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ORIGINAL ARTICLE

First DNA analysis of pill scarabs (Coleoptera: Hybosoridae: Ceratocanthinae) reveals multiple paraphyly of Afrotropical *Philharmostes*

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Key words. Coleoptera, Hybosoridae, Ceratocanthinae, Ceratocanthini, DNA barcode, ITS2, 28S, phylogeny, forest litter, taxonomy, *Philharmostes ballerioi* sp. n., Afrotropical Region, Eastern Arc Mountains

Abstract. This paper is the first attempt to resolve relationships among the Ceratocanthinae: Ceratocanthini pill scarab beetles using DNA sequences. It is focused on the *Philharmostes* group of seven Afrotropical genera: *Baloghianestes* (3 spp.), *Callophilharmostes* (1 sp.), *Carinophilharmostes* (1 sp.), *Chaetophilharmostes* (1 sp.), *Cryptophilharmostes* (3 spp.), *Petrovitzostes* (1 sp.) and *Philharmostes* (31 spp.). A phylogenetic analysis of 46 terminals and alignment of 2,913 bp from one mitochondrial and two nuclear fragments corroborates monophyly of this group, but rejects that of *Philharmostes*, the largest genus. The latter is paraphyletic with respect to at least four other smaller genera and consists of at least three distantly related clades. One of them, formed by *Philharmostes ballerioi* sp. n. from the Tanzanian Nguru (the type locality) and Kaguru Mountains, is sister to the rest of the entire *Philharmostes* group. The nominal genus *Philharmostes* is, therefore, a waste-basket taxon for accommodating members of this group that lack the distinct characters of the smaller genera. Pending further research, the phylogenetically inadequate generic taxonomy of the *Philharmostes* group is not modified. Molecular clock analysis estimates separation of the mitochondrial lineages of two known populations of the new species at about 2.2 Ma, which corresponds with recurring shrinkage and expansion of African rainforest caused by climatic fluctuations during the Pleistocene. Adults of all nominal ingroup genera are illustrated along with male and female body parts of the new species. Diagnostic and/or synapomorphic morphological characters of the *Philharmostes* group of genera are revised. Habitus images and other supplementary information on all sequenced specimens are available online at dx.doi.org/10.5883/DS-VGDS001 and dx.doi.org/10.5883/DS-VGDS004.

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INTRODUCTION

Ceratocanthini (the largest of the three currently recognized tribes in the subfamily Ceratocanthinae) constitutes a pantropical clade of 38 extant nominal genera with some 358 species (Ballerio & Grebennikov, 2016). They are best known for their ability to conglobate (= to pack their body into a tight spheroid) using interlocking exoskeletal structures, thus earning the entire subfamily the colloquial name "pill scarabs" (Howden & Gill, 2000). Their adults are mainly found in tropical forests at low altitudes. Flightless species are sometimes abundantly sifted from forest floor litter, while volant species are sampled by canopy fogging or using flight intercept traps. Adults and larvae of pill scarabs occur in rotten trees (Choate, 1987), sometimes in close proximity with ants and/or termites, however, this has never been documented as biologically significant (Parker, 2016). All three main tropical regions of the World (Neotropical, Afrotropical and those in Asia and Australia) have highly distinct pill scarab faunas with no genera in common. Until now, most research on these beetles has been predominantly descriptive and not DNA-based, with only three quantitative morphological studies addressing their phylogeny (Grebennikov et al., 2004; Ballerio, 2016; Ballerio & Grebennikov, 2016). The most representative phylogenetic analysis to date (Ballerio & Grebennikov, 2016) strongly supports monophyly of the tribe but is inconclusive on the internal grouping. The only exception was the consistently recovered clade of Afrotropical genera informally termed the *Philharmostes* group (= PhG), which forms the focus of this paper.

When first recognized (Ballerio, 2000), PhG contained (and still contains) seven nominal genera (current number of species is given in parentheses): *Baloghianestes* Paulian, 1968 (4, Fig. 1A), *Callophilharmostes* Paulian, 1968 (1, Fig. 1B), *Carinophilharmostes* Paulian, 1968 (1, Fig. 1C), *Chaetophilharmostes* Paulian, 1977 (1, Fig. 1D), *Cryptophilharmostes* Ballerio, 2000 (2, Fig. 1E), *Petrovitzostes* Paulian, 1977 (1, Fig. 1F) and *Philharmostes*

Kolbe, 1895 (31, Figs 2A–E, 4, 5). Monophyly of PhG appears likely because of the number of shared diagnostic characters (Ballerio, 2000, 2001) and its relatively high bootstrap support (85% in Ballerio & Grebennikov, 2016). The largest PhG genus (*Philharmostes*) is, however, hypothesized to be paraphyletic with respect to at least one (*Baloghianestes*, see Ballerio & Grebennikov, 2016) and perhaps more, small genera. This scenario is indeed likely, since species of all six small PhG genera and those of *Philharmostes* are sympatric and their biological preferences are similar. Moreover, three of the four monotypic genera (*Callophilharmostes*, *Carinophilharmostes*, *Chaetophilharmostes*) were established for nominal species originally described in *Philharmostes* (Paulian, 1968, 1977), which is reflected in their unwieldy names. It seems likely, therefore, that the species-rich genus *Philharmostes* is a paraphyletic waste-basket taxon used for those members of PhG that lack distinct diagnostic characters of the six other small genera.

This paper was triggered by the availability of many representatives of PhG recently collected in Africa, mainly Tanzania. Freshly collected specimens of four small genera of PhG, along with numerous "typical" *Philharmostes* from most of their distribution (West, East and South Africa, as well as Madagascar, map in Fig. 3) offered an opportunity of testing the taxonomically-implied reciprocal monophyly of nominal genera. Furthermore, two populations from the adjacent Nguru and Kaguru mountains in Tanzania (map in Fig. 6) belonging to a new species which in the present classification should be included in *Philharmostes* are not similar to the rest of the genus (smaller in size, nearly spherical when enrolled and lacking dorsal eyes). The first and main goal of this paper is, therefore, to perform the first DNA-based phylogenetic analysis of Ceratocanthini pill scarabs focussing on PhG and to test two hypotheses: monophyly of the entire group and that of its largest genus *Philharmostes*, particularly with respect to the small genera; and to determine the placement of the new Nguru/Kaguru species. The second goal of this paper is to formally describe this new species and, considering its newly hypothesized sister-group relationships with the rest of PhG (see Results), thoroughly illustrate its external and internal structures for use in future comparative morphological studies. The third goal, also triggered by the remarkably ancient relationships of this new species, is to review all diagnostic and/or synapomorphic morphological characters of PhG, as previously defined, and to determine which of them might be detected in the new species. The fourth and final goal is to test reciprocal monophyly of both neighbouring populations of the new species and to date their separation. This is intriguing, because this date might indicate when the currently small and compact wet rainforests on Nguru and Kaguru became separated by hot and dry savannah (presently the gap is about 65 km). Overall, this paper strives to shed new phylogenetic light on Ceratocanthini, a diversified, easy-to-recognize and relatively neglected branch of the scarabaeoid beetles.

MATERIAL AND METHODS

Specimen sampling and deposition

The majority of the specimens of Ceratocanthini included in this study were sifted from forest litter in Tanzania (list of localities and sample codes are in Grebennikov, 2017; Grebennikov & Heiss, 2018). In addition, (mainly outgroup) specimens were opportunistically accumulated from a variety of sources (see Acknowledgments). Unless otherwise stated, all herein reported specimens are stored in the Canadian National Collection of Insects, Arachnids and Nematodes in Ottawa, Canada (CNC, curator P. Bouchard).

Preliminary DNA barcoding

A total of 149 freshly sampled Afrotropical Ceratocanthini (and those received from other sources) were preliminary sorted into morphospecies and DNA barcoded (658 bp of COI-5' sequenced; Hebert et al., 2003a, b; all data are not shown). DNA barcoding followed the standard protocol of the "Canadian Centre for DNA Barcoding" at the University of Guelph, Canada (CCDB, http://www.ccdb.ca). Alignment of the protein-coding COI was trivial and did not result in insertions/deletions (= indels), stop codons or frame shifts. Their Neighbour Joining (NJ) clustering (topology is not shown) was performed online using the Barcode of Life database (= BOLD, Ratnasingham & Hebert, 2007) engine (http://www.boldsystems.org/).

Three-marker dataset

Select DNA barcoded specimens representing all the terminal PhG clusters (= putative species) in the NJ tree were additionally sequenced for two nuclear ribosome-coding regions: internal ribosomal spacer 2 (ITS2) and 28S rDNA (Table 1). Among seven nominal genera of PhG (= ingroup, for the purpose of this phylogenetic analysis), DNA sequences from 32 terminals were obtained, including multiple representatives of all three non-monotypic genera (*Baloghianestes*, *Cryptophilharmostes*, *Philharmostes*) and the type species of two monotypic genera (*Carinophilharmostes* and *Petrovitzostes*). No DNA-grade specimens could be obtained for the genera *Callophilharmostes* and *Chaetophilharmostes* so these taxa were not included in the analysis. To test the monophyly of PhG and place it into a broader phylogenetic framework, 12 other Ceratocanthini non-PhG genera, each represented by a single terminal, were added to the dataset. Monophyly of Ceratocanthini and their placement within monophyletic Hybosoridae are well established based on analyses utilizing larval morphology (Grebennikov & Scholtz, 2004; Grebennikov et al., 2004), DNA (Ocampo & Hawks, 2006) and adult morphology (Ballerio & Grebennikov, 2016). This hypothesis is herein accepted a priori by adding to the matrix two representatives of non-Ceratocanthini Hybosoridae (Table 2) to root the topology. All laboratory work was done in CCDB using protocols and primers described in Grebennikov (2018). All relevant laboratory data (such as electropherograms, sequences, specimen images and their localities) are available online in BOLD dataset dx.doi.org/10.5883/DS-VGDS001; the GenBank accession numbers are listed in Table 2. All DNA sequences used in this study were newly generated.

Table 1. 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).

Fragment	#	Min	Max	Aligned	Positions
CO1-5P	45	576	658	658	1 to 658
ITS2	44	231	876	1514	659 to 2172
28S	44	284	648	741	2173 to 2913

Table 2. DNA fragments and their GenBank accession numbers of the 46 terminals used in the phylogenetic analyses. All included Ceratocanthinae belong to the nominotypical tribe Ceratocanthini.

Voucher	Subfamily	Species	Country	CO1	ITS2	28S
3669	Hybosorinae	*Cryptogenius* sp.	Bolivia	MH778064	MH777800	MH777844
3705	Ceratocanthinae	*Philharmostes basilewskyi*	Tanzania	MH778074	MH777811	MH777854
3710	Ceratocanthinae	*Philharmostes* sp.	Tanzania	MH778057	MH777793	MH777837
3712	Ceratocanthinae	*Philharmostes werneri*	Tanzania	MH778099	MH777835	MH777879
3718	Ceratocanthinae	*Philharmostes pseudumbratilis*	Tanzania	MH778091	MH777829	MH777871
3722	Ceratocanthinae	*Cryptophilharmostes mahunkai*	Tanzania	MH778059	MH777795	MH777839
3723	Ceratocanthinae	*Cryptophilharmostes mahunkai*	Tanzania	MH778088	MH777826	MH777868
3742	Ceratocanthinae	*Philharmostes* sp.	Tanzania	MH778078	MH777815	MH777858
3756	Ceratocanthinae	*Cryptophilharmostes* sp.	Tanzania	MH778082	MH777819	none
3763	Ceratocanthinae	*Cryptophilharmostes merkli*	Tanzania	MH778058	MH777794	MH777838
3767	Ceratocanthinae	*Cryptophilharmostes merkli*	Tanzania	MH778060	MH777796	MH777840
7013	Ceratocanthinae	*Philharmostes pseudumbratilis*	Tanzania	MH778080	MH777817	MH777860
7017	Ceratocanthinae	*Philharmostes grebennikovi*	Tanzania	MH778062	MH777798	MH777842
7027	Ceratocanthinae	*Philharmostes ornatus*	Tanzania	MH778065	MH777801	MH777845
7055	Ceratocanthinae	*Philharmostes ballerioi* sp. n.	Tanzania	MH778071	MH777808	MH777852
7058	Ceratocanthinae	*Cryptophilharmostes* sp.	Tanzania	MH778066	MH777803	MH777847
7070	Ceratocanthinae	*Philharmostes ballerioi* sp. n.	Tanzania	MH778061	MH777797	MH777841
7131	Ceratocanthinae	*Petrovitzostes guineensis*	Cameroon	MH778090	MH777828	MH777870
8417	Ceratocanthinae	*Philharmostes interruptus*	South Africa	MH778068	MH777805	MH777849
8907	Ceratocanthinae	*Pseudosynarmostes mitsinjo*	Madagascar	MH778069	MH777806	MH777850
8933	Ceratocanthinae	*Philharmostes* sp.	Tanzania	MH778098	MH777834	MH777878
8934	Ceratocanthinae	*Philharmostes* sp.	Tanzania	MH778085	MH777822	MH777864
8978	Ceratocanthinae	*Philharmostes* sp.	Tanzania	MH778095	MH777832	MH777875
9116	Ceratocanthinae	*Melanophilharmostes poggii*	Eq. Guinea	MH778087	MH777824	MH777866
9213	Ceratocanthinae	*Baloghianestes oribatidiformis*	Eq. Guinea	MH778086	MH777823	MH777865
9412	Ceratocanthinae	*Baloghianestes* sp.	Ghana	MH778093	none	MH777873
9456	Ceratocanthinae	*Synarmostes humilis*	Comoros	MH778075	MH777812	MH777855
9459	Ceratocanthinae	*Madrasostes* sp.	Malaysia	MH778097	MH777833	MH777877
9468	Ceratocanthinae	*Aneilobolus* sp.	South Africa	MH778096	none	MH777876
9474	Ceratocanthinae	*Pseudopterorthochaetes demirei*	Cameroon	MH778070	MH777807	MH777851
9476	Ceratocanthinae	*Carinophilharmostes vadoni*	Cameroon	MH778079	MH777816	MH777859
9480	Ceratocanthinae	*Baloghianestes oribatidiformis*	Cameroon	MH778094	MH777831	MH777874
9490	Ceratocanthinae	*Baloghianestes anceps*	Cameroon	MH778056	MH777792	MH777836
9491	Ceratocanthinae	*Baloghianestes oribatidiformis*	Cameroon	MH778089	MH777827	MH777869
9602	Ceratocanthinae	*Philharmostes* sp.	Ghana	MH778084	MH777821	MH777863
9611	Ceratocanthinae	*Nesopalla iviei*	Puerto Rico	MH778083	MH777820	MH777862
9614	Ceratocanthinae	*Astaenomoechus* sp.	Panama	MH778067	MH777804	MH777848
9617	Ceratocanthinae	*Cyphopisthes* sp.	Philippines	MH778073	MH777810	none
9709	Ceratocanthinae	*Philharmostes* sp.	South Africa	MH778076	MH777813	MH777856
9710	Ceratocanthinae	*Philharmostes* sp.	Tanzania	MH778063	MH777799	MH777843
9716	Ceratocanthinae	*Ceratocanthus amazonicus*	French Guiana	MH778081	MH777818	MH777861
9720	Ceratocanthinae	*Congomostes hintelmanni*	Cameroon	MH916833	MH777802	MH777846
9755	Ceratocanthinae	*Germarostes* sp.	Mexico	MH778072	MH777809	MH777853
9756	Anaidinae	*Anaides laticollis*	Mexico	none	MH777825	MH777867
9760	Ceratocanthinae	*Philharmostes* sp.	Madagascar	MH778077	MH777814	MH777857
9761	Ceratocanthinae	*Philharmostes* sp.	Madagascar	MH778092	MH777830	MH777872

Alignment of ribosomal markers

Alignment of the ITS2 and 28S sequences was done using the MAFFT 7 online platform (Katoh et al., 2002; Katoh & Toh, 2008a) and the Q-INS-i algorithm (Katoh & Toh, 2008b) utilising the secondary structure information and resulted in the introduction of 638 and 93 indels, respectively (Table 1). To minimize bias, no parts of the alignments were excluded from the analysis. Three aligned single-fragment datasets were concatenated using Mesquite 3.11 (Maddison & Maddison, 2011) into a matrix of 46 terminals and 2,913 aligned positions containing 45% of the completely undetermined characters (mainly due to numerous indels in ITS2).

Analysis of the three-marker dataset (= phylogenetic analysis)

Phylogenetic analysis was done on the CIPRES Science Gateway online platform (Miller et al., 2010) using the Maximum Likelihood (ML) method. Phylogenetic trees were obtained using RAxML 7.2.7 (Stamatakis, 2006), with default parameters, unless otherwise stated. The concatenated matrix was partitioned into three fragments (Table 1) and an independent GTR + G model (the only one implemented in RAxML) was applied to each data partition. The best scoring ML tree was selected among 1000 searches on the original alignment with different randomized parsimony starting trees. Support values were obtained based on 1000 bootstrap replicates (Felsenstein, 1985; Stamatakis et al., 2008).

Temporal analysis of DNA barcodes

The second analysis was designed to test the reciprocal monophyly of the Nguru and Kaguru populations of the herein described new species and if so, to estimate when they ceased exchanging genetic material. Lacking fossils and unambiguous biogeographical events to calibrate the phylogeny, a uniform substitution rate was implemented. A flat molecular clock of 0.018 nucleotide substitutions per site per million years per lineage (subs/s/Myr/l) was applied to the DNA barcode fragment, which is in agreement with results obtained for other beetles (Papadopoulou et al., 2010;

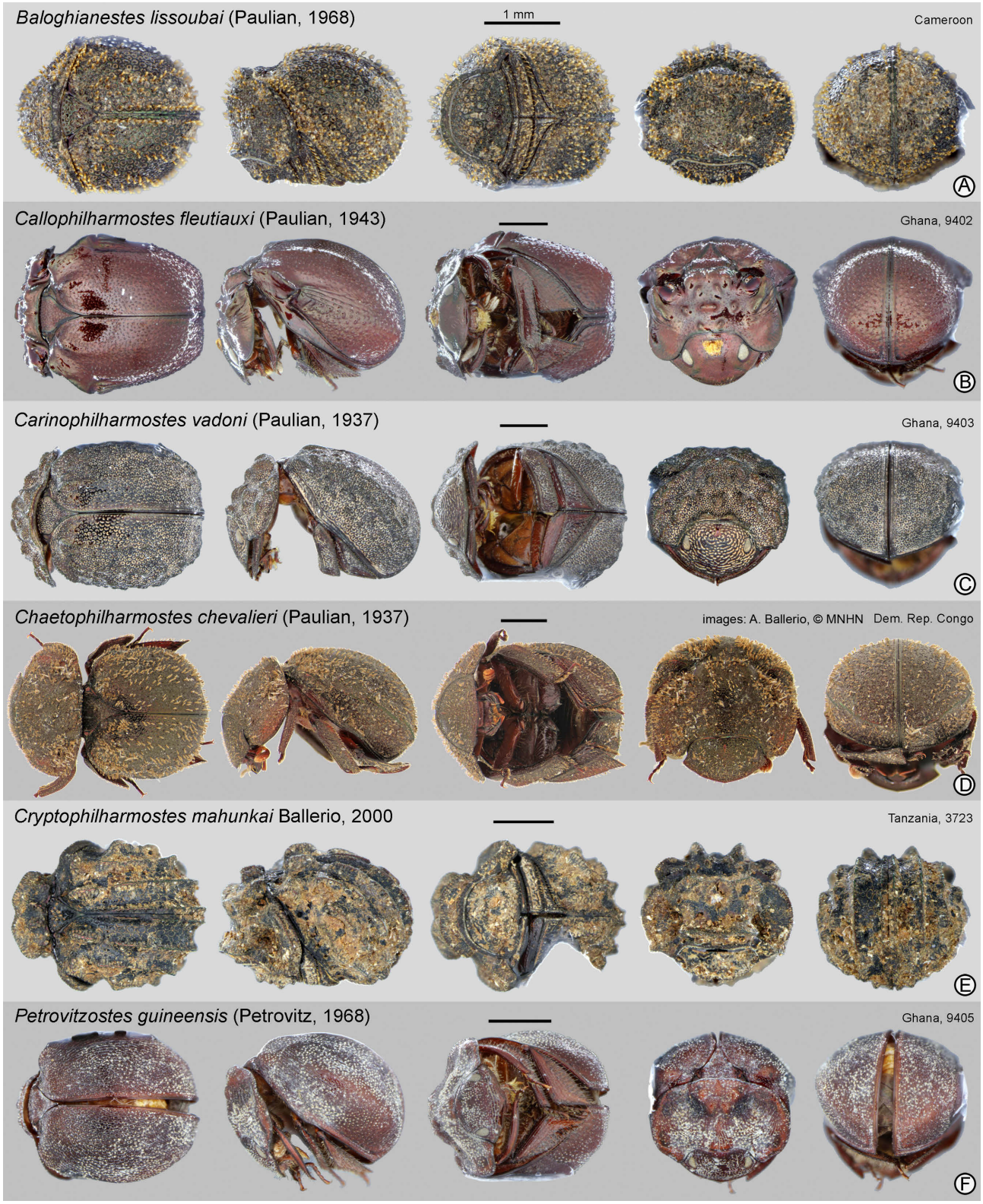

Fig. 1. Non-*Philharmostes* representatives of the pill scarab *Philharmostes* group of genera (Coleoptera: Hybosoridae: Ceratocanthinae). The specimen of *Chaetophilharmostes chevalieri* depicted (not seen) is stored in the Muséum National d'Histoire Naturelle (Paris, France) and bears the following label: "Congo Belge, P.N.G. Mission H. De Saeger, II/gc/8, 30-IV-1952, H. De Saeger, 3405".

Andújar et al., 2012). A matrix was formed from 13 barcodes of the new species, of which six and seven were for the Nguru and Kaguru populations, respectively. Bayesian phylogenetic analysis in BEAST 1.8 (Drummond et al., 2012) was used to simultaneously estimate an ultrametric phylogenetic tree and ages of diversification. The TN93 evolutionary model (estimated in MEGA 7, Kumar et al., 2016) was applied and the MCMC chains ran for 10 million generations. Consensus trees were estimated using TreeAnnotator (Drummond et al., 2012) after discarding 25% of the initial trees as a burn-in fraction, after checking ESS of likeli-

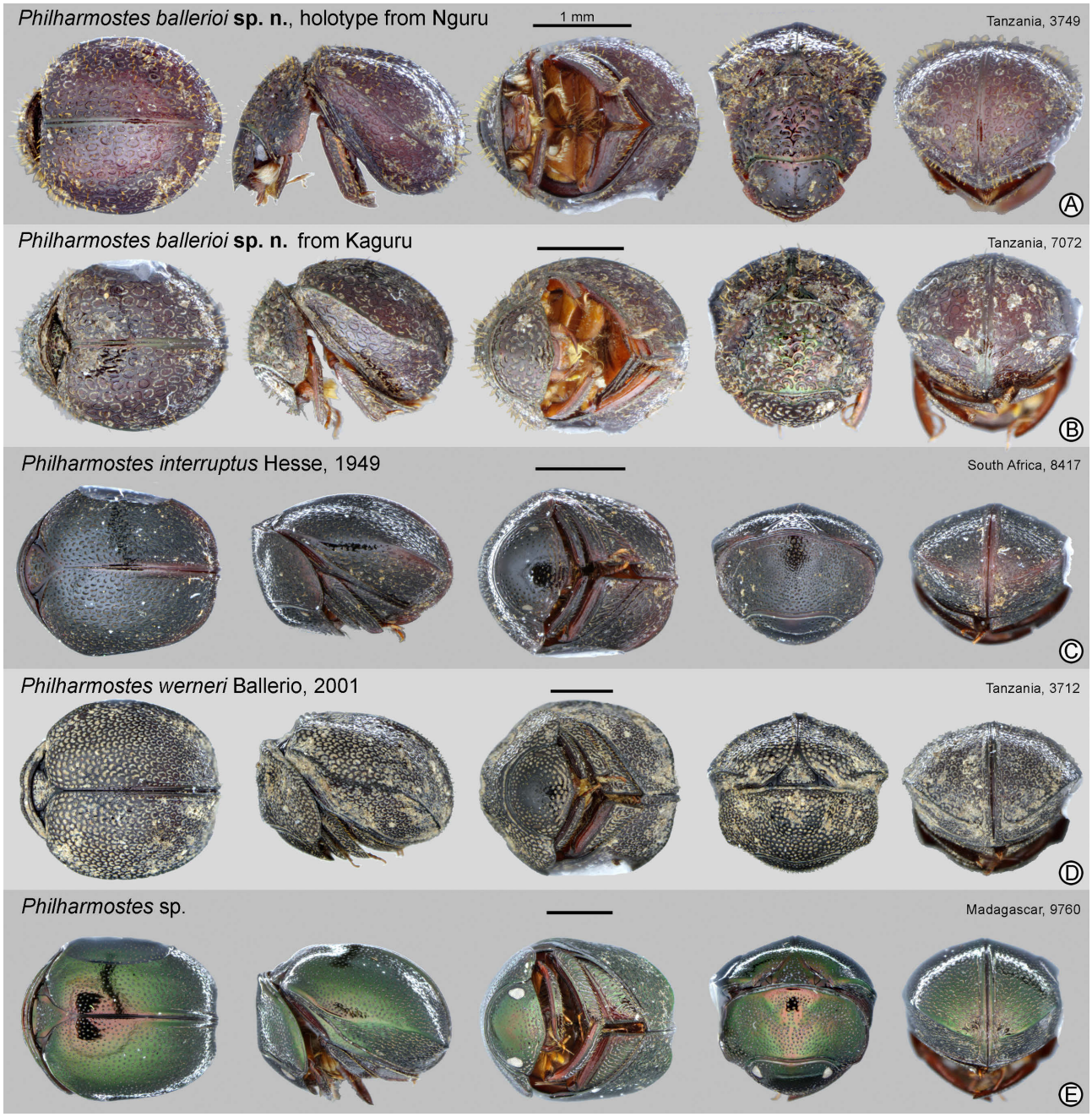

Fig. 2. Nominal *Philharmostes* representatives of the *Philharmostes* group of genera (Coleoptera: Hybosoridae: Ceratocanthinae).

hood, evolutionary rates and root age values, and ensuring that the tree likelihood values had reached a plateau. Posterior probabilities were used as a measure of node support. No monophyletic groups were enforced prior to the analysis, and the rooting was done on the longest branch.

Morphological study

In order to illustrate the adult morphology of the members of PhG, two imaging strategies were followed. Firstly, high-resolution habitus images were generated for specimens of all seven nominal genera of PhG (Figs 1A–E, 2A–F). These images were of dry-mounted specimens and based on multiple imaging with subsequent digital deep-focus stacking. Unlike the majority of earlier pill scarab illustrations of artificially descended (= unrolled, flattened) specimens (see, for example, Ballerio & Grebennikov, 2016), the images are of enrolled specimens, as they are most commonly seen by humans. Secondly, and in view of its phylogenetic position as the sister to the rest of PhG (see Results), morphological structures of the herein newly described species are extensively illustrated and those of both sexes compared. For this purpose, male (paratype 3751) and female (paratype 7055) of *P. ballerioi* sp. n. were disarticulated and extensively imaged (Figs 4A–Y, 5A–J). Disarticulated body parts were photographed submerged in glycerol, while the genitalia of both sexes were first macerated in a warm 5–10% solution of KOH in water and then stained in Chlorazol black. Since the newly described species cannot be reliably sexed without dissecting the genitalia, which resulted in significant damage to the body, the holotype was selected from structurally intact and, therefore, unsexed specimens. No specimens from Kaguru Mts. were dissected.

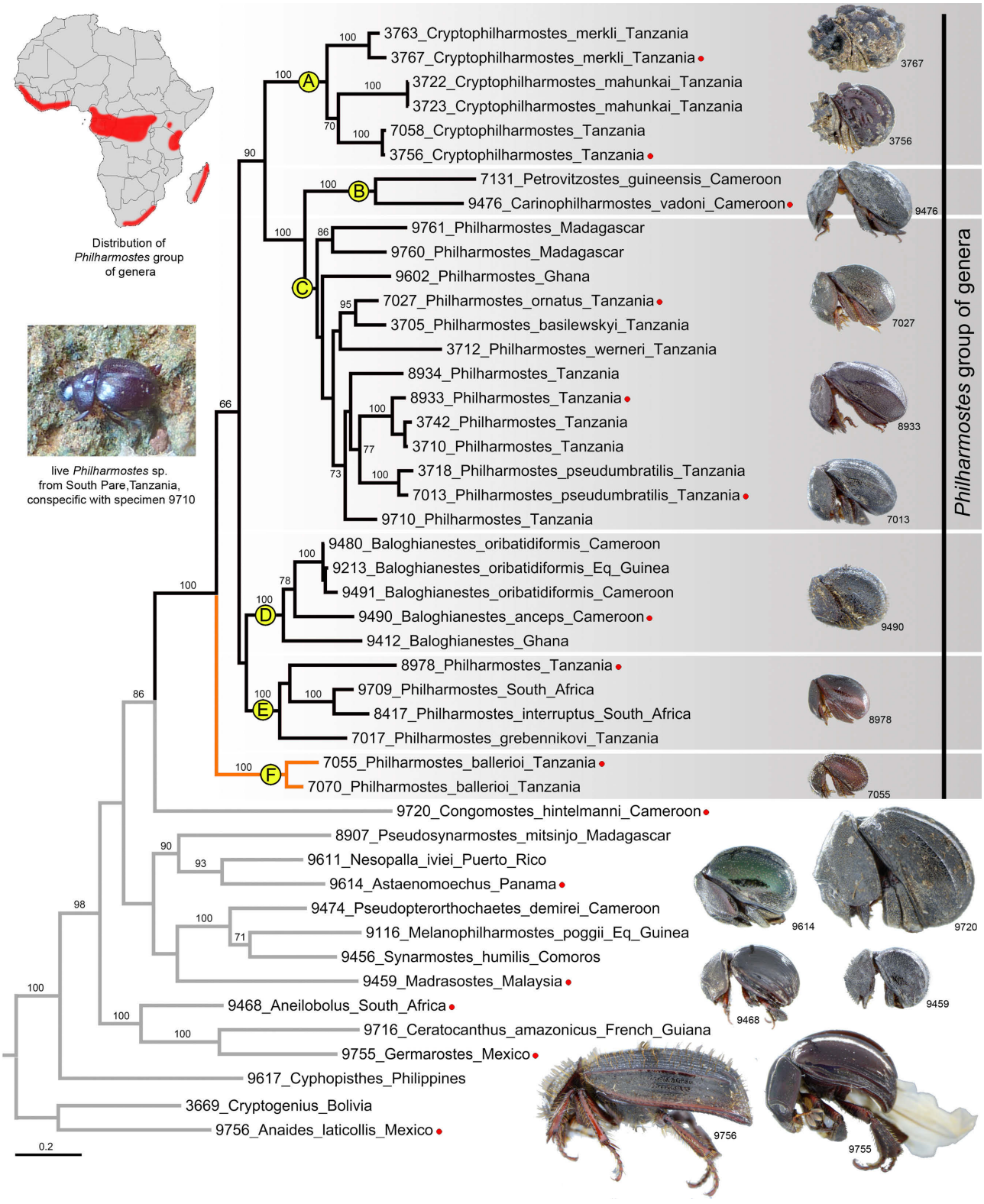

Fig. 3. Maximum Likelihood inference phylogram of Ceratocanthini pill bugs, which reveals that the genus *Philharmostes* is paraphyletic with respect to other members of the monophyletic *Philharmostes* Group (Analysis 1). Orange branch represents the herein described new species from Tanzania, sister to the rest of the *Philharmostes* Group. Digits at internodes are bootstrap values > 65%. Map indicates known distribution of the *Philharmostes* Group with all of its seven nominal genera found in the tropical belt of continental Africa, while only the nominal *Philharmostes* is additionally known from both South Africa and Madagascar (approximated from Ballerio & Grebennikov, 2016). Red dots denote imaged specimens (to scale).

Morphological terms

The terms "genal canthus" and "dorsal ocular area" (Ballerio & Grebennikov, 2016), which refer to the horizontally oriented ridge completely dividing (in some members of PhG) the compound eye, are consistently referred to as "interocular bridge" and "dorsal eye", respectively, in line with their usage in other

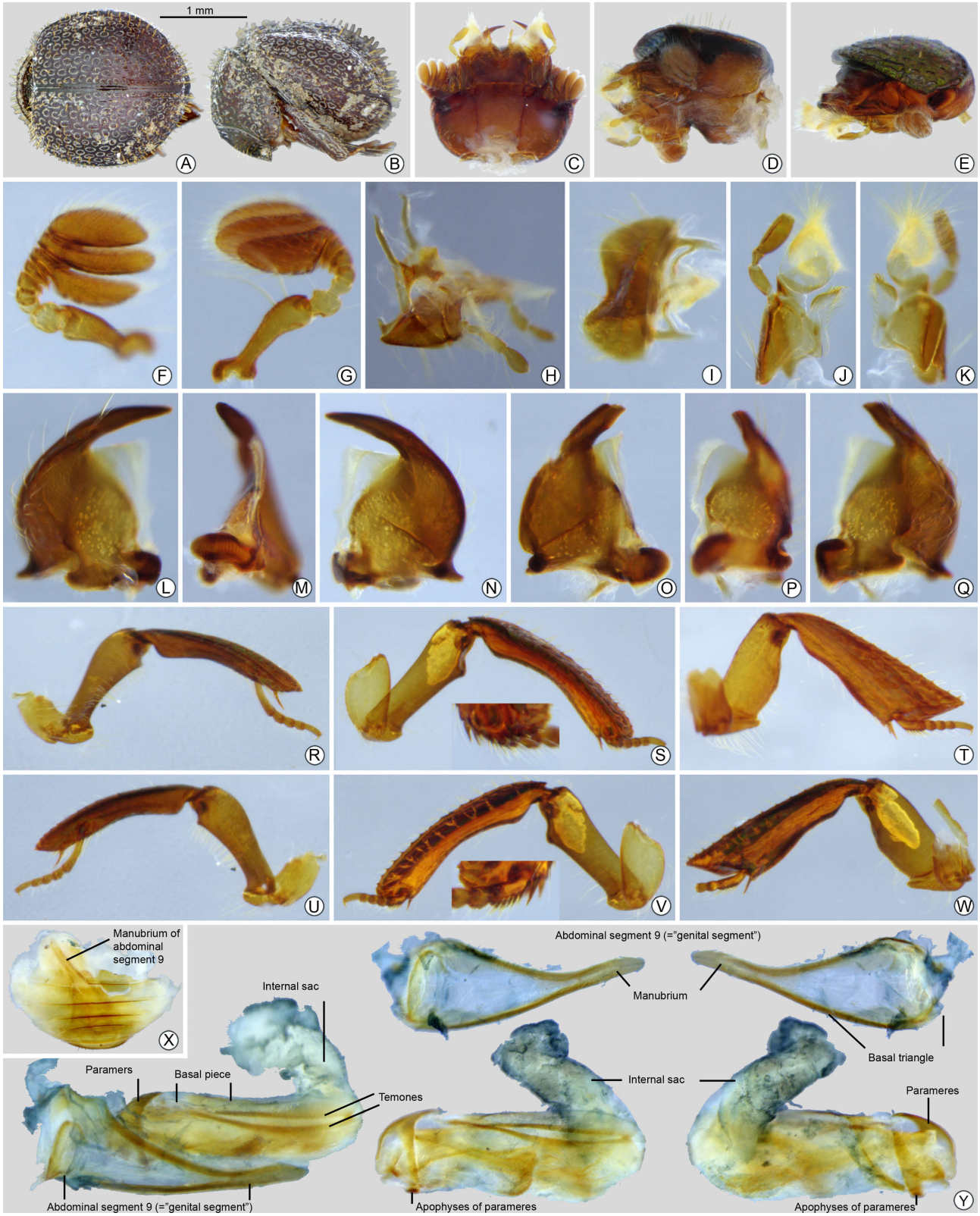

Fig. 4. *Philharmostes ballerioi* sp. n., male paratype 3751, habitus (A–B) and body parts (C–X). C–E – head, ventral (C), left latero-ventral (D) and left latero-frontal (E); F–G – left antenna, dorsal (F) and ventral (G); H – labium and epipharynx, right latero-fronto-dorsal; I – labrum, left latero-dorsal; J–K – left maxilla, dorsal (J) and ventral (K); L–N – left mandible, dorsal (L), mesal (M) and ventral (N); O–Q – right mandible (apex broken), ventral (O), mesal (P) and dorsal (Q); R–W – legs, fore (R, U), middle (S, V, with inserts of tibial apices) and hind (T, W), posterior (R–T) and anterior (U–W); X – abdomen, ventral; Y – male genitalia.

beetles (i.e. Gyrinidae: Beutel et al., 2017). Terminology of male genitalia and associated structures mainly follows D'Hotman & Scholtz (1990), therefore the term "basal piece" is used instead of "phalobasis" (Ballerio, 2016). "Genital segment" of D'Hotman & Scholtz (1990) is, however, referred to by its anatomical term "abdominal segment 9" (exact homology of its sclerites is un-

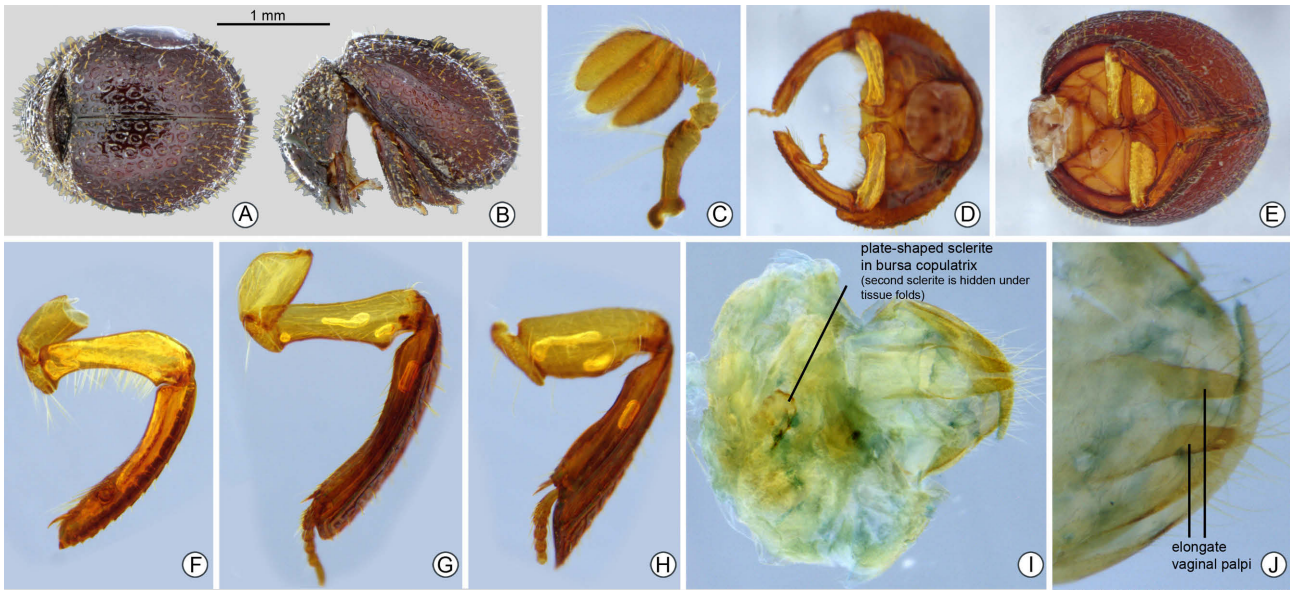

Fig. 5. *Philharmostes ballerioi* sp. n., female paratype 7055, habitus (A–E) and body parts (C–J). C – left antenna, ventral; C – prothorax, ventro-posterior; E – hind body, ventral; F–H – left fore (F, tarsus broken), middle (G) and hind (H) legs, anterior; I–J – female genitalia.

known); its structures referred to as "manubrium" and "basal triangle" (i.e. Ballerio et al., 2011) are shown in Fig. 4Y. "Apophyses of parameres" (Fig. 4Y) are illustrated in Ballerio (2000, Fig. 6). All other morphological terms are the same as in Ballerio & Grebennikov (2016), including "enrolment coaptations", which defines beetles' ability to fold their body into a tight spheroid without gaps or protruding appendages.

RESULTS

The ML phylogenetic analysis of 46 terminals analyzed using the concatenated 2,913 bp matrix produced a well-resolved topology (Fig. 3). This analysis recovered the monophyletic PhG (bootstrap support 100%) sister (86%) to the Afrotropical genus *Congomostes* Paulian, 1968. Monophyletic PhG consists of six clades (A–F, Fig. 3), all having 100% support (except clade C with 56%). The genera *Cryptophilharmostes* and *Baloghianestes* (clades A and D, respectively) are monophyletic (both with 100%). The genus *Philharmostes* consists of three not most closely related clades (C, E and F); *Philharmostes* from Madagascar form the basal dichotomy of clade C, while clades E and F consist of small-bodied species. The genera *Petrovitzostes* and *Carinophilharmostes*, each represented by a single terminal, form clade B (100%) sister (100%) to *Philharmostes* clade C; the resulting clade B + C is sister (90%) to the genus *Carinophilharmostes*. The herein described new species (clade F) is sister (66%) to the rest of PhG.

The Bayesian temporal analysis of 13 terminals of the new species using the 658 bp DNA barcode matrix, recovered two geographically structured clades from the Nguru and Kaguru Mountains (Fig. 6), both with 100% posterior probabilities. Separation of their mitochondrial strains is dated at 2.21 million years ago (Ma), while diversification within them started 0.45 and 0.13 Ma, respectively (Fig. 6).

Genus *Philharmostes* Kolbe, 1895

Kolbe, 1895: 344.

Type species. *Philharmostes aeneoviridis* Kolbe, 1895 (designated by Fairmaire, 1899: 471).

Philharmostes ballerioi sp. n.

Figs 2A, B, 4A–Y, 5A–J, 6

ZooBank taxon LSID:
1347B76F-E691-4A48-8CB2-64A7598B9163

Diagnosis. In the key to genera of Afrotropical pill scarabs (Ballerio & Grebennikov, 2016) this species is identified as belonging to the *Philharmostes* Group (couplets 15–20) by having (1.) the enrolment coaptation and (2.) broadly arcuate protibia, with the outer margin nearly even and without distinct teeth. Lacking the unique dorsal head trichome of *Callophilharmostes*, this species in lacking a dorsal eye will not key out to *Philharmostes* (all species of which have a dorsal eye), but to *Baloghianestes* (because of its similarly shaped dorsal head contour, which is unlike that of the quite dissimilar *Carinophilharmostes*). The new Tanzanian species differs from the exclusively West African *Baloghianestes* by having an even, uninterrupted and sharp lateral carina on each elytron; in the latter genus it is either absent (the type species) or notably serrate in dorsal view (other species). As its phylogenetic position is far outside *Baloghianestes*, it might be warranted to erect a new genus for this species; this, however, is postponed until its relationships are better understood. The new species is, therefore, provisionally assigned to the (already) non-monophyletic *Philharmostes* (see Discussion).

Description. Holotype (unsexed, Fig. 2A): body length in dorsal view and in an enrolled position 2.05 mm; DNA data as in Table 2; enrollment coaptation (= ability to conglobate) present; dorsal surface of head on each side without dorsal eye; protibiae broadly arcuate, their outer margin smooth and without distinct teeth; lateral carina on each elytron even, uninterrupted and sharp. Both sexes with antennae consisting of nine antennomeres (Figs 4F,

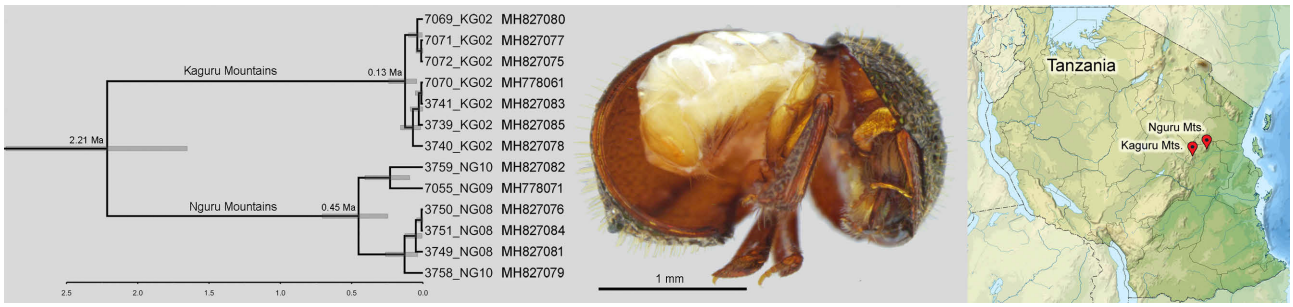

Fig. 6. Ultrametric time tree dating the separation of the Nguru and Kaguru populations of *Philharmostes ballerioi* sp. n. obtained using BEAST and 0.018 subs/s/Myr/l (Analysis 2). Numbers on the time scales are million years before present. Node bars represent 95% confidence interval of age estimate. Terminal numbers are specimen number (first four digits) followed by litter sifting sample code and GenBank accession number. The six sequenced Nguru specimens are the types, specimen 3749 is the holotype. Illustrated paratype 3758 has right elytron removed to reveal complete lack of hind wings. Map illustrates both known localities of *P. ballerioi* sp. n.

G; 5C); hind wings absent (Fig. 6). Male (Figs 4A–Y) with aedeagus weakly sclerotized, without distinguishable pseudosclerites in internal sac (Fig. 4Y), with seemingly asymmetrical parameres bearing basal apophyses (Fig. 4Y, this character is poorly understood), median lobe apparently absent (Fig. 4Y). Female (Figs 5A–J) externally indistinguishable from male (including apices of mesotibiae); genitalia with two plate-shaped sclerites in bursa copulatrix (Fig. 5I) and with elongate vaginal palpi (Fig. 5J). Specimens from Kaguru are similar to those from Nguru, except they are slightly bigger (by about 5–15% in each dimension); DNA barcodes of six Nguru and six Kaguru specimens as in Fig. 6.

Type material. Only the six sequenced specimens from Nguru Mts. (Fig. 6) were designated as types. Holotype (No. 3749, unsexed, CNC): "TANZANIA, Nguru Mts. at Mhonda, 6°06′24″S 37°31′48″E, 3.i.2012, 1254 m, sift.21, V. Grebennikov", "CNCCOLVG00003749". Paratypes (all CNC): 3750, 3751 (dissected male) – same data as holotype; 7055 (dissected female) – same data as holotype except "6°06′22″S 37°32′38″E, 4.i.2012, 967 m, sift.22"; 7058, 7059 – same data as holotype except "6°04′26″S, 37°32′38″E, 5.i.2012, 623 m, sift.23". All paratypes also bear the "CNCCOLVG0000NNNN" numbers (where NNNN is the specimen number) identifying sequenced specimens.

Additional material. 18 specimens from Nguru and 7 from Kaguru (CNC; two Nguru specimens marked with asterisks are in the collection of A. Ballerio, Brescia, Italy). 3670, 3671, 3672, 3673, 3674, 7062, 7063, 7064, 7065* and one* not numbered: "TANZANIA, Nguru Mts. at Turiani, S06°04′10″ E037°33′03″, 29.x.2010, 711 m, sifting01, V. Grebennikov". 7066, 7067, 7068: Same data except "S06°06′24″ E037°31′48″, 3.xi.2010, 1236 m, sifting05". 3752, 3753, 7054, 7056, 7057: "TANZANIA, Nguru Mts. at Mhonda, 6°06′22″S 37°32′38″E, 4.i.2012, 967 m, sift.22, V. Grebennikov". 3739, 3740, 3741, 7069, 7070, 7071, 7072: "TANZANIA, Kaguru Mts. at Masenge vil., 6°22′32″S 36°55′54″E, 28.xii.2011, 1875 m, sift.17, V. Grebennikov". All sequenced Kaguru specimens (see Fig. 6) also bear the "CNCCOLVG0000NNNN" numbers (where NNNN is the specimen number).

Type locality. Tanzania, Nguru Mts., near Mhonda, 6°06′24″S, 37°31′48″E, 1254 m.

Etymology. This species is named after Alberto Ballerio in recognition of his extensive work on pill scarabs that prompted my interest in this tribe.

Distribution. Known only from the Nguru and Kaguru mountains in Tanzania (Fig. 6), two discrete sky-islands of wet, closed-canopy rainforests separated by at least 65 km of arid savannah and forming a part of the exceptionally biodiverse chain of the Eastern Arc Mountains. Altitude: 623–1,229 m at Nguru and 1875 m at Kaguru.

DISCUSSION

Monophyly and sister group of the *Philharmostes* group of genera

The herein recovered and strongly supported monophyly of the *Philharmostes* group of pill scarab genera is not surprising, since it was twice hypothesized based on morphological similarities (Ballerio, 2000, 2001), and then supported by a morphology-based quantitative analysis (Ballerio & Grebennikov, 2016). The sister group relationships between PhG and a clade represented here by *Congomostes* is more controversial. The bootstrap support of this clade (86%) is moderate, while the analysis is lacking representatives of the 21 remaining nominal Ceratocanthini genera. Previously, PhG was recovered as sister to a weakly supported clade of eight Afrotropical and Neotropical genera (Ballerio & Grebennikov, 2016), five of which are included in the present analysis and do not form a clade. Considering these discrepancies, it is best to conclude that the sister group of PhG remains unknown.

Morphological synapomorphies of the *Philharmostes* group of genera

Since *P. ballerioi* sp. n. is sister to the rest of PhG, it offers an opportunity to review known morphological synapomorphies of the clade in the expectation that some of them might be detected and others not in the new species. The former, therefore, should be interpreted as those possessed by the Most Recent Common Ancestor (MRCA) of the herein re-defined PhG (that is, including *P. ballerioi* sp. n.), while the latter likely evolved later, during the period corresponding to the internode between the basal-most dichotomy of PhG and before MRCA of the clade consisting of PhG minus *P. ballerioi* sp. n. (= clades A–E in Fig. 3).

Six adult morphological characters were originally used to phenetically define PhG (cited verbatim from Ballerio, 2000; of these characters 2, 3, 4 and 6 were noted as unique

within pill scarabs): (1.) "aedeagus with parameres slightly asymmetrical and weakly sclerotized" (Fig. 4Y); (2.) "female genital segment elongate and with genital palpi elongate and narrow, fringed with long hairs" (Figs 5I–J); (3.) "female bursa copulatrix usually with two or more symmetrical, sclerotized, echinulate, sub circular plates" (Fig. 5I); (4.) "protibiae, at least in the male, regularly and broadly curved outwards, with outer margin smooth or finely serrate" (Figs 4R, U); (5.) "hind wings: M-Cu loop absent"; (6.) "hind wings: apical detached vein very long and close to first complete anal vein". Other than the two hind wing characters inapplicable to the wingless *P. ballerioi* sp. n., all these characters are present in the new species. Three vaguely defined characters added later (Ballerio, 2001: "shape and sculpturing of labrum", "shape and sculpturing of distal epipharynx" and "vestiture of galear brush of maxillae") lack exact definition and cannot be unambiguously interpreted. Recently, Ballerio & Grebennikov (2016) summarized known adult morphological diversity of pill scarabs by scoring 107 characters, of them 10 optimised (either unambiguously, or under slow or quick optimisation) as synapomorphic for PhG: (1.) distal longitudinal furrow on labrum present (Fig. 4I); (2.) wing vein MP4 longer than half the length of CuA; (3.) distal part of wing vein MP4 bent towards CuA; (4.) short proximal expansion of vein CuA3 + 4 present; (5.) protibiae curved (Figs 4R, U); (6.) dentation on outer side of distal third of protibiae absent (Figs 4R, U); (7.) basal apophyses on parameres present (Fig. 4Y); (8.) vaginal palpi elongate, at least twice as long as wide (Figs 5I, J); (9.) sclerites on bursa copulatrix present (Fig. 5I); (10.) sclerites on bursa copulatrix plate-like (Fig. 5I; for this character see "Correction added in the proofs" on page 52, Ballerio & Grebennikov, 2016). Except for the three hind wing characters, all morphological synapomorphies of PhG are present in *P. ballerioi* sp. n., which, therefore, renders clade A–E (= the rest of PhG, Fig. 3) without a synapomorphy.

Inadequate generic taxonomy of the *Philharmostes* group of genera

The analysis presented indicates that the existing taxonomic arrangement of PhG in seven nominal genera is non-cladistic and must be modified. The topology depicted in Fig. 3 shows that the nominal genus *Philharmostes* is a waste-basket taxon used to accommodate distantly related and morphologically uniform members of PhG, while its easy-to-diagnose smaller lineages are artificially elevated in at least four nominal genera (and likely six, since the genera *Callophilharmostes* and *Chaetophilharmostes* were not included in this analysis). This situation is commonly encountered in various branches of the Tree of Life, when a plethora of historical names are scrutinized phylogenetically. In such situations three nomenclatorial revisions are possible. Firstly, one may apply the oldest genus-group name (*Philharmostes* in this case) to the entire PhG and, consequently, synonymize all six smaller nominal genera with the latter. Secondly, one may split the nominal *Philharmostes* into at least three smaller genera corresponding to clades C, E and F (Fig. 3). The third solution is to apply a tree-based biological nomenclature approach of the PhyloCode, when rank-free taxon names are linked directly to clades (de Queiroz & Gauthier 1990, 1992; Cantino & de Queiroz, 2014), as recently implemented among anole lizards (Dactyloidae, Poe et al., 2017). With pill scarabs, however, none of these solutions seems timely, since the clade remains in a state of phylogenetic obscurity, with hardly any among its 38 nominal genera phylogenetically sound. Considering that the very first herein implemented DNA-based analysis revealed a non-phylogenetic taxonomy, similar results might be eventually found among these beetles. In such a situation the conservative solution is to adhere to the current interim generic taxonomy (Ballerio & Grebennikov, 2016) and continue testing the taxonomically implied groups until a well-resolved and densely sampled tree of pill scarabs becomes available for a taxonomic rearrangement.

Philharmostes ballerioi sp. n., sister to the rest of the *Philharmostes* group of genera

Clades such as that formed by *P. ballerioi* sp. n., i.e., much smaller in number of species compared to their sister clades (and often in their geographical area), are often erroneously called "basal" (but see Krell & Cranston, 2004), "primitive" or "ancestral" (but see Omland et al., 2008). *Philharmostes ballerioi* sp. n. is yet another such example, even if at a shallower level, being the only known representative of the lineage sister to the rest of the entire PhG embracing 41 extant species. Discovery and/or phylogenetic interpretation of such deeply-nested organisms are of particular evolutionary significance, since following the principle of parsimony, their properties indicate ancestral states for binary characters having both states in the diversified sister clade. At least two characters of *P. ballerioi* sp. n. were likely acquired convergently with those of some PhG members, namely (1.) antennae consisting of nine antennomeres (since their number in the sister clade varies between seven and ten, and the latter state is widespread in other pill scarabs and likely has been present in MRCA of PhG) and (2.) complete lack of hind wings (other PhG have hind wings varying between entirely absent and fully functional, and the latter state is widespread in other pill scarabs and likely has been present in MRCA of PhG). These hypotheses will be tested and their list likely expanded once still inadequately known adult morphological characters of PhG (particularly understudied genitalia of both sexes contributing only seven among 107 characters in Ballerio & Grebennikov, 2016) become optimized on an inclusive and well-supported PhG phylogenetic tree.

Philharmostes ballerioi sp. n.: phylogeographical aspects

The discovery of the phylogenetically significant new species sister to a much larger clade was made in the Nguru and Kaguru mountains (Fig. 6), two adjacent localities in the Eastern Arc Mountains (= EAM) in Tanzania (Fig. 6) renowned for their exceptionally high biodiversity (Lovett & Wasser, 1993). The orographic effect of these highlands results in a reliable source of rainfall from the

nearby Indian Ocean, and the moisture is sufficient to support sky-islands of rainforest on the slopes of the EAM, which sharply contrast with the surrounding hot and dry lowlands. Such conditions enabled the moisture-dependent biota on EAM to survive throughout the dramatic climatic fluctuations in the Pliocene-Pleistocene, when Afrotropical rainforest repeatedly shrank to about 10% of its present size (Fig. 4 in Hamilton & Taylor, 1991). The recently radiated neoendemics and species-poor clades of relics form two main groups of EAM endemics (Fjeldså & Lovett, 1997), and the new species likely belong to the latter. These considerations indicate that the separation between *P. ballerioi* sp. n. and the rest of PhG might predate the Miocene uplift of the central African plateau. The latter event caused aridification of the East African climate and fragmentation of the rainforest belt which extended throughout the entire continent since at least the Middle Cretaceous (Fig. 9.24 in Kirk-Spriggs & Muller, 2017).

Lack of the hind wings in adults of the new *Philharmostes* indicates reduced dispersal capacity. Flightlessness is thought to be positively selected for in the island-type EAM forest, since it prevents the aridity-intolerant organisms from being swept by wind into nearby dry inhospitable areas (Grebennikov, 2008). The presence of the new species in at least two EAM localities is, therefore, likely a result of vicariance of their once widespread ancestor, rather than a relatively recent founder dispersal (Heads, 2014). This hypothesis is consistent with the reciprocal monophyly of both populations (Fig. 6) and the time of their separation estimated at about 2.21 Ma, when both currently isolated Nguru and Kaguru forests might have been connected during at least one of many recurring wet periods concurrent with the Pliocene-Pleistocene interglacial cycles.

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