

**Article title:** Origin of Madagascan Scarabaeini dung beetles (Coleoptera: Scarabaeidae): dispersal from Africa.

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**Short title:** Colonisation of Madagascar by Scarabaeini

## **Abstract**

Madagascar, the world's fourth largest island, has a long history of isolation (160 MY) and a wide range of climates and ecosystems which have in turn resulted in high levels of endemism across different taxonomic levels. Although Madagascar has a rich dung beetle fauna that belongs to various tribes only three species of the Scarabaeini are found there, namely *Scarabaeus viettei*, *S. radama* and *S. sevoistra*. These three species are superficially quite distinctive and have, consequently, had a relatively tortured taxonomic history since the first was described in 1896. The morphological differences between these species resulted in them being placed in different genera at different times. However, currently, based on cladistic analysis, they are all classified in the genus *Scarabaeus*. In this study, two of the species, *S. viettei* and *S. radama*, were included in a phylogenetic analysis based on two mitochondrial gene regions - cytochrome oxidase I (COI) and 16S rRNA - and a 247 morphological and behavioural dataset of 23 members of the Scarabaeinae. A Bayesian phylogram supports the monophyly of the genus *Scarabaeus*, with the two species from Madagascar appearing sister to three species of *Scarabaeus* from south-west Africa. Estimated times of divergence based on published mutation rates of 0.012 and 0.0075 for COI indicate that a shared African/Madagascan origin occurred around 15.18 or 24.15 MYA, respectively. This study is another example in support of Madagascan fauna having an African origin with colonisation having occurred via dispersal as opposed to ancient vicariant events.

**Keywords** Molecular phylogeny, morphology, divergence time, biogeography, Madagascar, vicariance/dispersal

## Introduction

The origin of the fauna and flora of Madagascar has long intrigued biologists. The exceptionally high levels of endemism at different taxonomic levels (Myers et al. 2000) and the island's long separation from Africa have led to speculation that much of the biota belongs to ancient lineages that evolved *in situ* after vicariance from ancestral forms. Since it is well established geologically that the island separated from Africa as long ago as 160 million years before present (De Wit 2003), a vicariant origin for the biota implies extreme ages of the groups. However, recent studies, many of which were based on molecular analyses and related dating programmes, have returned much younger ages for both plants and animals, pointing unequivocally to dispersal [mainly from Africa] of the ancestral lineages of much of extant Madagascan biota (Yoder & Nowak 2006).

The dung beetle (Scarabaeidae: Scarabaeinae) fauna of Madagascar has high levels of endemism at generic and species level but no endemic higher taxa are present (Scholtz et al. 2009). Although the subtribe Helictopleurina, one of three belonging to the widespread tribe Oniticellini, has long been considered endemic to Madagascar (Davis et al. 2002), recent phylogenetic studies have implied that the validity of the three subtribes is doubtfully justifiable (Monaghan et al. 2007; Wirta et al. 2008). Four dung beetle tribes occur on Madagascar: Canthonini, with seven endemic genera and about 200 species; Oniticellini with two endemic genera comprising 65 species of *Helictopleurus* and one belonging to the monotypic genus *Heterosyphus*; Onthophagini with six (all belong to the cosmopolitan genus *Onthophagus*); and Scarabaeini with three *Scarabaeus* species. The Canthonini, as presently constituted, is a large Gondwanan tribe but various recent studies have

provided unequivocal evidence that the group is polyphyletic (Philips et al. 2004; Monaghan et al. 2007). Onthophagini is the largest dung beetle tribe, with members on all continents. Scarabaeini is mainly African, but with elements in the Mediterranean - Palearctic and Oriental regions.

The Madagascan Canthonini and *Helictopleurus* have recently been subjected to phylogenetic studies and the ages of founding on the island have been estimated (Wirta & Montreuil 2008; Wirta et al. 2008; Wirta et al. 2009). Madagascan Canthonini appear to stem from at least three independent colonisation events (Monaghan et al. 2007, Wirta et al. 2009). Orsini et al. (2007), using a 2 % divergence per million years calibration of branch lengths, estimated the ages of each with the “oldest” group apparently having radiated at about 14 million years ago (MYA), the next diverged around 10.4 MYA, while three terminal, related generic lineages diverged at 5.6, 9.3 and 12.3 MYA, respectively. Orsini et al. (2007) justified the apparent young ages in terms of the prevailing hypothesis (Paulian 1987) that the canthonine fauna on the island must have colonised Madagascar during the Cretaceous (144-65 MYA). However, their evidence of multiple colonisation events during the Miocene is much more plausible in view of their own and other recent phylogenetic studies of the Scarabaeinae (Scholtz et al. 2009). On the other hand using a Bayesian coalescent approach Wirta et al. (2009) estimate the ‘oldest’ Canthonini colonisation event to have occurred between 64 and 40 (86/26) MYA and the most recent at 30 to 19 (63/11) and 23 to 15 (35/9) MYA, respectively. The estimated time of divergence of the ancestral *Helictopleurus* from an African relative is thought to have occurred between 44 MYA (29/64) and 28 (18/39) MYA (upper and lower 95% credibility limits given in brackets). The time to the most common ancestor of all *Helictopleurus* was estimated to have occurred between 35 (25/44) and

23 (17/29) MYA, suggesting that radiation of *Helictopleurus* started some 5 MY after colonisation (Wirta et al. 2008).

The tribe Scarabaeini consists of approximately 150 species in three genera. *Scarabaeus* is by far the largest genus, with about 135 species, *Pachysoma* has 13 and *Pachylomerus* two. There is little doubt that the tribe is monophyletic (Philips et al. 2004; Monaghan et al. 2007) and phylogenetic analysis of the tribe provided strong support for monophyly of the genera (Forgie et al. 2005, 2006). *Scarabaeus* has been divided into four morphologically, behaviourally and phylogenetically distinct subgenera, the nominate subgenera, *Scarabaeus* s. str., *Kheper*, *Sceliages* and *Scarabaeolus* (Forgie et al. 2005, 2006). *Scarabaeus* and *Kheper* species are widespread in Africa, the Mediterranean - Palaearctic and Oriental regions, although most species occur in Africa. Members of the other two subgenera are restricted to Africa, with most species found in southern Africa. The three *Scarabaeus* species on Madagascar belong to the nominate subgenus *Scarabaeus*.

Although only three species of Scarabaeini are known from [south-western, arid] Madagascar, they are morphologically very distinct and have, consequently, had a fairly variable taxonomic history, with each of them having been considered to belong to different genera at various times [synonyms in brackets]: *Scarabaeus* [*Actinophorus* – Shipp 1896] *radama* Fairmaire 1895; *S.* [*Madateuchus*] *viettei* Paulian 1938; *S.* [*Neateuchus* – Gillet 1911, later moved to *Neomnematium* – Janssens 1938] *sevoistra* Alluaud 1902. The genera were subsequently synonymised with *Scarabaeus*: *Actinophorus* by Felsche (1901); *Neomnematium* by Holm & Scholtz (1979); *Madateuchus* and *Neateuchus* by (Mostert & Scholtz 1986). The validity of these taxonomic decisions was subsequently supported by cladistic analysis based on morphological characters for the flightless species, *S. sevoistra* (Harrison & Philips

2003), and the winged species, *S. viettei* (Forgie et al. 2005). *Scarabaeus radama* has yet to be included in a phylogenetic study. The question of the relationships of the Madagascan Scarabaeini is therefore particularly interesting as they were postulated to have evolved independently on the island after vicariance from an African ancestor, possibly as early as the Cretaceous (Paulian 1987). Although two of the species have superficially been considered in various phylogenetic studies over the last two decades, none of the studies has attempted to explain the history of their presence on the island.

The Scarabaeini were recently subjected to phylogenetic analyses based on a morphological data-set (Forgie et al. 2005) as well as a combined morphological and molecular data-set (Forgie et al. 2006). In this study, we add morphological and molecular (cytochrome oxidase I (COI) and 16S rRNA) data-sets to those of Forgie et al. (2005, 2006) for two Madagascan species, *S. radama* and *S. viettei*, and test for relationship amongst the studied taxa using the combined data-set. We also calculated the possible times of origin of the tribe, and of the Madagascan species in an attempt to elucidate whether Madagascan Scarabaeini stem from a single or multiple colonising events in the island's geographic history.

## **Materials and Methods**

### *Taxa*

The two endemic Madagascan Scarabaeini, *Scarabaeus radama* Fairmaire and *S. viettei* (Paulian), were collected using pitfall traps baited with fish. *Scarabaeus radama* was sampled from three localities in south and south-western Madagascar.

*Scarabaeus viettei* was found in a single locality on the west coast of Madagascar. All the localities were in dry forest areas at low elevation, <150 meters asl. *Scarabaeus sevoistra* Alluaud is only known from a small series of individuals collected during the early 1900's. As far as we are aware all subsequent attempts to collect the species have failed, this study being no exception.

Sequences for 23 Scarabaeinae species were obtained from GenBank (accession numbers – 16S: AF499690 – AF499713; COI: AF499750 – AF499773) (Forgie et al. 2006). These include; *Circellium bacchus* (Fabricius - Canthonini), *Eucranium arachnoides* Brullé (Eucraniini), *Heliocopris hamadryas* (Fabricius - Dichotomiini), and the following Scarabaeini: *Scarabaeus (Kheper) nigroaeneus* (Boheman), *S. (Kh) subaeneus* (Harold), *Scarabaeus proximus* (Péringuey), *S. proboscideus* (Guérin), *S. (Scarabaeolus) bohemani* Harold, *S. (Scarabaeolus) flavicornis* (Boheman), *S. (Scarabaeolus) rubripennis* (Boheman), *S. galenus* (Westwood), *S. goryi* Castelnau, *S. rugosus* (Hausman), *S. rusticus* (Boheman), *S. satyrus* (Boheman), *S. westwoodi* Harold, *S. zambesianus* Péringuey, *Sceliages adamastor* (Serville), *Sceliages brittoni* zur Strassen, *Sceliages hippias* Westwood, *Pachylomerus femoralis* Kirby, *Pachysoma bennigseni* Felsche and *P. hippocrates* M'Leay.

Species from the genus *Aphodius*, which has been proposed as the sister taxon of the Scarabaeinae (Browne & Scholtz 1999; Monaghan et al. 2007), were used as outgroup representatives in the analysis.

### *DNA extraction and sequencing*

The specimens of *S. radama* and *S. vittei* were preserved in 95% ethanol or dried prior to DNA extraction and sequenced for the mitochondrial gene regions COI and 16S rRNA. For details of the extraction, amplification and sequencing protocols see Orsini et al. (2007). The primers and their references are listed in Table 1.

### *Morphological characters*

Morphological characters for *S. radama* and *S. viettei* were defined and scored according to Forgie et al. (2005). Of a total of 247 characters, 244 were morphological characters from the sclerotised internal and external structures, while three were biological characters. For details on the characters scored see Forgie et al. (2005).

### *Phylogenetic analyses*

The sequences were aligned with Clustal W (Thompson et al. 1994). The evolutionary models for the two gene regions were selected with FindModel (Tao et al. 2008). A combined dataset analysis (COI – 1197 bp, 16S rRNA – 401 bp and 247 morphological characters), using a single representative from each species (GenBank accession numbers GU305940 – GU305943), was performed using Bayesian inference with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The data were partitioned by gene region (COI and 16S rRNA) and morphology. The analyses were run for 10 million generations. The parameter values and the trees were summarised



after discarding topologies prior to the stabilisation of the likelihood value. Two independent analyses with two runs each were done to check topological convergence and homogeneity of posterior probabilities.

### *Estimating divergence times*

The COI region was used to estimate the time of divergence of the closest African relative and of each of the Madagascan species. A Bayesian lognormal relaxed clock estimation of divergence times was performed with Beast version 1.5.2 (Drummond & Rambaut 2007). Time to most recent common ancestor (TMRCA) was estimated under the log normal uncorrelated model (relaxed molecular clock), assuming the Yule speciation model with all estimates utilising the GTR + I + G model of substitution. Two independent Markov chains were run for 10 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).

## Results and Discussion

### *Phylogenic considerations*

Bayesian analysis returned a relatively well resolved tree, supporting the monophyly of the African and Madagascan *Scarabaeus* (Fig. 1). The two Madagascan *Scarabaeus* species were supported as sister to each other, and they, in turn were sister to a group of southern African species, *S. proboscideus*, *S. satyrus* and *S. zambesianus*, albeit with moderate support. In a cladistic analysis based on 64 morphological characters Harrison & Philips (2003) placed *S. sevoistra* sister to *S. galenus* and *S. proboscideus*. Morphologically the flightless *S. sevoistra* possesses many apomorphies that closely ally it to its fully winged sister species *S. radama*, *S. viettei* and *S. proboscideus* (Harrison & Philips 2003; Harrison et al. 2003). It does, however, possess a suite of convergently evolved characters shared by other flightless taxa within the Scarabaeinae. Examples of this are seen in *S. scholtzi* Mostert and Holm (from Somalia), *S. ritchei* (MacLeay) (from Libya) and the genus *Eucranium* (from Argentina) which occur in similar habitats but are geographically isolated. Interestingly, *S. proboscideus* and *S. sevoistra* were both considered to belong to the genus *Neateuchus* at one stage, mainly because of the shared, prominent tubercle on the front of the head.

## *Origins of Madagascan fauna*

Madagascar's biota are not only highly diverse, endemic and threatened but show a distinct signature of evolution in isolation which make it one of the world's biodiversity hotspots (Yoder & Nowak 2006). Interestingly, Madagascar also exhibits a skewed representation of biota in that certain taxa are either poorly represented or completely absent, whereas other taxonomic groups show unparalleled diversity (Yoder & Nowak 2006). Mechanisms put forward to describe the origins of the Madagascan fauna include; vicariance following the breakup of Gondwana (ca. 160 MYA); vicariance following the breakup of India and Madagascar (Indo-Madagascar) (ca. 88 MYA); vicariance following the submersion of land bridges linking Indo-Madagascar to Antarctica and South America (ca. 80 MYA) and oceanic colonisation during the Cenozoic (Noonan & Chippindale 2006a; Yoder & Nowak 2006; Evans et al. 2008). Each of these mechanisms is compatible with contrasting phylogenetic and temporal patterns. Gondwanan vicariance, Indo-Madagascan vicariance and the land bridge model imply ancient divergence dates (>80 MYA), while the dispersal model implies divergence dates randomly distributed within the time frame of evolution of the studied taxa (Yoder et al. 2003). Added to this vicariance can be inferred if the phylogenetic history of a group is congruent with the known sequence of vicariant events (for examples of fishes (Sparks 2004; Sparks & Smith 2004, 2005); reptiles (Noonan & Chippindale 2006a); snakes (Noonan & Chippendale 2006b); frogs (Evans et al. 2008); turtles (Vargas-Ramirez et al. 2008)) while dispersal can be inferred when a phylogeny reveals lineages from one geographic area nested within lineages from another area (for examples of fig wasps (Kerdelhue et al. 1999); Lepidoptera (Torres et al. 2001; Zakharov et al. 2004); Amphibians (Vences et al.

2003); carnivorans, lemurs, tenrecs and rodents (Yoder et al. 2003; Poux et al. 2005); snakes (Nagy et al. 2003; Noonan & Chippindale 2006a); allodapine bees (Fuller et al. 2005); minnow mayflies (Monaghan et al. 2005); dung beetles (Wirta et al. 2009)).

Estimated divergence times for the Madagascan and African *Scarabaeus* species lie between 15.18 and 24.15 MYA (Table 2). Even when one examines the extreme boundaries of the 95% credibility intervals for the split the minimum and maximum divergence estimates lie between 11.89 and 29.83 MYA (Table 2). All our estimates of divergence times post-date a Gondwanan split of Africa and Madagascar, the split between Madagascar and India as well as that of the land bridge connections. The distance between Madagascar and Africa has been relatively constant at *c.* 450km since *c.* 80 MYA. McCall (1997) suggested there might have been a land bridge connection between Africa and Madagascar 45 to 26 MYA but this is poorly supported (Ali & Huber 2010). However, had the land bridge existed it would have subsided well before the *Scarabaeus* dispersal event suggested by our analysis and could therefore not have aided this dispersal across the Mozambique Channel. Our phylogenetic analyses indicates that the two Madagascan *Scarabaeus* species are nested within the African *Scarabaeus* species, albeit with moderate support. In the absence of *Scarabaeus* from India these findings indicate that the two Madagascan *Scarabaeus* would probably have evolved from an African ancestor that dispersed across the ocean to Madagascar via a single colonisation event. Added to which, there are several indications that dung beetles are actually excellent dispersers. Charles Darwin recorded in “*The voyage of the Beagle*” (1839) finding living “*Scarabaeus*” floating at sea about 40 km from the mouth of the Rio Plata in South America. On the other hand, some modern groups of dung beetles such as the genera *Copris*, *Sisyphus* and *Onthophagus*, which are thought to have originated in Africa

(Davis et al. 2002; Emlen et al. 2005; Monaghan et al. 2007), have dispersed quickly and over vast distances and are now widespread on several continents. The ancestor of the Sisyphini genus *Nesosisyphus*, with four species endemic to the Indian Ocean island of Mauritius, must have dispersed there, probably from Africa, over an unbelievable distance of several thousand kilometres, since the island is of volcanic origin and a mere 8 MY old (Vinson 1951; Matola et al. 2007). The endemic canthonine, *Nesovinsonia*, likewise, must originate from a trans-oceanic dispersal event (Scholtz et al. 2009; Ali & Huber 2010).

### *Scarabaeus diversification*

The range of major biomes in Madagascar is said to resemble that of large continental landmasses. This and the unpredictable rainfall patterns are thought to have accounted for the evolution of certain taxa and the rareness of others (Vences et al. 2009). The endemic Madagascan dung beetle fauna is dominated at the species level by the Canthonini and *Helictopleurus* while the Scarabaeini are poorly represented. Approximately 80% of Madagascar's extant dung beetle fauna is centred in the eastern forests, where they are known to feed on lemur dung and carrion, while less than 20% occur in the drier vegetation on the west (Hanski et al. 2008; Scholtz et al. 2009). The three species of *Scarabaeus* are restricted to the dry southern and north-western areas of Madagascar (Rahagalala et al. 2009). Considering the number of close African relatives of the Madagascan *Scarabaeus* living and thriving in arid habitats, we may ask why have the Scarabaeini not radiated, as have the Canthonini and *Helictopleurus*. Davis & Scholtz (2001) show that a suitable climate and number of dung types are the two principal ecological factors influencing dung beetle tribal,

generic and species richness patterns. The dominant global distribution pattern within the Scarabaeini is centred on drier areas at high latitudes (Davis et al. 2002; Davis et al. 2008). Considering that the African taxa, sister to the Madagascan *Scarabaeus*, are arid adapted this may have predisposed them to the arid areas of Madagascar (south-western coast). Limited diversification of mammal dung types - pellets and small odiferous droppings being the main dung source on Madagascar - (Davis et al. 2002) and therefore limited food availability (Rahagalala et al. 2009) may have been an added driving force in their observed lack of radiation.

In conclusion this study contributes to the growing amount of recent evidence that dispersal is one of the mechanisms having affected the biota of Madagascar. These results also demonstrate the high vagility of insects. However, the challenge now remains to determine what factors may promote or have promoted this dispersal.

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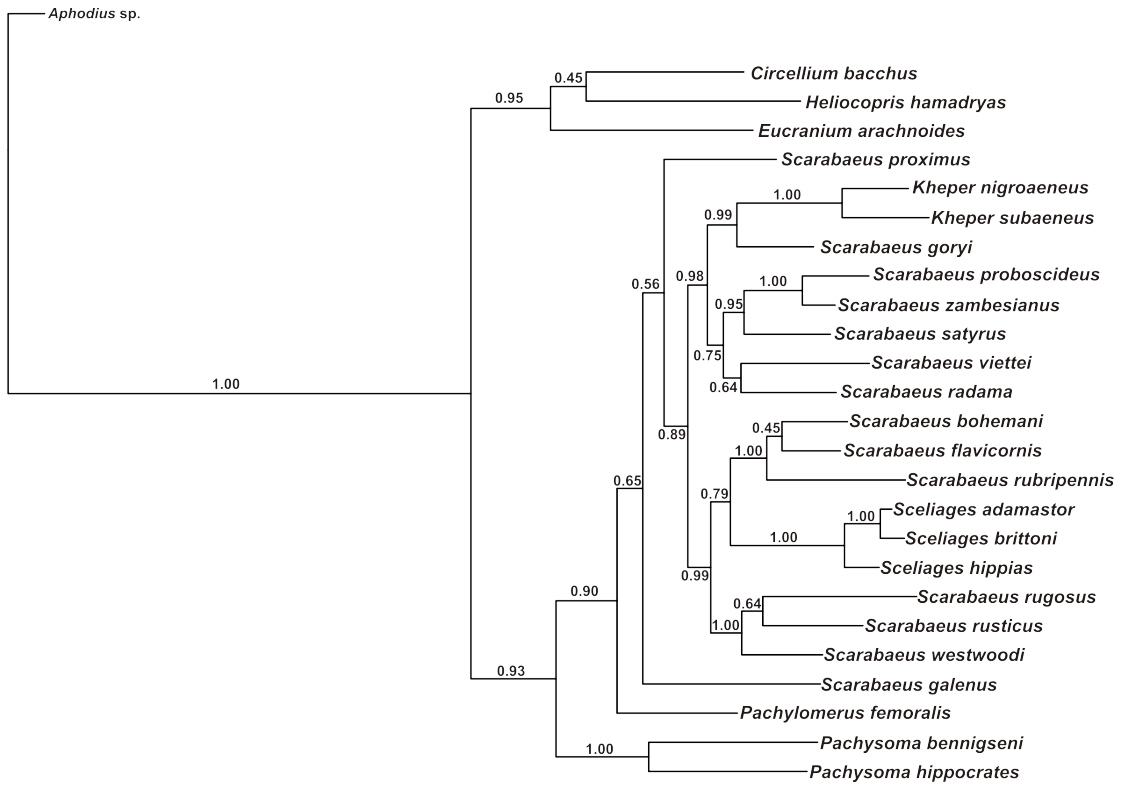


Figure 1. Combined Bayesian phylogram (datasets include: COI, 16s rRNA and morphology) with posterior probabilities

**Table 1.** Summary of oligonucleotide primers used in this study

<b>Locus</b>	<b>Primer name and sequence</b>	<b>Length</b>	<b>Reference</b>
Cytochrome oxidase I	C1-J-2183 (5'CAACATTTATTTTGATTTTTTGG 3')	23mer	Simon <i>et al.</i> , (1994)
	TL2-N-3014 (TCCAATGCACTAATCTGCCATATTA 3')	25mer	Simon <i>et al.</i> , (1994)
	C1-J-1751 (5' GGATCACCTGATATAGCATTCCC 3')	23mer	Simon <i>et al.</i> , (1994)
	C1-N-2191 (5' CCCGGTAAAATTAATAAACTTC 3')	26mer	Simon <i>et al.</i> , (1994)
16s rRNA	16sf (luisa) (5' ATGTCTTTTTGAKWATAATWTAAAG 3')	25mer	Orsini <i>et al.</i> , (2007)
	16sr (luisa) (5' ACGCTGTTATCCCTAAGGTAATTT 3')	24mer	Orsini <i>et al.</i> , (2007)

Table 2. Time estimates in million years (MY) for the most recent common ancestor (tMRCA) for 0.0075 and 0.012 % per million years for the lineages Scarabaeini and the South Africa/Madagascar split, with 95% upper and lower estimates.

Phylogenetic lineage	Mean rate	tMRCA mean	95% upper	95% lower
Scarabaeini*	0.0075	40.45	52.59	29.97
	0.012	25.38	32.84	18.53
South African/Madagascar split	0.0075	24.15	29.83	18.87
	0.012	15.18	18.78	11.89

\* Scarabaeini includes the following taxa *Kheper nigroaeneus*, *Kh. subaeneus*, *Scarabaeus proximus*, *S. proboscideus*, *S. (Scarabaeolus) bohemani*, *S. (Scarabaeolus) flavicornis*, *S. (Scarabaeolus) rubripennis*, *S. galenus*, *S. goryi*, *S. rugosus*, *S. rusticus*, *S. satyrus*, *S. westwoodi*, *S. zambesianus*, *Sceliages adamastor*, *Sceliages brittoni*, *Sceliages hippias*, *Pachylomerus femoralis*, *Pachysoma bennigseni* and *P. hippocrates*

§ South African lineage includes the following taxa *S. proboscideus*, *S. satyrus*, *S. zambesianus*

§ Madagascar lineage includes the following taxa *S. viettei* and *S. radama*