

# A molecular phylogeny of the African Scarabaeinae (Coleoptera: Scarabaeidae)

SUKOLUHLE MLAMBO \*, CATHERINE L. SOLE & CLARKE H. SCHOLTZ

Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028 Pretoria, South Africa; Sukuluhle Mlambo \* [smlambo@zoology.up.ac.za]; Catherine L. Sole [clssole@zoology.up.ac.za]; Clarke H. Scholtz [chscholtz@zoology.up.ac.za] — \*Corresponding author

Accepted 25.vi.2015.

Published online at [www.senckenberg.de/arthropod-systematics](http://www.senckenberg.de/arthropod-systematics) on 07.viii.2015.

## Abstract

Dung beetles of the subfamily Scarabaeinae have a worldwide distribution, with the Afrotropical region, the putative origin of the diversification of Scarabaeinae, having the richest diversity. We use partial sequences from two ribosomal (16S, 28S) and two protein coding genes (COI, CAD) to examine the relationships among 55 genera, representing more than half of the genera in the region. Taxa were sampled to maximize representation of dung beetle morphological and ecological diversity in all nine tribes that occur in Africa. We estimated the divergence times of the tribes to determine relative ages. The phylogenetic hypothesis of tribal and generic relationships was found to largely concur with that of a recent molecular study done at a global scale, suggesting earliest diverging lineages which are quite distinct from the ones traditionally recognized. Thus recent calls for a new classification for Scarabaeinae are supported. We suggest possible changes to the classification, corroborate the likely African origin of the subfamily and provide support for fungus-feeding as the most likely ancestral feeding habit in the Scarabaeinae.

## Key words

Afrotropical, Scarabaeinae, molecular, phylogeny, divergence.

## 1. Introduction

The subfamily Scarabaeinae constitutes a group of dung beetles encompassing approximately 5700 species (SCHOLTZ et al. 2009). These beetles have a world-wide distribution with most being associated with moist herbivore dung. Some, however, utilise a variety of other dung types and even non-dung food sources. Dung is a highly nutritious but patchy and ephemeral resource, characteristics that, together with intense competition at the dung pat, have led to the evolution of an impressive range of morphological attributes (such as horns, modifications of the tibiae, bright metallic colours, complex sculpture) and behaviours, in terms of feeding and nesting in dung beetles (DAVIS & SCHOLTZ 2001). Scarabaeines are of biological interest for these attributes and for their important role in ecosystem functioning (NICHOLS et al. 2008; BROWN et al. 2010).

Scarabaeinae is a monophyletic group morphologically defined by twelve shared apomorphic character states of hindwing articulation (BROWNE & SCHOLTZ 1998) and also strongly supported by molecular evidence (PHILIPS et al. 2004; OCAMPO & HAWKS 2006; MONAGHAN et al. 2007). It is generally assumed that the sister group of the Scarabaeinae is the similar dung-feeding Aphodiinae (PHILIPS et al. 2004; MONAGHAN et al. 2007; BROWNE & SCHOLTZ 1999). The 227 genera in Scarabaeinae have been grouped in a number of different ways with earlier classifications having been based on personal intuition or morphology from limited data sets (ZUNINO 1983; 1985). Traditionally, the subfamily has been divided into two groups based on either their rolling or tunnelling behaviour (BALTHASAR 1963; CAMBEFORT 1991a). Rollers construct balls of dung and bury them at a distance

from the source for feeding and breeding, while tunnelers bury dung beneath or near the dung pat. JANSSENS (1949) grouped the scarabaeines into six tribes: rolling Eurysternini and Scarabaeini, the latter with subtribes Eucraniina, Canthonina, Gymnopleurina, Scarabaeina and Sisyphina; and tunnelling Onthophagini, Onitini, Oniticellini and Coprini, the latter with subtribes Dichotomiina, Phanaeina and Ennearabdina. BALTHASAR'S (1963) division of the group (therein ranked as a family, Scarabaeidae) was into two subfamilies with six tribes each, as follows: Coprinae with tribes Coprini, Ateuchini, Phanaeini, Oniticellini, Onitini and Onthophagini; and Scarabaeinae with tribes Eucraniini, Eurysternini, Deltophilini, Gymnopleurini, Scarabaeini and Sisyphini. This grouping by BALTHASAR (1963) was followed by many workers on the Scarabaeinae. These include HANSKI & CAMBEFORT (1991), who further envisioned the Deltophilini and Ateuchini (formerly Dichotomiini) as the primitive or "old" tribes that gave rise to the rest of the rollers and tunnelers, respectively. The tribes Scarabaeini, Gymnopleurini, Eucraniini, Eurysternini, Onitini and Phanaeini were considered to be "intermediate" in age (CAMBEFORT 1991a). Sisyphini were considered the most modern/derived of the rollers and Coprini, Oniticellini and Onthophagini the most recently evolved tunnelers (CAMBEFORT 1991a). It was, however, an intuitive classification, lacking in phylogenetic support (MEDINA et al. 2003; PHILIPS et al. 2004; MONAGHAN et al. 2007). The current classification divides the subfamily into 11 tribes (BOUCHARD et al. 2011): Ateuchini, Deltophilini, Eucraniini, Gymnopleurini, Oniticellini (including former Eurysternini as a subtribe), Onitini, Coprini, Onthophagini, Phanaeini, Scarabaeini, and Sisyphini.

Early phylogenetic studies such as that of ZUNINO (1983), using morphological data for cladistic analyses, indicated problems with the roller/tunneller division; there was evidence of tunneller groups nested within the rollers. In addition, the tribes Deltophilini and Ateuchini did not appear to arise from basal phylogenetic dichotomies. MONTREUIL (1998) used 42 adult morphological characters for his study of the Ateuchini and Coprini and found the two tribes to be non-monophyletic. He suggested major changes to scarabaeine classification. The tribe previously known as Dichotomiini was renamed Ateuchini Perty after reassigning some genera to Coprini.

Another phylogenetic analysis to raise serious doubts about the then accepted dung beetle classification (BALTHASAR 1963) was that by PHILIPS et al. (2004). Their study was based on 200 morphological characters and 50 taxa covering all 12 tribes recognised then, sampled from six biogeographical regions. The tribes comprise Deltophilini and Ateuchini, which are widespread with their main generic diversity in the southern continents (Neotropical, Afrotropical and Australia); Eucraniini, Phanaeini and Eurysternini restricted to the Americas; Scarabaeini, Gymnopleurini and Onitini showing an Afro-Eurasian distribution; and the cosmopolitan Onthophagini, Sisyphini, Oniticellini and Coprini (DAVIS & SCHOLTZ 2001). PHILIPS et al.'s (2004) phylogeny deviated widely

from the two-clade scenario, with the relationships within the ingroup differing from the earlier mentioned studies. Rollers and tunnelers were intermixed within the phylogenetic tree and, although some Ateuchini genera originated from basal dichotomies within the tree, others did not. There was, however, generally poor support for most nodes at the base of their tree, with no evidence for the monophyly of the ateuchines, coprines and deltochilines. Monophyly was supported to some extent for the remaining nine tribes (an average of only two genera per tribe were used). From this study it was concluded that the Scarabaeinae ancestor was a tunneller, with all other clades having evolved directly or indirectly from an Ateuchini-like ancestor, and rolling having evolved independently a number of times. Furthermore, the study suggests that according to biogeographical evidence, some Scarabaeinae taxa may have been present in the Mesozoic, consistent with fossil evidence from a study by KRELL (2006) suggesting a late Mesozoic origin for dung beetles. SCHOLTZ & CHOWN (1995) on the other hand had proposed the Cenozoic epoch for the most recent dung beetle ancestor. In a recent morphological study TARASOV & GÉNIER (2015) used 110 taxa and 205 characters to hypothesize a phylogeny that is consistent with the results from different studies while also suggesting new relationships especially among Deltophilini and Dichotomiini.

The Scarabaeinae study by VILLALBA et al. (2002) was the first to use molecular data on the group. It was based on the DNA sequences of the mitochondrial cytochrome oxidase I and II genes (COI, COII). Thirty-three species representing the seven tribes and all the genera occurring in the Iberian Peninsula were used in the analysis. VILLALBA et al. (2002) showed a contradiction to the accepted classification, with Coprini, a tunneller, placed among the rolling tribes in their phylogeny, though with poor support. This study, however, relied solely on mitochondrial genes whereas the current trend is to use both mitochondrial and nuclear genes to produce a more reliable phylogenetic tree. Mitochondrial genes are fast evolving and useful for comparisons of closely related taxa while nuclear genes are slowly evolving and suitable for comparisons of distantly related taxa (SIMON et al. 1994). Furthermore, it is desirable to use several markers of independent evolutionary history (WAHLBERG & WHEAT 2008).

OCAMPO & HAWKS (2006) used two ribosomal nuclear genes for their reconstruction of the scarabaeine phylogeny. They included genera from all tribes except Sisyphini and Gymnopleurini. Again inconsistencies were found among sister relationships of the tribes.

To date, MONAGHAN et al. (2007) have performed the most extensive molecular phylogenetic study on the Scarabaeinae using 214 species, sampled from across the world. They used three gene regions, two mitochondrial (COI and 16S) and one nuclear (28S domain 3). This study can be compared to that of PHILIPS et al. (2004) in that it showed that the relationships within the group are not as simple and clear-cut as previously postulated. They found that rolling has not evolved on a single occasion within the group and some of the most "basal tribes"

are not monophyletic. Although the phylogenetic analysis of MONAGHAN et al. (2007) used a large number of taxa from all the continents, there was little to no support for most of the deeper relationships between taxa, which did little to resolve the evolutionary questions within the Scarabaeinae.

One of the major findings in MONAGHAN et al. (2007) was the biogeographical distinction in the dung beetle lineages. Each region has endemic clades, which suggests biogeographical isolation over a long time period. SOLE & SCHOLTZ (2010) used sequences from two mitochondrial and three nuclear genes to construct a phylogeny of the African genera of the “ancestral” tribes Deltochilini and Ateuchini. They teased out the previously poorly resolved relationships among the genera of these tribes using 8 of the 9 genera of Ateuchini and 17 of 23 deltochiline genera. With evidence from recent phylogenetic studies suggesting Africa as the origin of the diversification of Scarabaeinae (PHILIPS et al. 2004; MONAGHAN et al. 2007), they also estimated divergence times for the subfamily. Neither tribe was recovered as monophyletic but three well-supported lineages were obtained. An Eocene origin was estimated for the most recent dung beetle ancestor.

It is clear that although a number of phylogenetic analyses have been undertaken using both morphological and molecular characters, different and often contradictory conclusions have been reached. Until there is consensus, the cladograms should therefore not be translated into classification or used to definitely interpret evolutionary history. In essence, the major contentious systematic issues in the Scarabaeinae can be summarised as follows: (1) the traditional classification of the Scarabaeinae into rollers and tunnellers appears to be unfounded and has little phylogenetic support (PHILIPS et al. 2004; MONAGHAN et al. 2007; ZUNINO 1985; SOLE & SCHOLTZ 2010); (2) non-monophyly of the tribes Ateuchini, Coprini, Deltochilini and Onthophagini (MONTREUIL 1998; PHILIPS et al. 2004; MONAGHAN et al. 2007) is a source of confusion leading to (3) uncertain sister-group relationships within the Scarabaeinae and (4) lack of consensus on the time of divergence of the group among morphological (PHILIPS et al. 2004) and molecular (FORGIE et al. 2006; OCAMPO & HAWKS 2006; SOLE & SCHOLTZ 2010) data sets.

The present study is aimed at reconstructing the genealogical ties between the tribes of Scarabaeinae on the African continent (excluding Madagascar) as well as estimating the time of divergence since they last shared a common ancestor. To the two tribes in the SOLE & SCHOLTZ (2010) study, we added genera from the remaining seven African tribes and, for continuity, used four (16S, CO1, 28SD2 and CAD) of the five gene regions they utilized in their study, to construct a phylogeny of Scarabaeinae in an effort to untangle its history on the continent. (The fifth gene region, 28SD3, had very few parsimony informative sites and was therefore excluded.)

The Afrotropical region represents the richest in dung beetle diversity, with more than 2000 species (SCHOLTZ et al. 2009), of which about 670 are found in southern

Africa. Here we use a large sample of more than half of the known African genera. We compared our phylogeny to that of the African taxa in the study of MONAGHAN et al. (2007) and to other recently proposed phylogenies (PHILIPS et al. 2004; FORGIE et al. 2005). Additionally, we examine evolutionary trends among the African scarabs.

## 2. Methods

### 2.1. Taxon sampling

We obtained samples of species from all the nine tribes of Scarabaeinae that occur in Africa. An attempt was made to include as many as possible of the African genera. In-group taxa include 55 out of the 105 genera found in Africa. Two species of *Aphodius* Illiger, 1798 were used as out-group taxa (MONAGHAN et al. 2007; SOLE & SCHOLTZ 2010). The choice of this taxon as a valid out-group was based on the phylogenetic study of Scarabaeoidea (BROWN & SCHOLTZ 1999) (see Table 1 for taxa details). All voucher specimens are deposited with the Scarab Research Group, University of Pretoria, South Africa.

### 2.2. Gene sampling

As our focus lay in resolving both the relationships between the genera as well as the deeper branches of the tree we used two genes from the fast-evolving mitochondrion: cytochrome oxidase subunit I (COI) and the large ribosomal subunit (16S), as well as two from the slower-evolving nucleus: a portion of the rRNA large subunit – 28S rRNA domain 2 (28SD2) – and the CPSase region of carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase (CAD). The DNA sequences generated in this study have been submitted to GenBank (see Table 1 for accession numbers). For details on genera and sequences used for the tribes Ateuchini and the majority of the Deltochilini (see SOLE & SCHOLTZ 2010). The specimens were field collected in dung or dung/carrion baited traps and then preserved in absolute ethanol. All the specimens in this study were collected in Southern Africa.

### 2.3. Molecular protocol

Fifty-five Scarabaeinae genera were available for sequencing. We used the same DNA extraction, amplification and sequencing protocols applied by MLAMBO et al. (2011). One to three individuals of each species, according to their availability, were sequenced for four gene regions COI, 16S, 28SD2 and CAD. For CAD, additional primers CD439F/CD688R and CD667F (WILD & MADISON 2008) for nested PCR were used for taxa that were difficult to amplify (all used primers are listed in Table 2).

**Table 1.** Scarabaeinae taxa studied (all from Africa) and their Genbank numbers. \* indicates specimens used in the combined data analysis.

| Taxon                      | Collecting Locality     | ID    | GenBank accession numbers |          |          |          |
|----------------------------|-------------------------|-------|---------------------------|----------|----------|----------|
|                            |                         |       | COI                       | 16S      | 28S      | CAD      |
| <b>Tribe Coprini</b>       |                         |       |                           |          |          |          |
| <i>Copris1</i>             | Utrecht                 | CCU1* | JN804613                  | JN804686 | JN804759 | —        |
| <i>Catharsius1</i>         | Utrecht                 | CJU1  | JN804618                  | —        | —        | —        |
| <i>Catharsius2</i>         | Bosbokstrand            | CFB1* | —                         | JN804687 | JN804760 | JN804572 |
| <i>Catharsius3</i>         | Bosbokstrand            | CFB2  | —                         | JN804688 | JN804761 | —        |
| <i>Catharsius4</i>         | Bosbokstrand            | CFB3  | —                         | JN804689 | JN804762 | —        |
| <i>Litocopris1</i>         | Kruger National Park    | LTC1  | JN804639                  | JN804711 | JN804781 | JN804583 |
| <i>Litocopris2</i>         | Kruger National Park    | LTC2* | JN804640                  | JN804712 | JN804782 | JN804584 |
| <i>Litocopris3</i>         | Kruger National Park    | LTC3  | JN804639                  | —        | —        | JN804585 |
| <i>Metacatharsius1</i>     | Geselskop Farm          | MEG1* | JN804647                  | JN804718 | JN804788 | JN804589 |
| <i>Metacatharsius2</i>     | Geselskop Farm          | MEG2  | JN804648                  | JN804719 | JN804789 | JN804590 |
| <i>Metacatharsius3</i>     | Geselskop Farm          | MEG3  | JN804649                  | JN804720 | JN804790 | —        |
| <i>Metacatharsius4</i>     | Geselskop Farm          | MMG1* | JN804650                  | JN804721 | JN804791 | JN804591 |
| <i>Metacatharsius5</i>     | Geselskop Farm          | MMG2  | —                         | —        | JN804792 | JN804592 |
| <b>Tribe Gymnopleurini</b> |                         |       |                           |          |          |          |
| <i>Allogymnopleurus1</i>   | Tswalu Kalahari Reserve | ALG1* | JN804607                  | JN804678 | JN804751 | JN804566 |
| <i>Allogymnopleurus2</i>   | Tswalu Kalahari Reserve | ALG2  | JN804608                  | JN804679 | JN804752 | JN804567 |
| <i>Allogymnopleurus3</i>   | Tswalu Kalahari Reserve | ALG3  | JN804609                  | JN804680 | JN804753 | —        |
| <i>Garreta1</i>            | Kruger National Park    | GRA1  | JN804630                  | JN804703 | JN804773 | JN804579 |
| <i>Garreta2</i>            | Kruger National Park    | GRA2  | JN804631                  | JN804704 | JN804774 | JN804580 |
| <i>Garreta3</i>            | Kruger National Park    | GRA3* | JN804632                  | JN804705 | JN804775 | JN804581 |
| <i>Gymnopleurus1</i>       | Kruger National Park    | GMP1  | JN804627                  | JN804700 | —        | —        |
| <i>Gymnopleurus2</i>       | Kruger National Park    | GMP2* | JN804628                  | JN804701 | JN804772 | —        |
| <i>Gymnopleurus3</i>       | Kruger National Park    | GMP3  | JN804629                  | JN804702 | —        | —        |
| <b>Tribe Onitini</b>       |                         |       |                           |          |          |          |
| <i>Anonychonitis1</i>      | Tswalu Kalahari Reserve | ANY1  | JN804610                  | JN804681 | JN804754 | JN804568 |
| <i>Anonychonitis2</i>      | Tswalu Kalahari Reserve | ANY2* | JN804611                  | JN804682 | JN804755 | JN804569 |
| <i>Anonychonitis3</i>      | Tswalu Kalahari Reserve | ANY3  | JN804612                  | JN804683 | JN804756 | —        |
| <i>Cheironitis1</i>        | Tswalu Kalahari Reserve | CHR1* | JN804616                  | JN804690 | JN804763 | JN804573 |
| <i>Cheironitis2</i>        | Tswalu Kalahari Reserve | CHR2  | JN804617                  | JN804691 | JN804764 | —        |
| <i>Heteronitis1</i>        | Tswalu Kalahari Reserve | HTN1  | JN804636                  | JN804708 | JN804778 | —        |
| <i>Heteronitis2</i>        | Tswalu Kalahari Reserve | HTN2* | JN804637                  | JN804709 | JN804779 | JN804582 |
| <i>Heteronitis3</i>        | Tswalu Kalahari Reserve | HTN3  | JN804638                  | JN804710 | JN804780 | —        |
| <i>Onitis1</i>             | Tswalu Kalahari Reserve | OTC1  | —                         | —        | JN804798 | —        |
| <i>Onitis2</i>             | Tswalu Kalahari Reserve | OTS1* | JN804656                  | —        | —        | —        |
| <i>Onitis3</i>             | Tswalu Kalahari Reserve | OTS2* | JN804657                  | JN804729 | —        | —        |
| <b>Tribe Oniticellini</b>  |                         |       |                           |          |          |          |
| <i>Cyptochirus1</i>        | Tswalu Kalahari Reserve | CYP1* | JN804620                  | JN804693 | JN804766 | JN804574 |
| <i>Euoniticellus1</i>      | Grahamstown             | ETA1* | JN804626                  | JN804699 | JN804771 | JN804578 |
| <i>Liatongus1</i>          | Tswaing Nature Reserve  | LTG1  | —                         | JN804713 | JN804783 | JN804586 |
| <i>Liatongus2</i>          | Tswaing Nature Reserve  | LTG2  | JN804642                  | JN804714 | JN804784 | JN804587 |
| <i>Liatongus3</i>          | Tswaing Nature Reserve  | LTG3* | JN804643                  | JN804715 | JN804785 | JN804588 |
| <i>Oniticellus1</i>        | Kruger National Park    | ONT1  | JN804652                  | JN804723 | JN804794 | JN804593 |
| <i>Oniticellus2</i>        | Kruger National Park    | ONT2  | JN804653                  | JN804724 | JN804795 | JN804594 |
| <i>Oniticellus3</i>        | Kruger National Park    | ONT3* | JN804654                  | JN804725 | JN804796 | JN804595 |
| <i>Tiniocellus1</i>        | Kruger National Park    | TCL1  | JN804674                  | —        | JN804819 | JN804605 |
| <i>Tiniocellus2</i>        | Kruger National Park    | TCL2* | JN804675                  | JN804746 | JN804820 | —        |
| <i>Tiniocellus3</i>        | Kruger National Park    | TCL3  | JN804676                  | JN804747 | JN804821 | —        |
| <b>Tribe Onthophagini</b>  |                         |       |                           |          |          |          |
| <i>Caccobius1</i>          | Kruger National Park    | CCB1* | JN804614                  | JN804684 | JN804757 | JN804571 |
| <i>Caccobius2</i>          | Kruger National Park    | CCB2  | JN804615                  | JN804685 | JN804758 | —        |
| <i>Digitonthophagus1</i>   | Tswalu Kalahari Reserve | DTP1  | JN804621                  | JN804694 | —        | —        |
| <i>Digitonthophagus2</i>   | Tswalu Kalahari Reserve | DTP2* | JN804622                  | JN804695 | JN804767 | —        |
| <i>Onthophagus1</i>        | Kruger National Park    | ENT1  | JN804623                  | JN804696 | JN804768 | JN804575 |
| <i>Onthophagus2</i>        | Kruger National Park    | ENT2  | JN804624                  | JN804697 | JN804769 | JN804576 |
| <i>Onthophagus3</i>        | Kruger National Park    | ENT3* | JN804625                  | JN804698 | JN804770 | JN804577 |
| <i>Hylonthophagus1</i>     | Kruger National Park    | HTA2* | JN804634                  | JN804706 | JN804776 | —        |
| <i>Hylonthophagus2</i>     | Kruger National Park    | HTA3  | JN804635                  | JN804707 | JN804777 | —        |

Table 1 continued.

| Taxon                           | Collecting Locality      | ID    | GenBank accession numbers |          |          |          |
|---------------------------------|--------------------------|-------|---------------------------|----------|----------|----------|
|                                 |                          |       | COI                       | 16S      | 28S      | CAD      |
| <b>Tribe Onthophagini</b>       |                          |       |                           |          |          |          |
| <i>Milichus1</i>                | Kruger National Park     | MCH1  | JN804645                  | JN804716 | JN804786 | —        |
| <i>Milichus2</i>                | Kruger National Park     | MCH2* | JN804646                  | JN804717 | JN804787 | —        |
| <i>Onthophagus4</i>             | Grahamstown              | OTH1* | JN804655                  | JN804728 | JN804799 | JN804596 |
| <i>Onthophagus5</i>             | Kruger National Park     | OPV1  | —                         | —        | JN804797 | —        |
| <i>Phalops1</i>                 | Kruger National Park     | PLP1* | JN804660                  | JN804732 | JN804800 | JN804599 |
| <i>Phalops2</i>                 | Kruger National Park     | PLP2  | JN804661                  | JN804733 | JN804801 | JN804600 |
| <i>Phalops3</i>                 | Kruger National Park     | PLP3  | JN804662                  | JN804734 | JN804802 | JN804601 |
| <i>Proagoderus1</i>             | Kruger National Park     | PRS1  | JN804663                  | —        | —        | JN804602 |
| <i>Proagoderus2</i>             | Kruger National Park     | PRS2  | JN982326                  | JN804735 | JN804803 | JN804603 |
| <i>Proagoderus3</i>             | Kruger National Park     | PRS3* | JN804664                  | JN804736 | JN804804 | JN804604 |
| <b>Tribe Scarabaeini</b>        |                          |       |                           |          |          |          |
| <i>Scarabaeus Scarabaeus1</i>   | Kruger National Park     | SBF1* | JN982324                  | JN804737 | JN804805 | JN819270 |
| <i>Scarabaeus Scarabaeus2</i>   | Kruger National Park     | SBF2  | JN982325                  | JN804738 | JN804806 | —        |
| <i>Scarabaeus Scarabaeus3</i>   | Kruger National Park     | SBP1* | —                         | JN804739 | JN804807 | JN819269 |
| <i>Scarabaeus Scarabaeus4</i>   | Kruger National Park     | SBZ1* | —                         | —        | JN804808 | JN819268 |
| <i>Scarabaeus Kheper1</i>       | Kruger National Park     | SKB1  | —                         | JN804740 | JN982328 | JN819271 |
| <i>Scarabaeus Kheper2</i>       | Kruger National Park     | SKB2* | JN804665                  | —        | JN804809 | —        |
| <i>Scarabaeus Kheper3</i>       | Kruger National Park     | SKB3  | JN804666                  | —        | —        | —        |
| <i>Scarabaeus Scarabaeolus1</i> | Tswalu Kalahari Reserve  | SSB1* | JN804671                  | JN804743 | JN804814 | —        |
| <i>Scarabaeus Scarabaeolus2</i> | Tswalu Kalahari Reserve  | SSB2  | JN804672                  | JN804744 | JN804815 | —        |
| <i>Scarabaeus Sceliages1</i>    | Tswalu Kalahari Reserve  | SSS1  | —                         | JN982329 | JN804816 | —        |
| <i>Scarabaeus Sceliages2</i>    | Tswalu Kalahari Reserve  | SSS2* | JN804673                  | JN804745 | JN804817 | —        |
| <i>Scarabaeus Sceliages3</i>    | Tswalu Kalahari Reserve  | SSS3  | —                         | JN982330 | JN804818 | —        |
| <i>Pachylomera1</i>             | Tswalu Kalahari Reserve  | PLF1* | JN804658                  | JN804730 | —        | JN819267 |
| <i>Pachylomera2</i>             | Tswalu Kalahari Reserve  | PLF2  | —                         | JN804731 | —        | —        |
| <i>Pachylomera3</i>             | Tswalu Kalahari Reserve  | PLF3  | JN804659                  | —        | —        | —        |
| <i>Pachysoma1</i>               | Leipoldtville            | LA08* | AY965170                  | JN804749 | JN804823 | JN804597 |
| <i>Pachysoma2</i>               | Leipoldtville            | SK04  | AY965190                  | JN804750 | JN804824 | JN804598 |
| <i>Pachysoma3</i>               | Leipoldtville            | KK04  | AY965161                  | —        | JN804825 | —        |
| <b>Tribe Sisyphini</b>          |                          |       |                           |          |          |          |
| <i>Sisyphus1</i>                | Kruger National Park     | SPF1  | JN804668                  | —        | JN804811 | —        |
| <i>Sisyphus2</i>                | Kruger National Park     | SPF2* | JN804669                  | JN804742 | JN804812 | JN819266 |
| <i>Sisyphus3</i>                | Kruger National Park     | SPF3  | JN804670                  | —        | JN804813 | —        |
| <i>Neosisyphus1</i>             | Tswalu Kalahari Reserve  | NSP1* | JN804651                  | JN804722 | JN804793 | JN819265 |
| <b>Tribe Canthonini</b>         |                          |       |                           |          |          |          |
| <i>Aliuscanthoniola1</i>        | Ntsubane Forest          | SMA1* | JN804667                  | JN804741 | JN804810 | —        |
| <i>Silvaphilus1</i>             | Riviersonderend Mountais | TIN1* | JN804677                  | JN804748 | JN804822 | JN804606 |

## 2.4. Phylogenetic analysis and dating

The sequences were viewed in Chromas version 2.0, assembled and edited in CLC Bio Main Workbench 5 (<http://www.clcbio.com>), and subsequently aligned in MAFFT version 6 (KATO & TOH 2008) using default settings. Thereafter the alignment of sequences was checked manually. The aligned data consisted of 2748 nucleotides (nt) as follows: COI mtDNA  $\approx$  748 nt / 369 parsimony informative sites (PI); 16S rRNA  $\approx$  368 nt / 230 PI, 28S D2 rRNA  $\approx$  698 nt / 263 PI and CAD  $\approx$  899 nt / 478 PI.

The phylogenetic relationships among Scarabaeinae sequences were inferred using maximum parsimony (MP) implemented in PAUP\*4.010b (SWOFFORD 2003). MrBayes version 3.1.2 (RONQUIST & HUELSENBECK 2003)

was used to infer a Bayesian phylogram (MB). Phylogenetic trees were generated for the combined gene region dataset.

For the MP analysis all characters were equally weighted and unordered and tree statistics calculated excluding uninformative sites. Heuristic searches with 10 random addition sequences were used for each of 1000 bootstrap replicates. Partitioned Bremer Support (pbs) (BAKER et al. 1998) and Partition Congruence Indices (PCI) (BROWER 2006) implemented in PAUP\* were applied using TreeRot version 3 (SORENSEN & FRANZOSA 2007) to test for congruence among the DNA sequences. Conflict was identified and the relative contributions of the different gene regions to the phylogenetic tree were determined. When there is no conflict between partitions PCI and PBS values are equal and as conflict increases,

**Table 2.** Primers used for PCR amplification.

| Locus                | Primer name and sequence (5'–3') |                                     | Reference                 |
|----------------------|----------------------------------|-------------------------------------|---------------------------|
| Cytochrome oxidase 1 | C1-J-2183                        | CAACATTTATTTTGATTTTTGG              | SIMON et al. (1994)       |
|                      | TL2-N-3014                       | TCCAATGCACTAATCTGCCATATTA           | SIMON et al. (1994)       |
| 16S rRNA             | 16Sb2                            | TTAATCCAACATCGAGG                   | VOGLER et al. (1993)      |
|                      | LR-N-13398                       | CGCCTGTTAAACAAAACAT                 | SIMON et al. (1994)       |
| 28S rRNA domain 2    | D2-3551                          | CGTGTGCTTGATAGTGCAGC                | GILLESPIE et al. (2005)   |
|                      | D2-4057                          | TCAAGACGGGTCCTGAAAGT                | GILLESPIE et al. (2005)   |
| CAD                  | 54F                              | GTNGTNTTYCARACNGGNATGGT             | MOULTON & WIEGMANN (2004) |
|                      | 680R                             | AANGCRCTCNCGNACMACYTCRTAYTC         | MOULTON & WIEGMANN (2004) |
|                      | 338F                             | ATGAARTAYGGYAATCGTGGHCAYAA          | WINTERTON et al. (2010)   |
|                      | 654R                             | TCYTTCCANCCYTYARSGATTRTC            | WINTERTON et al. (2010)   |
|                      | CD439F                           | TTCAGTGTACARTTYCAYCCHGARCAYAC       | WILD & MADDISON (2008)    |
|                      | CD668R                           | ACGACTTCATAYTCNACYTCYTTCCA          | WILD & MADDISON (2008)    |
|                      | CD688R                           | TGTATACCTAGAGGATCDACRTTYTCCATRTTRCA | WILD & MADDISON (2008)    |

PCI values decrease until they become negative (BROWER 2006).

MB analysis allows the data to be partitioned and optimal models of nucleotide substitution applied to each partition. The model of nucleotide substitution for each gene region was assessed using the Akaike Information Criteria (AIC) in MrModeltest version 3.7 (NYLANDER et al. 2004) (Table 3). Ten million generations were run with 3 cold and 1 heated chain with trees sampled every 100 generations. Posterior probabilities were calculated after discarding the initial 20% as burn-in. Two independent runs were performed for each analysis.

In addition, to take RNA secondary structure into consideration, LocARNA (SMITH et al. 2010) was used to align the 16S and 28S sequences. LocARNA is a tool that simultaneously folds and aligns RNA sequences. The program FASconCAT version 1.0 (KUCK & MEUSEMANN 2010) was used to concatenate the alignments of the RNA sequences, including information on their secondary structures, and the protein coding regions. Bayesian analyses were performed in MrBayes which can simultaneously analyze partitioned datasets using both conventional DNA models and mixed RNA/DNA substitution models.

The program Beast version 1.6.1 (DRUMMOND & RAMBAUT 2007), which uses MCMC estimation, was used to estimate ages of nodes. The majority consensus tree inferred from MrBayes was used as guidance for topology. The published rates of evolution of 0.0075 and 0.012 mutation changes per million years were used to cover the range of rates reported (SOLE & SCHOLTZ 2010; BROWER 1994; JUAN et al. 1995; FARRELL 2001; SMITH & FARRELL 2005; WIRTA et al. 2008). Lineage age was estimated under the log normal uncorrected model assuming the Yule speciation for all estimates using the GTR+I+G model (Table 3). The analyses were run for 50 million generations starting from a random tree and the results were analyzed with the programme Tracer version 1.3 (FARRELL 2001). The analyses were run twice to validate consistency of the time estimates between runs.

**Table 3.** Estimated model parameters and data characteristics for 16S, COI, 28S domain 2, CAD.

| Gene                        | COI     | 16S     | 28S     | CAD     |
|-----------------------------|---------|---------|---------|---------|
| Length (bp)                 | 748     | ≈368    | ≈698    | 934     |
| Best model                  | GTR+I+G | GTR+I+G | GTR+I+G | GTR+I+G |
| FreqA                       | 0.3064  | 0.3907  | 0.1957  | 0.4470  |
| FreqC                       | 0.1948  | 0.1250  | 0.2832  | 0.0979  |
| FreqG                       | 0.1699  | 0.0861  | 0.3083  | 0.0318  |
| FreqT                       | 0.3289  | 0.3983  | 0.2128  | 0.4233  |
| rA-C                        | 1.12739 | 0.4211  | 1.4131  | 0.6856  |
| rA-G                        | 5.1234  | 5.3172  | 2.1257  | 8.8656  |
| rA-T                        | 0.6673  | 1.5486  | 2.2605  | 0.4700  |
| rC-G                        | 1.5038  | 0.4825  | 0.5878  | 2.1632  |
| rC-T                        | 5.5907  | 1.0305  | 3.6351  | 8.2627  |
| rG-T                        | 1.0000  | 1.0000  | 1.0000  | 1.0000  |
| Pinv                        | 0.2422  | 0.1884  | 0.2822  | 0.2936  |
| alph                        | 0.6625  | 0.5663  | 0.6576  | 0.2862  |
| Parsimony informative sites | 369     | 230     | 263     | 478     |

The use of these “standard” substitution rates is controversial as researchers use different combinations of mitochondrial genes and different time scales. This is despite the fact that, for example, the arthropod substitution rate of 0.012 mutation changes per million years is based on short sequences and a small sample size of closely related species (WIRTA et al. 2008). PONS et al. (2010) point out that for deep level phylogenies age estimations using COI, the most frequently used gene region, may be erroneous, suggesting the use of NAD2, NAD4 and NAD5, which exhibit more homogenous rates among codon positions compared to COI. Without reliable fossil evidence, however, researchers continue to use these rates. Moreover COI has become a standard gene region to sequence as it is used for bar-coding (MOULTON & WIEGMANN 2004) and costs for sequencing additional gene regions may be prohibitive. For this study, the gene region COI only, was used.

### 3. Results and discussion

#### 3.1. Congruence of genes

An assessment of the relative contribution of each data partition of the tree indicated that the gene partitions COI, D2 and CAD contributed some conflict to 4 nodes each, while 16S contributed conflict to only one node (Table 4). All the 68 nodes in the parsimony phylogram had positive PCI values, which indicate congruence among the gene regions.

#### 3.2. Phylogenetic analysis

Here we present a phylogenetic analysis of the nine tribes that make up the African Scarabaeinae. Taxon sampling was comprehensive and included a number of genera that have not been used in recent analyses of the subfamily (PHILIPS et al. 2004; MONAGHAN et al. 2007). Earlier analyses made little provision for many in the African group, a number of which may have been unknown or poorly studied at the time. These include recently described genera such as *Silvaphilus* and *Aliuscanthoniola* and other poorly known genera for example *Namakwanus* and *Outenikwanus*, which are amongst some of the earliest diverging lineages.

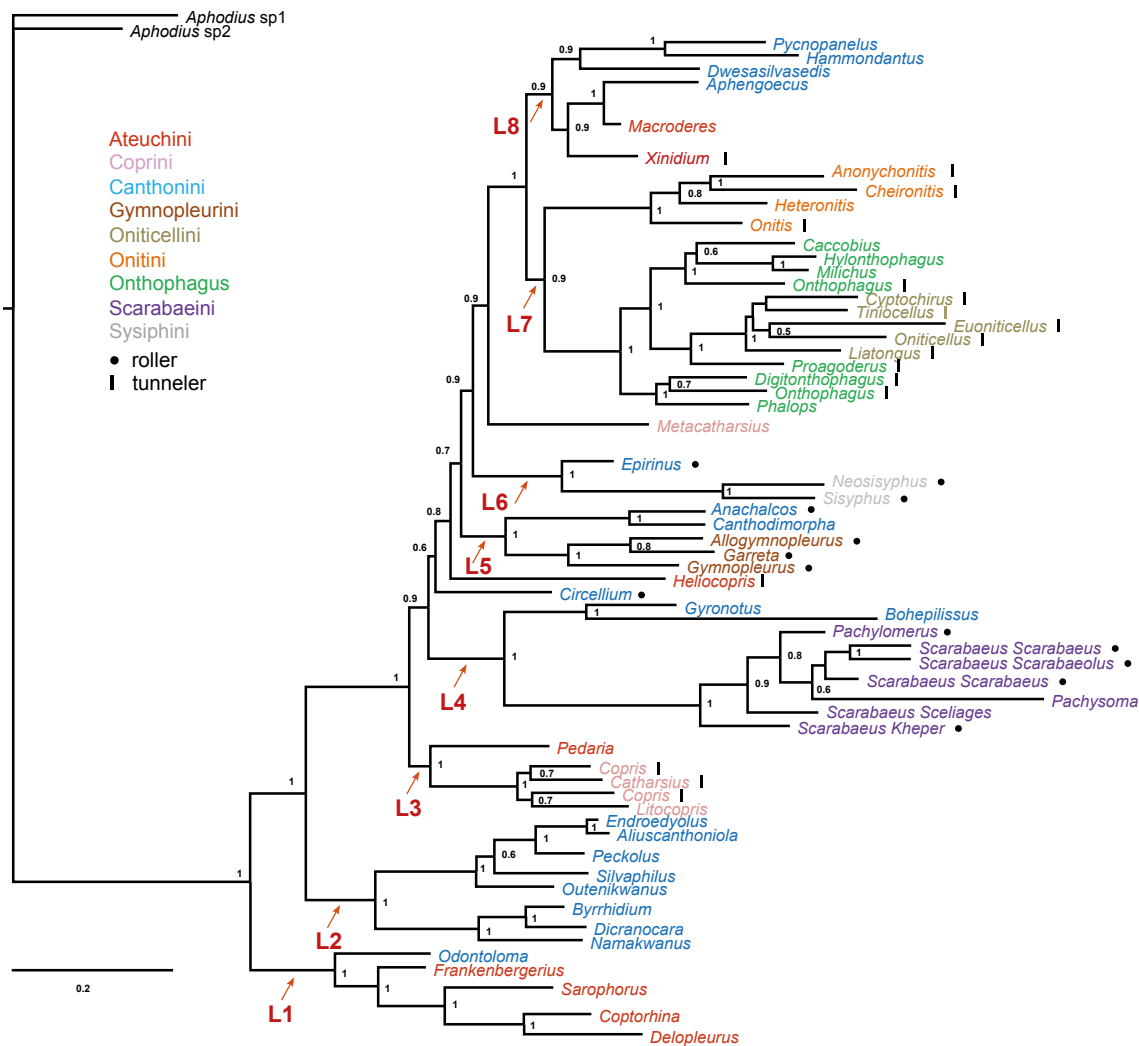
The two phylogenetic analyses (MP, MB) using conventional DNA models (MAFFT alignment) yielded trees that exhibit essentially congruent topologies with varying statistical support for tree nodes (Figs. 1, 2). The MP strict consensus of 12 most parsimonious trees is presented in Fig. 2. Tree length is 11374 and CI/RI is 0.217/0.494. In the MB analysis with RNA secondary structure taken into consideration, a tree with similar topology was returned, but support for the deeper nodes in particular, was reduced (Fig. 3).

#### 3.3. High-rank relationships

As expected from the results of previous analyses on the group, the five tribes Scarabaeini, Gymnopleurini, Sisyphini, Oniticellini and Onitini are monophyletic in all analyses. Addition of taxa compared to SOLE & SCHOLTZ (2010) did not alter the composition of Lineages 1 (L1) and 2 (L2) in Fig. 1 as these remain the same as those obtained by SOLE & SCHOLTZ (2010) and have strong support (MB posterior probability pp = 1.0, MP bootstrap bs = 99% and pp = 1.0, bs = 97% respectively). Lineage L1 comprises *Odontoloma* Boheman, 1857, *Frankenbergerius* Balthasar, 1938, *Sarophorus* Erichson, 1847, *Coptorhina* Hope, 1830, *Delopleurus* Erichson, 1857 (all are ateuchines, except *Odontoloma*). Lineage L2 contains *Namakwanus* Scholtz & Howden, 1987, *Dicranocara* Frolov & Scholtz, 2003, *Byrrhidium* Harold, 1869, *Outenikwanus* Scholtz & Howden, 1987, *Silvaphilus* Roets & Oberlander, 2010, *Peckolus* Scholtz & Howden,

**Table 4.** Bremer support indices calculated from the parsimony analysis. Node numbers correspond to those on the parsimony phylogram (Fig. 2).

| Node # | PBS values |           |          |           | Bremer (bs) | PCI   |
|--------|------------|-----------|----------|-----------|-------------|-------|
|        | pbs (COI)  | pbs (16S) | pbs (D2) | pbs (CAD) |             |       |
| 1      | 10         | 20        | 3        | 14        | 47          | 50.0  |
| 2      | 3          | 18        | 12       | 7         | 40          | 43.0  |
| 3      | 3          | 39        | 28       | 7         | 77          | 80.0  |
| 4      | -12        | 16        | -22      | 39        | 21          | 20.8  |
| 5      | 0          | 9         | 0        | 4         | 13          | 16.0  |
| 6      | -5         | 17        | -1       | 25        | 36          | 38.7  |
| 7      | 4          | 22        | 10       | 49        | 85          | 88.0  |
| 8      | 11         | 18        | 20       | 17        | 66          | 69.0  |
| 9      | 11         | 14        | 9        | 46        | 80          | 83.0  |
| 10     | 13         | 6         | 4        | 2         | 25          | 28.0  |
| 11     | 12         | 42        | 13       | 56        | 123         | 126.0 |
| 12     | 14         | 49        | 4        | 17        | 84          | 87.0  |
| 13     | 8          | 13        | 2        | 2         | 25          | 28.0  |
| 14     | -3         | 25        | -15      | 39        | 46          | 48.2  |
| 15     | -1         | 6         | -13      | 42        | 34          | 36.2  |
| 16     | 16         | 24        | 14       | 4         | 58          | 61.0  |
| 17     | 6          | 11        | 37       | -30       | 24          | 24.5  |
| 18     | 9          | 0         | 7        | 2         | 18          | 21.0  |
| 19     | 11         | 12        | 7        | 4         | 34          | 37.0  |
| 20     | 15         | 3         | 26       | 6         | 50          | 53.0  |
| 21     | 17         | 8         | 16       | 2         | 43          | 46.0  |
| 22     | 9          | 46        | 13       | 14        | 82          | 85.0  |
| 23     | 8          | 3         | 10       | -6        | 15          | 17.2  |
| 24     | 6          | 17        | 5        | 7         | 35          | 38.0  |
| 25     | 9          | 9         | 8        | 4         | 30          | 33.0  |
| 26     | 7          | 12        | 24       | 11        | 54          | 57.0  |
| 27     | 9          | 3         | 8        | 2         | 22          | 25.0  |
| 28     | 9          | 3         | 8        | 2         | 22          | 25.0  |
| 29     | 9          | 26        | 11       | 14        | 60          | 63.0  |
| 30     | 11         | -2        | 8        | 2         | 19          | 21.8  |
| 31     | 3          | 6         | 6        | 0         | 15          | 18.0  |
| 32     | 4          | 8         | 6        | 4         | 22          | 25.0  |
| 33     | 11         | 16        | 20       | 4         | 51          | 54.0  |
| 34     | 12         | 8         | 1        | 9         | 30          | 33.0  |
| 35     | 7          | 9         | 15       | 27        | 58          | 61.0  |
| 36     | 9          | 3         | 8        | 2         | 22          | 25.0  |
| 37     | 9          | 9         | 8        | 4         | 30          | 33.0  |
| 38     | 9          | 9         | 7        | 4         | 29          | 32.0  |
| 39     | 2          | 9         | 8        | 4         | 23          | 26.0  |
| 40     | 0          | 4         | 10       | 0         | 14          | 17.0  |
| 41     | 13         | 1         | 8        | 5         | 27          | 30.0  |
| 42     | 9          | 9         | 8        | 4         | 30          | 33.0  |
| 43     | 9          | 3         | 8        | 2         | 22          | 25.0  |
| 44     | 9          | 9         | 8        | 4         | 30          | 33.0  |
| 45     | 9          | 9         | 8        | 4         | 30          | 33.0  |
| 46     | 18         | 7         | 11       | -4        | 32          | 34.8  |
| 47     | 5          | 39        | 5        | 0         | 49          | 52.0  |
| 48     | 9          | 9         | 8        | 4         | 30          | 33.0  |
| 49     | 9          | 4         | 11       | 0         | 24          | 27.0  |
| 50     | 3          | 15        | 17       | 4         | 39          | 42.0  |
| 51     | 5          | 4         | 15       | 0         | 24          | 27.0  |
| 52     | 24         | 30        | 2        | 2         | 58          | 61.0  |
| 53     | 2          | 4         | 7        | 0         | 13          | 16.0  |
| 54     | 2          | 4         | 7        | 0         | 13          | 16.0  |
| 55     | 6          | 21        | 12       | 13        | 52          | 55.0  |
| 56     | 9          | 9         | 8        | 4         | 30          | 33.0  |
| 57     | 8          | 47        | 1        | 4         | 60          | 63.0  |
| 58     | 17         | 25        | 8        | 2         | 52          | 55.0  |
| 59     | 9          | 25        | 10       | 12        | 56          | 59.0  |
| 60     | 11         | 13        | 12       | 12        | 48          | 51.0  |
| 61     | 9          | 9         | 14       | 22        | 54          | 57.0  |
| 62     | 17         | 4         | 18       | 2         | 41          | 44.0  |
| 63     | 2          | 19        | 23       | 5         | 49          | 52.0  |
| 64     | 11         | 6         | 5        | 6         | 28          | 31.0  |
| 65     | 0          | 5         | 15       | 6         | 26          | 29.0  |
| 66     | 2          | 9         | 8        | 4         | 23          | 26.0  |
| 67     | 10         | 22        | 14       | -3        | 43          | 45.9  |
| 68     | 2          | 9         | 8        | 4         | 23          | 26.0  |



**Fig. 1.** Bayesian phylogram (conventional DNA models used) with posterior probabilities (where  $\geq 0.5$ ) for the nine African Scarabaeinae tribes.

1987, *Aliuscanthoniola* Deschodt & Scholtz, 2008 and *Endroedyolus* Scholtz & Howden, 1987 (all are deltochines). Lineage 2 (L2) has two well-supported lineages within it, one of which is made up of *Namakwanus*, *Dicranocara* and *Byrrhidium* while the other consists of *Outenikwanus*, *Silvaphilus*, *Peckolus*, *Aliuscanthoniola* and *Endroedyolus*. Lineage L3 consists of all of the genera from the tribe Coprini (only *Metacatharsius* Paulian, 1939 lacking) as well as the genus *Pedaria* Castelnau, 1832, traditionally classified as an ateuchine (pp = 1.0, bs = 66%). Lineage L4 includes a monophyletic Scarabaeini and the deltochiline genera *Gyronotus* van Lansberge, 1874 and *Bohepilissus* Paulian, 1975 (pp = 1.0, bs = 100%). The genera *Circellium* Latreille, 1825, a deltochiline, and *Heliocopris* Hope, 1837, an ateuchine, form two distinct separate lineages. In Lineage L5, a monophyletic Gymnopleurini is sister to two deltochiline genera, *Anachalcos* Hope, 1837 and *Canthodimorpha* Davis, Scholtz & Harrison, 1999 (pp = 1.0). Sysiphini genera *Sisyphus* Latreille, 1807 and *Neosisyphus* Müller, 1942, sister to the deltochiline genus *Epirinus* Reiche, 1841 (pp = 1.0, bs = 66%), form Lineage L6. *Metacatharsius*,

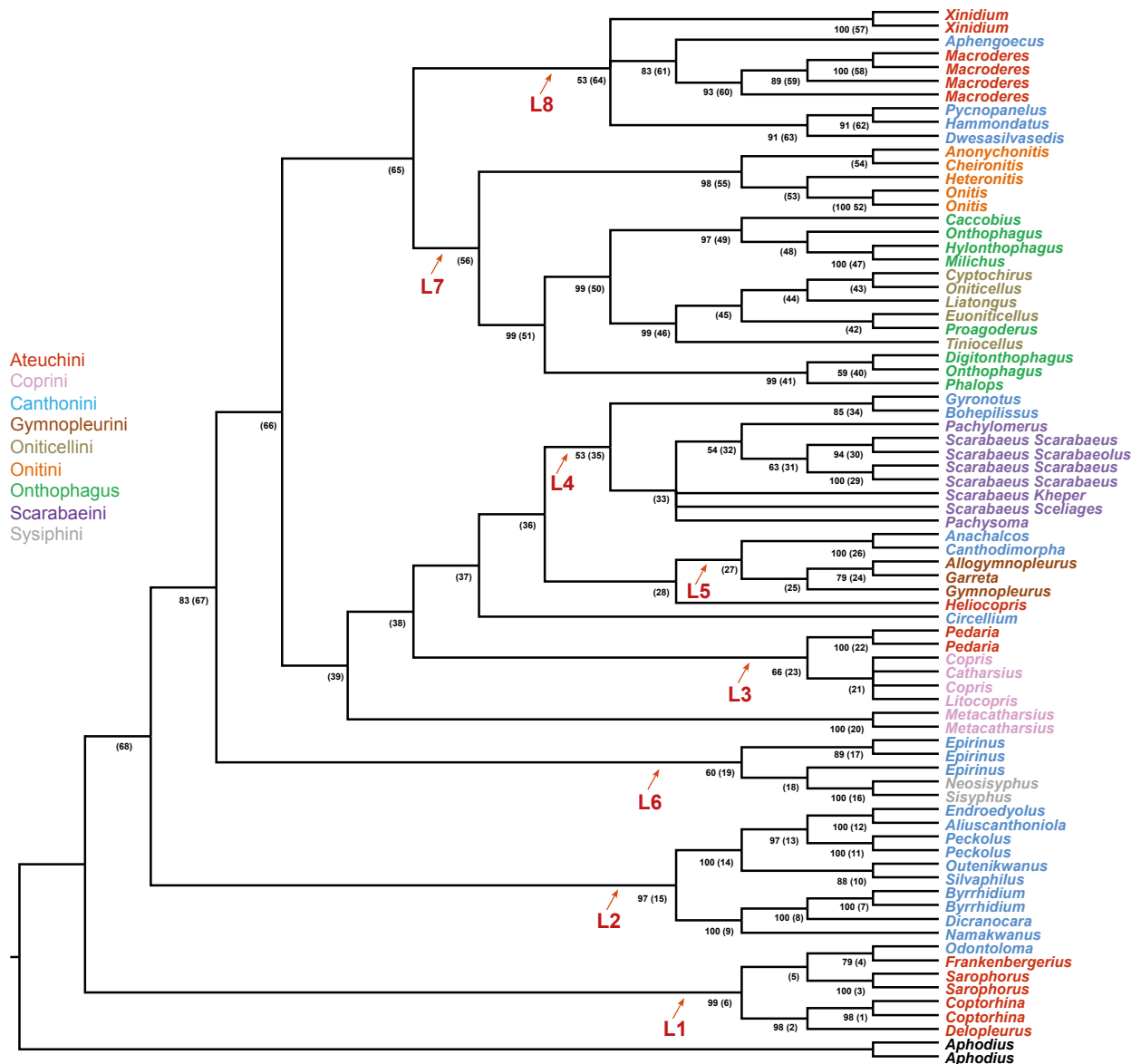
a separate lineage, is sister to all the genera in Lineages L7 and L8.

Within Lineage L7 a monophyletic Onitini (pp = 1.0, bs = 96%) is sister to Onthophagini (pp = 1.0, bs = 99%), with Oniticellini (pp = 1.0, bs = 99%) nested within it. Lineage L8 (pp = 1.0, bs = 53%) has two sister lineages within it: one contains the deltochiline genera *Pycnopanelus* Arrow, 1931, *Hammondantus* Cambefort, 1978 and *Dwesasilvasedis* Deschodt & Scholtz, 2008; in the sister lineage are *Aphengoecus* Péringuey, 1901, a deltochiline, and the two ateuchine genera *Macroderes* Westwood, 1876 and *Xinidium* Harold, 1869.

### 3.4. Estimation of divergence times

Our analysis places the earliest split in the subfamily in Africa between 42 (32/53) and 27 (20/35) MYA for the rates of 0.0075 and 0.012 substitutions /site / MY respectively (upper and lower 95% confidence intervals in brackets). Confidence intervals are wide for the slower mutation rate (0.0075). The posterior distributions of a number of pa-





**Fig. 2.** Strict consensus phylogram of 12 most parsimonious trees found for the combined dataset (two mitochondrial and two nuclear genes) with bootstrap support (where  $\geq 50\%$ ). Numbers in brackets correspond to node numbers in Table 4 for partitioned Bremer support.

rameters were poor (low ESS values); therefore, there is doubt about the validity of these age estimates. For this reason, discussion will be based on the higher evolutionary rate. In Fig. 4 are the estimates of selected lineage ages based on a BEAST analysis of COI sequence data.

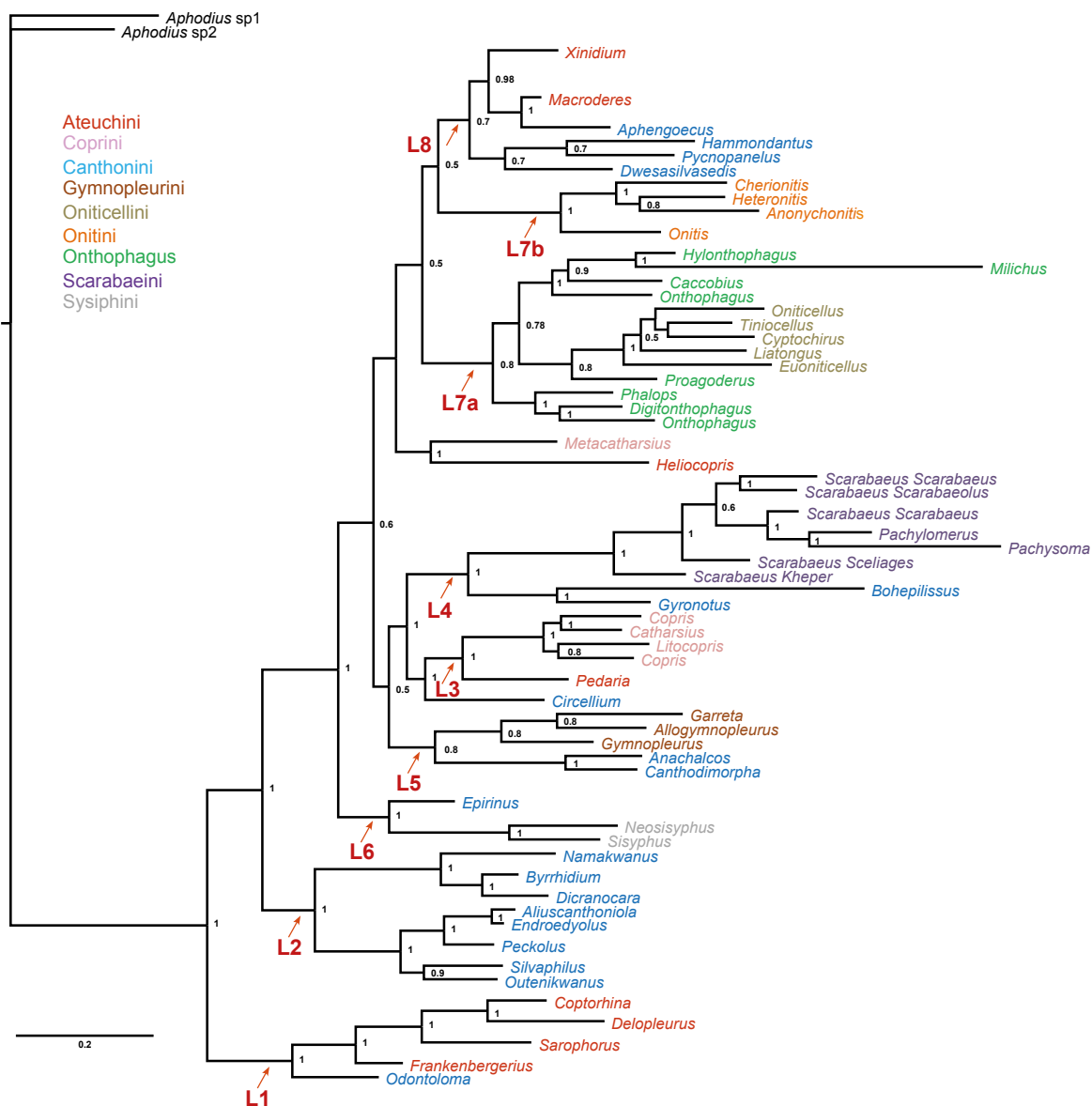
### 3.5. Phylogeny

Below we compare our phylogenetic results (Fig. 1) with those on African taxa in MONAGHAN et al. (2007), and to some extent, other recent molecular phylogenies and the morphological analysis of PHILIPS et al. (2004). Table 5 provides information on the number of species, feeding characteristics, habitats and size of taxa included in this study. Generally there is congruence between our tree and that of MONAGHAN et al. (2007) providing a reliable basis for the evolutionary analysis of different traits. Both

phylogenies support the monophyly of the tribes Scarabaeini, Sisyphini, Gymnopleurini, Onitini and Oniticellini. MONAGHAN et al. (2007) found Coprini, Ateuchini, Deltophilini and Onthophagini to be polyphyletic with three, four, four and three lineages, respectively. In the present study, the same tribes form two, five, eight and three lineages respectively.

#### 3.5.1. Gymnopleurini

There are three genera of the tribe in Africa, namely *Gymnopleurus* Illiger, 1803, *Allogymnopleurus* Janssens, 1940 and *Garreta* Janssens, 1940. In the studies of VILLALBA et al. (2002) Gymnopleurini is sister to Scarabaeini. Our analysis returns a sister relationship with the deltochiline genera *Anachalcos* and *Canthodimorpha*; this is congruent with MONAGHAN et al.'s (2007) phylogeny, which places Gymnopleurini in a clade that is sister to



**Fig. 3.** Bayesian phylogram (all data, with RNA coding regions analysed using structural models) with posterior probabilities (where  $\geq 0.5$ ) for the nine African Scarabaeinae tribes.

*Anachalcos*. The genera in this lineage (5) are all rollers except *Canthodimorpha*, whose food exploitation activities and diet are unknown. *Canthodimorpha* is a monotypic and flightless forest genus whereas *Anachalcos* is one of the few deltochilines that is fairly widely distributed. It is nocturnal and food association varies with species ranging from carrion to herbivore and omnivore dung.

MONAGHAN et al.'s (2007) phylogeny suggests divergence in the order *Gymnopleurus* then *Garreta* + *Allogymnopleurus*. Our dating analysis also suggests that *Gymnopleurus* is the earliest diverging genus among the three.

### 3.5.2. Sisyphini

This tribe consists of three genera, two of which occur in Africa. All the species in Sisyphini are rollers. In our phylogeny Sisyphini is sister to the rolling deltochiline

genus *Epirinus*. The two are in turn sister to a clade comprising the genus *Metacatharsius* and all the genera from the tribes Onthophagini, Onitini, Oniticellini and an at-euchine/deltochiline group (labeled lineage 8). A similar relationship is shown in MONAGHAN et al.'s study (2007) where *Epirinus* is sister to a clade comprising Sisyphini, Onitini, Onthophagini and Oniticellini. PHILIPS et al. (2004) found similar patterns. We found Sisyphini to be the most recently diverged tribe with an age of 12 My (7.7/16.8 My), which is in line with CAMBEFORT's (1991a) categorization of the tribe as a "modern" roller.

### 3.5.3. Scarabaeini

A number of recent phylogenetic analyses have included this tribe (PHILIPS et al. 2004; MONAGHAN et al. 2007; FORGIE et al. 2005, 2006; SOLE et al. 2011) and none has questioned its monophyly. In both MONAGHAN et al.'s (2007) and

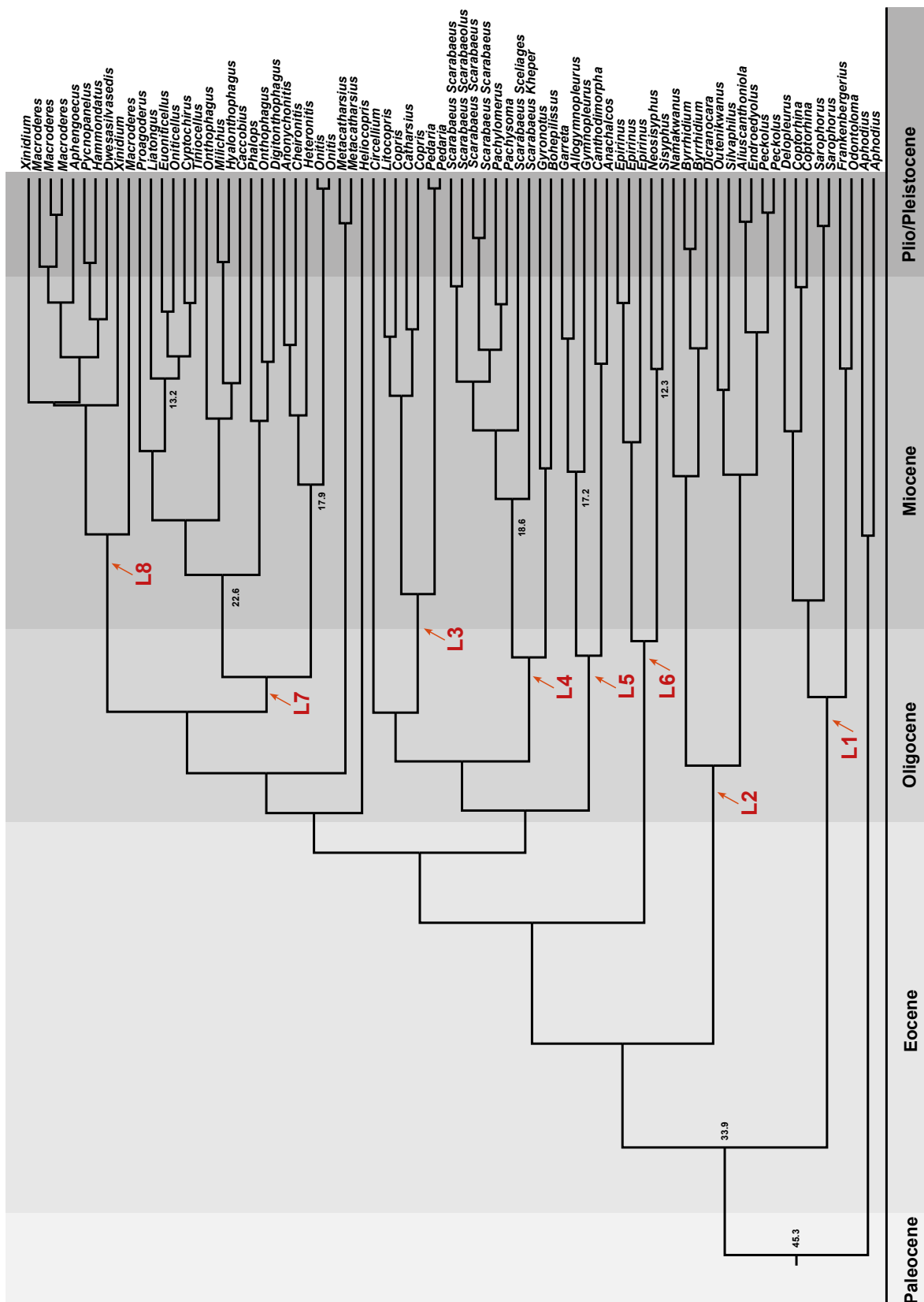


Fig. 4. Divergence times for the African Scarabaeinae based on mutation rate of 0.012 mutation changes per million years for COI.

PHILIPS et al.'s (2004) analyses Scarabaeini was sister to a clade that included the monotypic deltochiline genus *Circellium*. In our analysis the tribe formed a sister relationship with deltochiline genera *Gyronotus* and *Bohepilissus*.

The genus *Pachysoma* Macleay, 1821 has had a variable taxonomic history. It has been considered a genus (FERREIRA 1953), synonymized with *Scarabaeus* Linnaeus, 1758 (MOSTERT & HOLM 1982), considered a subgenus

of *Scarabaeus* (FORGIE et al. 2005; HARRISON & PHILIPS 2003) and currently has full generic status (FORGIE et al. 2006). SOLE et al. (2007) also supported the idea of *Pachysoma* as a genus. Interestingly, as with *Kheper* Janssens, 1940 and *Sceliages* Westwood, 1837, it groups as sister to *Scarabaeus* species in our study confirming its classification as a genus. This also raises the question about *Sceliages* and *Kheper*, once considered to be genera, to have that level re-instated.

It was originally postulated that the Scarabaeini was an old group with Madagascan representatives as old as the Mesozoic separation of the island (DAVIS et al. 2008). A recent study has estimated the African and Madagascan Scarabaeini taxa have a shared origin of between 24 and 15 mya (SOLE et al. 2007). FORGIE et al. (2005) estimated the origin of the Scarabaeini at between 23 and 9.8 mya. Both studies used 16S and COI sequence data but different analytical methods. Our estimate of their origin is approximately 17 mya (12.7/21.9 mya) falling within the estimates of both FORGIE et al. (2005) and SOLE et al. (2007).

#### 3.5.4. Onitini

The genera of Onitini form a monophyletic lineage which is sister to the Onthophagini and Oniticellini. Onitini has 17 genera that occur in Africa. All species are tunnellers and coprophagous (SCHOLTZ et al. 2009). *Onitis* and *Cheironitis* van Lansberge, 1875 are species-rich whereas the remaining genera have no more than five species each. The monophyly of Onitini has not been questioned with recent analyses, both molecular (MONAGHAN et al. 2007; VILLALBA et al. 2002) and morphological (PHILIPS et al. 2004) data, having confirmed the tribe to be sister to Onthophagini and Oniticellini. It was suggested by CAMBEFORT (1995), cited in SCHOLTZ et al. (2009), that the genera associated with non-ruminant dung diverged earlier than those associated with the dung of ruminants (in tandem with the respective herbivores). The genera here are too few, however, to draw any concrete conclusions.

#### 3.5.5. Oniticellini

Oniticellini is divided into four subtribes. The Helictopleurina are restricted to Madagascar, Eurysternina to the Neotropics whereas Drepanocerina and Oniticellina are represented in Africa. There has been much work recently to stabilize the systematics of the Afrotropical fauna in this tribe (BARBERO et al. 2009a,b; 2011). In the present study one drepanocerine genus, *Cyptochirus* Lesne, 1900 and four oniticellines *Tiniocellus* Péringuey, 1901, *Euoniticellus* Janssens, 1953, *Oniticellus* Serville, 1828 and *Liatongus* Reitter 1893, form a monophyletic group that is nested within Onthophagini. This group is sister to the genus *Proagoderus* van Lansberge, 1883, an onthophagine.

In our Bayesian analysis and that of OCAMPO & HAWKS (2006) the tribe Oniticellini was found to be a monophy-

letic group nested within Onthophagini whereas MONAGHAN et al. (2007) found both Onitini and Oniticellini to form monophyletic clades within Onthophagini. Our MP analysis returns Onthophagini paraphyletic in respect to Oniticellini; and Oniticellini paraphyletic in respect to *Proagoderus*. In their study, WIRTA et al. (2008) found both Onthophagini and Oniticellini to be paraphyletic with an Onitini species branching off within the two tribes. This instability points to a need for further examination of these relationships.

#### 3.5.6. Onthophagini

Onthophagini is the most species-rich tribe in the subfamily Scarabaeinae with more than 2500 species. The genera are widely distributed in Africa. Onthophagini is made up of mainly tunneling genera, although, some small-bodied species are kleptocoprids, relying on dung buried by other dung beetles for breeding. Examples include some *Cleptocaccobius* Cambefort, 1984, *Caccobius* Thomson, 1859 and *Hylonthophagus* Palestrini, 1989 species. Within the tribe, there is also ant or termite-association in some genera such as *Haroldius* Boucomont, 1914 (not included in the present study).

The Onthophagini in our analysis are paraphyletic. *Phalops* Erichson, 1847, *Digitonthophagus* Balthasar, 1959 and *Onthophagus* form a clade that is sister to the rest of the genera, consistent with the findings of MONAGHAN et al. (2007).

#### 3.5.7. Coprini

Coprini is a tunneling tribe, which according to its traditional constitution comprised 10 genera of which seven occur in Africa. Three of the genera in our analysis form a natural group that is sister to *Pedaria*, with *Metacatharsius*, the fourth genus, forming a distant distinct lineage. The separation of these genera was unexpected since the African *Metacatharsius* and *Catharsius* Hope, 1837 in MONAGHAN et al.'s (2007) analysis grouped together.

#### 3.5.8. Ateuchini and Deltochilini

For a long time there has been doubt concerning the monophyly of Deltochilini (PHILIPS et al. 2004; FROLOV & SCHOLTZ 2003a; SCHOLTZ & HOWDEN 1987). In addition, many of the African deltochiline genera are either monotypic or have only a few species and are therefore likely to be relicts of otherwise extinct lineages (FROLOV & SCHOLTZ 2003a). There has been similar instability within Ateuchini, that is, mis-classification due to para- or polyphyly of the tribe. The Afrotropical region has nine Ateuchini genera with 126 species. Previous phylogenetic studies have confirmed its polyphyletic nature since the first detailed study of Coprini and Ateuchini by MONTREUIL (1998).

There is congruence between our findings and those of MONAGHAN et al. (2007) and PHILIPS et al. (2004) concerning the position of *Coptorhina* + *Sarophorus* Erich-

son, 1847 being in the most basally originating lineages. *Sarophorus* shares a number of apparently synapomorphic characters with *Coptorhina* and *Delopleurus* Erichson, 1847 (FROLOV & SCHOLTZ 2003b). Our analysis and that of SOLE & SCHOLTZ (2010) concur with the relatedness of these genera; however, there is need for further molecular and morphological studies to determine the relationship between this group of four genera and *Odontoloma*, which is currently placed among the deltochilines. *Odontoloma*, a genus with an African distribution range has some characters similar to certain onthophagines. Therefore, its placement in Deltocilini was uncertain (HOWDEN & SCHOLTZ 1987).

Three genera in the earliest diverging lineage, *Coptorhina*, *Delopleurus*, and *Frankenbergerius* Balthasar, 1938, feed on and breed in fungi while most species of *Sarophorus* are known to prefer detritus (very old carrion and dung remains) (DAVIS et al. 2008). The fifth genus in the lineage, *Odontoloma* has species recorded from dung, carrion and rotting fungus (HOWDEN & SCHOLTZ 1987). CAMBEFORT (1991b) and SCHOLTZ & CHOWN (1995) hypothesized that fungus feeding was the ancestral feeding pattern of dung beetles with a later change to feeding on dung. With the beetles depending nutritionally on the microbes within the substrate, the change would have been easy (SCHOLTZ & CHOWN 1995; CAMBEFORT 1991b).

The flightless genera *Byrrhidium*, *Namakwanus* and *Dicranocara* form a distinct sub-lineage in our analysis. The close relationship among these genera alluded to by FROLOV & SCHOLTZ (2003a,b) is confirmed in our molecular study. *Byrrhidium*, *Namakwanus* and *Dicranocara* each have three species and all but one species of *Byrrhidium* are only known from deposits of dung pellets in rock hyrax (*Procapra capensis* Pallas, 1766) middens (DESCHODT et al. 2007). These genera, together with many other deltochilines, are thought to be relicts of a fauna that was once widespread but have been forced into restricted environments and trophic specializations through increasing aridity. In addition, the feeding habits of this group fall in line with what CAMBEFORT (1991a) postulated, that the ‘taxa possibly turned to the dung of primitive small mammals that were forest dwellers’ giving the example of the Afro-Oriental genus *Panelus* Lewis, 1895 in Vietnam that feeds on rat faeces.

*Peckolus*, *Endroedyolus*, *Aliuscanthoniola*, *Outenikwanus* and the newly described monotypic *Silvaphilus* (ROETS & OBERLANDER 2010), are in a clade that is sister to *Byrrhidium*, *Namakwanus* and *Dicranocara*. Our molecular analysis confirms the close relationship among these taxa that is suggested by general morphology (ROETS & OBERLANDER 2010). These are all small (less than 5 mm) flightless forest endemics (DAVIS et al. 2008; DESCHODT et al. 2007; ROETS & OBERLANDER 2010). Our analysis and that of SOLE & SCHOLTZ (2010) validate the need for a new classification that will re-categorize these two monophyletic groups.

*Pycnopenelus* and *Hammondantus*, positioned in lineage 8, doubtfully belong to the tribe Deltocilini, and share a number of morphological characters with

Onthophagini (DAVIS et al. 2008). However, there is no implication of such a relationship in our analysis. Whereas the two genera are found in southwestern arid areas, they appear closely related to *Dwesasilvasedis*, a flightless forest endemic. This is an example of the diversity that occurs among closely related genera expressed by MONAGHAN et al. (2007). The other deltochiline in this lineage is *Aphengoecus*, a coastal Sandveld genus. It comprises two flightless species whose habitat is deep coastal sands and mountains. *Aphengoecus* appears closely related to *Macroderes*, an ateuchine. The genus *Xinidium* appears as a monophyletic genus also in the same lineage. It is also an ateuchine.

### 3.6. Evolution of dung-feeding

The food of the ancestral Scarabaeidae beetle has been proposed to have been fungus or fungus-enriched detritus but that a switch to mammal dung when the latter group started to radiate during the early Tertiary was one of the biological adaptations that contributed largely to the success of the group. In both Scarabaeinae and the sister group Aphodiinae, most species are coprophagous and breed in the dung of herbivorous animals but some are saprophagous in the adult stage, feeding on decaying and dead plant material. Some species are adapted for “hard saprophagy” and use hard organic substances such as leaf litter, dead wood, mushrooms and spores whereas the species adapted for “soft saprophagy” use liquid and semiliquid components of decaying vegetation such as vegetable juices, dissolved albuminous substances and/or bacterial albumens in decaying humus (STEBNICKA 2001). The coprophages are adapted to “soft saprophagy” feeding on the digestive juices, albuminous substances, fats, carbohydrates, mineral salts, vitamins and bacterial albumens within the dung. Essentially dung is the excreted food remains of animals made up of a mixture of physical, chemical and microbial components, whose nature is determined by the original food. While adults were shown in experiments by HOLTER et al. (2002) to utilize the nutritious liquid component, larvae feed on the fibrous fragments which consist mostly of cellulose (HALFFTER & MATTHEWS 1966).

Consequently, dung-feeding is considered to be a synapomorphy for the modern groups, with deviations such as feeding on vertebrate and invertebrate carrion and plant detritus, as is found in many groups, thought to have evolved secondarily. This assumption was implicitly accepted by the traditional arrangement of the groups into ancestral dung ball rollers (considered to be members of the tribe Deltocilini) and one of ancestral tunnellers (Dichotomiini, now Ateuchini). All other groups were assumed to have radiated from one of these groups. This presupposed that all extant dung beetle groups have ancestral dung-feeding in common in spite of some early papers (ZUNINO 1983) that pointed out that the genus *Coptorhina* is both basal and a fungus-feeder. The question that was never actually addressed, although it was

**Table 5.** Selected characteristics of the African Scarabaeinae taxa used in this study.

| <b>Taxon</b> (number of known species in brackets) | <b>Habitat</b>                     | <b>Size range</b> (mm) | <b>Food type/relocation</b>   | <b>Diel activity</b> |
|--|------------------------------------|------------------------|---|----------------------|
| <b>Tribe Canthonini</b>                            |                                    |                        |   |                      |
| <i>Aliuscanthoniola</i> (1)                        | Leaf litter in dense forest        | 2.5–2.6                | Unknown, trapped with pig dung  | unknown              |
| <i>Anachalcos</i> (9)                              | Forest to savanna                  | 14.5–38.0              | Moist dung  | nocturnal            |
| <i>Aphengoecus</i> (2)                             | Open scrubland                     | 4.1–5.9                | Dung, decomposing material  | unknown              |
| <i>Bohepilissus</i> (2)                            | Forest                             | 1.7–3.1                | Probably dung and decomposing material  | unknown              |
| <i>Byrrhidium</i> (3)                              | Succulent karoo to desert          | 10.5–13.0              | Herbivore and omnivore dung, hyrax pellets                                    | unknown              |
| <i>Canthodimorpha</i> (1)                          | Coastal dune forest                | 14.6–15.1              | Unknown   | unknown              |
| <i>Circellium</i> (1)                              | Dense shrubland                    | 22.0–50.0              | Rollers / moist dung  | diurnal              |
| <i>Dicranocara</i> (3)                             | Desert                             | 9.0–12.0               | Hyrax pellets, pick up with front legs and walk backwards                     | nocturnal            |
| <i>Dwesasilvasedis</i> (1)                         | Forest                             | 4.2–4.8                | Dung, decomposing material  | unknown              |
| <i>Endroedyolus</i> (1)                            | Forest                             | 3.0–4.0                | Probably dung and decomposing material  | unknown              |
| <i>Epirinus</i> (29)                               | Forest / savanna / grassland       | 3.0–13.5               | Carrion, herbivore and omnivore dung, probably decomposing material / rollers | diurnal              |
| <i>Gyronotus</i> (6)                               | Forest                             | 11.0–18.0              | Moist dung  | likely diurnal       |
| <i>Hammondantus</i> (1)                            | Desert                             | 5.0–6.0                | Kleptoparasites of <i>Pachysoma</i> nest                                      | diurnal              |
| <i>Namakwanus</i> (3)                              | Desert                             | 7.0–9.0                | Hyrax pellets   | unknown              |
| <i>Odontoloma</i> (20)                             | Forest / savanna / grassland       | 1.9–3.9                | Carrion, herbivore and omnivore dung  | diurnal              |
| <i>Outenikwanus</i> (1)                            | Forest                             | 1.6–2.1                | Probably dung and decomposing material  | unknown              |
| <i>Peckolus</i> (2)                                | Forest                             | 1.8–2.3                | Probably dung and decomposing material  | unknown              |
| <i>Pycnoperanus</i> (3)                            | Savanna                            | 3.0–4.5                | Dung  | diurnal              |
| <i>Silvaphilus</i> (1)                             | Forest                             | 4.2                    | Herbivore dung pellets  | unknown              |
| <b>Tribe Ateuchini</b>                             |                                    |                        |   |                      |
| <i>Coptorhina</i> (15)                             | Savanna to grassland               | 8.2–20.7               | Mushrooms   | diurnal              |
| <i>Delopleurus</i> (3)                             | Savanna to grassland               | 5.4                    | Mushrooms   | unknown              |
| <i>Frankenbergerius</i> (7)                        | Savanna to forest                  | 4.5–16.0               | Mushroom and dung   | diurnal              |
| <i>Heliocopris</i> (49)                            | Savanna to grassland               | 26.5–65.3              | Dung, tunnellers  | nocturnal            |
| <i>Macroderes</i> (14)                             | Succulent karoo                    | 7.5–13.0               | Dung, carry dung pieces with front legs and move backwards to burrows         | nocturnal            |
| <i>Pedaria</i> (56)                                | Savanna / forest / grassland       | 5.2–10.0               | Kleptoparasites of <i>Heliocopris</i> nest / dung                             | nocturnal            |
| <i>Sarophorus</i> (10)                             | Shrubland                          | 6.0–10.0               | Very old dung / carrion remains   | diurnal              |
| <i>Xinidium</i> (3)                                | Grassland to forest                | 8.0–13.0               | Dung, tunnellers  | nocturnal            |
| <b>Tribe Coprini</b>                               |                                    |                        |   |                      |
| <i>Copris</i> (105)                                | Forest, savanna, grasslands        | 8.5–27.3               | Omnivore / herbivore dung, tunnellers   | diurnal / nocturnal  |
| <i>Catharsius</i> (80)                             | Forest, savanna, grasslands        | 13.1–44.6              | Dung / tunnellers   | nocturnal            |
| <i>Metacatharsius</i> (62)                         | Arid and moist savannas            | 4.8–15.2               | Herbivore / omnivore dung   | nocturnal            |
| <i>Litocopris</i> (4)                              | Savanna, grasslands                | 10.3–13.6              | Old dung  | unknown              |
| <b>Tribe Onitini</b>                               |                                    |                        |   |                      |
| <i>Onitis</i> (130)                                | Forest, savanna, grasslands        | 12.0–24.9              | Dung, tunnellers  | diurnal / nocturnal  |
| <i>Heteronitis</i> (5)                             | Savanna                            | 24.9–38.8              | Elephant dung, tunnellers   | diurnal / nocturnal  |
| <i>Cheironitis</i> (10)                            | Savanna, grasslands                | 10.2–16.5              | Coarse-fibred dung, tunnellers  | diurnal              |
| <i>Anonychonitis</i> (1)                           | Savanna                            | 14.7–17.0              | Coarse-fibred dung, tunnellers  | unknown              |
| <b>Tribe Oniticellini</b>                          |                                    |                        |   |                      |
| <i>Oniticellus</i> (5)                             | Forest, savanna                    | 6.5–14.7               | Tunnellers / endocoprid; older, drier dung                                    | diurnal              |
| <i>Cyptochirus</i> (4)                             | Savanna, grasslands                | 8.3–10.4               | Herbivore dung, tunnellers  | diurnal              |
| <i>Tiniocellus</i> (4)                             | Savanna                            | 4.9–7.6                | Tunnellers  | diurnal              |
| <i>Euoniticellus</i> (15)                          | Savanna, grasslands                | 3.0–14.0               | Tunnellers / very fresh dung  | diurnal              |
| <i>Liatongus</i> (17)                              | Forest, grassland                  | 7.4–10.9               | Tunnellers / older, drier dung  | diurnal              |
| <b>Tribe Onthophagini</b>                          |                                    |                        |   |                      |
| <i>Onthophagus</i> (970)                           | Forest, savanna, grasslands        | 2.5–11.0               | Carrion, dung, fruit, fungi / tunnellers, some kleptocoprids                  | diurnal / nocturnal  |
| <i>Caccobius</i> (55)                              | Forest, savanna, grasslands        | 2.5–8.5                | Endocoprid / kleptocoprids  | diurnal / nocturnal  |
| <i>Hylonthophagus</i> (10)                         | Savanna                            | 6.8–10.3               | Endocoprid / kleptocoprids  | diurnal              |
| <i>Euonthophagus</i> (16)                          | Savanna, grasslands                | 5.7–9.6                | Herbivore dung / tunnellers   | dusk                 |
| <i>Milichus</i> (15)                               | Forest, savanna                    | 5.9–9.5                | Herbivore dung  | nocturnal            |
| <i>Digitonthophagus</i> (1)                        | Arid / moist savannas              | 12.1–14.4              | Herbivore dung, tunnellers  | nocturnal            |
| <i>Proagoderus</i> (107)                           | Savanna / grassland                | 10.0–22.0              | Omnivore dung / tunnellers  | diurnal              |
| <i>Phalops</i> (31)                                | Arid / moist savannas              | 6.6–13.0               | Herbivore dung / tunnellers   | diurnal              |
| <b>Tribe Scarabaeini</b>                           |                                    |                        |   |                      |
| <i>Scarabaeus</i> (110)                            | Arid / grassland / forest / desert | 7.3–48.5               | Dung / millipedes / rollers   | diurnal / nocturnal  |
| <i>Pachylomera</i> (2)                             | Arid to moist savanna              | 23.4–44.7              | Herbivore dung / rollers  | diurnal              |

Table 5 continued.

| Taxon (number of known species in brackets) | Habitat                      | Size range (mm) | Food type/relocation   | Diel activity |
|---|------------------------------|-----------------|--|---------------|
| <b>Tribe Scarabaeini (cont.)</b>            |                              |                 |  |               |
| <i>Pachysoma</i> (13)                       | Desert                       | 15.0–36.3       | Dry dung pellets / plant detritus, held by hind legs and dragged forward | diurnal       |
| <b>Tribe Sisyphini</b>                      |                              |                 |  |               |
| <i>Sisyphus</i> (20)                        | Forest / grassland / savanna | 4.0–11.6        | Omnivore / ruminant herbivore / rollers                                  | diurnal       |
| <i>Neosisyphus</i> (22)                     | Savanna                      | 6.7–14.2        | Omnivore / ruminant, elephant / rollers                                  | diurnal       |
| <b>Tribe Gymnopleurini</b>                  |                              |                 |  |               |
| <i>Gymnopleurus</i> (33)                    | Savanna                      | 7.8–14.0        | Dung / rollers   | diurnal       |
| <i>Allogymnopleurus</i> (20)                | Grassland / savanna          | 8.1–18.2        | Dung / rollers   | diurnal       |
| <i>Garreta</i> (11)                         | Forest / grassland / savanna | 10.7–24.0       | Dung / rollers   | diurnal       |

referred to by several workers, was whether *Coptorhina* is on a lineage of beetles that never developed dung-feeding habits, or whether it is a reversal to fungus-feeding from a dung-feeding ancestor. The latter hypothesis was the preferred one. We believe, however, that the overwhelming phylogenetic evidence now available unequivocally points to *Coptorhina* and its relatives, which are also fungus-feeders, being relictual members of a lineage that never deviated from their ancestral fungus-feeding habits.

### 3.7. Food and its relocation

One of the reasons for the success of dung beetles is the evolution of specialized ways of relocating dung. The two most well-known methods, rolling and tunneling, facilitate its quick removal from competition (HANSKI & CAMBEFORT 1991). Some dung beetles may be dwellers and others kleptocoprids (DOUBE 1990). There is much evidence for the plasticity of this trait alluded to in previous studies and dung beetles, although many have particular preferences, appear to be opportunists that use whatever dung type is available with a suitable relocation method associated with the habitat. Examples are the forward dragging of detritus and dry dung pellets on sand in *Pachysoma* (SCHOLTZ 1989), and carrying of pieces of dung by the “tunneller” genus *Macroderes* (DAVIS et al. 2008). This trait varies widely within tribes, showing no traceable pattern.

### 3.8. Loss of flight

In the African Scarabaeinae there has been loss of flight on a number of occasions. In some genera there are both flightless and flight capable species. Examples are the deltochiline genera *Epirinus* (SCHOLTZ & HOWDEN 1987; MEDINA & SCHOLTZ 2005; MLAMBO et al. 2011) and *Odontoloma* (DAVIS et al. 2008) as well as the scarabaeine genus *Scarabaeus* (FORGIE et al. 2005, 2006). There is no record of wing polymorphism within species in dung beetles but the condition is suspected in *Scarabaeus [Mnematidium] multidentatus*, which is fully winged but has morphological characters associated with flightless-

ness (FORGIE et al. 2005). It is hypothesized that adult females may resort to a flightless phase for greater reproductive potential.

Changes in a habitat or habitat isolation are generally thought to be the main factors that lead to flightlessness. The desert habitat likely drove the loss of flight in the earliest diverging flightless lineage comprising *Byrrhidium*, *Namakwanus* and *Dicranocara*. We also propose that a change in feeding habit from the assumed primitive saprophagy to coprophagy also contributed. IKEDA et al. (2008) suggest that a change in life-history such as food habit is a complex process involving other traits, hence, other evolutionary processes independent of change in habitat or isolation may result in flight loss.

### 3.9. Biogeography

The dates for the split of Scarabaeinae from Aphodiinae estimated in this study are consistent with those of SOLE & SCHOLTZ (2010) placing the event in the Paleocene era. This compares well with the dates calibrated by SCHOLTZ et al. (2009) for the phylogeny of MONAGHAN et al. (2007) but is contrary to the Mesozoic estimate from fossil evidence (KRELL 2006). MONTREUIL et al. (2010) describe a dung beetle fossil from lower Eocene amber but the group’s minimal age is not discussed. Dung beetle distribution is influenced by two ecological factors, namely climate and the number of dung types (DAVIS et al. 2002). SOLE & SCHOLTZ (2010) discuss the conditions that have led to the current distribution of various dung beetle groups on the continent.

While it was believed that the Scarabaeinae had a Gondwanaland origin (CAMBEFORT 1991a; DAVIS et al. 2002), MONAGHAN et al. (2007) in their analysis of the global fauna suggested that basal dichotomies in Africa gave rise to Scarabaeinae lineages in this and all other regions. Moreover, they asserted that most lineages in their phylogeny were of African origin. The present study however, cannot contribute to testing the hypothesis of an African origin as only African taxa are included.

Among the earliest diverging Scarabaeinae, the African taxa are sister to the rest (MONAGHAN et al. 2007). For the tribe Scarabaeini MONAGHAN et al. (2007) had only African species whereas the phylogenies of FORGIE

et al. (2005) and SOLE et al. (2011) included Oriental and Malagasy taxa. In these latter studies, the phylogenetic relationships of taxa together with their geographic distributions suggest that basal dichotomies took place in Africa. For the tribe Gymnopleurini, *Gymnopleurus* appears to be the earliest diverging genus (MONAGHAN et al. 2007). This genus has an Afrotropical and Palearctic distribution pattern. A similar situation is true for earliest diverging genera in Oniticellini. Helictopleurina, a subtribe of Oniticellini that is endemic to Madagascar, is thought to have colonized Madagascar from an African origin (WIRTA et al. 2008). MONAGHAN et al. (2007) found Helictopleurina most closely related to species from the Afrotropical-Oriental and Palearctic regions. BARBERO et al. (2009b) hypothesize that the ancestor of *Eodrepanus* Barbero, Palestrini & Roggero, 2009 in the subtribe Drepanocerina, was from the Afrotropical-Oriental region.

Among the onthophagine species sampled by MONAGHAN et al. (2007) the South African species *Phalops ardea* Klug, 1855 and *Digitonthophagus gazella* (Fabricius, 1787) appear to have diverged earliest, while TARASOV & SOLODOVNIKOV (2011) proposed an Afrotropical-Oriental ancestor for the Onthophagini they analyzed morphologically. The largest and most widespread genus among the Scarabaeinae, *Onthophagus* (DAVIS et al. 2008), was shown by EMLEN et al. (2005) to be of African origin.

All this evidence suggests Africa as the major diversification region for Scarabaeinae with subsequent dispersal to other biogeographical regions.

## 4. Conclusions

Some of the relative ages for the different tribes that were proposed by CAMBEFORT (1991a) have proven to be inaccurate. Among the tunnellers for example, the divergence of “intermediate” Onitini is more recent than that of Coprini, Onthophagini and Oniticellini. Moreover, although some of the genera in Ateuchini and Deltochilini are indeed “old”, the divergence estimates indicate that the terminal taxa in these tribes have diverged as recently as the tribes Onthophagini and Oniticellini. In addition, these taxa are very distantly related to the rest of the genera in their current tribes. We suggest morphological re-examination of these taxa with the use of novel characters and the assignment to a new tribe(s). SCOTLAND et al. (2003) suggest rigorous examination of few morphological characters in the framework of molecular phylogenies as the best way to integrate the strengths of molecular and morphological data. We recommend the same treatment for apparently oddly placed taxa such as *Pedaria* which is superficially similar to *Sarophorus* (FROLOV & SCHOLTZ 2003b) and *Circellium* and *Metacatharsius* with possible tribal reassignment. The enigmatic genus *Circellium* has no close relatives and has been placed in Scarabaeini in the past but is currently placed in the tribe Deltochilini

(DAVIS et al. 2008). The placement of this genus in our analysis has low support and therefore remains uncertain. Another challenge also remains to combine the results from this molecular study with morphological data to consolidate African dung beetle systematics. The findings, together with those from other biogeographical regions can then be used to gain a better understanding of worldwide dung beetle relationships.

## 5. Acknowledgements

We are grateful to all members of the Scarab Research Group for sample collection. Anonymous reviewers and the editor are thanked for their valuable comments. Funding: An NRF grant was awarded to CHS.

## 6. References

- BAKER R.H., YU X., DESALLE R. 1998. Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. – *Molecular Phylogenetics and Evolution* **9**: 427–436.
- BALTHASAR V. 1963. Monographie der Scarabaeidae und Aphodidae der Palaearktischen und Orientalischen Region (Coleoptera: Lamellicornia). Vols 1–3. – Verlag Tschechoslowakische Akademie der Wissenschaft, Prague.
- BARBERO E., PALESTRINI C., ROGGERO A. 2009a. Taxonomic study of *Drepanellus* gen. n. (Coleoptera, Scarabaeidae, Oniticellini), with the description of two new species. – *Deutsche Entomologische Zeitschrift* **56**: 251–265.
- BARBERO E., PALESTRINI C., ROGGERO A. 2009b. Systematics and phylogeny of *Eodrepanus*, a new Drepanocerine genus, with comments on biogeographical data (Coleoptera: Scarabaeidae: Oniticellini). – *Journal of Natural History* **43**: 1835–1878.
- BARBERO E., PALESTRINI C., ROGGERO A. 2011. *Tibiodrepanus tagliaferrii* – a new Afrotropical Drepanocerina species (Coleoptera: Scarabaeidae: Oniticellini), with notes on phylogeny and distribution of the genus. – *Zootaxa* **2923**: 27–47.
- BOUCHARD P., BOUSQUET Y., DAVIES A.E., ALONSO-ZARAZAGA M.A., LAWRENCE J.F., LYAL C.H.C., NEWTON A.F., REID C.A.M., SCHMITT M., ŚLIPIŃSKI S.A., SMITH A.B.T. 2011. Family-group names in Coleoptera (Insecta). – *ZooKeys* **88**: 1–972.
- BROWER A.V.Z. 2006. The how and why of branch support and partitioned branch support, with a new index to assess partition incongruence. – *Cladistics* **22**: 378–386.
- BROWER A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. – *Proceedings of the National Academy of Sciences of the USA* **91**: 6491–6495.
- BROWN J., SCHOLTZ C.H., JANEAU J.-L., GRELLIER S., PODWOJEWSKI P. 2010. Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. – *Applied Soil Ecology* **46**: 9–16.



- BROWNE J., SCHOLTZ C.H. 1998. Evolution of the scarab hindwing articulation and wing base: a contribution toward the phylogeny of the Scarabaeidae (Scarabaeoidea: Coleoptera). – *Systematic Entomology* **23**: 307–326.
- BROWNE J., SCHOLTZ C.H. 1999. A phylogeny of the families of Scarabaeoidea (Coleoptera). – *Systematic Entomology* **24**: 51–84.
- CAMBEFORT Y. 1991a. Biogeography and evolution. Pp 51–67 in: HANSKI I., CAMBEFORT Y. (eds), *Dung Beetle Ecology*. – Princeton University Press, NJ, USA.
- CAMBEFORT Y. 1991b. From saprophagy to coprophagy. Pp 22–35 in: HANSKI I., CAMBEFORT Y. (eds), *Dung Beetle Ecology*. – Princeton University Press, NJ, USA.
- DAVIS A.L.V., FROLOV A.V., SCHOLTZ C.H. 2008. The African Dung Beetle Genera. – Protea Book House, Pretoria.
- DAVIS A.L.V., SCHOLTZ C.H., PHILIPS T.K. 2002. Historical biogeography of scarabaeine dung beetles. – *Journal of Biogeography* **29**: 1217–1256.
- DAVIS A.L.V., SCHOLTZ C.H. 2001. Historical vs. ecological factors influencing global patterns of scarabaeine dung beetle diversity. – *Diversity and Distribution* **7**: 161–174.
- DESCHODT C.M., SCHOLTZ C.H. 2008. Systematics of South African forest-endemic dung beetles: new genera and species of small Canthonini (Scarabaeidae: Scarabaeinae). – *African Entomology* **16**: 91–106.
- DESCHODT C.M., KRYGER U., SCHOLTZ C.H. 2007. New taxa of relictual Canthonini dung beetles (Scarabaeidae: Scarabaeinae) utilizing rock hyrax middens as refuges in south-western Africa. – *Insect Systematics & Evolution* **38**: 361–376.
- DOUBE B.M. 1990. A functional classification for analysis of the structure of dung beetle assemblages. – *Ecological Entomology* **15**: 371–383.
- DRUMMOND A.J., RAMBAUT A. 2007. Beast: Bayesian Evolutionary Analysis Sampling Trees. – *BMC Evolutionary Biology* **7**: 214.
- EMLÉN D.J., MARANGELO I., BELL B., CUNNINGHAM C.W. 2005. Diversity in the weapons of sexual selection: horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). – *Evolution* **59**: 1060–1084.
- FARRELL B.D. 2001. Evolutionary assembly of the Milkweed Fauna: cytochrome oxidase I and the age of *Tetraopes* beetles. – *Molecular Phylogenetics & Evolution* **18**: 467–478.
- FERREIRA M.C. 1953. Monografia dos escarabaeios da Afirca do Sul. Tribo-Scarabaeini. I Parte Sub-tribo Pachysomides. – *Boletim da Sociedade de Estudos da Provincia de Mocambique* **23**: 1–85.
- FORGIE S.A., KRYGER U., BLOOMER P., SCHOLTZ C.H. 2006. Evolutionary relationships among the Scarabaeini (Coleoptera: Scarabaeidae) based on combined molecular and morphological data. – *Molecular Phylogenetics & Evolution* **40**: 662–678.
- FORGIE S.A., PHILIPS K., SCHOLTZ C.H. 2005. Evolution of Scarabaeini (Scarabaeidae: Scarabaeinae). – *Systematic Entomology* **30**: 60–96.
- FROLOV A.V., AKHMETOVA L.A., SCHOLTZ C.H. 2008. Revision of the obligate mushroom-feeding African “dung beetle” genus *Coptorhina* Hope (Coleoptera: Scarabaeidae: Scarabaeinae). – *Journal of Natural History* **42**: 1477–1508.
- FROLOV A.V., SCHOLTZ C.H. 2003. A new genus and species of dung beetle from southern Namibia (Coleoptera: Scarabaeinae). – *African Entomology* **11**: 297–299.
- FROLOV A.V., SCHOLTZ C.H. 2003. Revision of the Afrotropical dung beetle genus *Sarophorus* Erichson (Coleoptera: Scarabaeidae). – *African Entomology* **11**: 183–198.
- GILLESPIE J.J., MUNRO J.B., HERATY J.M., YODER M.J., OWEN A.K., CARMICHAEL A.E. 2005. A secondary structural model of the 28S rRNA expansion segments D2 and D3 for chalcidoid wasps (Hymenoptera: Chalcidoidea). – *Molecular Biology & Evolution* **22**: 1593–1608.
- HALFFTER G., MATTHEWS E.G. 1966. The natural history of dung beetles of the subfamily Scarabaeinae (Coleoptera: Scarabaeidae). – *Folia Entomologica Mexicana* **12–14**: 1–132.
- HANSKI I., CAMBEFORT Y. (eds) 1991. *Dung Beetle Ecology*. – Princeton University Press, NJ, USA.
- HARRISON J. DU G., PHILIPS T.K. 2003. Phylogeny of *Scarabaeus* (*Pachysoma* MacLeay) stat. nov., and related flightless Scarabaeini (Scarabaeidae: Scarabaeinae). – *Annals of the Transvaal Museum* **40**: 47–71.
- HOLTER P., SCHOLTZ C.H., WARDHAUGH K.G. 2002. Dung feeding in scarabaeines (tunnelers and endocoprids): even large dung beetles eat small particles. – *Ecological Entomology* **27**: 169–176.
- HOWDEN H.F., SCHOLTZ C.H. 1987. A revision of the Africa genus *Odontoloma* Boheman (Coleoptera: Scarabaeidae: Scarabaeinae). – *Journal of the Entomological Society of Southern Africa* **50**: 155–192.
- IKEDA H., TAKASHI K., KOHEI K., TOSHIO A. 2008. Evolutionary relationships among food habit, loss of flight, and reproductive traits: Life-history evolution in the Silphinae (Coleoptera: Silphidae). – *Evolution* **62**: 2065–2079.
- JANSENSSENS A. 1949. Contribution à l'étude des Coléoptères Lamellicornes. XIII. Table synoptique et essai de classification pratique des Coléoptères Scarabaeidae. – *Bulletin de la Institut Royal de Sciences Natural Belgique* **25**: 1–30.
- JUAN C., OROMI P., HEWITT G.M. 1995. Phylogeny of the genus *Hegerer* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from cytochrome oxidase I mitochondrial DNA sequences. – *Proceedings of the Royal Society of London* **261**: 173–180.
- KATO H., TOH H. 2008. Recent developments in the MAFFT multiple sequence alignment program. – *Briefings in Bioinformatics* **9**: 286–298.
- KRELL F.-T. 2006. Fossil record and evolution of Scarabaeoidea (Coleoptera: Polyphaga). – *Coleopterists Society Monograph* **5**: 120–143.
- KUCK P., MEUSEMANN K. 2010. FASconCAT: Convenient handling of data matrices. – *Molecular Phylogenetics and Evolution* **56**: 1115–1118.
- MEDINA C.A., SCHOLTZ C.H., GILL B.D. 2003. Morphological variation and systematics of *Canthon* Hoffmannsegg 1817, and related genera in New World Canthonini dung beetles (Coleoptera, Scarabaeinae). – *Mitteilungen aus dem Museum für Naturkunde Berlin, Deutsche Entomologische Zeitschrift* **50**: 23–68.
- MEDINA C.A., SCHOLTZ C.H. 2005. Systematics of the southern African genus *Epirinus* Reiche (Coleoptera: Scarabaeinae: Canthonini): descriptions of new species and phylogeny. – *Insect Systematics & Evolution* **36**: 145–160.
- MILLER M.A., PFEIFFER W., SCHWARTZ T. 2010. “Creating the CIPRES Science Gateway for inference of large phylogenetic trees” in *Proceedings of the Gateway Computing Environ-*

- ments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp. 1–8.
- MLAMBO S., SOLE C.L., SCHOLTZ C.H. 2011. Phylogeny of the African ball-rolling dungbeetle genus *Epirinus* Reiche (Coleoptera: Scarabaeidae: Scarabaeinae). – *Invertebrate Systematics* **25**: 197–207.
- MONAGHAN M.T., INWARD D.G., HUNT T.H., VOGLER A.P. 2007. A molecular phylogenetic analysis of the Scarabaeinae (dung beetles). – *Molecular Phylogenetics & Evolution* **45**: 674–692.
- MONTREUIL O. 1998. Analyse phylogénétique et paraphylie des Coprini et Dichotomiini (Coleoptera: Scarabaeidae). Scénario biogéographique. – *Annales de la Société Entomologique de France* **34**: 135–148.
- MONTREUIL O., GENIER F., NEL A. 2010. *Lobateuchus*, un nouveau genre de «bousier» fossile du nord de la France (Coleoptera: Scarabaeidae, Scarabaeinae). – *Annales de la Société Entomologique de France* **46**: 164–167.
- MOSTERT L.E., HOLM E. 1982. Notes on the flightless Scarabaeina (Coleoptera: Scarabaeidae) with a description of a new species. – *Cimbebasia (A)* **5**: 273–284.
- MOULTON J.K., WIEGMANN B.M. 2004. Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged eremoneuran Diptera (Insecta). – *Molecular Phylogenetics & Evolution* **31**: 363–378.
- NICHOLS E., SPECTOR S., LOUZADA J., LARSEN T., AMEZQUITA S., FAVILA M.E. 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. – *Biological Conservation* **141**: 1461–1474.
- NYLANDER J.A.A., RONQUIST F., HUELSENBECK J.P., NIEVES-ALDREY J.L. 2004. Bayesian phylogenetic analysis of combined data. – *Systematic Biology* **53**: 47–67.
- OCAMPO F.C., HAWKS D.C. 2006. Molecular phylogenetics and evolution of the food relocation behaviour of the dung beetle tribe Eucraniini (Coleoptera: Scarabaeidae: Scarabaeinae). – *Invertebrate Systematics* **20**: 557–570.
- PHILIPS K.T., PRETORIUS E., SCHOLTZ C.H. 2004. A phylogenetic analysis of dung beetles (Scarabaeinae : Scarabaeidae): unrolling an evolutionary history. – *Invertebrate Systematics* **18**: 53–88.
- PONS J., RIBERA I., BERTRANPETIT J., BALKE M. 2010. Nucleotide substitution rates for the full set of mitochondrial protein-coding genes in Coleoptera. – *Molecular Phylogenetics & Evolution* **56**: 796–807.
- RONQUIST F., HUELSENBECK J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics* **19**: 1572–1574.
- ROETS F., OBERLANDER K.C. 2010. *Silvaphilus*: a new relict forest-endemic Canthonini dung beetle genus from the Western Cape Province of South Africa (Coleoptera: Scarabaeidae: Scarabaeinae). – *African Entomology* **18**: 369–373.
- SCHOLTZ C.H. 1989. Unique foraging behaviour in *Pachysoma* (= *Scarabaeus*) *striatum* Castelnau (Coleoptera: Scarabaeidae): an adaptation to arid conditions? – *Journal of Arid Environments* **16**: 305–313.
- SCHOLTZ C.H., CHOWN S.L. 1995. The evolution of habitat use and diet in the Scarabaeoidea: a phylogenetic approach. Pp. 335–374 in: PAKALUK J., ŚLIPŃSKI A. (eds), *Biology, Phylogeny and Classification of Coleoptera. Papers Celebrating the 80<sup>th</sup> Birthday of Roy A. Crowson*. – Museum i Instytut Zoologii PAN, Warsaw.
- SCHOLTZ C.H., DAVIS A.V.L., KRYGER U. 2009. *Evolutionary Biology and Conservation of Dung Beetles*. – Pensoft Publishers, Bulgaria.
- SCHOLTZ C.H., HOWDEN H.F. 1987. A revision of the African Canthonina (Coleoptera: Scarabaeidae: Scarabaeinae). – *Journal of the Entomological Society of Southern Africa* **50**: 75–119.
- SCHOLTZ C.H., HOWDEN H.F. 1987. A revision of the southern African genus *Epirinus* Reiche (Coleoptera: Scarabaeidae: Scarabaeinae). – *Journal of the Entomological Society of Southern Africa* **50**: 121–154.
- SCOTLAND R.W., OLMSTEAD R.G., BENNETTE J.R. 2003. Phylogeny reconstruction: the role of morphology. – *Systematic Biology* **52**: 548–553.
- SIMON C., FRATI F., BECKENBACH A., CRESPI B., LIU H., FLOOK P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. – *Annals of the Entomological Society of America* **87**: 651–701.
- SMITH C.I., FARRELL B.D. 2005. Range expansions in the flightless longhorn cactus beetles, *Moneilema armatum*, in response to Pleistocene climate changes. – *Molecular Ecology* **14**: 1025–1044.
- SMITH C., HEYNE S., RICHTER A.S., WILL S., BACKOFEN R. 2010. Freiburg RNA Tools: a web server integrating IntaRNA, ExpaRNA and LocARNA. – *Nucleic Acids Research* **38**: sup-1W373-7.
- SOLE C.L., BASTOS A.D.S., SCHOLTZ C.H. 2007. Do individual and combined data analyses of molecules and morphology reveal the generic status of *Pachysoma* MacLeay (Coleoptera: Scarabaeidae)? – *Insect Systematics & Evolution* **38**: 311–330.
- SOLE C.L., SCHOLTZ C.H. 2010. Did dung beetles arise in Africa? A phylogenetic hypothesis based on five gene regions. – *Molecular Phylogenetics & Evolution* **56**: 631–641.
- SOLE C.L., WIRTA H., FORGIE S.A., SCHOLTZ C.H. 2011. Origin of Madagascan Scarabaeini dung beetles (Coleoptera: Scarabaeidae): dispersal from Africa. – *Insect Systematics & Evolution* **42**: 1–12.
- SORENSEN M.D., FRANZOSA E.A. 2007. TreeRot, version 3. – Boston University, Boston, MA.
- STAMATAKIS A., HOOVER P., ROUGEMONT J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. – *Systematic Biology* **75**: 758–771.
- STEBNICKA Z.T. 2001. Aphodiinae (Insecta: Coleoptera: Scarabaeidae). – *Fauna of New Zealand* **42**. 64 pp.
- SWOFFORD D.L. 2003. PAUP\*: Phylogenetic Analysis Using Parsimony (and other methods) Version 4. – Sinauer Associates, Sunderland, MA.
- TARASOV S., GÉNIER F. 2015. Innovative Bayesian and parsimony phylogeny of dung beetles (Coleoptera, Scarabaeidae, Scarabaeinae) enhanced by ontology-based partitioning of morphological characters. – *PLoS ONE* **10**(3): e0116671.
- TARASOV S.I., SOLODOVNIKOV A.Y. 2011. Phylogenetic analyses reveal reliable morphological markers to classify mega-diversity in Onthophagini dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). – *Cladistics* **27**: 1–39.
- VILLALBA S., LOBO J.M., MARTIN-PIERA F., ZARDOYA R. 2002. Phylogenetic relationships of Iberian dung beetles (Coleoptera: Scarabaeinae): Insights on the evolution of nesting behaviour. – *Journal of Molecular Evolution* **55**: 116–126.

- VOGLER A.P., DESALLE R., ASSMANN T., KNISLEY C.B., SCHULTZ T.D. 1993. Molecular population genetics of the endangered tiger beetle, *Cicindela dorsalis* (Coleoptera: Cicindelidae). – *Annals of the Entomological Society of America* **86**: 142–152.
- WAHLBERG N., WHEAT C.W. 2008. Genomic outposts serve the phylogenomic pioneers: Designing novel nuclear markers for genomic DNA extractions of Lepidoptera. – *Systematic Biology* **57**: 231–242.
- WILD A.L., MADDISON D.R. 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. – *Molecular Phylogenetics & Evolution* **48**: 877–891.
- WINTERTON S.L., HARDY N.B., WIEGMAN B.M. 2010. On wings of lace: phylogeny and Bayesian divergence time estimates of Neuropterida (Insecta) based on morphological and molecular data. – *Systematic Entomology* **35**: 349–378.
- WIRTA H., ORSINI L., HANSKI I. 2008. An old adaptive radiation of forest dung beetles in Madagascar. – *Molecular Phylogenetics & Evolution* **47**: 1076–1089.
- ZUNINO M. 1983. Essai préliminaire sur l'évolution des armures génitales des Scarabaeinae, par rapport à la taxonomie du groupe et à l'évolution du comportement de nidification (Col. Scarabaeidae). – *Bulletin de la Société Entomologique de France* **88**: 531–542.
- ZUNINO M. 1985. Las relaciones taxonomicas de los Phanaeina (Coleoptera: Scarabaeinae) y sus implicaciones biogeograficas. – *Folia Entomológica Mexicana* **64**: 101–115.

---

**Authors' contributions:** CHS and CLS were involved in the conception and design of the study. SM collected the molecular data, performed the phylogenetic analyses and drafted the manuscript as part of a PhD study. CLS and CHS revised the manuscript. All authors read and approved the final manuscript.