

MORPHOLOGY, FOSSILS AND MOLECULES ELUCIDATE
THE MYSTERY OF DUNG BEETLE (COLEOPTERA:
SCARABAEIDAE: SCARABAEINAE) EVOLUTION USING
NOVEL BAYESIAN AND PARSIMONY APPROACHES

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List of Papers

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II. TARASOV S., DIMITROV D. Multigene Phylogenetic Analysis using Model Adequacy Assessment redefines Dung Beetle (Coleoptera: Scarabaeidae: Scarabaeinae) Relationships and Classification. (*submitted to Cladistics*).

III. TARASOV S. A cybertaxonomic revision of a new dung beetle tribe Parachoriini **trib.n.** (Coleoptera: Scarabaeidae: Scarabaeinae) and its phylogenetic assessment using molecular and morphological data. (*submitted to PeerJ*).

IV. TARASOV S., VAZ-DE-MELLO F., KRELL F., DIMITROV D. A Review and Phylogeny of Scarabaeine Dung Beetle Fossils (Coleoptera: Scarabaeidae: Scarabaeinae) with Description of Two *Canthochilum* Species from the Dominican Amber. (*submitted to Systematic Entomology*).

Abstract

A plethora of studies published over the last two decades produced numerous contradictory phylogenies leading to the lack of consensus on phylogenetic history in dung beetles. Thus, due to those contradictions, the community of dung beetle systematists could not use advantages of current phylogenetic techniques to improve the largely artificial classification established a half-century ago. Moreover, those conflicting phylogenies, along with unstudied fossils, have significantly hampered our understanding of dung beetle evolutionary history. In this thesis, I revisit the phylogenetics, attempt to improve the classification and explore fossil evidence of dung beetles through the acquisition of global morphological and molecular data in the framework of traditional and novel methods for their phylogenetic analysis.

The global morphological (134 species, 232 characters) and molecular (8 genes 547 terminals) datasets were analyzed using parsimony, maximum-likelihood and Bayesian inference. To improve phylogenetic inference, I employed three innovative approaches: (1) use of the positional congruence index to eliminate characters supporting taxon instability in parsimony-based morphological phylogenies, (2) partitioning of morphological matrices using anatomy ontologies and (3) use of Bayesian posterior prediction for selecting data partitions in molecular analysis. The second is the Bayesian approach that assigns characters from different anatomical regions to different partitions, thus allowing to model heterogeneous evolutionary rates, improving model fit and reducing biases in phylogenetic reconstruction. The third approach fits model to data, then uses the estimated parameters to simulate replicated data, which are then compared to the observed data, thus allowing adequacy assessment of a model. The partitions in molecular analyses were selected based on the adequacy of the fitted model, which in turn resulted in improved phylogenetic inference. Both the molecular and morphological analyses yielded consistent results concerning the shallow and deeper nodes in the resulting phylogenetic trees, which supported the definition of new taxonomic concepts for three highly polyphyletic dung beetle tribes and stabilized dung beetle classification. Additionally, a new tribe, *Parachoriini* **trib.n.**, was recognized to accommodate the new concept of the Oriental genus *Parachorius* **sensu novo** (= *Cassolus* **syn.n.**), which was revised using the cybertaxonomic tool 3i.

The examination of 33 described dung beetle fossils revealed that only 21 of these (two herein described as new) can be reliably referred to dung beetles. Three best-preserved fossils were integrated into the morphological matrix to elucidate their phylogenetic

placement. Based on reliable fossils, the dung beetle minimal age shifts to the Eocene (53 Ma), while present-day dung beetle genera had already evolved by the Oligocene–mid Miocene. However, the biogeographic pattern of the molecular and morphological phylogenies suggests a Late Gondwanian origin of dung beetles, which corroborates the Upper Cretaceous age of origin inferred by a recent global phylogeny of Coleoptera.

1. Introduction

Dung beetles from the subfamily Scarabaeinae (Fig. 1) are a charismatic group of insects that comprises ~ 6200 species feeding mainly on mammalian excrements distributed around the globe. Ever since my school days, these creatures have fascinated me and I started my journey in studying their systematics and evolution while attending high school in my hometown.

Dung beetles are appealing – they come up in a tremendous diversity of shapes and colors and are often armed with various forms of horns. Beside that, they promise to revolutionize biology by acting as a model group, used by scientists to discover new patterns in the ecology and development of organisms as well as to monitor our environment. It is a rule of thumb that the majority of biological phenomena can be interpreted only if we know their evolutionary history. Dung beetles, despite an intensive array of investigations, have been concealing the mystery of their evolution over the last thirty years, the solution of which is fundamental to their use as model organisms.

Four years ago, I received an opportunity to conduct a PhD on the intriguing subject

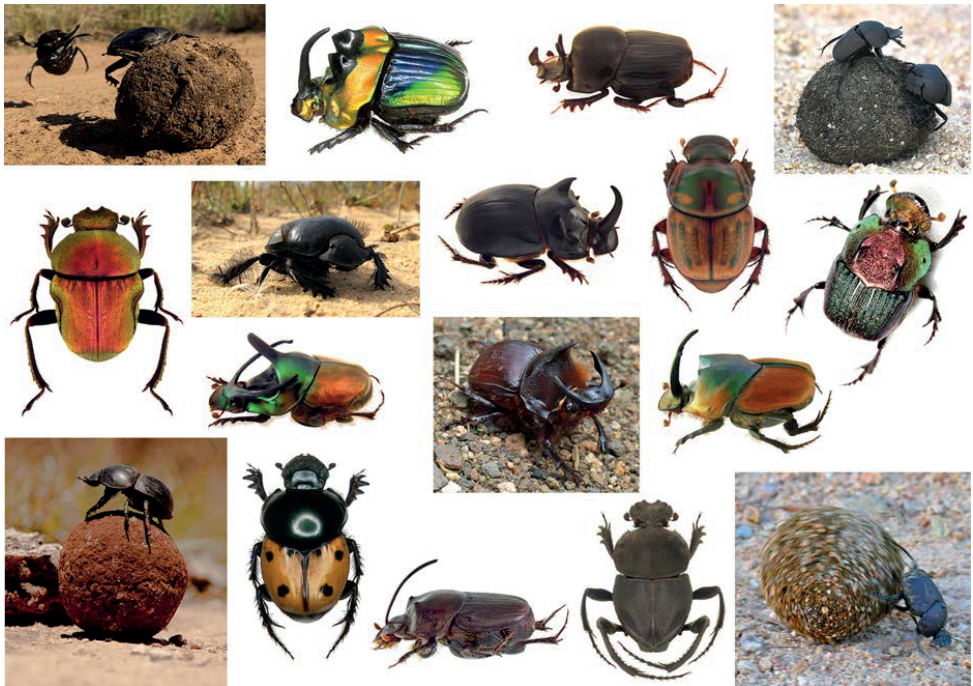


Figure 1. The example of dung beetle diversity produced using species pictures from www.flickr.com under CC BY-SA 2.0 license (<https://creativecommons.org/licenses/by-sa/2.0/>).

of the systematics and evolution of dung beetles, the results of which I am providing in this thesis. During this endeavor, I aimed at investigating and putting pieces of evidence from different fields of biology together in order to reconstruct the evolutionary history of dung beetles.

Below, I describe the main findings of my work. I begin with a short introduction to the importance of dung beetles, then move on to the challenges which motivated this thesis and next proceed with the work implemented during the course of my PhD.

1.1. Dung beetles in 50 seconds: their ecological and biological importance

As mentioned above, dung beetles are often used as model organisms in biology and ecology and they have charismatic behavioral and biological features, which make them stand out among other animals. The peculiarity of dung beetles can be quickly comprehended in 50 seconds – the average time needed to read the most striking facts about them.

- With over 6000 species of which 30–50% are still undescribed, dung beetle diversity exceeds the diversity of extant mammals (~5500 species) and approaches that of birds (~9800 species).
- Besides striking species diversity, dung beetles are also strikingly abundant: a single pile of fresh elephant dung can contain up to 7000 scarabaeine individuals.
- Dung beetles are extremely popular beetles in science: they are the second most cited subfamily of beetles on Google Scholar (Paper I, Fig. 1).
- The global ecosystem service of dung recycling provided by dung beetles is valued at \$380 million annually in the US (Losey and Vaughan, 2006).
- Beside utilization of animal excrements, dung beetles provide a number of other important ecosystem services, namely, they are involved in nutrient cycling, bioturbation, enhancement of plant growth, secondary seed dispersal, parasite suppression, fly control, trophic regulation and pollination (Nichols et al., 2008).
- Dung beetles, based on their behavior, are classified into tunnelers, dwellers and rollers. The first two bury dung beneath dung pads and live inside dung, respectively. The third are the most remarkable of them all; the rollers roll dung into balls and then bury the ball away from the dung pad for feeding and breeding needs, a behavioral trait that made them (namely *Scarabaeus*) a sacred animal in ancient Egypt.
- Dung beetles are the only known animals, beside humans, that use the Milky Way for navigation (Dacke et al., 2013)

- Cheap collection and processing of dung beetle samples single out these beetles among other invertebrates for various types of ecological studies (Gardner et al., 2008).
- Dung beetles, due to their ecological sensitivity, are used for assessing human impact (Spector, 2006; Scholtz et al., 2009b)
- The IUCN Red List Index program selected dung beetles, amongst few other invertebrate taxa, to measure the risk of biodiversity loss worldwide (Baillie et al., 2008; Nichols and Gardner, 2011).
- The Australian dung beetle project is worth special attention. Native Australian dung beetles are adapted to only feed on the dung of marsupials, the native Australian mammals. The native dung beetles were not able to utilize the dung of cattle that was relatively recently introduced to Australia by the Europeans. Undecomposed dung began to accumulate on pastures in high amounts, which stimulated pollution, uncontrolled breeding of pestilent flies and worms and the reduction of grazing areas for cattle. To fight this problem, dung beetles from Africa, which were capable of utilizing cattle dung, were introduced to Australia (Bornemissza, 1976).
- Male competition armed some species of dung beetles with extravagant head horns (Fig. 1). The scarabaeine genus *Onthophagus*, due to the dramatic diversity of horns among its species, is used as a model group in developmental biology and ecological development (Scholtz et al., 2009a; Moczek, 2011).

1.2. Background

When, four years ago, I started my PhD, 7 morphology-based and 6 molecular-based key phylogenies dealing with higher-level relationships in dung beetles had been already published (Zunino, 1983; Montreuil, 1998; Pretorius et al., 2000; Villalba et al., 2002; Philips et al., 2004; Ocampo and Hawks, 2006; Monaghan et al., 2007; Vaz-de-Mello, 2007b; Vaz-de-Mello, 2007a; Wirta et al., 2008; Sole and Scholtz, 2010; Wirta et al., 2010; Bai et al., 2011; Mlambo et al., 2013). However, their results, despite shared similarities, were largely contradicting, therefore hampering consensus on the dung beetle evolutionary history. All the findings inferred by those phylogenies can be summarized into two categories. First, the very early branching splits were well-corroborated among the studies, while intermediate and deep splits remained inconsistent or weakly supported. Second, seven out of twelve dung beetle tribes (Scholtz et al., 2009b) were recovered as monophyletic or nearly so, while three tribes Deltochilini (Canthonini), Ateuchini (Dichotomiini) and Coprini, have always been inferred

highly polyphyletic as their genera emerge in different parts of the dung beetle phylogenetic tree (Vaz-de-Mello, 2007b; Sole and Scholtz, 2010; Tarasov and Génier, 2015). It is noteworthy that those polyphyletic tribes comprised more than half of the described scarabaeine genera, thus resulting in a highly artificial classification of dung beetles.

This unsolved puzzle of dung beetle evolutionary history motivated me to re-tackle the morphological and molecular phylogenies from a different perspective that was based on assembling new, larger datasets and the application of novel approaches to their analysis. Additionally, the instability of the taxonomic concepts for the polyphyletic tribes stressed the need of new taxonomic limits for those problematic taxa. This part of my PhD thesis is implemented in **Papers I** and **II** dealing with morphological and molecular phylogenies of dung beetles, respectively. The revision of the tribal concepts is provided in **Paper II**.

Due to the revision of these tribal concepts, two scarabaeine genera from the Oriental Region, *Parachorius* and *Cassolus*, fell in the group of genera without a tribal placement. Over their taxonomic history, their tribal placement was ambiguous and was frequently changing depending on different authors. By adding these genera to the global molecular and morphological matrix from my previous studies, I attempted to test the hypothesis of their close relationship and, at the same time, assess their obscure phylogenetic placement, which was done in **Paper III**. This phylogenetic study inspired me to conduct a taxonomic investigation of museum and recent material from the Oriental Region, which revealed many undescribed species in *Parachorius sensu novo*. This, in turn, inspired me to revise *Parachorius sensu novo* taxonomically, which I did using the cybertaxonomic tool 3i in **Paper III** as well.

Fossils are critical for calibrating the timing of evolutionary events and elucidating relationships within clades of organisms. Despite the intensive phylogenetic scrutiny in dung beetles, their fossils remain almost entirely unexplored. My **Paper IV** aims at filling up this gap by reviewing and phylogenetically revising scarabaeine fossils in order to elucidate calibrating points for dating dung beetle phylogeny and assess dung beetle fossil diversity.

To sum up, the aim of my PhD work splits into three interlinked fields reflecting various aspects of dung beetle evolutionary history. Specifically, I focused on the following points: (1) assembly of large-scale anatomical and molecular data along with application of innovative phylogenetic approaches to reconstruct the evolutionary tree of dung beetles, (2) development of a new phylogeny-based classification, partially through application of cybertaxonomy, (3) revision of dung beetle fossils to elucidate the timing of dung beetle evolution.

2. Material and methods

2.1. Morphological data

The data matrix for **Paper I** comprises 110 taxa, 4 of which are outgroups. The ingroup taxa were sampled to cover the described taxonomic, biogeographic, and morphological diversity. They belong to all 12 tribes and to 101 genera of the subfamily which represents 37% of the total generic diversity. In **Paper IV**, three fossil species were integrated into the matrix from **Paper I**. Twenty-one species (primarily from *Parachorius sensu novo*) and 27 characters were added to the morphological matrix in **Paper III**.

For the morphological analyses, all possible characters of external and internal morphology were scored. The data resulting matrices are available on MorphoBank ([http://www.morphobank.org/projects/2286, 2184 and 1157](http://www.morphobank.org/projects/2286,2184%20and%201157)).

2.2. Molecular data

Taxon sample. For **Paper II**, a total of 530 specimens of dung beetles (Scarabaeinae) belonging to 137 genera representative for all 12 tribes and biogeographic regions were sampled. 95 specimens from 72 species were sequenced specifically for this study. The outgroup comprised 17 terminals from 10 genera of the Scarabaeidae subfamilies Chironinae, Aegialiinae and Aphodiinae which are the closest relatives of Scarabaeinae.

For **Paper III**, three terminals from *Parachorius sensu novo* and one *Panelus* species were added to the molecular matrix from **Paper II**.

Molecular markers. In the molecular analyses, 8 phylogenetically informative markers were used: 16s ribosomal RNA (16s), 18s ribosomal RNA (18s), 28s ribosomal RNA domain 2 (28sD2), 28s ribosomal RNA domain 3 (28sD3), cytochrome c oxidase I (COI), carbamoylphosphate synthetase (CAD), topoisomerase I (TP1) and wingless (Wg). The information on voucher specimens is given in the supplementary material (Paper II, Table S1 and Paper III, Table S1).

2.3. Dung beetle ontology

The simplified ontology database of dung beetles was constructed *de novo* in Microsoft Access, while the tree-like graph of anatomy relationships was constructed in R (Team, 2012) using exported entities from the Microsoft Access database.

The anatomical ontology is presented as a tree-graph to reflect the hierarchical relations among anatomical elements and was used to guide partitioning of the character

matrix in Bayesian analyses. In this tree-like graph, terminal branches correspond to the characters in the character matrix, and nodes represent anatomical regions where the characters or the other nodes (anatomical regions) of a lower hierarchical level are located.

2.4. Cybertaxonomy

The cybertaxonomic revision was powered by 3i Interactive Key and Taxonomic Database Software (<http://dmitriev.speciesfile.org/index.asp>). In comparison to numerous other cybertaxonomic tools, 3i is powerful software that incorporates all necessary features for producing taxonomic revisions and interactive keys.

2.5. Phylogenetic analyses

The majority of the computer calculations were run on the High Performance Computing cluster Abel at USIT, the University of Oslo.

Morphological analyses. The parsimony analysis was conducted in TNT ver. 1.1 (Goloboff et al., 2008) under equal and implied weights. The Bayesian analyses of morphology were run in MrBayes version 3.2.2. (Ronquist et al., 2012) using the $Mk+I$ model.

Molecular analyses. Initially, the entire dataset was split into 28 a priori data blocks. This was done based on the secondary structure (loops and stems regions) for each rDNA gene and based on domain structure and codon position for each protein-coding gene. The domain structure was obtained from the InterPro database (Jones et al. 2014; Mitchell et al. 2014).

For the phylogenetic analyses, the sequences were aligned in MAFFT (Kato and Standley 2013). The software Partition Finder (Lanfear et al., 2014) was used to elucidate the optimal partitioning scheme. The aligned and partitioned dataset was analyzed using traditional methods for phylogenetic inference – Maximum likelihood (ML), Bayesian inference (BI) and Direct optimization (DO). The ML analyses were run in RAxML version 8.0.26 (Stamatakis, 2014), while BI analyses were performed in MrBayes version 3.2.2. and ExaBayes version 1.4.1 (Aberer et al., 2014). The assessment of model adequacy using Bayesian posterior assessment (BPA) was done in PuMA (Brown and ElDabaje, 2009) using data from the MrBayes runs. Molecular analyses under DO were conducted in the computer program POY v 5.1.1b (Wheeler et al., 2014).

3. Results

3.1. Phylogeny of dung beetles (Papers I & II)

The phylogeny of dung beetles was reconstructed using morphological (**Paper I**) and molecular (**Paper II**) data.

Morphological phylogeny. For the morphological phylogenetic analysis, I attempted a thorough investigation of morphology for a large taxon sample sufficiently representing biogeographic and taxonomic diversity. The resulting morphological dataset was the largest known for scarabaeines: it comprised 110 taxa and 205 characters, and was analyzed with parsimony and a novel Bayesian method that uses anatomy ontology for matrix partitioning.

The preliminary parsimony runs yielded quite unresolved consensus that gave a hint that the dataset contained conflicting characters supporting instability of some taxa, which, in turn, yielded numerous equally parsimonious trees. I used the protocol of Pol and Escapa (2009) that was designed to detect unstable taxa and characters supporting that instability. Usually, this protocol is used for detecting and exclusion of unstable taxa. The assessment of characters supporting instability indicated that instability in many identified cases is caused by the same characters. Thus, the characters most frequently supporting instability were excluded and a second set of analyses was run and produced a well-resolved consensus.

The Bayesian method was applied with a novel approach that uses anatomy ontology for matrix partitioning. Partitioning is one of the ways to account for rate heterogeneity among characters, thus representing a more accurate model of evolutionary processes. Anatomy ontology describes relationships between anatomical organs of an organism. These relationships, in a simplified way, can be represented with a tree-like graph that was used to generate different parameter-partition schemes in the morphological matrix. Such partitioning procedure tends to assign characters from the same anatomical location to the same partition, on the assumption that characters of the same anatomical region undergo similar evolutionary dynamics, thus enabling to model heterogeneity in evolutionary rates among different partitions. Ontology partitioning generates multiple partitioning schemes, which were assessed using Bayes factor. This approach, beside phylogenetic inference that models heterogeneous evolutionary rates, also allows testing hypotheses about character evolution. Specifically, I used it to address the question of whether a morphological dataset can be reasonably partitioned and whether characters on the same anatomical region evolve at similar rates.

The model assessment revealed that partitioning of morphology does not significantly affect topology but improves model fit that can improve parameter estimation. The improved model fit indicated that characters on the same anatomical region tend to evolve at similar rates.

Molecular phylogeny. The molecular analysis used the largest dung beetle dataset ever assembled, with a well-represented global biogeographic and phylogenetic sample of dung beetles. This dataset was based on GenBank data in combination with a large number of newly sequenced data and consisted of 8 gene regions and 547 terminals. In order to account for the sensitivity of the results, I used the entire arsenal of traditional phylogenetic methods, namely, DO (as implemented in POY), ML and BI. Phylogenetic inference in model-based methods (ML and BI) uses statistical models for modeling a substitution process, which frequently poorly reflect the reality of the evolutionary process (Lemmon and Moriarty, 2004; Brown, 2014). Thus, the application of such inadequate models can severely bias the phylogenetic inference. Therefore, in model-based molecular analyses, I aimed at explicitly testing model adequacy using BPA. The molecular partitions in which the substitution models were identified as not adequately depicting the processes that generated the data were excluded from further analyses, which in turn improved the phylogenetic inference.

3.2. Corroborated results from molecular and morphological analyses lead to a new classification of dung beetles (Paper II)

The consistency between molecular and morphological phylogenies allowed the definition of new systematic concepts for the problematic tribes which have been constantly demonstrated to be polyphyletic. By following this consistency and the principle of monophyly, the taxonomic limits of Deltochilini (Canthonini), part of the Ateuchini, and Coprini were significantly narrowed and the tribe Pinotini (Dichotomiini) was revalidated. These changes were performed to accommodate only those genera that emerge in the same clade as the respective type genera of the problematic tribes. Additionally, the concept of the monophyletic tribe Sisyphini was expanded to accommodate the genus *Epirinus*, which was previously assigned to Deltochilini. In order to provide an effective identification of these new tribal concepts, the synapomorphies identified in the morphological phylogeny were used to formulate their diagnoses. Such delimitation of the tribal concepts left many genera without a tribal placement, i.e. unclassified (*incertae sedis*) within the scarabaeine subfamily.

However, the introduced taxonomic changes stabilized the tribal classification of dung beetles and at the same time provided a perspective toward its further elaboration.

3.3. New dung beetle tribe Parachoriini trib. n. (Paper III)

Over the years, the genera *Parachorius* and *Cassolus* had never been deemed to be closely related and did not stand out among the bulk of other phylogenetically challenging genera, up until recently when a putative intermediate form was found providing evidence for their close affiliation (Tarasov and Keith, 2011). *Parachorius* and *Cassolus* were integrated in the molecular and morphological character matrices from **Papers I, IV and II**, which were then analyzed using both parsimony and ML methods. The molecular matrix contained only a limited sample of *Parachorius* and *Cassolus* aiming at exclusively assessing their higher-level phylogenetic placement. In contrast, the morphological matrix contained the full set of species from those genera, which allowed elucidation of the relationships among their species. Interestingly, both analyses converged on strongly supporting monophyly of *Parachorius* + *Cassolus*. Moreover, the morphological analysis recovered *Parachorius* nested within *Cassolus*. This supported the synonymy between *Parachorius* and *Cassolus*, and since *Parachorius* was the senior synonym, I proposed a new concept of this genus, i.e. *Parachorius sensu novo* (= *Cassolus syn. n.*). Additionally, both phylogenetic analyses revealed the isolated position of *Parachorius sensu novo*; in all the analyses it was placed separately from all other known scarabaeine tribes. I followed the same taxonomic principles of monophyly and diagnosability, which were ascertained in **Paper II**, and placed *Parachorius sensu novo* in a newly recognized tribe, Parachoriini trib. n. The phylogenetic analysis of the morphology helped to formulate its diagnosis, which allows its straightforward separation from other dung beetle tribes.

3.4. Cybertaxonomic revision of *Parachorius sensu novo* (Paper III)

The second part of **Paper III** taxonomically revises *Parachorius sensu novo* whose phylogenetic position was assessed in the previous chapter. The revision of *Parachorius sensu novo* was powered by the cybertaxonomic tool 3i. This software was used to facilitate the process of taxonomic revision by providing an efficient way for managing taxonomic and distributional data, generating species descriptions, formulating differential diagnoses, drawing distribution maps and constructing keys to species. Prior to my work, the genera *Parachorius* and *Cassolus* comprised 8 and 9 species, respectively. The revision resulted in

the description of 7 new species, raising the total number of species in *Parachorius sensu novo* to 19. Additionally, the revision resulted in the synonymy of three species names. Two species originally described in *Cassolus* were placed in the Afro-Oriental genus *Panelus*. Morphological investigation of *Panelus* and *Macropanelus* suggested that the generic rank of *Macropanelus* must be lowered to subgeneric rank within the genus *Panelus* (i.e., *Panelus* = *Macropanelus* **syn.n.**).

3.5. Dung beetle fossils and timing of scarabaeine evolution (Paper IV)

The fossil record of Scarabaeinae comprised 35 described fossils, of which two new species of *Canthochilum* from Dominican amber were described in **Paper IV**. In that paper, scarabaeine fossils were assessed based on the original descriptions and by examining type specimens as well as their illustrations where possible. This assessment revealed that only 21 fossil species can be reliably placed in Scarabaeinae, whereas the placement of the remaining 14 fossils within Scarabaeinae should be taken as doubtful due to their poor preservation, which precludes an exact assessment of their taxonomic placement. Interestingly, two scarabaeine fossils from the Cretaceous, hitherto considered the oldest, were identified as doubtful Scarabaeinae, suggesting that they cannot be used in the assessment of scarabaeine minimal age. In turn, one fossil from Oise amber in France was identified as the earliest reliable dung beetle, which set the minimal Scarabaeinae age at the Eocene (53 Ma).

Timing of dung beetle origin is controversial: Cenozoic versus Mesozoic (Davis et al., 2002; Scholtz et al., 2009a). The Eocene minimal age does not rule out the Mesozoic origin of dung beetles, which is supported by a recent global phylogeny of Coleoptera using calibration points from other beetle families and inferring dung beetle origin at the Upper Cretaceous (McKenna et al., 2015). The Upper Cretaceous origin is also supported by the biogeographical patterns in my molecular and morphological phylogenies, which emphasize the key role of the Gondwana break up in shaping the present distribution of dung beetles. However, the present-day dung beetle lineages seem to have become established by the late Oligocene–mid Miocene, as can be deduced from the presence of only extant genera in the fossil record from that geological time.

To provide the explicit usage of fossils data in the phylogenetic framework, I integrated the oldest known and two described *Canthochilum* species in the global morphological matrix from my previous study (**Paper I**) to elucidate their relationships and make them available as calibrating points for future phylogenetic studies. Specifically, these

three species were scored in the matrix as they represent the best preserved specimens, while the fragmentary preservation of the others discourages their inclusion in phylogenetic studies. Both parsimony and BI were used to analyze the phylogenetic placement of the fossils. All methods yielded consistent results, where the two fossils of *Canthochilum* were recovered in a clade with the extant species of *Canthochilum*, and the oldest known fossil came up in a clade with the extant genera *Ateuchus* and *Aphengium*.

Interestingly, the habitus of one fossil *Canthochilum* looks extremely similar to that of an extant *Canthochilum* from Hispaniola. The age of this fossil, aligned with that of Dominican amber (~16 Ma), points to a slow rate of morphological evolution in this lineage.

4. General Discussion

4.1. Dung beetle phylogeny

Present molecular and morphological phylogenies, the core of this thesis, yielded consistent results that recovered new clades and corroborated results from some earlier studies (Ocampo and Hawks, 2006; Monaghan et al., 2007; Vaz-de-Mello, 2007b; Wirta et al., 2008; Sole and Scholtz, 2010; Mlambo et al., 2013). Both phylogenies largely converge in supporting very congruent topologies at the shallow and deep splits but many intermediate nodes remain poorly supported. However, the agreement between these molecular and morphological analyses allowed establishing new concepts for the hitherto polyphyletic tribes, revalidation of one tribe and recognition of a new tribe. The consensus of the two phylogenies yields six major phylogenetic groups summarized below.

1. Basal scarabaeine lineages. This group, corroborated by molecules and morphology, comprises Afro-Oriental genera that do not seem to form monophyletic group but rather represent a grade of lineages branching off early in the phylogeny of dung beetles. This group includes the following well-known genera and their numerous close relatives: *Paraphytus*, *Byrrhidium*, *Dicranocara*, *Haroldius*, *Sarrophorus*, *Odontoloma*, *Peckolus*.

2. Onitini + (Onthophagini + Oniticellini). Morphology suggest sister group relationships between Onthophagini and Oniticellini, while molecules place the latter within the former and support their sister group relationships with Onitini.

3. Pinotini (=Dichotomiini), Eucraniini, Phanaeini, Ateuchini. The monophyly of these tribes is corroborated by molecules and morphology, except that morphology does not fully support monophyly of Eucraniini, while molecules challenge the monophyly of Ateuchini. Additionally, molecular and morphological analyses also recover these tribes as

closely related. This is also supported by biogeography as all of them occur exclusively in the Americas.

4. Endemic Australasian genera. The clade comprising endemic Australasian genera is similar between molecular and morphological analyses; although in both cases endemic Australasian genera do not form strictly monophyletic groups. Morphology, unlike molecules, recovers three Australasian lineages. Of them one comprises the majority of Australasian genera whereas the other two consist of the remaining morphological outliers. Molecules support monophyly of Neotropical *Uroxys* + *Bdelyroscopy* and all Australasian genera (except *Boletoscapter*). Both results show evidences for close relationships among all Australasian genera, however a simultaneous analysis of morphology and molecules is desirable to further clarify these relationships.

5. Deltochilini, Coprini, Gymnopleurini, Scarabaeni+Circellium, Eurysternini, Sisyphini, Parachoriini. This group includes tribes whose monophyly is supported by both molecules and morphology, however the position of these tribes in the dung beetle tree is unresolved. The tribe Parachoriini is recognized and revised in this thesis.

6. Independent genera. Numerous remaining genera which do not fall in any of the above-mentioned categories form separate phylogenetic lineages, many of which deserve status of separate tribes.

4.2. Methods

The novel methods applied in the thesis, specifically partitioning using anatomy ontology and selection of molecular partitions using BPA improve parameters and topological inferences. However, their generalized implementation does not always seem straightforward. Ontology partitioning requires a well-developed anatomy ontology that is, by far, absent for many groups of animals. Available BPA software precludes handling of gaps or missing data and requires their elimination prior to the BPA analysis. However this problem may be overcome by further developments of the BPA toolbox.

5. Conclusions and future prospects

Prior to this study the phylogenetics of dung beetles consisted of 13 key but contradictory phylogenies. By using new global molecular and morphological data and innovative analytical approaches, I revisited the phylogenetics of dung beetles attempting to improve our knowledge of their relationships and build a revised classification based on

natural groups. This naturally raises two questions: whether my work substantially improved the phylogeny, and whether it managed to solve all existing problems. The answer to the first question is definitely yes, while the answer to the second is no, as many intermediate nodes still remain poorly resolved. This stresses the need for acquiring more, primarily molecular, data to resolve the remaining problematic branches of the dung beetles tree of life. However, at the same time, the convergence of molecular and morphological phylogenies generated during the work on this thesis has paved the way toward developing a new classification of dung beetles and allowed the reassessment of taxonomic concepts for the three extremely polyphyletic dung beetle tribes. This stabilizes dung beetle classification by making those tribes monophyletic and efficiently diagnosable, even though many genera were left unclassified. Additionally, a new tribe Parachoriini **trib. n.**, was described to accommodate the unclassified dung beetles assigned to the Oriental genus *Parachorius sensu novo*. The tribal classification of the remaining unclassified genera should be addressed by future studies, in which the present results can be directly used to split the scarabaeine phylogenetic tree into monophyletic taxonomic units. In addition, the examination of dung beetle fossils provided in this thesis identified reliable calibration points, which, along with the phylogenetic study of these fossils, lay out a firm basis for a future total evidence analysis that will allow global assessment of dung beetle evolutionary dynamics.

6. The disclamation of nomenclatural acts and new names proposed in this thesis

This thesis should not be considered a valid publication with regards to all proposed nomenclature acts and new names. Thus, I disclaim them according to the Articles 8.2 and 8.3 of ICZN (1999).

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