. **3.** – Hind tibiae, additional apical projection near inner apical spur (in at least some males): absent (Figs 7B, 8B, 9B): 0; present (Figs 5B, 6B): 1. **4.** – Pilosity on elytral declivity (excluding that on elytral protuberances), whether similar to that on the rest of elytra; similar: 0; dissimilar, notably denser and longer (Fig. 9A, except abraded specimens): 1. **5.** – Ratio of length to width of sclerotized part of aedeagus, dorsal view: one (aedeagus subquadrate as in Figs. 5E, 6E, 9E or notably unsclerotized, as in Fig. 8E): 1; two (aedeagus elongate, Fig. 7E): 2. **6.** – Numerous (about 10) "teeth" inside internal sac of aedeagus: absent (Figs 5E–F, 6E–F, 8E–F, 9E–F): 0; present (Figs 7E–F): 1. **7.** – Tegminal apodeme: absent (Figs 6F, 8F) or indistinguishably short (Figs 5F, 9F): 0; present, consisting of two rods (Fig. 7F): 1.

Character:	1	2	3	4	5	6	7
Taxon:							
T. amphion	0	1	1	0	1	0	0
T. canthus	1	1	1	0	1	0	0
T. clytius	2	0	0	0	2	1	1
T. iphitus	1	0	0	0	1	0	0
T. telamon	2	0	0	1	1	0	0

data available to conclude that both species taxonomically belong to the genus *Typoderus* and are, therefore, herein transferred to the later as *Typoderus distinctus* (Hoffmann, 1968) **comb. nov.** and *Typoderus baloghi* (Hoffmann, 1968) **comb. nov.**

= *Neoanchonidium* Hoffmann, 1968 syn. nov.

Hoffmann 1968: 24 (as subgenus of *Anchonidium* Bedel, 1884; species included: *distinctum*). Type species: *Anchonidum distinctum* Hoffmann, 1968 by monotypy.

Available evidence. Same as that presented above for *Subanchonidium*.

4.2.2. *Typoderus amphion* sp. nov.

http://zoobank.org/6AADC254-6C4A-451D-A61F-861B68A-8D118

Figs. 1–4, 5A–H.

Species diagnosis and description. Holotype male (Figs. 5A–H), length between anterior edge of pronotum and elytral apex 5.7 mm; DNA barcode: MH917894. Diagnostic combination of morphological characters as in
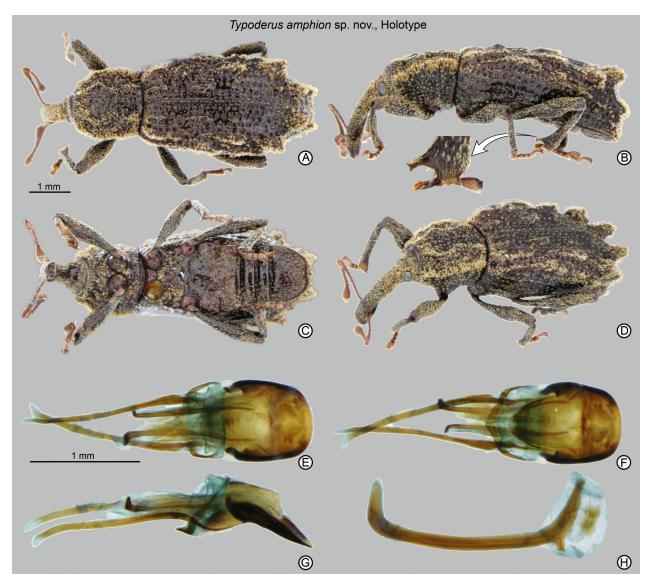
Figure 5. Male holotype of *T. amphion* **sp. nov.**, habitus (**A**: dorsal, **B**: left lateral, **C**: ventral, **D**: left fronto-lateral), aedeagus (**E**: dorsal, **F**: ventral, **G**: right lateral) and **H**: sternite 9.

Table 4. Sister clade to *T. canthus* **sp. nov.** and *T. telamon* **sp. nov.** (Fig. 3). Externally similar to sympatric *T. canthus* **sp. nov.**; can be distinguished by presence of additional protuberance on elytral interstria 2–3 located on elytral declivity nearest to elytral apices (Fig. 5B versus Fig. 6B).

Material examined. Holotype male (CNC; Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada): "CAMEROON, Mt. Oku, 6.2216 10.5063, 2273m, 23.xi.2014, sift34, local collector", "CNCCOLVG00008341". Paratypes (CNC): 2, as in Fig. 3; specimen 8342: same data as holotype; specimen 8918: "CAMEROON, Mt. Oku, 6.2273 10.5202, 2243m, 24.xi.2014, sift35, local collector".

Distribution. Known only from Mt. Oku in Cameroon, in sympatry with *T. canthus* **sp. nov.** Elevation: 2,234–2,273 m.

Etymology. Amphion, from ancient Greek mythology, one of the Argonauts, brother of Asterius; noun in apposition.

4.2.3. *Typoderus canthus* **sp. nov.**

http://zoobank.org/60B40925-24E1-4C43-B72F-1333F2E1-B3AE

Figs. 1–4, 6A–H.

Species diagnosis and description. Holotype male (Figs. 6A–H), length between anterior edge of pronotum and elytral apex 5.1 mm; DNA barcode: MH917899. Diagnostic combination of morphological characters as in Table 4. Sister clade to *T. telamon* **sp. nov.** (Fig. 3).

Material examined. Holotype male (CNC): "CAMEROON, Mt. Oku, 6.2337 10.4980, 2090m, 26.xi.2014, sift36, local collector", "CNCCOLVG00008376". Paratypes (CNC): 1, as in Fig. 3; specimen 8343: "CAMEROON, Mt. Oku, 6.2216 10.5063, 2273m, 23.xi.2014, sift34, local collector".
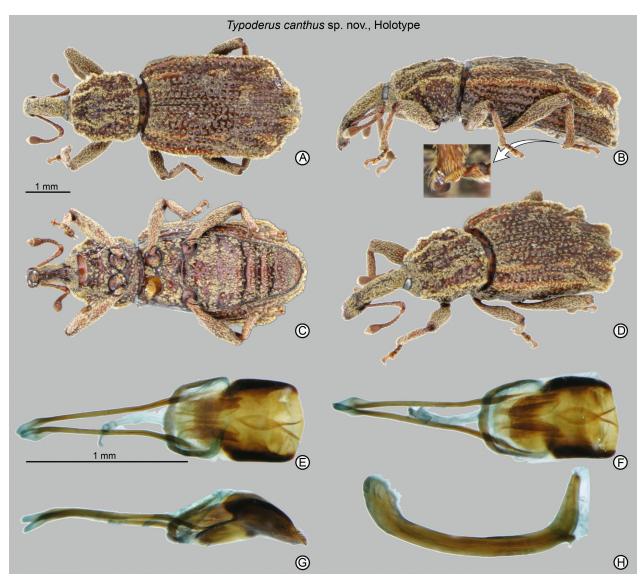
Figure 6. Male holotype of *T. canthus* **sp. nov.**, habitus (**A**: dorsal, **B**: left lateral, **C**: ventral, **D**: left fronto-lateral), aedeagus (**E**: dorsal, **F**: ventral, **G**: right lateral) and **H**: sternite 9.

Distribution. Known only from Mt. Oku in Cameroon, in sympatry with *T. amphion* **sp. nov.** Elevation: 2,090–2,273 m.

Etymology. Canthus, from ancient Greek mythology, one of the Argonauts, killed by a shepherd in Libya; noun in apposition.

4.2.4. *Typoderus clytius* sp. nov.

http://zoobank.org/F148AFA0-7C11-44F0-8A8A-4FECEB-5F22A7

Figs. 1–4, 7A–H.

Species diagnosis and description. Holotype male (Figs. 7A–H), length between anterior edge of pronotum and elytral apex 5.6 mm, DNA barcode: MH981205. Diagnostic combination of morphological characters as in

Table 4. Sister clade to four other CVL *Typoderus* (Fig. 3). Externally similar to allopatric *T. telamon* **sp. nov.**; can be distinguished by pilosity on elytral declivity similar in density and length to than on the rest of elytra (Fig. 7A versus Fig. 9A).

Material examined. Holotype male (CNC): "CAMEROON, Mt. Cameroon, 4.1175, 9.0718, 1079m, 28.xii.2015, sift.CM07, V.Grebennikov", "CNCCOLVG00009731". Paratypes (CNC): 4, as in Fig. 3; specimen 9511: same data as holotype; specimens 9375, 9376, 9732: "CAMEROON, Mt. Cameroon, 4.1001, 9.0629, 638m, 28.xii.2015, sift. CM06, V.Grebennikov".

Distribution. Known only from Mt. Cameroon in Cameroon; no sympatric congeners are known. Elevation: 638–1,079 m.

Etymology. Clytius, from ancient Greek mythology, one of the Argonauts, master archer, killed together with his brother Iphitus by Heracles; noun in apposition.
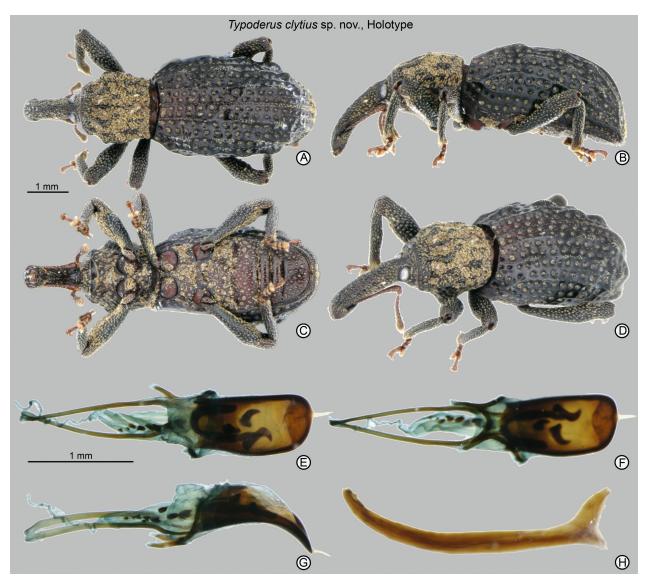
Typoderus clytius sp. nov., Holotype

Figure 7. Male holotype of *T. clytius* **sp. nov.**, habitus (**A**: dorsal, **B**: left lateral, **C**: ventral, **D**: left fronto-lateral), aedeagus (**E**: dorsal, **F**: ventral, **G**: right lateral) and **H**: sternite 9.

4.2.5. *Typoderus iphitus* sp. nov.

http://zoobank.org/83775EEA-6CA3-47FB-99BB-0B6EEC75-2B35

Figs. 1–4, 8A–H.

Species diagnosis and description. Holotype male (Figs. 8A–H), length between anterior edge of pronotum and elytral apex 4.6 mm, DNA barcode: MH917891. Diagnostic combination of morphological characters as in Table 4. Sister clade to *T. amphion* **sp. nov.**, *T. telamon* **sp. nov.** and *T. clytius* **sp. nov.** (Fig. 3).

Material examined. Holotype male (CNC): "CAMEROON, Mt. Kupe, 4.8017, 9.7015, 1977m, 29.xii.2015, sift.CM15, V.Grebennikov", "CNCCOLVG00009722". Paratypes (CNC): 4, as in Fig. 3; specimens 9556, 9723 and 9724: same data as holotype; specimen 9531: "CAMEROON, Mt. Kupe, 4.8193, 9.7075, 1525m, 29.xii.2015, sift.CM13, V.Grebennikov".

Distribution. Known only from Mt. Kupe in Cameroon, in sympatry with *T. telamon* **sp. nov.** Elevation: 1,525–1,977 m.

Etymology. Iphitus, from ancient Greek mythology, one of the Argonauts, brother of Clytius; noun in apposition.

Comment. Being morphologically indistinguishable and sympatric, it is more parsimonious to treat all specimens assigned to this species as the same biological entity, despite a relatively deep intraspecific divergence (Figs. 2–4).

4.2.6. *Typoderus telamon* sp. nov.

http://zoobank.org/73A586EF-632B-4BEE-B3F1-B7A45A9-6E799
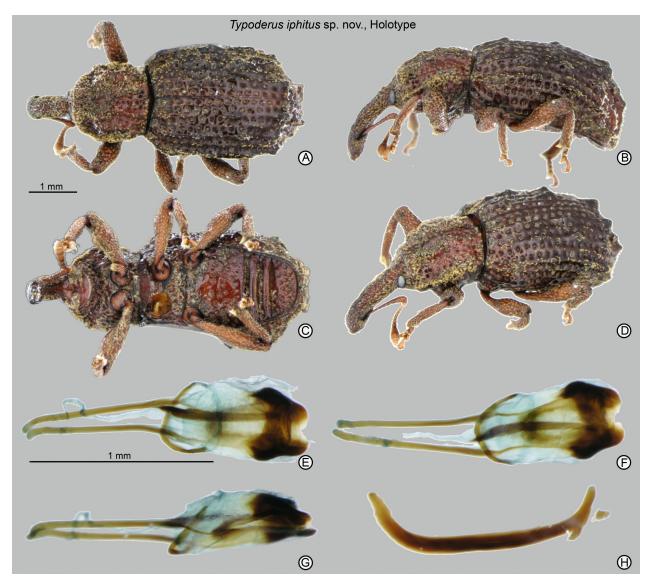
Figs. 1–4, 9A–H.

Figure 8. Male holotype of *T. iphitus* **sp. nov.**, habitus (**A**: dorsal, **B**: left lateral, **C**: ventral, **D**: left fronto-lateral), aedeagus (**E**: dorsal, **F**: ventral, **G**: right lateral) and **H**: sternite 9.

Species diagnosis and description. Holotype male (Figs. 9A–H), length between anterior edge of pronotum and elytral apex 6.5 mm, DNA barcode: MH917901. Diagnostic combination of morphological characters as in Table 4. Sister clade to *T. canthus* **sp. nov.** (Fig. 3).

Material examined. Holotype male (CNC): "CAMEROON, Mt. Kupe, 4.8193, 9.7075, 1525m, 29.xii.2015, sift.CM13, V.Grebennikov", "CNCCOLVG00009533". Paratypes (CNC): 4, as in Fig. 3; specimen 9534: same data as holotype; specimens 9657, 9729 and 9730: "CAMEROON, Mt. Kupe, 4.8017, 9.7015, 1977m, 29.xii.2015, sift.CM15, V.Grebennikov".

Distribution. Known only from Mt. Kupe in Cameroon, in sympatry with *T. iphitus* **sp. nov.** Elevation: 1,525–1,977 m.

Etymology. Telamon, from ancient Greek mythology, one of the Argonauts, father of Ajax the great; noun in apposition.

4.2.7. Inter- and infra-specific variability

The morphological interspecific variability is reflected by the character states in table 4. Several characters also vary within species, for an example the colour of the beetles varies depending on the age of the specimens. A freshly emerged beetle is paler than the colour referred to in character 1 (table 4). Similarly, not all specimens have the additional apical projection near inner apical spur in the hind tibiae referred to in character 3, nevertheless it is coded as present if at least some males have it. Lastly, the similarity of the pilosity on the elytral declivity to that on the rest of the elytra referred to in character 4 cannot be assessed in abraded specimens. The observed molecular variability within the newly described *Typoderus* species is consistent with the somewhat arbitrarily 1–2% threshold (Hebert et al. 2003a), although *T. iphitus* shows a deep split into two BINs differing by as much as 2.9%, indicating the possibility that it might consist of two cryptic species.
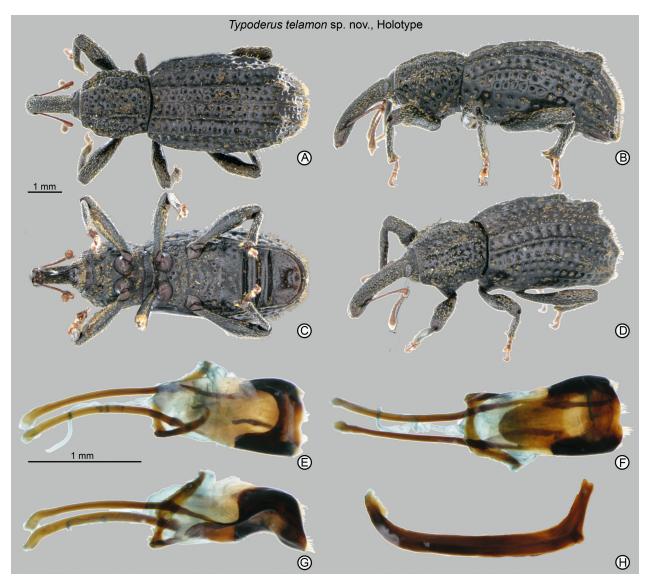
Figure 9. Male holotype of *T. telamon* **sp. nov.**, habitus (**A**: dorsal, **B**: left lateral, **C**: ventral, **D**: left fronto-lateral), aedeagus (**E**: dorsal, **F**: ventral, **G**: right lateral) and **H**: sternite 9.

5.   Conclusions

This is the first report of *Typoderus* weevils from Cameroon, where unnamed congeners are likely to exist. Thus, species-level identification of these beetles might be far from straightforward. A field biologist must collect as many *Typoderus* specimens as possible, in order to asses two characters with intraspecific variation (Table 4, particularly the colour of specimens). For determination of recently hatched pale individuals, molecular data from as in this work might be necessary.

This study highlights two taxonomic challenges or, rather, severe practical limitations of standard taxonomic procedures when performed in inadequately known and likely exceptionally diverse clades, such as *Typoderus* weevils. Firstly, for more than a decade the type specimens anchoring two genus-group names (the subgenera of *Anchonidium* herein synonymised with *Typoderus*) are not returned to a public institution, rendering both names vulnerable to misinterpretation. Secondly, even if

perhaps not as diverse as the weevil genus *Trigonopterus* with well over a thousand unnamed species (Riedel et al. 2013), many dozens of unnamed *Typoderus* have emerged during extensive litter sampling in Tanzania (Grebennikov 2017, 2019b). The effort to merely document this vast new diversity following the current taxonomic procedures is in itself an overwhelming task, for which no resources seem readily available. Being restricted by uncertainties surrounding old taxonomic names while facing a long list of new ones to introduce, I question when one shall find time and energy to utilize this yet-to-be-fine-tuned taxonomy as a tool for evolutionary biological studies? Perhaps similarly to astronomy, biology at present rapidly generates exponentially increasing amount of factual biodiversity data, for which the classical information storage system, the Linnaean taxonomy, is gradually becoming inadequate (comparably to the Messier or other historical catalogues of astronomic objects, presently of mainly educational interest). Will emerging DNA-based identifiers, such as BINs, take over species names? Only the future will tell.

6.   Acknowledgements

7.   References

Abadi S, Azouri D, Pupko T, Mayrose I (2019 Model selection may not be a mandatory step for phylogeny reconstruction. Nature Communication 10(934): 11 pp. https://doi.org/10.1038/s41467-019-08822-w

Andújar C, Serrano J, Gómez-Zurita J (2012) Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. BMC Evolutionary Biology 12(40): 16 pp. https://doi.org/10.1186/1471-2148-12-40

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DL (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389–3402. https://doi.org/10.1093/nar/25.17.3389

Bocak L, Grebennikov V.V, Masek M (2013) A new species of *Dexoris* (Coleoptera: Lycidae) and parallel evolution of brachyptery in the soft-bodied elateroid beetles. Zootaxa 3721: 495–500. https://doi.org/10.11646/zootaxa.3721.5.5

Bauzà-Ribot MM, Juan C, Nardi F, Oromí P, Pons J, Jaume D (2012) Mitogenomic phylogenetic analysis supports continental-scale vicariance in subterranean thalassoid crustaceans. Current Biology 22(21): 2069–2074. https://doi.org/10.1016/j.cub.2012.09.012

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from mitochondrial DNA evolution. PNAS 91: 6491–6495. https://doi.org/10.1073/pnas.91.14.6491

Burke K (2001) Origin of the Cameroon Line of Volcano-Capped Swells. The Journal of Geology 109: 349–362.

Butcher BA, Smith MA, Sharkey MJ, Quicke DLJ (2012) A turbo-taxonomic study of Thai *Aleiodes* (*Aleiodes*) and *Aleiodes* (*Arcaleiodes*) (Hymenoptera: Braconidae: Rogadinae) based largely on COI barcoded specimens, with rapid descriptions of 179 new species. Zootaxa 3457: 1–232.

De Queiroz K (2007) Species concepts and species delimitation. Systematic Biology 56: 879–886. https://doi.org/10.1080/10635150701701083

Demenocal PB (2004) African climate change and faunal evolution during the Pliocene–Pleistocene. Earth and Planetary Science Letters 220: 3–24. https://doi.org/10.1016/S0012-821X(04)00003-2

Deuve T, Cruaud A, Genson G, Rasplus J-Y (2012) Molecular systematics and evolutionary history of the genus *Carabus* (Col. Carabidae). Molecular Phylogenetics and Evolution 65: 259–275. https://doi.org/10.1016/j.ympev.2012.06.015

Déruelle B, Ngounouno I, Demaiffe D (2007) The 'Cameroon Hot Line' (CHL): a unique example of active alkaline intraplate structure in both oceanic and continental lithospheres. Comptes Rendus Geosciences 339: 589–600. https://doi.org/10.1016/j.crte.2007.07.007

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. https://doi.org/10.1093/molbev/mss075

Fjeldså J, Lovett JC (1997) Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. Biodiversity and Conservation 6: 325–346. https://doi.org/10.1023/A:1018356506390

Gremann C (2020) On *Anchonidium* Bedel, 1884 sensu stricto, with descriptions of two new species from the Iberian peninsula (Coleoptera, Curculionidae: Molytinae). Revue Suisse de Zoologie 127: 27–41. https://doi.org/10.35929/RSZ.0004

Grebennikov VV (2015) Wingless *Paocryptorrhinus* (Coleoptera: Curculionidae) rediscovered in Tanzania: synonymy, four new species and a mtDNA phylogeography. Bonn Zoological Bulletin 64: 1–15.

Grebennikov VV (2016) The genus *Prothrombosternus* (Coleoptera: Curculionidae: Molytinae) rediscovered: a male from Rubeho Mountains, Tanzania. Zootaxa 4171: 170–174. https://doi.org/10.11646/zootaxa.4171.1.7

Grebennikov VV (2017) Phylogeography and sister group of *Lupangus*, a new genus for three new flightless allopatric forest litter weevils endemic to the Eastern Arc Mountains, Tanzania (Coleoptera: Curculionidae, Molytinae). Fragmenta entomologica 49: 37–55. https://doi.org/10.4081/fe.2017.229

Grebennikov VV (2018) Re-defined *Aparopionella* from Afrotropical forest floors comprises 20 poorly known nominal species (Coleoptera, Curculionidae, Molytinae). Entomologische Blätter und Coleoptera 114: 219–231.

Grebennikov VV (2019a) First DNA analysis of pill scarabs (Coleoptera: Hybosoridae: Ceratocanthinae) reveals multiple paraphyly of Afrotropical *Philharmostes* and detects its basal dichotomy formed by a new wingless species. European Journal of Entomology 116: 52–63. https://doi.org/10.14411/eje.2019.006

Grebennikov VV (2019b) Independent morphological and genetic diversification in close geographic and phylogenetic proximity: *Typoderus* weevils (Coleoptera) in the Uluguru Mountains, Tanzania. Arthropod Systematics and Phylogeny 77: 251–265. https://doi.org/10.26049/ASP77-2-2019-04

Grebennikov VV (2019c) Miocene uplift and Pleistocene forest connectivity drove the evolution of large-bodied Afrotropical pill scarabs (Coleoptera: Hybosoridae: *Afrocloetus* and *Congomostes*). Arthropod Systematics and Phylogeny 77: 417–431. https://doi.org/10.26049/ASP77-3-2019-3

Grebennikov VV (2020) *Tazarcus*, a new phylogenetically unplaced genus of two flightless weevils with metapleural ridge from the Eastern Arc Mountains, Tanzania (Coleoptera: Curculionidae: Molytinae). Zootaxa 4766: 421–434. https://doi.org/10.11646/zootaxa.4766.3.2

Grebennikov VV, Heiss E (2018) Survey and DNA barcoding of flat bugs (Hemiptera: Aradidae) in the Tanzanian Forest Archipelago reveal a phylogeographically structured fauna largely unknown at species level. European Journal of Entomology 115: 512–523. https://doi.org/10.14411/eje.2018.051

Hamilton AC, Taylor D (1991) History of climate and forests in tropical Africa during the last 8 million years. Climatic Change 19: 65–78. https://doi.org/10.1007/978-94-017-3608-4_8

Hebert PDN, Ratnasingham S, DeWaard JR (2003a) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society B: Biological Sciences 270: 96–99. https://doi.org/10.1098/rsbl.2003.0025

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003b) Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert PDN, Ratnasingham R, Zakharov EV, Telfer AC, Levesque-Beaudin V, Milton MA, Pedersen S, Jannetta P, DeWaard R (2017) Counting animal species with DNA barcodes: Canadian insects. Philosophical Transactions of Royal Society B 371: 20150333, 10 pp. https://doi.org/10.1098/rstb.2015.0333

Hoffmann A (1968) The scientific results of the Hungarian soil zoological expedition to the Brazzaville-Congo. 32. Espèces de la famille Curculionidae (Coleoptera). Opuscula Zoologica, Budapest 8: 11–29.

Jendek E, Grebennikov VV (2011) *Agrilus* (Coleoptera, Buprestidae) of East Asia. Jan Farkač, Prague, 362 pp. Available from: https://www.researchgate.net/publication/269700572_Agrilus_Coleoptera_Buprestidae_of_East_Asia/download (accessed August 2019)

Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics bbx108: 7 pp. https://doi.org/10.1093/bib/bbx108

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis Version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Kuraku S, Zmasek CM, Nishimura O, Katoh K (2013) aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with en-hanced interactivity. Nuclear Acid Research 41: W22–W28. https://doi.org/10.1093/nar/gkt389

Lees DC, Kawahara AY, Rougerie R, Ohshima I, Kawakita A, Bouteleux O, De Prins J, Lopez-Vaamonde C (2014) DNA barcoding reveals a largely unknown fauna of Gracillariidae leaf-mining moths in the Neotropics. Molecular Ecology Resources 14: 289–296. https://doi.org/10.1111/1755-0998.12178

Maddison DR (2012) Phylogeny of *Bembidion* and related ground beetles (Coleoptera: Carabidae: Trechinae: Bembidiini: Bembidiina). Molecular Phylogenetics and Evolution 63: 533–576. https://doi.org/10.1016/j.ympev.2012.01.015

Maddison DR, Maruyama M (2018) Phylogenetic relationships and convergent evolution of ocean-shore ground beetles (Coleoptera: Carabidae: Trechinae: *Bembidion* and relatives). Systematic Entomology 44: 39–60. https://doi.org/10.1111/syen.12307

Maley J (1996) The African rain forest – main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. Proceedings of the Royal Society of Edinburgh 104B: 31–73. https://doi.org/10.1017/S0269727000006114

Marshall GAK (1953) On a collection of Curculionidae (Coleoptera) from Angola. Publicações Culturais da Companhia de Diamantes de Angola 16: 98–119.

Meierotto S, Sharkey MJ, Janzen DH, Hallwachs W, Hebert PDN, Chapman EG, Smith MA (2019) A revolutionary protocol to describe understudied hyperdiverse taxa and overcome the taxonomic impediment. Deutsche Entomologische Zeitschrift 66: 119–145. https://doi.org/10.3897/dez.66.34683

Miller M, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, pp. 1–8.

Miralles A, Bruy T, Wolcott K, Scherz MD, Begerow D, Beszteri B, Bonkowski M, Felden J, Gemeinholzer B, Glaw F, Glöckner FO, Hawlitschek O, Kostadinov I, Nattkemper TW, Printzen C, Renz J, Rybalka N, Stadler M, Weibulat T, Wilke T, Renner SS, Vences M (2020) Repositories for taxonomic data: where we are and what is missing. Systematic Biology 69: 1231–1253. https://doi.org/10.1093/sysbio/syaa026

Near TJ, Eytan RI, Dornburg A, Kuhn KL, Moore JA, Davis MP, Wainwright PC, Friedman M, Smith WL (2012) Resolution of ray-finned fish phylogeny and timing of diversification. PNAS 109: 13698–13703. https://doi.org/10.1073/pnas.1206625109

O'Grady PM, Desalle R (2018) Phylogeny of the genus *Drosophila*. Genetics 209: 1–25. https://doi.org/10.1534/genetics.117.300583

Papadopoulou A, Anastasiou I, Vogler AP (2010) Revisiting the insect mitochondrial molecular clock: the mid-aegean trench calibration. Molecular Biology and Evolution 27: 1659–1672. https://doi.org/10.1093/molbev/msq051

Papadopoulou A, Knowles LL (2015) Genomic tests of the species-pump hypothesis: recent island connectivity cycles drive population divergence but not speciation in Caribbean crickets across the Virgin Islands. Evolution 69: 1501–1517. https://doi.org/10.1111/evo.12667

Plana V (2004) Mechanisms and tempo of evolution in the African Guineo-Congolian rainforest. Philosophical Transactions of the Royal Society B: Biological Sciences 359: 1585–1594. https://doi.org/10.1098/rstb.2004.1535

Popper K (1959) The Logic of Scientific Discovery. Basic Books, New York, 484 pp.

Rambaut A (2019) FigTree. Ver. 1.4.0. Program and Documentation. URL http://tree.bio.ed.ac.uk/software/figtree [accessed 05 July 2019].

Ratnasingham S, Hebert PDN (2007) BOLD: the barcode of life data system. Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) System. PLoS ONE 8(7): e66213, 16 pp. https://doi.org/10.1371/journal.pone.0066213

Renner SS (2016) A return to Linnaeus's focus on diagnosis, not description: The use of DNA characters in the formal naming of species. Systematic Biology 65: 1085–1095. https://doi.org/10.1093/sysbio/syw032

Reusch AM, Nyblade AA, Wiens DA, Shore PJ, Ateba B, Tabod CT, Nnange JM (2010) Upper mantle structure beneath Cameroon from body wave tomography and the origin of the Cameroon volcanic line. Geochemistry, Geophysics, Geosystems 11: 1–17. https://doi.org/10.1029/2010GC00320

Riedel A, Sagata K, Suhardjono YR, Tänzler R, Balke M (2013) Integrative taxonomy on the fast track – towards more sustainability in biodiversity research. Frontiers in Zoology 10: 15. https://doi.org/10.1186/1742-9994-10-15

Shin S, Clarke DJ, Lemmon AR, Lemmon EM, Aitken AL, Haddad S, Farrell BD, Marvaldi AE, Oberprieler RG, McKenna DD (2017) Phylogenomic data yield new and robust insights into the phylogeny and evolution of weevils. Molecular Biology and Evolution 35: 823–836. https://doi.org/10.1093/molbev/msx324

Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. URL: http://www.simplemappr.net [accessed 05 July 2019].

Šípek P, Grebennikov V, Gill B (2009) Afromontane *Coelocorynus* (Coleoptera: Scarabaeidae: Cetoniinae): larval descriptions, biological notes, phylogenetic analysis and polyphyly of Trichiini. European Journal of Entomology 106: 95–106.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758–771. https://doi.org/10.1080/10635150802429642

Summers MM, Al-Hakim II, Rouse GW (2014) Turbo-taxonomy: 21 new species of Myzostomida (Annelida). Zootaxa 3873: 301–344. https://doi.org/10.11646/zootaxa.3873.4.1

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739. https://doi.org/10.1093/molbev/msr121

Tänzler R, Van Dam MH, Toussaint EFA, Suhardjono YR, Balke M, Riedel A (2016) Macroevolution of hyperdiverse flightless beetles reflects the complex geological history of the Sunda Arc. Scientific Reports 6: 18793. https://doi.org/10.1038/srep18793

Taylor PJ, Maree S, Cotterill FPD, Missoup AD, Nicolas V, Denys C (2014) Molecular and morphological evidence for a Pleistocene radiation of laminate-toothed rats (*Otomys*: Rodentia) across a volcanic archipelago in equatorial Africa. Biological Journal of the Linnean Society 113: 320–344. https://doi.org/10.1111/bij.12317

Tilbury CR (2018) Chameleons of Africa: An Atlas, Including the Chameleons of Europe, the Middle East and Asia. Chimaira Buchhandelsgesellschaft, Frankfurt am Main, 643 pp.

Vences M (2020) The promise of next-generation taxonomy. Megataxa 1: 35–38. https://doi.org/10.11646/megataxa.1.1.6

Voelker G, Outlaw RK, Bowie RCK (2010) Pliocene forest dynamics as a primary driver of African bird speciation. Global Ecology and Biogeography 19: 111–121. https://doi.org/10.1111/j.1466-8238.2009.00500.x

Voss E (1965) Mission zoologique de l'I.R.S.A.C. en Afrique Orientale (P. Basilewsky et N. Leleup, 1957) Résultats scientifiques. Cinquième partie. Coleoptera Curculionidae II (Schluss). Annales du Musée Royal de l'Afrique Centrale, Tervuren (Série in 8°, Zoologie) 138: 293–377.

Weirauch C, Forthman M, Grebennikov V, Baňař P (2017) From Eastern Arc Mountains to extreme sexual dimorphism: systematics of the enigmatic assassin bug tribe Xenocaucini (Reduviidae: Tribelocephalinae). Organisms Diversity and Evolution 17: 421–445. https://doi.org/10.1007/s13127-016-0314-2

Wheeler QD, Knapp S, Stevenson DW, Stevenson J, Blum SD, Boom BM, Borisy GG, Buizer JL, de Carvalho MR, Cibrian A, Donoghue MJ, Doyle V, Gerson EM, Graham CH, Graves P, Graves SJ, Guralnick RP, Hamilton AL, Nanken J, Law W, Lipscomb DL, Lovejoy TE, Miller H, Miller SJ, Naeem S, Novacek MJ, Page LM, Platnick NI, Porter-Morgan H, Raven PH, Solis MA, Valdecasas AG, Van der Leeuw S, Vasco A, Vermeulen N, Vogel J, Walls RL, Wilson EO, Wooley JB (2012) Mapping the biosphere: exploring species to understand the origin, organization and sustainability of biodiversity. Systematics and Biodiversity 10: 1–20. https://doi.org/10.1080/14772000.2012.665095

Zink RM (2004) The role of subspecies in obscuring avian biological diversity and misleading conservation policy. Proceedings of the Royal Society London Series B: Biological Sciences 271: 561–564. https://doi.org/10.1098/rspb.2003.2617

Zimkus BM, Gvoždík V (2013) Sky islands of the Cameroon Volcanic Line: a diversification hot spot for puddle frogs (Phrynobatrachidae: *Phrynobatrachus*). Zoologica Scripta 42: 591–611. https://doi.org/10.1111/zsc.12029