

# Affinities of the Canthonini dung beetles of the Eastern Arc Mountains

Sukoluhle Mlambo<sup>\*</sup>, Catherine L. Sole and Clarke H. Scholtz

Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa.

<sup>\*</sup>Corresponding author: [mlambo@mitsong.org.za](mailto:mlambo@mitsong.org.za); tel. - +27 12 0000040; fax - +27 12 3227939

## Abstract

The Eastern Arc Mountains (EAM) consist of thirteen separate mountain blocks running from southern Kenya through eastern Tanzania in an arc shape. Organisms occurring in the forests of these mountains are known for their high levels of endemism. Some of these organisms have their closest relatives in distant geographic regions. In this study molecular phylogenetic methods, based on partial sequences of one nuclear (28S) and two mitochondrial (COI and 16S) genes, are used to determine the relationships of three Scarabaeinae genera (tribe Canthonini) endemic to the EAM. *Janssensantus* and *Tanzanolus* are found to have a sister relationship within a lineage of south-eastern African genera while *Madaphacosoma*'s closest ties are with Oriental and Madagascan taxa *Ochicanthon* and *Epactoides* respectively. Divergence time estimates suggest a Miocene origin for the ancestral lineages of the three genera with *Janssensantus* and *Tanzanolus* separating in the Pleistocene. Our results provide evidence for a Madagascan origin for the EAM genera.

## Key words

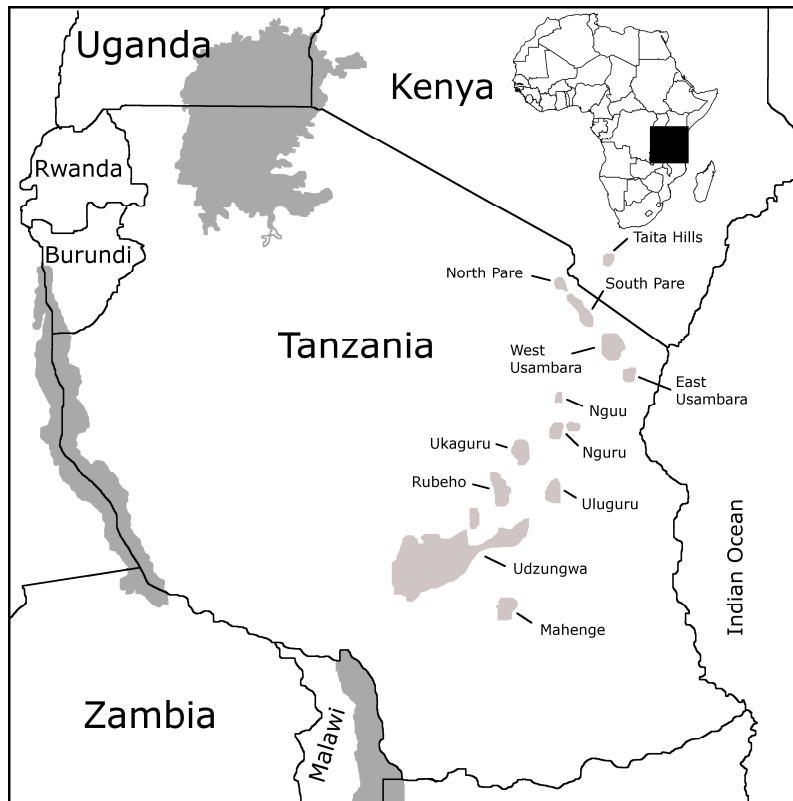
Eastern Arc Mountains, Madagascar, Oriental, dung beetles, endemic, phylogenetic.

## Introduction

The Eastern Arc Mountain region (EAM) is one of eight biodiversity hotspots in Africa, qualifying for this status due to its extraordinary high concentration of endemic species (Myers et al. 2000). The mountains are part of a geologically ancient chain

consisting of 13 blocks that stretch about 900 km from southern Kenya through south-central Tanzania (Lovett, 1985) (see Figure 1). They are formed mainly from pre-Cambrian rocks that were uplifted approximately 100 million years ago. Climatic conditions are thought to have been fairly stable for the past 30 million years and it is this stability, high rainfall and fragmentation of the mountain blocks that are thought to have resulted in forests that are ancient and biologically diverse (Griffiths 1993). There has however been recent re-uplift of the Eastern Arc Mountains blocks and despite the relative climatic stability and long-term persistence of forests in the region compared with other parts of Africa, there have been changes. These could be the origin of range fragmentations and endemism in particular mountain blocks.

Three dung beetle genera *Tanzanolus*, *Janssensantus* and *Madaphacosoma*, are endemics of the EAM and belong to the dung beetle tribe Canthonini. Members belonging to the “ancient” polyphyletic tribe Canthonini in the subfamily Scarabaeinae have a worldwide distribution, but are most abundant and diverse in Africa, with about 44% of the world genera (Davis *et al.* 2008). Climate, vegetation and a wide range of vertebrate dung types are some of the major factors that have contributed to their successful diversification on the continent (Davis and Scholtz 2001). Africa is the putative origin of the subfamily (Philips *et al.* 2004; Monaghan *et al.* 2007, Sole and Scholtz 2010; Mlambo *et al.* submitted) and hosts ancient lineages of the tribes Dichotomiini and Canthonini.



**Fig. 1** Eastern Arc Mountains in Tanzania and Kenya

The African canthonines, which are monophyletic (Monaghan *et al.* 2007), appear to be represented by relicts of once more widespread fauna, with many genera being monotypic or having small numbers of species which are almost exclusively restricted to localised areas of forest patches (Deschodt and Scholtz 2008). Correspondingly, the endemics of the EAM region show relictual and restricted distributions, being confined to small montane forest patches. *Janssensantus pauliani*, one of the two species in the genus is found in the East Usambara Mountains while the other, *J. inexpectatus*, has only been collected from the Uluguru Mountains. *T. leleupi* is the only species of *Tanzanolus* and is restricted to high altitudes on the Uluguru Mountains. *Madaphacosoma* also comprises a single species, *M. basilewskyi*, also restricted to the Uluguru Mountains (Figure 1).

*Madaphacosoma* has had a complex taxonomic history and its relationship with the Oriental and Madagascan genera, *Ochicanthon* and *Epactoides* respectively, remains uncertain. Formerly the genus *Madaphacosoma* comprised three species, one African and the other two Madagascan, the latter now synonymised with *Epactoides*. The African species was first described within the Oriental genus *Phacosoma* Boucomont 1914, now *Ochicanthon* Vaz-de-Mello 2003. It was later transferred to the Madagascan Canthonine genus *Madaphacosoma* Paulian 1975. Scholtz and Howden (1987) point out that the African genus is likely misidentified as it differs markedly from the Madagascan species. In addition, Madagascan canthonine genera are endemic to the island. The Madagascan *Madaphacosoma* together with three other genera, *Phacosomoides*, *Sikorantus* and *Aleiantus* have since been synonymised with *Epactoides* (Wirta and Montreuil 2008) as the descriptions of the former four were based on inconsistent morphological characters without specific diagnoses for the genera.

In this study, molecular data from three gene regions were used to determine the relationships amongst the three endemic genera of the Eastern Arc Mountains, their ancestry and their relationships with the rest of the African Scarabaeinae genera. The three EAM genera all differ significantly in appearance and they are not expected to be closely related. Some genera within the polyphyletic Canthonini have close relationships with genera in the tribe Dichotomiini which is also polyphyletic. The latter tribe was therefore included in the analysis together with *Ochicanthon* from Indonesia and the Madagascan Canthonini genera *Epactoides*, *Nanos*, *Arachnodes*, *Pseudoarachnodes* and *Apotolamprus*.

## **Materials and methods**

### *Taxa*

Sequences for COI, 16S and 28S domain 3 from 42 African, 5 Madagascan (*Apotolamprus cyanescens*, *Arachnodes andriai*, *Pseudoarachnodes hanskii*, *Nanos bimaculatus* and *Epactoides helenae*) and one Oriental Scarabaeinae species (*Phacosoma punctatum* were obtained from Genbank (Monaghan *et al.* 2007; Sole and Scholtz 2010; Wirta *et al.* 2010; Mlambo *et al.* submitted). The African species represent the

Dichotomiini genera *Coptorhina* Hope 1830, *Delopleurus* Erichson 1847, *Frankenbergerius* Balthasar 1938, *Heliocopris* Hope 1837, *Macroderes* Westwood 1876, *Pedaria* Castelnau 1832, *Sarophorus* Erichson 1847, and *Xinidium* Harold 1869.

Canthonini genera included were *Aliuscanthoniola* Deschodt & Scholtz 2008, *Anachalcos* Hope 1837, *Aphengoecus* Péringuey 1901, *Byrrhidium* Harold 1869, *Canthodimorpha* Davis, Scholtz and Harrison 1999, *Circellium* Latreille 1825, *Dicranocara* Frolov and Scholtz 2003, *Dwesasilvasedis* Deschodt and Scholtz 2008, *Endroedyolus* Scholtz and Howden 1987, *Epirinus* Reiche 1841, *Gyronotus* van Lansberge 1847, *Hammondantus* Cambefort 1978, *Namakwanus* Scholtz and Howden 1987, *Odontoloma* Boheman 1857, *Outenikwanus* Scholtz and Howden 1987, *Peckolus* Scholtz and Howden 1987, *Pycnopanelus* Arrow 1931 and *Silvaphilulus* Roets & Oberlander 2010 .

The Eastern Arc Mountain taxa included are *Janssensantus* sp., *Madaphacosoma* sp. and *Tanzanolus* sp. which were collected from forest litter (Grebennikov pers. comm.). *Janssensantus* was sampled from Eastern Usambara and Nguru Mountains, *Tanzanolus* from Eastern Usambara, Nguru and Kaguru, and *Madaphacosoma* from Uluguru Mountains. The sequences were submitted to genebank under accession numbers KC928067 - KC928077. As Aphodiinae have been proposed as the sister taxon of Scarabaeinae (Browne and Scholtz 1999), species from the genus *Aphodius* were used as an outgroup in the analysis.

#### *DNA extraction and sequencing*

The specimens of *Tanzanolus*, *Janssensantus* and *Madaphacosoma* were preserved in 95% ethanol before DNA extraction and sequencing for the mitochondrial gene regions COI and 16S and the nuclear rRNA 28S Domain 3. These three regions were used to align the study with those of Wirta *et al.* 2008 and Monaghan *et al.* 2007. For details of the extraction, amplification, primers and sequencing protocols see Sole and Scholtz 2010. (The nuclear gene regions CAD and 28S domain 2 were excluded from the present analysis as they were not used in the Wirta *et al.* 2008 and Monaghan *et al.* 2007 studies from which sequences for the Madagascar and Asian taxa were obtained.)

### *Phylogenetic Analysis*

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 and Toh 2008) using the default settings. Thereafter the sequences were checked manually. The aligned data consisted of 1450 bp ( $\approx$ 362 bp from 16S, 749 bp from COI, and  $\approx$ 339 bp from 28S D3). The phylogenetic relationships among the sequences were inferred using MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001). Bayesian 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). Ten million generations were run with 3 cold and one heated chain with trees sampled every hundred generations. Posterior probabilities were calculated after discarding the initial 20% as burn-in. Two independent runs were performed for each analysis.

### *Estimating divergence times*

The COI region was used to estimate the time of divergence of the east African and the Madagascan species from the rest of the African species. A Bayesian lognormal relaxed clock estimation of divergence times was performed with Beast version 1.5.2 (Drummond and Rambaut, 2007). Time to most recent common ancestor ( $t$  MRCA) was estimated under the log normal uncorrelated model (relaxed molecular clock), assuming the Yule speciation model with all estimates utilising the HKY model of substitution. Two independent Markov chains were run for 50 million iterations using a random starting tree. The program TRACER version 1.5 (Rambaut & Drummond 2007) was used to assess the convergence between runs and posterior probabilities of the estimates. Published mutation rates of 0.0075 and 0.012 mutational changes per million years were used, respectively, to cover the range of rates reported for COI mtDNA (Brower 1994; Juan *et al.* 1995; Farrell 2001; Smith & Farrell 2005; Wirta *et al.* 2008, Sole and Scholtz, 2010; Sole *et al.*, 2011).

## Results and discussion

### *Phylogeny*

The Bayesian analysis returned a fairly well resolved phylogeny with good support for most lineages. Inclusion of the Tanzanian genera has resulted in a topology that is somewhat different from previous analyses (Sole and Scholtz 2010; Mlambo *et al.* submitted) in that the group under study divides into two well-supported monophyletic lineages A and B (Figure 2). Interestingly, lineage B, consists solely of genera currently classified as canthonines, whilst the other, though mixed, shows members of the two tribes Canthonini and Dichotomiini, separating out in natural groups – Lineages C, D and F. The exception is lineage G, where *Heliocopriss* is the only dichotomiine among canthonines. Compared with previous studies, relationships among the taxa in Lineage C have remained stable as have lineage B taxa, except for the addition of *Odontoloma*, with weak support, and *Hammondantus*.

### *Eastern Arc Mountain and Madagascan genera*

Our analysis revealed that the EAM genera do not form a monophyletic assemblage: *Madaphacosoma* is genetically closer to endemic genera of Madagascar and Asia, *Epactoides* and *Ochicanthon* respectively, than to *Tanzanolus* and *Janssensantus*. *Madaphacosoma* appears to be one of many EAM taxa that are representative of old lineages whose closest relatives are geographically very distant (Fjeldsa and Lovett 1997). *Tanzanolus* and *Janssensantus* appear as sister to *Circellium*, a south-east African endemic, in a lineage that is far removed from *Madaphacosoma*, *Epactoides* and *Ochicanthon*. From this analysis *Tanzanolus* and *Janssensantus*, whose last common ancestor lived 1.9-3.0 mya, have closer links to southeastern African forest genus *Gyronotus* and the more widespread genera *Canthodimorpha* and *Anachalcos*.

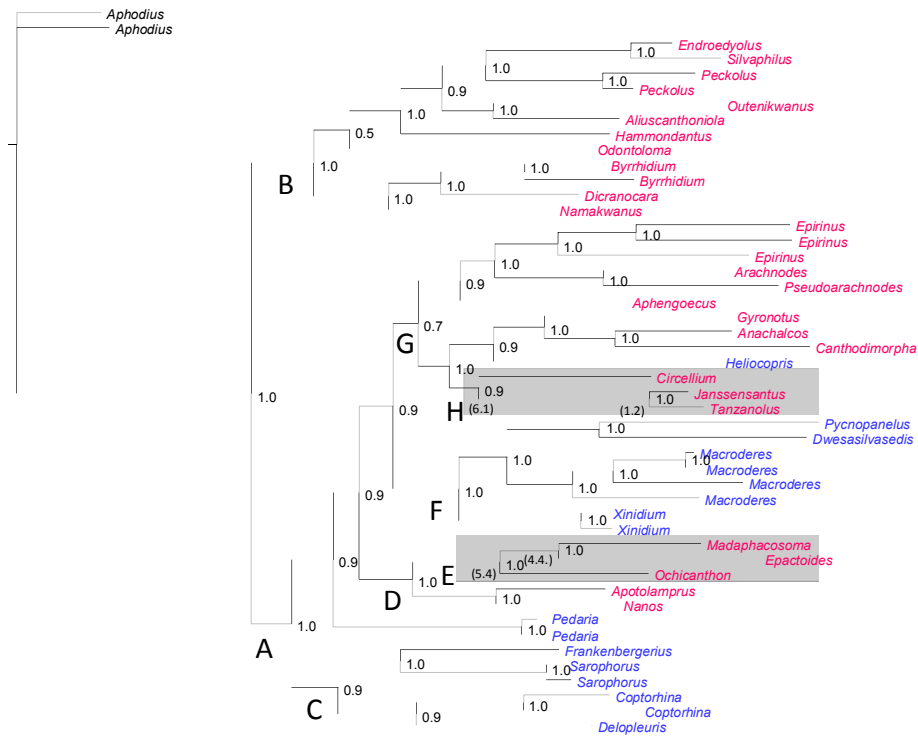
**Table 1** Estimates of divergence times (MYA).

<b>Lineage</b>	<b>Estimate for evolutionary rate 0.0075 (upper/lower 95% confidence intervals)</b>	<b>Estimate for evolutionary rate 0.012 (upper/lower 95% confidence intervals)</b>
E	8.6 (6.9/10.3)	5.4 (4.3/6.5)
H	9.2 (7.5/12.2)	6.1 (4.7/7.6)

Furthermore, although the three EAM genera are associated with the Pleistocene epoch, there is a considerable age difference among them, with *Madaphacosoma* diverging 4.4 mya while *Janssensantus* and *Tanzanolus* have a more recent divergence date, 1.2 mya

From the phylogram in Figure 2 and the Beast analysis Table 1, *Ochicanthon* diversified before either *Madaphacosoma* or *Epactoides*, whose last common ancestor lived 4.5-7.0 (late Miocene / early Pliocene) mya (much lower than Wirta *et al.*'s [2008] 38-24 mya). A common ancestor at node E diverges into both the common ancestor of Madagascan *Epactoides* and Afrotropical *Madaphacosoma*, and the oriental genus *Ochicanthon*. With the divergence at the preceding and historically earlier node D comprising two lineages each containing Madagascan genera (*Nanos/Apotolamprus* and *Epactoides*), a Madagascan origin is likely for both *Madaphacosoma* and *Ochicanthon*. *Ochicanthon* has 47 species distributed in moist forests (Latha *et al.* 2011). The genus consists of small to medium-sized canthonines and appears to be associated with carrion and /or dung (Krikken and Huijbregts 2007). Five montane species in the genus are flightless. It is likely that there was divergence between Madagascar and the Orient (Node E) followed by divergence of the Madagascar element between Madagascar and Africa. Dispersal from a Madagascan origin has been suggested for chameleons, with oceanic dispersal into Africa (Raxworthy *et al.* 2002).





**Fig. 2** Bayesian phylogram from the MrBayes analysis of study taxa. Lineages with EAM genera are shaded grey, Canthonini names are red, and Dichotomiini blue. Posterior probabilities are indicated at nodes. In brackets at selected nodes are divergence times estimated using mutational rates of 0.012 changes per million years.

Like the Eastern Arc mountain region, Madagascar is one of the world's biodiversity hotspots due to the high diversity, endemism and the evolution in isolation of its biota (Yoder and Nowak, 2006). Madagascar became isolated from mainland Africa 160–158 Mya and from India 80 Mya (Briggs, 2003; de Wit, 2003). Four Scarabaeinae tribes occur in Madagascar - Canthonini, Scarabaeini, Onthophagini and Oniticellini. Together they have more than 250 species endemic to the island (Wirta *et al.* 2010). Recent studies have indicated that Madagascan Scarabaeini and Oniticellini originated from African ancestors (Sole *et al.* 2011; Wirta *et al.* 2008). The island's Canthonini colonised Madagascar in three separate events (Wirta *et al.* 2010).

Finally, *Tanzanolus*, *Janssensantus* and *Madaphacosoma* are among many endemic lineages inhabiting the highly fragmented and threatened rain forests of Eastern Africa. With many being species-poor and having small distributional ranges they are vulnerable to extinction, which would result in the loss of substantial phylogenetic diversity. Human activities, through resource over-exploitation and alteration of natural habitats (Brooks *et al.* 2002; Hall *et al.* 2008) are among the major factors driving extinctions. Urgent conservation efforts are required (Myers *et al.* 2000; Myers 2003) to preserve the biodiversity in the forest patches of the EAM.

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