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A new hypothesis on the evolution of the hybosorid beetle capacity to conglobate their bodies into a tight ball (Coleoptera: Scarabaeoidea)Vasily V. GREBENNIKOV¹, Andrew B.T. SMITH²¹Canadian Food Inspection Agency, 960 Carling Ave., Ottawa, ON, K1A 0Y9, Canada – vasily.grebennikov@canada.ca²Research Division, Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, ON, K1P 6P4, 1740 Chemin Pink, Gatineau, QC, J9J 3N7 Canada – asmith@nature.ca**Abstract**

Although best known for the capacity to fully conglobate their bodies into a “perfect” spheroid, a few Ceratocanthinae (= pill scarabs; Coleoptera: Hybosoridae) have drastically different body architecture. Six South American species, or 1.5% of species diversity, have straight bodies incapable of any conglobation, while some 20% of species can achieve only incomplete body conglobation with legs and abdomen partly exposed. Historically, both latter character states were considered as ancestral and transitional, respectively, in the irreversible evolutionary “progression” towards full-body conglobation. Here we use molecular sequence data to hypothesize a new clade uniting all bodily straight and incompletely conglobate pill scarabs. Significantly, this clade is nested within a clade otherwise consisting of species capable of full-body conglobation. This topology implies that the most recent common ancestor of all pill scarabs had a capacity of full-body conglobation. Consequently, we re-interpret the pill scarab straight and partly conglobate body architectures as secondary reversals to the ancestral condition from the fully conglobate state. If so, the tribe Ceratocanthini uniting 98% of pill scarab species is rendered paraphyletic by two remaining and much smaller South American tribes, Ivieolini and Scarabatermitini. The latter contains three and five rare species, respectively, all of them supposedly termitophilous and all herein illustrated.

Keywords: DNA, phylogeny, Scarabaeoidea, *Ivieolus*, termitophily, body architecture, evolution, reversal.**Introduction**

A dramatic change in the body architecture, i.e., the general posture and appearance of an organism, bespeaks a novel and overwhelming selective pressure. Distinguishing between primary versus secondary origin of similar body architecture is, therefore, both evolutionary important and intellectually captivating. Examples range between iconic, such as the fish-like appearance of the aquatic tetrapods (e.g., cetaceans, ichthyosaurs, and mosasaurs) or secondarily quadruped dinosaurs (such as *Diplodocus* Marsh, 1878 or *Triceratops* Marsh, 1889; e.g., McPhee et al. 2018) to little known even among biologists, such as the secondarily legless parasitic arthropods (e.g., pancrustacean *Pentastomia* or “tongue worms” infesting the upper respiratory tract of amniotes; Junker & de Klerk-Lorist 2020). Such dramatic reversals in the body architecture are often misunderstood until the growing body of evolutionary evidence precipitates their dramatic re-interpretation. In this paper, we offer our novel data and inductive reasoning pointing towards yet another case from this illustrious cohort, this time among the scarab beetles capable to roll their bodies into a tight ball.

This study is focused on the pantropical subfamily Ceratocanthinae (= pill scarabs) (Coleoptera: Hybosoridae), a clade of 44 extant genera and 366+ species (Ballerio & Grebennikov 2016; Grebennikov 2021). The monophyly of this subfamily is consistently supported with larval (Grebennikov et al. 2004) and adult (Ballerio & Grebennikov 2016) morphological characters, as well as in DNA analyses (Ocampo & Hawks 2006; Grebennikov 2021). The sister group of Ceratocanthinae is likely the hybosorid subfamily Liparochrinae (Ocampo 2006; Ocampo & Hawks 2006) containing two genera and 48 named species distributed between Indonesia and Australia (Matthews & Ballerio 2019).

Body architecture in pill scarabs is most remarkable. Most of them can roll themselves (= conglobate) into a nearly perfect ball, a spheroid with no appendages, gaps, or membranes exposed (Figs 1A–B). This morphological and behavioural trait is unique among 31,500+ named species of monophyletic Scarabaeoidea (Bouchard et al. 2017) and resembles those of armadillos (Cingulata) or pill bugs (Isopoda: Armadillidiidae), hence the colloquial name (Howden & Gill 2020). In pill scarabs, however, not only the body but also all six markedly flattened tibiae interlock

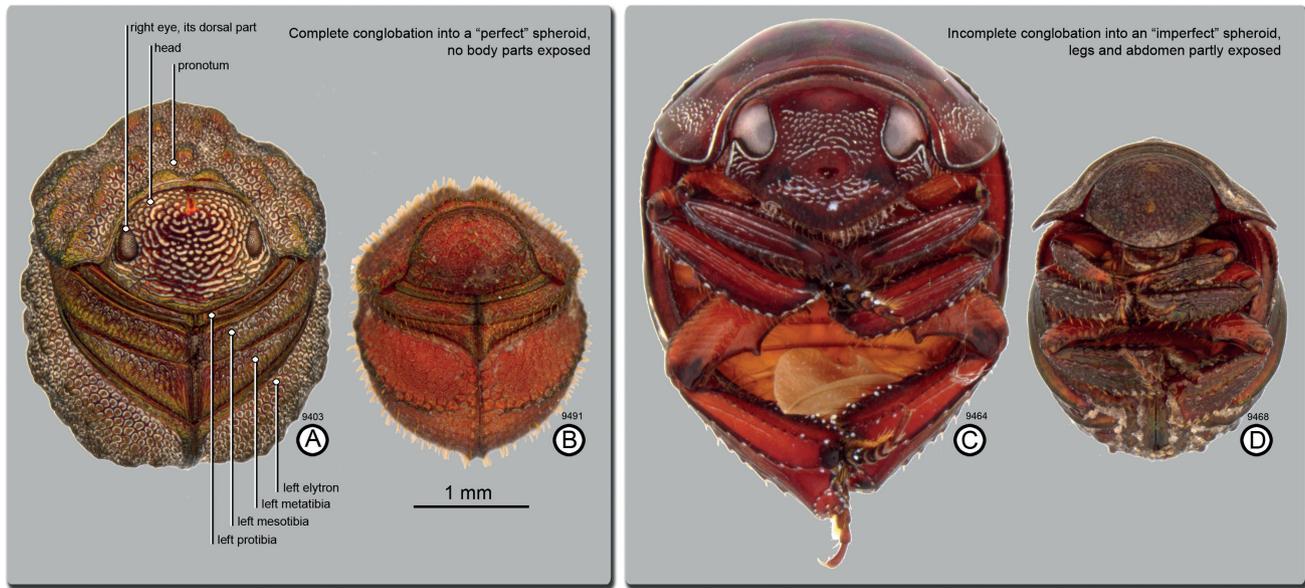


Fig. 1 – Habitus of pill-scarabs (Ceratocanthinae), tribe Ceratocanthini; ventral view. **A**, *Carinophilharmostes vadoni*; **B**, *Baloghianestes oribatidiformis*; **C**, *Germarostes* sp., **D**, *Aneilobolus* sp. Four-digit numbers are specimen IDs. Images are to scale.

their surfaces to form an unbroken spheroid (Figs 1A–B). The structural complexity of these beetles is, therefore, unparalleled in the whole of Animalia, extant or extinct.

Perhaps 20% of pill scarabs, although capable of conglobating, are unable to form an unbroken “perfect” spheroid, leaving the abdomen and legs partly exposed (Figs 1C–D). These beetles belong to eight genera (Ballerio & Grebennikov 2016), all of the tribe Ceratocanthini: *Acanthocero-des* Péringuey, 1901 (3 spp., South Africa), *Aneilobolus* Hesse, 1948 (4 spp., South Africa), *Aulisostes* Howden & Gill, 2000 (2 spp., Colombia and Brazil), *Ceratocanthoides* Paulian, 1982 (monotypic, South America), *Cloetotus* Germar, 1843 (3 spp., South America), *Germarostes* Paulian, 1982 (71 spp. between Canada and Argentina) and *Martinezostes* Paulian, 1982 (5 spp. from Chile and Argentina).

Within the family Hybosoridae, therefore, one can distinguish at least three seemingly ordered stages of the same character: degree of body deflexion/conglobation:

- none, head and pronotum are not deflexed; no specific exoskeletal modifications (all Hybosoridae other than Ceratocanthinae and Liparochrinae, as well as all genera of the ceratocanthine tribes Ivieolini and Scarabatermitini, with the exception of *Trachycrusus* Howden & Gill, 1995; see below);
- incomplete conglobation: head and pronotum can be strongly deflexed to form an “imperfect” spheroid; legs and abdomen partly exposed (Liparochrinae, eight aforementioned genera of Ceratocanthini, and *Trachycrusus* of the tribe Scarabatermitini);
- complete conglobation: head, pronotum, elytra, and all tibiae interlock to form a “perfect” spheroid; no other parts of the body are exposed (32 genera of Ceratocanthinae: Ceratocanthini).

Predictably, the historical intuitive interpretation of these three character states was linear (= ordered, additive), irreversible, and evolutionary “directed” towards

Table 1. Taxonomic composition of non-Ceratocanthini pill scarabs. Tribe abbreviations “Iviol.”: Ivieolini; “Scarab.”: Scarabatermitini. HT, AL, and PT indicate holotypes, allotypes, and paratypes in either the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC) or the Canadian Museum of Nature (CMN).

Tribe	Genus	Species	Distribution	CNC	CMN
Iviol.	<i>Ivieolus</i> Howden & Gill, 1988	<i>I. brooksi</i> Howden & Gill, 2000	French Guiana	1	none
Iviol.	<i>Ivieolus</i> Howden & Gill, 1989	<i>I. inflaticollis</i> Howden & Gill, 2001	Ecuador	none	HT, 2PT
Iviol.	<i>Ivieolus</i> Howden & Gill, 1990	<i>I. pseudoscutellatus</i> Howden & Gill, 1988	Guyana, Ecuador, Venezuela	none	7PT
Scarab.	<i>Scarabaeinus</i> Silvestri, 1940	<i>S. termitophilus</i> Silvestri, 1940	Brazil	none	none
Scarab.	<i>Scarabatermes</i> Howden, 1973	<i>S. amazonensis</i> Howden, 1973	Colombia	3	none
Scarab.	<i>Trachycrusus</i> Howden & Gill, 1995	<i>T. lescheni</i> Howden & Gill, 1995	Colombia, Ecuador, Peru	none	4PT
Scarab.	<i>Trachycrusus</i> Howden & Gill, 1995	<i>T. striatulus</i> Howden & Gill, 1995	Peru	none	HT, 2PT
Scarab.	<i>Xenocanthus</i> Howden & Gill, 1988	<i>X. singularis</i> Howden & Gill, 1988	Colombia, Venezuela	none	HT, AL, 4PT

morphologically more and more “specialized” ball-forming bodies (Howden & Gill 1988a). The origin of the capacity of pill scarabs to conglobate is, however, both obscure and controversial. Only three phylogenetic analyses can offer an indirect interpretation of the evolutionary events with respect to body architecture. The older hypothesis suggests that the incomplete body conglobation evolved from the straight bodies before the most recent common ancestor (MRCA) of pill scarabs. It is so because all members of the sister clade, the subfamily Liparochrinae, are distinctly capable of bending their head and pronotum in a ventral direction and, therefore, forming an “imperfect” ball, with abdominal apex and appendages partly exposed (Fig. 6). Moreover, some Liparochrinae have mesotibiae and metatibiae notably flattened (as in the genus *Antiochrus* Sharp, 1873; Matthews & Ballerio 2019), which can also be seen as a pre-adaptation towards body conglobation. Since no other hybosorid or other scarab beetles display this capacity to conglobate, it can be treated as an autapomorphic trait supporting the Ceratocanthinae and Liparochrinae clade (Ocampo 2006; Ocampo & Hawks 2006). Conversely, capacity to the partial body conglobation was optimized as parallelisms in both Liparochrinae and Ceratocanthinae: Ceratocanthini (Ballerio & Grebennikov 2016). Ballerio & Grebennikov (2016) also suggested that full body conglobation is a unique evolutionary novelty within Ceratocanthinae. All three topologies implied that the incompletely conglobating pill scarabs minority (Figs 1C–D) represent the evolutionary “intermediate” state between the straight-bodied ancestors and “highly evolved” fully conglobate majority.

This simplified morphological pattern contains, however, at least one significant uncertainty. The pill scarabs capable to conglobate, either completely or incompletely, form about 90% of the genera and 95% of species in the subfamily. These “typical” conglobating organisms are all united in the tribe Ceratocanthini. The remaining minority of pill scarabs consists of six straight-bodied poorly-known species attributed to four genera and two partly conglobate species of the genus *Trachycrusus* Howden & Gill, 1995 (Figs 2–4). These “atypical” pill scarabs are classified into two exclusively South American tribes of Ceratocanthinae: Ivieolini and Scarabatermitini (Figs 2–4; Table 1). At least four reasons contribute to the exceeding obscurity of these organisms. Firstly, non-Ceratocanthini Ceratocanthinae are rarely collected. The first of these species was named 80 years ago (Silvestri 1940), with the rest was described between 1973–1995, in a series of papers by Henry Howden (Jameson & Ratcliffe 2006 and Table 1). Excepting a single published record of each of four species (Howden 1971; Ballerio 2014; Arias-Buriticá & Vaz-de-Mello 2015; Ballerio & Grebennikov 2016), all of these elusive beetles until now remain publicly known only from the

Table 2. DNA fragments used in the 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
COI-5P	76	468	658	658	1 to 658
ITS2	69	206	654	1583	659 to 2241
28S	79	284	671	940	2242 to 3181

type series (although hundreds of *Ivieolus*, and the lesser number of *Trachycrusus*, were collected using flight intercept traps; Alberto Ballerio, personal communication). Secondly, owing to the rarity of the specimens, none has been included in the emerging DNA-based phylogenetic framework of Ceratocanthinae (Grebennikov 2019a, b; 2021). Thirdly, available evidence suggests that at least some, if not all, of Ivieolini and Scarabatermitini are termitophilous (Ballerio & Grebennikov 2016). This biological trait is rare among scarab beetles and reliably known only in Aphodiinae (Scarabaeidae, e.g., Tangelder & Krikken 1982; Kakizoe & Maruyama 2015), while being strongly suggested also in the genus *Penichrolucanus* Deyrolle, 1863 (Lucanidae; Bartolozzi 1989). Fourthly, adults of all eight species of Ivieolini and Scarabatermitini are so unusually shaped, that it was not until 1988 when Howden & Gill (1988a, 1988b) deciphered their pill scarab affinities (envisioned in Howden 1973). Both Ivieolini and Scarabatermitini are, therefore, phylogenetically intriguing, alas inadequately known “atypical” pill scarabs, who either do not conglobate at all or do it only partially (*Trachycrusus*).

The only phylogenetic treatment of Ivieolini and Scarabatermitini was restricted to adult morphological characters (Ballerio & Grebennikov 2016). In it, Ivieolini and Scarabatermitini formed a clade supported by 13 unambiguously optimized synapomorphies (Fig. 5), three of them unique (embossed sculpturing on pronotum; vertical procoxae; abdominal adult physogastry). The latter character, although not obvious in dry-mounted specimens (Figs. 2–3), was scored after observing the deflexed abdominal folds in softened specimens, which are suggestive of the capacity to inflate the abdomen. The monogeneric tribe Ivieolini was recovered nested among four Scarabatermitini genera, thus rendering the latter taxon paraphyletic (Ballerio & Grebennikov 2016). Concurrent with intuitive expectations and the straight body architecture prevailing among these beetles, the clade of Ivieolini plus Scarabatermitini form the sister group to strongly supported and conglobating Ceratocanthini. This topology reinforced the earlier notion (Howden & Gill 1988a; Ocampo 2006; Ocampo & Hawks 2006) that the process of body conglobation went from none (straight bodies), through partial and to complete. In other words, all straight (Figs 2–3) or incompletely (Figs 1C–D) conglobate pill scarabs represented the ancestral character state and not a secondary reversal.

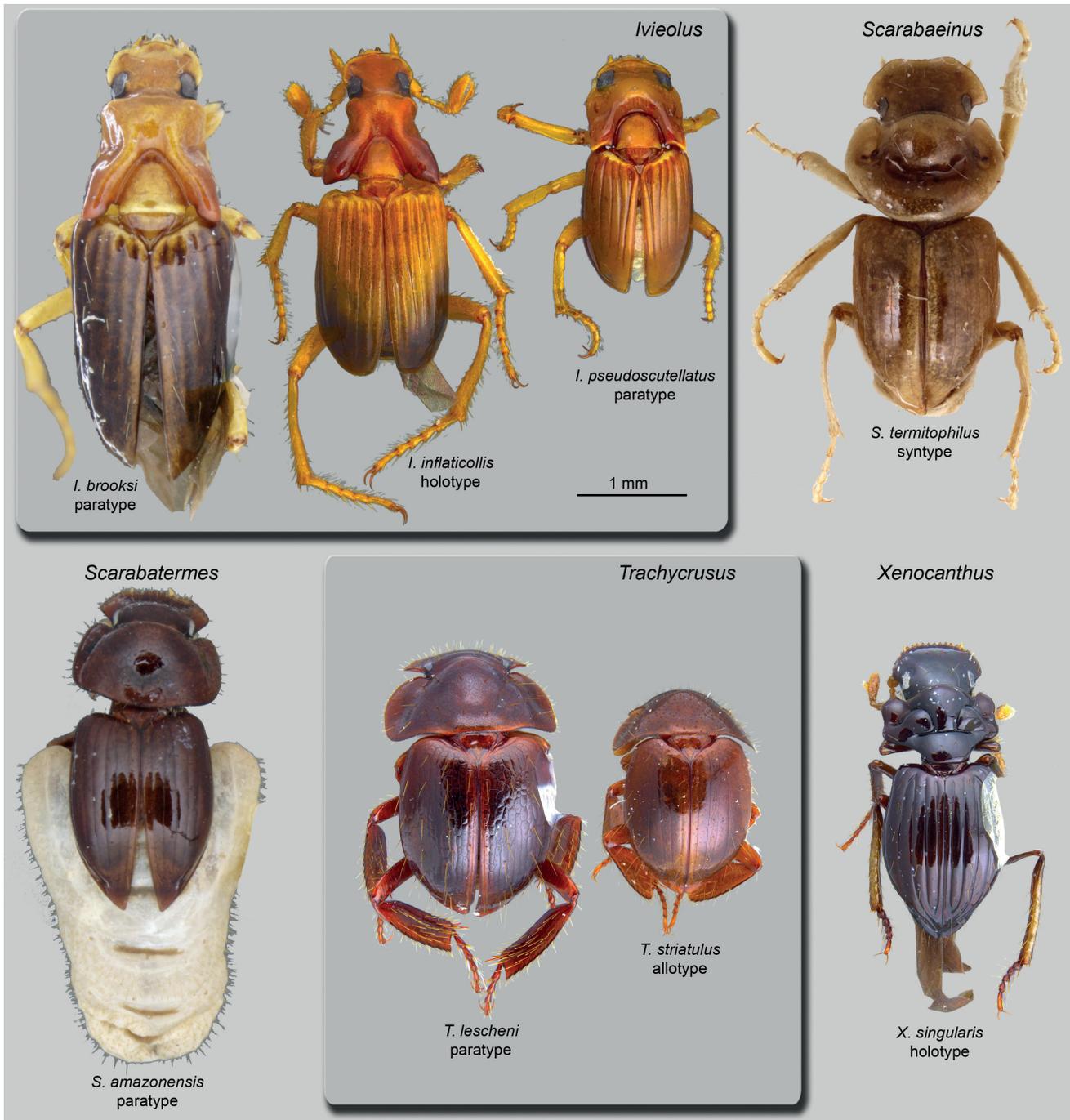


Fig. 2 – Habitus of pill-scarabs (Ceratocanthinae), all named species of the tribes Iviolini (*Ivieolus*) and Scarabatermitini (the rest); dorsal view. *Scarabaeinus termitophilus* image and copyright: Alberto Ballerio, used with permission. Images are to scale.

This paper was prompted by the availability of the DNA-grade adult specimen of *Ivieolus pseudoscutellatus* Howden & Gill, 1988 (Fig. 5), the first to represent the mysterious Iviolini and Scarabatermitini hypothesized clade on the DNA-based tree. Deciphering phylogenetic affinities of *Ivieolus* within Hybosoridae was bound to shed new light on the evolution of body architecture. Specifically, if *Ivieolus* emerges as the sister of the rest of the subfamily, as suggested by the morphology-based analysis (Ballerio & Grebennikov 2016),

this will reinforce the only existing notion of the primary (not secondary) nature of the straight bodies of the termitophilous minority of Ceratocanthinae. Unexpectedly, our newly generated molecular placement of *Ivieolus* deviated greatly from this original expectation and, therefore, triggering a drastic re-interpretation of the evolution of the body architecture within Hybosoridae. This, together with documenting all that is currently known on Iviolini and Scarabatermitini, form the primary and the secondary goals of this paper, respectively.

Material and methods

This study is pivoted on an adult specimen of *Ivieolus pseudoscutellatus* (Fig. 5) collected in Ecuador (Orellana, Yasuni Research Station, 0.668° S 76.662° W, 311 m, flight intercept trap, Louisiana State Arthropod Museum team, August 12, 2008; the first country record). The beetle is deposited in the Canadian Museum of Nature (Ottawa, Canada); its unique identifier is CNCCOLVG00010301 (hereafter referred to as specimen 10301). In all other methodological aspects our study closely follows those of Grebennikov (2019a, b; 2021), therefore detailed material and method descriptions might be found there. Three DNA fragments were sequenced and analysed (Table 2, fragment abbreviations are in brackets): mitochondrial cytochrome c oxidase I (COI, the DNA barcode fragment, Hebert *et al.* 2003), nuclear internal transcribed spacer 2 (ITS2) and nuclear 28S ribosomal DNA (28S). Sequencing of DNA was performed at the Canadian Centre for DNA Barcode using their standard protocols. The primers used are listed in Table 2 in Grebennikov (2017). All details on the laboratory work (such as DNA extraction, amplification, PCR protocols), as well as images of the original electropherograms, habitus images, and locality data for all 80 specimens sequenced for this analysis, are available online in the Barcode of Life Database (BOLD, Ratnasingham & Hebert 2007) public dataset at doi.org/10.5883/DS-VGDS23.

This work re-uses the Hybosoridae-only 73 terminal dataset (Grebennikov 2021) by adding to it seven newly sequenced Ceratocanthinae terminals, including *Ivieolus pseudoscutellatus* (Table 3). The non-Ceratocanthinae outgroup consisted of four Liparochrinae and three Anaidinae terminals. The root was placed between Anaidinae and the rest of the topology, consistently with morphological and molecular topologies by Ocampo (2006) and Ocampo & Hawks (2006), respectively.

Alignment of all three DNA fragments was done separately using the online MAFFT Q-INS-i algorithm (Kato *et al.* 2017; <https://mafft.cbrc.jp/alignment/server/>). Unlike ITS2, alignments of COI and 28S were trivial and required no subsequent edition. Two short ITS2 fragments concisely misaligned (MW340095 and MW340101) and were, therefore, excluded; the dataset was then re-aligned.

Inconsistently sequenced 5'-end and 3'-end of the ITS2 alignment were trimmed of 19 and 25 positions, respectively. Additionally, the 3'-end of ITS2 of the following three fragments were manually trimmed of 73, 6, and 27 misaligned nucleotides, respectively: MH777818, MH777824, and MW340096. No internal parts of DNA fragments were removed before the analysis, even if consisting mainly of insertions/deletions (indels, particularly frequent in ITS2).

Three aligned single-fragment datasets (Table 1) were concatenated using Mesquite 3.61 (Maddison & Maddison 2020) into a matrix of 3181 positions. Unrooted topology was built using a maximum likelihood (ML) approach, as implemented in CIPRES Science Gateway online platform (Miller *et al.* 2010; <http://www.phylo.org>, tool "RAxML-HPC2 on XSEDE") and using RAxML version 8 (Stamatakis 2014) algorithm. To balance the complexity and speed of the analysis, the CAT approximation to the GTR + G nucleotide substitution model was applied independently for each of the three partitions. Support values were generated based on 1000 bootstrap replicates (Stamatakis *et al.* 2008) and categorized as strong ($\geq 95\%$), moderate ($< 95\%$ and $\geq 75\%$), and weak ($< 75\%$, while clade is present on the consensus tree). The tree was visualized in FigTree v1.4.4. (Rambaut 2020).

Besides the sequenced specimen of *I. pseudoscutellatus* (Fig. 5), we studied the total of 27 specimens of Iviolini and Scarabatermitini, all deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes and the Canadian Museum of Nature (Ottawa, Ontario, Canada) (Table 1). We saw and took pictures of representatives of all but one named non-Ceratocanthini Ceratocanthinae (Figs 2–4). The exception is *Scarabaeinus termitophilus* Silvestri, 1940, which has a well-documented type series (Ballerio 2000). Both herein reproduced images of this species were taken, and made available to us, by Alberto Ballerio. The photographed specimens of this species were not measured, therefore their sizes in Figs 2–3 are approximate (while the original description state adult body length 4–6 mm; Silvestri 1940).

Results

The ML analysis produced a moderately- to a well-resolved tree (Fig. 6) much resembling that in Grebennikov

Table 3. DNA fragments and their GenBank accession numbers of seven newly sequenced Ceratocanthinae.

Voucher	Genus and/or species	Country	COI	ITS2	28S
CNCCOLVG00010301	<i>Ivieolus inflaticollis</i>	Ecuador	MW996549	MW996543	MW996556
CNCCOLVG00010310	<i>Astaenomoechus americanus</i>	Belize	MW996550	none	MW996557
CNCCOLVG00010311	<i>Germarostes</i>	Belize	MW996551	MW996544	MW996558
CNCCOLVG00010312	<i>Ceratocanthus</i>	Belize	MW996546	MW996540	MW996553
CNCCOLVG00010321	<i>Germarostes globosus</i>	United States	MW996547	MW996541	MW996554
CNCCOLVG00010360	<i>Germarostes</i>	Bolivia	MW996548	MW996542	MW996555
CNCCOLVG00010361	<i>Germarostes posticus</i>	Chile	MW996545	MW996539	MW996552

(2021); the newer analysis enriching and re-using the dataset of the earlier one. All representatives of Ceratocanthinae and Liparochrinae formed a strongly supported cluster, making the rooting of the tree unambiguous. The main novelty of our study is the newly detected and strongly supported clade uniting representatives of all three different pill scarab body types: straight (*I. pseudoscutellatus*), incomplete (nine terminals, genera *Aneilo-*

bolus and *Germarostes*), and complete conglobation (two terminals of *Ceratocanthus* White, 1842). Internal nodes within this new clade are poorly resolved, with the genera *Ceratocanthus* and *Germarostes* rendering each other paraphyletic. This new clade lacks a well-supported sister group and is nested within the strongly supported monophyletic Ceratocanthinae, otherwise consisting of species capable of full body conglobation.

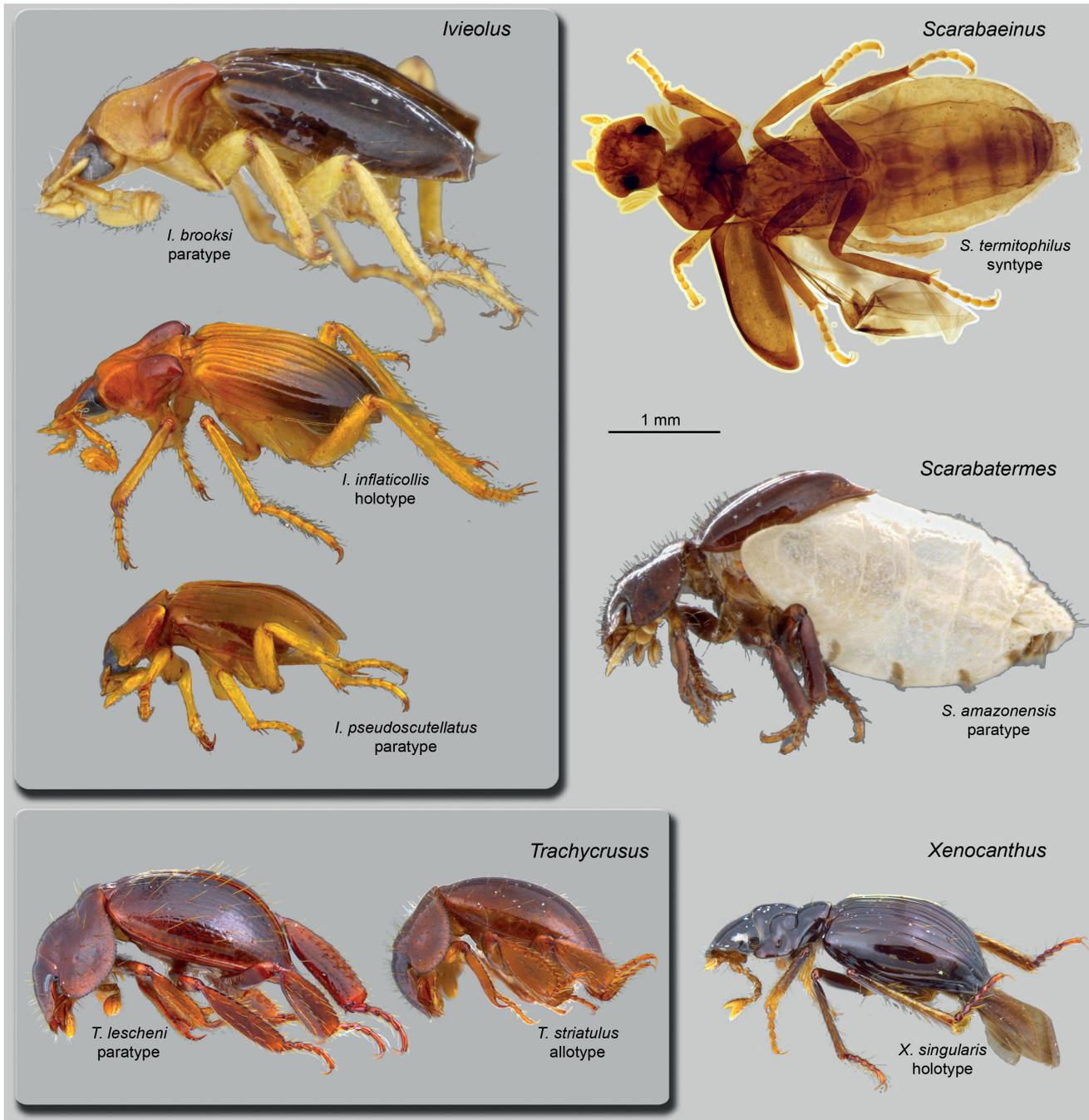


Fig. 3 – Habitus of pill-scarabs (Ceratocanthinae), all named species of the tribes Iviolini (*Ivieolus*) and Scarabatermitini (the rest); left lateral view (except *Scarabaeinus termitophilus* imaged in dorsal view, with right elytron and hind wing removed, in transparent light on a permanent microscope slide in an unknown medium resembling Canada Balsam; note the long “lateral gland arising from the first abdominal segment” [Howden 1971] inwardly and parallel to left metatibiae; image and copyright Alberto Ballerio, used with permission). Images are to scale.

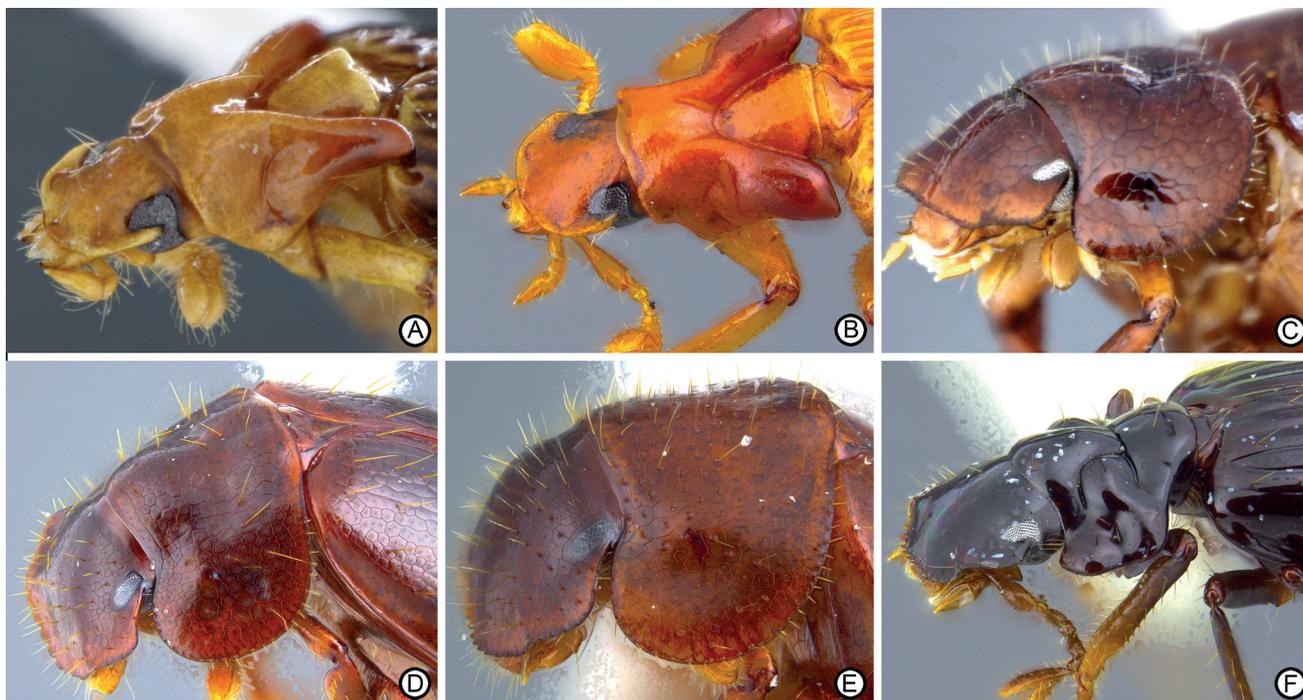


Fig. 4 – Heads and pronota of pill scarabs (Ceratocanthinae), tribes Ivieolini and Scarabatermitini. Images are not to scale.

Discussion

Tree robustness. Even though our phylogenetic tree (Fig. 6) is ambiguous in some respects (see below), it is highly suggestive of the sequence of evolutionary events leading towards the currently observed diversity of Hybosoridae body architecture: straight, incompletely or completely conglobate. This credulity is pivoted on the detection of the new and strongly supported Ceratocanthinae sub-clade uniting all three body types; of them the straight-bodied pill scarabs (Ivieolini and Scarabatermitini) are for the first time represented on the DNA tree. Notably, this new and strongly supported clade is nested within the rest of the strongly supported Ceratocanthinae, and this rest consisting exclusively of the completely conglobating species. This, together with monophyletic Ceratocanthinae forming a dichotomy with incompletely conglobating Liparochrinae, permits a significant fine-tuning and re-interpretation of the evolutionary direction of changes in the Hybosoridae body architecture, as either implied (Ocampo 2006; Ocampo & Hawks 2006) or explicitly hypothesised (Ballerio & Grebennikov 2016) before our work.

Straight bodied and incompletely conglobate pill scarabs represent evolutionary reversals. Most significantly, and unlike any earlier thought, neither straight-bodies *Ivieolus* (and by extension none among the straight-bodied Ivieolini and Scarabatermitini, as well as incompletely conglobating *Trachycrusus*) nor incompletely conglobating *Aneilobolus* and *Germarostes* (and by extension none of the remaining six incompletely

conglobating Ceratocanthini genera; see Introduction) represent the ancestral character states. The most parsimonious interpretation of our results (Fig. 6) implies that both body types are secondarily reversals from their once completely conglobating ancestors. Indeed, the origin of complete body conglobation, as implied by our results (Fig. 6) likely took place not within the Ceratocanthinae (as hypothesised by Ballerio & Grebennikov 2016), but somewhere on the internode between the most recent common ancestor (MRCA) of the subfamily and that of the more inclusive Ceratocanthinae and Liparochrinae clade; the latter being already incompletely conglobate (Fig. 6). This conclusion represents the main novelty of our study.

Alternative interpretations of changes in body architecture are less likely. At least four other interpretations of the same topology (Fig. 6), while possible, are notably less parsimonious. The primary (versus secondary) straight body of *Ivieolus* (as hypothesized in Ballerio & Grebennikov 2016) seems unlikely because this implies non-monophyly of three herein strongly supported clades (Ceratocanthinae plus Liparochrinae; Ceratocanthinae and the newly detected clade; Fig. 6). Primary incompletely conglobate bodies of pill scarabs, such as *Aneilobolus* and *Germarostes* (as hypothesized in Ballerio and Grebennikov 2016) are also unlikely and imply non-monophyly of two herein strongly supported clades (Ceratocanthinae and the newly detected clade; Fig. 6). The double origin of incomplete conglobation (as hypothesized in Ballerio & Grebennikov 2016) contradicts earlier findings (Ocampo

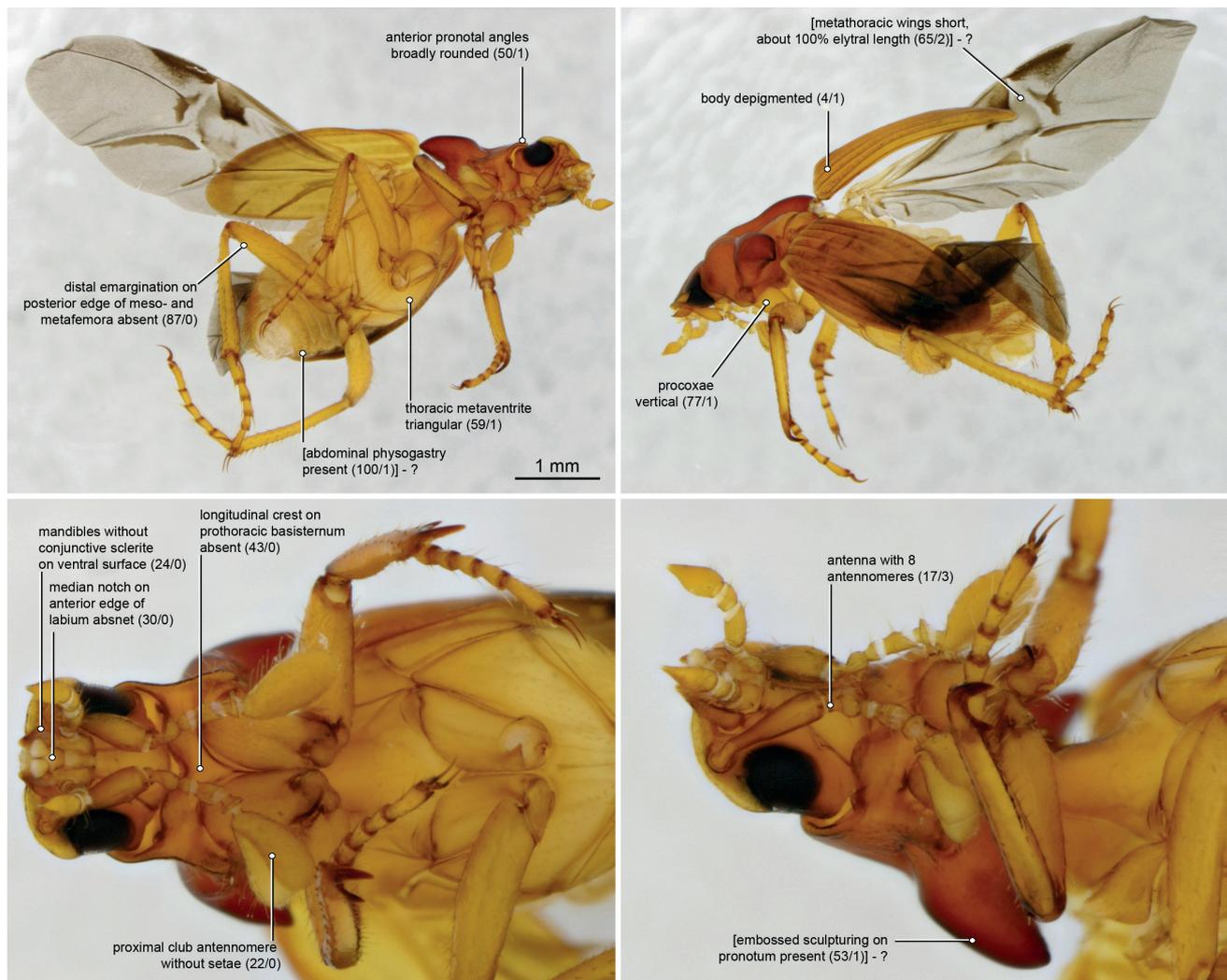


Fig. 5 – The herein sequenced specimen 10301 of *Ivieolus pseudoscutellatus* illustrating all 13 unambiguously optimized morphological apomorphic characters in support of the Ivieolini plus Scarabatermitini clade (character/state numbering follows Ballerio & Grebennikov 2016). Three of these characters (in square brackets and marked with a question mark) need critical re-assessment and perhaps rescoring.

2006; Ocampo & Hawks 2006) and implies non-monophyly of the Ceratocanthinae plus Liparochrinae clade (Fig. 6). Finally, the never-published hypothesis of the double origin of complete body conglobation in pill scarabs (*Ceratocanthus* plus *Ceratocanthopsis* Paulian, 1982 versus the rest of such genera; Alberto Ballerio, personal communication), although accounting for notable morphological dissimilarities between both groups (Ballerio & Grebennikov 2016), requires a reversal to the straight body in *Ivieolus*. Significantly, this hypothesis suggests the independent and therefore highly unlikely acquisition of morphologically complex interlocking structures characteristic in two clades of conglobating species. All in all, the likeliest interpretation of the resulting tree (Fig. 6) suggests at least four evolutionary events concerning the changes in the body architecture in Hybosoridae: (1) origin incomplete body conglobation before the MRCA of Ceratocanthinae + Liparochrinae; (2) origin of complete body conglobation before the MRCA of Ceratocanthinae,

followed by at least two reversals, to incompletely conglobate and straight bodies within Ceratocanthinae (*Aneilobolus* plus *Germarostes* versus *Ivieolus*, respectively).

Tree uncertainties. Perhaps the most significant uncertainty of our tree is the poorly resolved divergence events within the newly detected pill scarab subclade. This includes the counterintuitive non-monophyly of *Ceratocanthus*, as well as dubious sister groups of *Ivieolus* and incompletely conglobate African genus *Aneilobolus*. These uncertainties, however, do not weaken the credibility of the existence of the entire and well-supported clade.

Tribal classification of pill scarabs. The current tribal arrangement of the subfamily Ceratocanthinae into three tribes, Ceratocanthini, Ivieolini, and Scarabatermitini (Ballerio & Grebennikov 2016) sharply disagrees with the available phylogenetic evidence. Ballerio & Grebennikov (2016) demonstrated that monogenetic Ivieolini are nested

within monophyletic Scarabatermitini, rendering the latter paraphyletic. Our present topology (Fig. 6) suggests that the tribe Ceratocanthini, by far the largest in the subfamily, is paraphyletic to at least Ivieolini (Fig. 6). We postpone changes in the classification when better resolved and denser sampled trees become available.

Trachycrusus, a morphologically “intermediate” genus. Images of both *Trachycrusus* species (Figs 2–4) suggest that, although scored as straight bodied in Ballerio & Grebennikov (2016), these species are capable of at least partial body conglobation. When described, the genus was noted to “bridge the gap between” (Howden & Gill 1995)

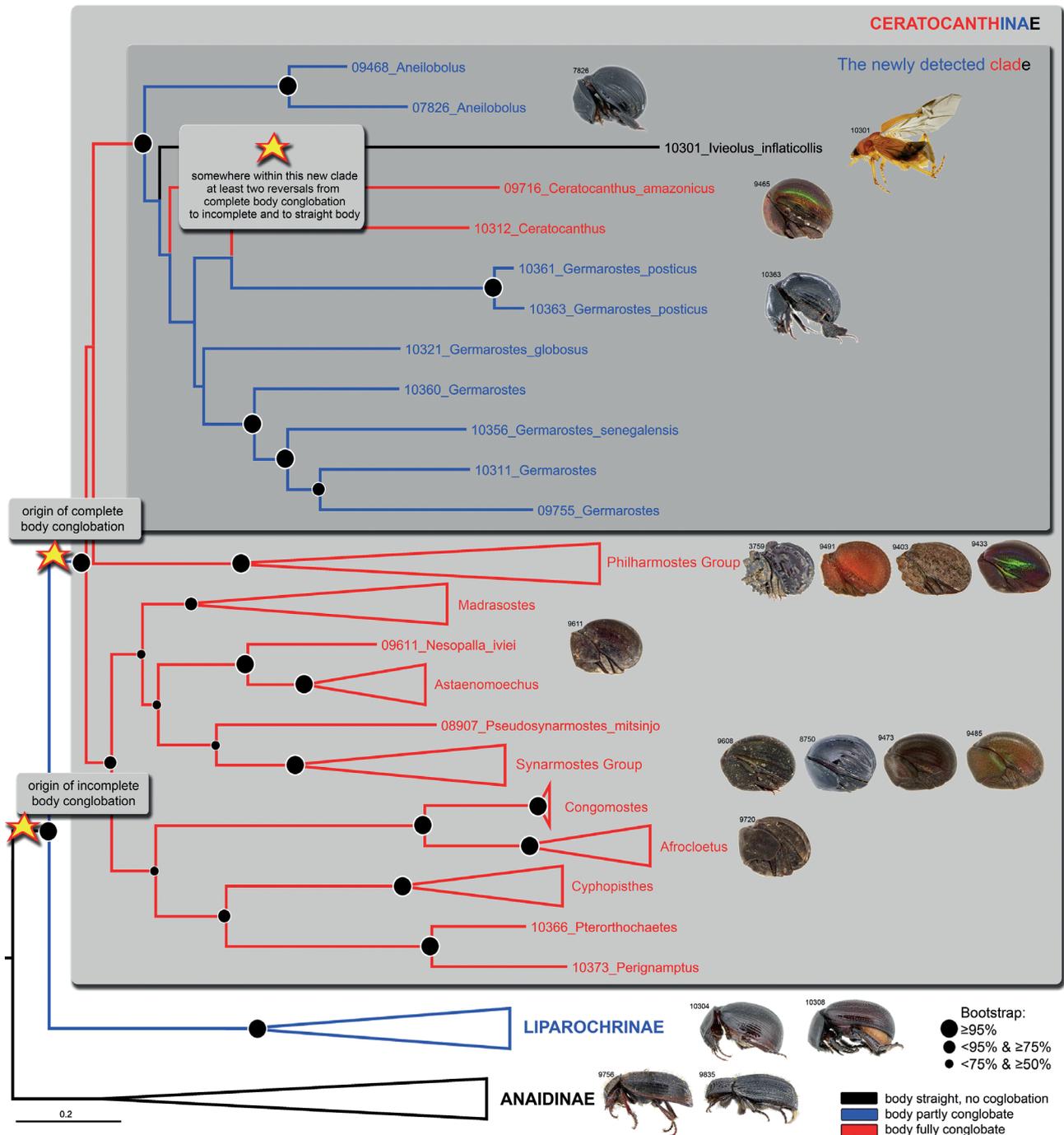


Fig. 6 – Maximum likelihood tree of pill scarabs (Ceratocanthinae), as reconstructed by RAXML from the three-fragment concatenated matrix. Two main clades are recognized, both with strong support: Ceratocanthinae and its subordinate clade consisting of pill scarabs with three different types of body architecture. Three types of body architecture are colour-coded and at least three of their changes marked with numbered starts. The exact position of the reversal from complete body conglobation to incomplete and/or straight body is ambiguous because of the lack of higher resolution with the newly pill scarab hypothesized clade. Habitus images are not to scale; four- or five-digit numbers are specimen IDs.

“typical” conglobating Ceratocanthini and non-conglobating rest of the subfamily. All known specimens of *Trachycrusus* were collected by flight intercept traps, leaving its supposed termitophily (Ballerio & Grebennikov 2016) untested. It is intriguing to test if, as circumstantial evidence suggests, this genus is a sister to the rest of the Iviolini and Scarabatermitini clade (as indicated by *Trachycrusus* flattened meso- and metatibiae, unique to this clade), and all of them nested within Ceratocanthini.

Is termitophily a credible hypothesis for all members of the supposed Iviolini and Scarabatermitini clade?

Of five non-Ceratocanthini pill scarab genera (Figs. 2–4) likely forming a clade (Ballerio & Grebennikov 2016), live specimens of only two monotypic genera were found in direct association with termites: *Scarabaeinus* (Silvestri 1940) and *Scarabatermes* (Howden 1973). Only these two genera unquestionably display hypertrophic (‘physogastric’) abdomen (Figs. 2–3); a morphological trait correlating with termitophily in beetles (Pisno et al. 2019), including scarabs (Krikken 2008). The remaining three genera are known only from the flight-intercept-trap records and their termitophily as assumed on circumstantial evidence, as is frequently done with the rarely collected and also supposedly termitophilous Aphodiinae (e.g., Vårdal & Forshage 2010; Kakizoe et al. 2021). The current answer to the question is, therefore, “likely yes, but verification is still required”.

If the origin of Iviolini and Scarabatermitini clade is indeed linked with termitophily, then the age of the crown group might be compared with the maximal known age of termitophily. Termite eusociality was the first to evolve on Earth, is estimated at 150 Ma, and pre-dates the origin of the termite crown group (Chouvenc et al. 2017). Counter-intuitively, the fossil evidence of termitophily is relatively very recent and no such fossils are known from earlier than 19 Ma (Cao et al. 2017; with a single and contested 99 Ma exception, Cao et al. 2017; Yamamoto et al. 2017). Having only a single, poorly resolved and supposedly termitophilous pill scarab on our molecular tree, we are unable to infer the age of the clade it represents and, therefore, cannot discuss the temporal aspect of its evolution.

Factors potentially driving the evolution of body architecture in Hybosoridae. Finally, it is alluring to speculate on what might be the main force driving hybosorid beetles towards the structurally complex and presumably functionally highly restrictive complete body conglobation. This question is likely unanswerable because no hypothesis can be put to a sufficiently rigorous test. Perhaps, for this reason, the early authors were reluctant to speculate on this subject. Besides, the timeline of Hybosoridae evolution is poorly known. The fossil records of pill scarabs (reviewed in Ballerio & Grebennikov 2016; Poinar & Ballerio 2017) offers no evidence that

the crown Ceratocanthinae are older than the Oligocene. The placement of the *Mesoceratocanthus tuberculifrons* Nikolajev, Wang, Liu, & Zhang, 2010 impression fossil from the Lower Cretaceous of China as a sister clade of *Ivieolus* (Nikolajev et al. 2010) was done outside the phylogenetic framework and requires a reinterpretation. The only relevant DNA-based age estimation suggests that monophyletic Hybosoridae separated from its sister (Glaphyridae plus Ochodaeidae) 130–69 million years ago (Ma) (fig. S9 in McKenna et al. 2015). Among other things, this vague temporal framework of pill scarab evolution is coincidental with two other potentially relevant factors: a Paleogene diversification and ecological dominance of ants (Hymenoptera: Formicidae) (Wilson & Hölldobler 2005; Barden 2017), and the occurrence of termitophily (Cao et al. 2017). The Ballerio & Wagner (2005) report of completely conglobating pill scarabs coexisting with super-abundant ants in the tree canopy in Uganda, although relevant to the recent fauna, also points to ants as a significant factor. Hopefully, these vague assumptions can later be put to a test.

Concluding remarks

Our results, although far from being conclusive, completely reorganise existing thoughts on the evolution of body conglobation in Hybosoridae. Most significantly, we advocate that the MRCA of pill scarabs was already capable of complete body conglobation, with a subsequent reversal to incompletely conglobating and straight-bodied species, some of the latter termitophilous. This drastic re-interpretation of the long-established and seemingly intuitive earlier and morphologically-inspired thoughts might seem too radical. In this respect, the novelty of our result might be comparable to other and perhaps better-publicized studies in vertebrate evolution. Examples include the conclusion that “worm snakes” (Scolelephidia) are paraphyletic with respect to “typical snakes” (Alethinophidia), implying the burrowing (= fossorial, underground) origin of the latter (Miralles et al. 2018), or breaking apart the century-old “classical” group of Saurischia dinosaurs and suggesting the independent origin of hypercarnivory in superficially similar, but only distantly related Herrerasauridae and the iconic mega-predator *Tyrannosaurus rex* Osborn, 1905 (Theropoda; Baron et al. 2018). Will any of these three reinterpretations last? Only time will tell.

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