Phylogenetics of the keratin-feeding beetle family Trogidae (Coleoptera: Scarabaeoidea)

By

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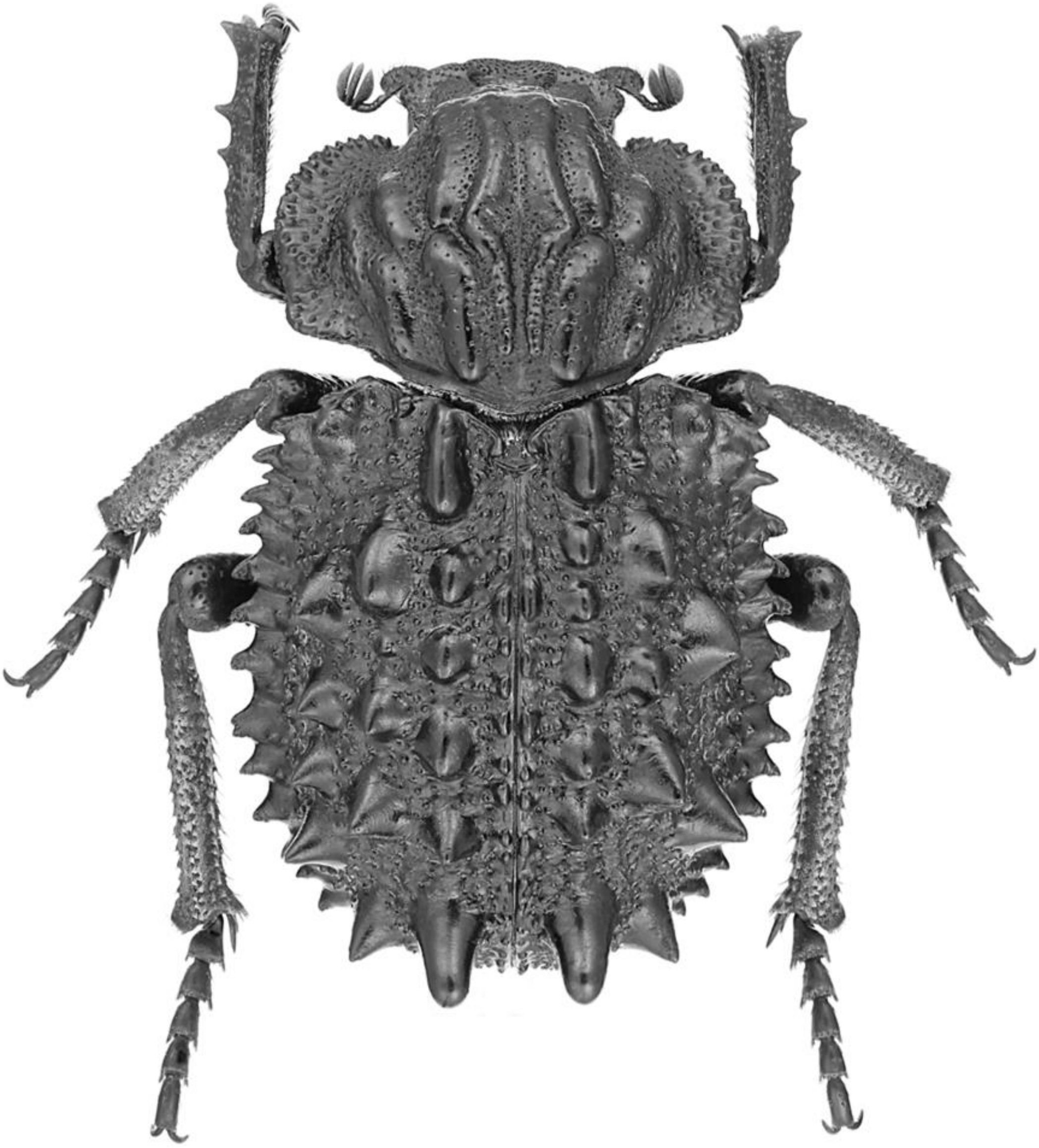

Omorgus (*Omorgus*) *gigas* (Harold, 1872)

Photograph by: W.P. Strümpher

Summary

Trogidae constitute a monophyletic and biologically unique family within the Scarabaeoidea, being the only keratinophagous group in its superfamily. Traditionally, the family has been divided into three distinctive genera, *Omorgus* Erichson, *Polynoncus* Burmeister, and *Trox* Fabricius. Although the taxonomy of the group is relatively well studied, recently changes to the existing classification have been proposed without the family as currently constituted being subjected to phylogenetic analyses. In this study I present the first molecular phylogeny for this cosmopolitan family, based on three partially sequenced gene regions: 16SrRNA, 18S rRNA and 28S rRNA domain 2. Analyses resolved relationships between and within genera and subgenera that are largely congruent with existing taxonomic hypotheses based on morphology. I recovered four well-supported radiations: *Polynoncus*, *Omorgus*, Holarctic *Trox* and Afrotropical *Phoberus*. One of the more surprising results was the monophyly of *Phoberus*. Based on the evidence, I propose reinstating the genus *Phoberus* to accommodate all Afrotropical (including Madagascan endemic) species. The results re-emphasise the validity of Scholtz's (1986a) classification system for the genus *Omorgus* and its subgenera and provide evidence of an African origin for Madagascan trogids. Divergence analysis was able to date the major events for the origin of extant lineages of Trogidae. The subsequent diversification of the major lineages is largely attributed to Pangaean and Gondwanan vicariance events in the Mid-Jurassic and early Late Cretaceous, respectively. The separation of the landmasses resulted in the evolution of four distinct groups: *Omorgus*, *Polynoncus*, *Trox* and *Phoberus*.

The monophyletic subgenus *Phoberus* MacLeay is of particular interest; about one-third (14 of 40) of the species in the Afrotropical region have lost the ability to fly. To gain insights into the evolution of flightlessness in this clade, phylogenetic relationships among species were inferred using molecular sequence data. Four partial sequences of four genes (COI, 16S, 18S and 28S domain 2) were obtained for 27 species, covering all of the recognised species-groups. Estimated times of divergence were based on published mutation rates for COI. The results recovered eight monophyletic lineages, supporting the morphology-based subdivisions of the genus. Flightlessness evolved at least five times within the subgenus. There is no strong support for the re-acquisition of functional wings once they are lost. Diversification, and the present relictual distributions, in African *Phoberus* can be linked to climatic and geological events associated with the Miocene and Pliocene Climate Optimum in southern Africa. The evolution of flightlessness in *Phoberus* is consistent with the habitat stability hypothesis. All flightless species occupy refugial areas with relatively long-term environmental stability. *Phoberus* is a promising model group for studying evolutionary trends relating to flightlessness, speciation and biogeography.

Flightlessness is a counterintuitive evolutionary development, given the many advantages of flight. To interpret the diversification in the flightless *Phoberus capensis* Scholtz, the phylogenetic relationships among several populations of *P*. *capensis* are investigated. Phylogenetic relationships among populations were inferred using molecular sequence data that suggested three distinct evolutionary lineages, which was also supported by morphological characters. Divergence time estimates suggest a Pliocene-Pleistocene diversification. Based on these results, it is suggested that *P. capensis* experienced climatically-driven allopatric speciation with sheltered Afrotemperate forests and high mountain peaks serving as important refugia in response to climatic exacerbations. The *P. capensis* complex thus represents a speciation process in which flight-restricted populations evolved in close allopatry, possibly as recently as the Pleistocene. Two of the three divergent and geographically distinct lineages are described as novel species.

This study represents the most recent comprehensive work on the Trogidae. On the basis of this study, it is proposed that taxonomic changes to the generic classification of the family be made. Trogidae are formally divided into two subfamilies, Omorginae and Troginae. The subgenus *Phoberus* is restored to genus rank to include all the Afrotropical species, and *Afromorgus* is confirmed at subgeneric rank. The genus *Madagatrox* is synonymised with *Phoberus.*

Table of contents

Preface

This thesis comprises six chapters. The first and last chapters provide a brief introduction of the study group and a general discussion respectively. The four intervening chapters have been compiled as separate manuscripts for publication, resulting in the unavoidable repetition of certain methodological aspects. Each chapter contains its own set of references and supporting information.

The publication status of each data chapter is summarized below.

Chapter 2

Strümpher, W.P., Sole, C.L., Villet, M.H. & Scholtz, C.H. (2014) Phylogeny of the family Trogidae (Coleoptera: Scarabaeoidea) inferred from mitochondrial and nuclear ribosomal DNA sequence data. *Systematic Entomology* **39**(3), 548-562. DOI: 10.1111/syen.12074.

Chapter 3

Strümpher, W.P., Villet, M.H., Sole, C.L. & Scholtz, C.H. (in prep.) Evolution of flightlessness in the Afrotropical (*Trox*) *Phoberus* (Coleoptera: Trogidae).

Chapter 4

Strümpher, W.P., Sole, C.L., Villet, M.H. & Scholtz, C.H. (2015) Allopatric speciation in the flightless *Phoberus capensis* (Coleoptera: Trogidae) group, with description of two new species. *Insect Systematics and Evolution* (Accepted 9 December 2015).

Chapter 5

Strümpher, W.P., Villet, M.H. & Scholtz, C.H. (2015) Overview and revision of the extant genera and subgenera of Trogidae (Coleoptera: Scarabaeoidea). *Insect Systematics and Evolution.* DOI 10.1163/1876312X-46052133.

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Most importantly, I would like to express my admiration to my family for their unconditional love and support in all ways. I am deeply indebted to my wife Hermien Viljoen. Thank you for your patience, encouragement and love. I dedicated this thesis to you.

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Declaration

I, Werner Petrus Strümpher, declare that the thesis, which I hereby submit for the degree Doctorate of Philosophy (Entomology) at Rhodes University, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:

Date:

Chapter 1

Overview of the biology, taxonomy and biogeography of the Trogidae McLeay (Coleoptera: Scarabaeoidea)

> *“Not to know the Trox is quite excusable, for the insect has never been much talked about. It is an obscure creature, overlooked by the historian. When impaled in a collector’s box, it ranks close to the Dung Beetles, just after the Geotrupes. Its mean and earthy attire denotes a digger. But what precisely is its calling?”*

(Excerpt from the: The Beaded *Trox*, by J. Henri Fabre)

Overview

The Trogidae MacLeay is a cosmopolitan family of the Scarabaeoidea (Scholtz, 1986a). The family comprises some 330 species (Scholtz, 1982; Smith, 2003; Pittino, 2006; Zidek, 2013) that primarily inhabit the temperate and arid savanna regions of the world. Adult trogids are small to medium-sized (4 - 30 mm) beetles, dark grey to black, and can easily be identified by their heavily sculptured (and usually dirt-encrusted) pronotum and elytra, the ten-segmented antennae with a three-jointed club and their five visible abdominal sternites. They exhibit thanatosis when alarmed or disturbed, retracting their legs and head and remaining motionless. Their cryptic appearance and ability to feign death are effective ways for these beetles to escape potential predators (Ratcliffe, 1991). Another unique and perhaps independently acquired feature of adult trogids is their ability to stridulate (Grimaldi & Engel, 2005). They achieve this by rubbing a coarse plate (plectrum), situated on the outer surface of the first ventral segment of the abdomen against a file on the inside margin of their elytra (Vaurie, 1955; Lawrence & Britton, 1991). The larvae are typically scarabaeiform, white or cream, and immediately recognisable by their heavily sclerotized black cranium and prominent sclerotized shields on either side of the prothorax. Legs are four-segmented, well developed, and lacking the stridulatory apparatus present in some related taxa. It is the only family in the Scarabaeoidea in which all known larvae possess well developed lateral ocelli (Baker, 1968; Scholtz, 1990a).

The apparent obscurity of these beetles can be attributed to their remarkable feeding specialisation. Adults and larvae of all known species are considered true keratinophagous (keratin-digesting) beetles. Members are closely associated with carrion and are more often than not among the last insects to visit the dried remains of dead animals where adults and larvae may be found feeding on various sources of keratinous matter (hair, skin, hooves and nails) and are extremely well adapted (morphologically, behaviourally and physiologically) to exploit the ephemeral and patchy distribution of animal remains in nature (Baker, 1968; Scholtz, 1980, 2000; Scholtz & Caveney, 1988, 1992; Friedländer & Scholtz, 1993; Strümpher *et al.,* 2014). The whole life cycle is completed at, or beneath, the food source. Females burrow into the soil under the food source, where they deposit their eggs. Under ideal conditions the larvae hatch after six to ten days and burrow vertically to the soil surface, where they begin feeding. They pass through three instars in three to four weeks before moulting to the pupal stage. After another two weeks, the adult beetles emerge (Baker 1968; Scholtz & Caveney, 1988).

Keratin-feeding specialisation

The fibrous protein keratin is an important structural component of hair, fur, hooves and feathers. It is a complex polypeptide which is highly resistant to biodegradation due to the supercoiled helical structure of the polypeptides and the numerous disulphide cross-linked bonds between adjacent polypeptide chains. The disulphide linkages, responsible for the rigid structure of keratin, also make it highly insoluble (Hughes & Vogler, 2006; Tombolato *et al*., 2010). Of the insects, only some clothes moth (Tineidae) larvae, bird lice (Mallophaga), and adult and larval hide and museum beetles (Dermestidae) are adapted to eating keratin. Trogids are the only members of the Scarabaeoidea capable of digesting it (Scholtz, 1980, 1986a; Scholtz & Caveney, 1988; Hughes & Vogler, 2006).

Detailed studies of keratin-feeding by insects are mainly restricted to pest species of fabrics [reviewed by Baker and Bry (1987)]. The species studied so far that can digest keratin are all omnivorous; in addition to keratin they also feed on a range of dry plant and animal matter. This has led to speculation that keratin only provides some of the nutrients required for growth but that other food elements need to be acquired from other sources. Interestingly, keratin digestion in these insects is purely by proteinase activity in the presence of strong mid-gut reducing conditions and there is no evidence that keratin digestion is dependent on microbial gut symbionts (Baker & Bry, 1987; Waterhouse, 1957).

No study to date has addressed keratin digestion in trogids, although there are anecdotal observations about the mechanism. Even though keratin makes up the primary component of trogids' diets, additional nutrients or resources are required for survival, since beetles fed only sterilized keratin starve. These additional food elements are most probably acquired from food sources associated with naturally-occurring animal remains, probably in the form of micro-organisms (Scholtz pers comm.).

Enzymatic digestion by microbial gut symbionts (mutualists) is one possible mechanism of keratin digestion. Trogid guts have a rich microflora of protozoans, fungi and bacteria (Scholtz pers comm.) and extra-cellular keratinases have been isolated from several micro-organisms, so there is a strong likelihood that some of these organisms assist in breaking down the keratin by the production of keratinase. Another possible contribution to the chemical breakdown of keratin is the presence of unusually high pH in the midguts (Waterhouse, 1957). The strong disulphide cross-linked bonds between adjacent polypeptide chains, that are responsible for the rigid structure of keratin, are known to de-link at high pH (Waterhouse, 1957; Hughes & Vogler, 2006).

The evolution of a mutualistic relationship between microbes and trogids is not an unlikely scenario. Mutualistic interactions have resulted in major adaptive radiations in insects (Price *et al*., 2011) and especially in the Scarabaeoidea (Scholtz & Chown, 1995). Mutualism in general refers to a relationship or bond between two species, which is beneficial to both; and one classic example of a mutualistic relationship is that of fungus-cultivating termites (Price *et al*., 2011). Mutualism is an important creative evolutionary force. A new mutually beneficial relationship between two species potentially enables them to step into a new adaptive zone or ecological niche (Price *et al*., 2011).

Trogids (adults and larvae) feed almost exclusively on keratin; an attribute unique in the superfamily. Considering that the pleisiomorphic habitat for Scarabaeoidea larvae was clearly soil and the pleisiomorphic diet was humus (Scholtz & Chown, 1995), a shift from ancestral humus-feeding to feeding on keratin clearly represented a major evolutionary step into vacant “adaptive zones” or “niche space”. Dead organic matter (detritus) is considered an important environment that shaped the evolution of insects. Organic matter contains a multitude of micro-organisms pre-adapted to live in or on dead organic matter (e.g. wood, humus) (Price *et al*., 2011). Insects living in these environments would be exposed to multitudes of microbial organisms. Under these conditions the development of mutually beneficial relationships between the insects and microbes would proceed more rapidly than in the *de novo* evolution of the necessary traits, and result in more efficient exploitation of a novel ecological niche or resource (Price *et al*., 2011).

Furthermore, there is a substantial body of evidence to support the concept that humus-feeders are, in fact, so-called “deposit-feeders”, and are dependent on the rich array of microbes such as bacteria and fungi in the humus rather than the cellulose which makes up its bulk. Consequently, a switch from feeding on micro-organisms to feeding on keratin, would not have represented a large step if preceded by the ability to digest microflora, and a switch from feeding on decomposing plant matter to animal remains, is plausible. Keratolytic fungi (e.g. Gymnoascaceae) are common in keratin-enriched soils, on hair and feathers on the soil surface, in mammal burrows and bird nests (Cooke & Rayner, 1984). The plesiomorphic condition for adult scarabaeoids appears to be mycetophagy (Scholtz & Chown, 1995) and the switch to keratin-feeding via microbes as in larvae, is possible. What is clear, however, is that a switch to keratin-feeding very early in scarabaeoid evolution, provided trogids with a readily-available food source that is mostly free of competitors and this has remained so since their initial radiation.

Even though adults and larvae are associated with animal carcasses/remains, records also indicate an array of opportunistic feeding events on many other sources of animal matter. Some North American trogid species are found only in the nests of particular predatory birds (owls and vultures) where they feed on regurgitated pellets (Baker 1968; Vaurie 1955), while species from other regions have been collected only from mammalian carnivore dens (hyaenas) where they feed on carnivore faeces (Krell *et al*., 2003). Ratcliffe (1983) observed *T. hamatus* Robinson feeding on a scarab brood ball, and Leefmans (1932) documented feeding on bat guano in caves. Records of other atypical food sources include the horse-hair cushion of a motor-car seat (Vaurie 1955), old carpets and a felt hat (Robinson, 1941). Occasionally trogids can be found feeding on dung and even decomposing fungi and fish (Scholtz, 1980).

There are also records of some species feeding on insects. Young and Hamm (1985) reported adults of *O. suberosus* Fabricius feeding on dead armyworm caterpillars and Van Emden (1948) documented trogid larvae feeding on locust eggs. Moreover, some reports and field observations from Australia indicate that a group of large, flightless species appear to feed on insects rather than carrion. Surprisingly, none of these species appear to have been found at carrion, and attempts at feeding them on carcasses were unsuccessful (Houston *et al*., 2009; Strümpher & Scholtz, 2011). Recently Houston *et al*. (2009) confirmed that one of these large species, *O. rotundulus* Haaf from Western Australia, feeds on various insects (or insect remains), mostly ants and termites. This is most likely a much more readily available and dependable food source in desert areas than is carrion which, under most conditions, can be considered highly ephemeral. However, the larvae of these large species would require a large, dependable source of food to successfully complete their life-cycle and carcasses would appear more suitable than scattered insect remains. Consequently, it remains to be proven whether certain species of trogids prefer or depend on chitin rather than on keratin (Strümpher & Scholtz, 2011). The latter scenario would clearly be an important step into another vacant “adaptive zone” or “niche space”.

Systematics

The Trogidae are a well-defined family within the Scarabaeoidea. Several phylogenetic studies using morphological and molecular data have presented convincing evidence that Trogidae occupy a basal position in Scarabaeoidea and are closely related to: Passalidae Leach, Lucanidae Latreille, Bolboceratidae Mulsant and Pleocomidae LeConte (Browne *et al*., 1993; Browne & Scholtz, 1995, 1999; Smith *et al*., 2006; Hunt *et al*., 2007; McKenna & Farrell, 2009; Bai *et al*., 2012a, b; Ahrens *et al*., 2014).

Taxonomic history

The taxonomic history of the Trogidae extends back 257 years to when the first 'trogid' species, *Scarabaeus sabulosus* Linnaeus, was described. Since then numerous authorities have contributed to the knowledge of the family. The group is taxonomically well-defined and by the end of the nineteenth century all of the major taxonomic groups had been established.

Fabricius (1775) described the genus *Trox*, (from the Greek *trog*, which means to gnaw), and placed four species in the genus; *T. sabulosus* (Linnaeus) and three which he described, *T. suberosus* Fabricius, *T. spinicornis* Fabricius and *T. horridus* Fabricius. Latreille (1810) designated *T. sabulosus* as the type species of the genus (Vaurie, 1955). *Trox* was first reviewed by Herbst (1790), who included 13 species in his study, which was followed by a general revision by Fabricius (1792).

MacLeay (1819) proposed the family name Trogidae, and described the genus *Phoberus* to accommodate the large flightless southern African species, *T. horridus*. However, *Phoberus* was not generally recognised as a genus; Burmeister (1876) and Preudhomme de Borre (1886) considered *Phoberus* to represent a monotypic subgenus. Harold (1872) and Scholtz (1979a) considered *Phoberus* a synonym of *Trox*, whereas Haaf (1953) treated *Phoberus* only as a species group. Péringuey (1900), Arrow (1912) and, later, Scholtz (1980, 1982) treated *Phoberus* as a subgenus of *Trox*, but they differed in their views regarding inclusion of taxa in the subgenus.

In 1847 Erichson erected the genus *Omorgus* for two American *Trox* species, separating North American species into two genera, *Trox* and *Omorgus*; he reviewed the North American Trogidae in 1848. Le Conte (1854) followed Erichson. *Trox suberosus* was designated the type species of the genus by Lacordaire (1856) in his revision of the Trogidae. Other authors considered *Omorgus* either as synonymous with *Trox* (Lacordaire, 1856; Harold, 1872; Horn, 1874; Loomis, 1922) or as a subgenus (Burmeister, 1876; Péringuey, 1900, 1908; Gerstaecker, 1873; Arrow, 1912; Balthasar, 1936; Robinson, 1940; Scholtz, 1980, 1982). In her revisions of the New World *Trox*, Vaurie (1955, 1962) treated *Omorgus* as equivalent to the *suberosus* species-group. Haaf (1953, 1954a) and Scholtz (1979b) treated the species of *Omorgus* in Africa and Asia, and Namibia as species groups, the *radula-melancholicus*-group and the *radula*-group respectively. Baker (1968) restored *Omorgus* (for the *suberosus* species-group) to a valid genus based on morphological differences in larvae and adults between *Trox* and *Omorgus*.

The first comprehensive treatment of the world *Trox* was by Harold (1872). He provided the foundation for the classification of the family in his monograph, in which he described 30 new species and recognised about 100 species worldwide. He was the first author to provide keys and detailed descriptions to all the species included in his monograph; he also discussed morphological characters and made species comparisons. Harold, however, recognised only the genus *Trox*.

Burmeister (1876) reviewed trogids of Argentina and split them into different groups, *Omorgus*, *Chesas*, *Lagopelas* and *Polynoncus*, which he treated as subgenera of the genus *Trox*. *Chesas* and *Lagopelas* were later synonymised with *Omorgus* (Vaurie, 1962; Baker, 1968). *Polynoncus* has remained a well-defined group and all of its species are endemic to the Patagonian sub-region of South America. Other authors followed Burmeister and treated *Polynoncus* as a subgenus of *Trox* (Preudhomme de Borre, 1886; Arrow, 1912; Scholtz, 1982). Scholtz (1982) designated *Trox pedestris* Harold as the type species for the subgenus.

Péringuey (1900), in his revision of southern African *Trox*, provided the first identification and descriptions and illustrations of male genitalia which have since become essential for trogid classification.

Arrow (1912) placed three well-defined genera in the family: *Trox*, *Glaresis* Erichson and *Cryptogenius* Westwood. Petrovitz (1968) described the genus *Afroglaresis* in the Trogidae, to be later synonymised with *Glaresis* (Scholtz *et al*., 1987a). However, the inclusion of *Glaresis* and *Cryptogenius* in the family remained problematic (Scholtz, 1986a).

Until the mid-nineteen eighties Trogidae classification was based mainly on overall physical similarity of species and limited character sets and none of the earlier revisions addressed evolutionary patterns or relationships in the Trogidae. The problem faced in classifying Trogidae was whether to see (1) *Trox* as a large, variable genus with numerous species groups, (2) two genera (*Trox* Fabricius and *Omorgus* Erichson) or (3) a single genus with several distinct subgenera.

The first comprehensive classification to date for this group was by Scholtz (1986a), whose study attempted to infer relationships between and within genera based on shared, derived character states (synapomorphies) as per the cladistic method. The resulting phylogeny (Fig 1), based on 18 adult morphological characters considered important in trogid classification, divided the family into two distinct lineages, a basal *Trox* lineage (with two subgenera, *Trox* and *Phoberus*) and a derived lineage consisting of *Polynoncu*s and *Omorgus* (with three subgenera, *Omorgus* s. str., *Haroldomorgus* Scholtz and *Afromorgus* Scholtz). Within *Trox*, the nominal subgenus was characterised by fewer apomorphies than was *Phoberus*. Of the *Omorgus* subgenera, *Haroldomorgus* has the fewest apomorphies, *Omorgus* more and *Afromorgus* the most apomorphic states.

Scholtz’s (1986a) results indicated that the Afrotropical *Trox* species are polyphyletic and *Phoberus* was consequently re-established as a subgenus of *Trox*. He elevated *Polynoncus* and *Omorgus* to generic level. Since then *Omorgus* has been internationally recognised as a valid genus in the Trogidae (Ratcliffe, 1991; Jameson, 2002).

In addition, Scholtz also found there was no phylogenetic justification for the retention of *Glaresis* and *Cryptogenius* within the Trogidae because they shared no synapomorphies with *Trox*. Scholtz *et al*. (1987a, b) subsequently placed *Glaresis* in a monotypic family, Glaresidae Kolbe and transferred *Cryptogenius* to the Hybosoridae Erichson. The Glaresidae, however, are still treated, by some authors, as a subfamily of the Trogidae (Smith *et al*., 2006; Ratcliffe & Paulsen, 2008), although recent studies (Bai *et al*., 2012a, b; Ahrens *et al*., 2014) have clearly demonstrated their independent phylogenetic origins.

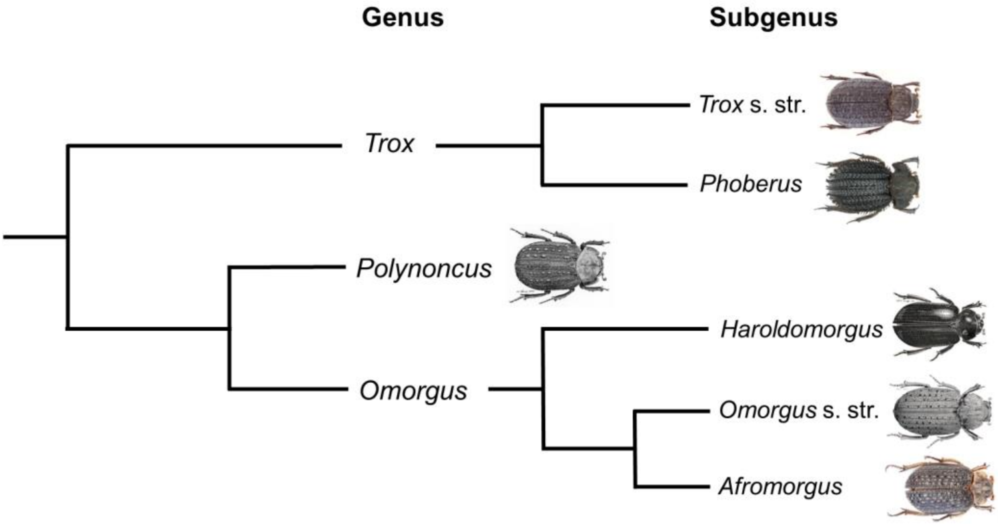

Figure 1. Phylogram of the Trogidae genera and subgenera based on a morphological dataset (Scholtz, 1986a). Images depict the type species of the genus or subgenus.

A series of studies that followed Scholtz (1986a) supported the above-mentioned findings: Scholtz & Peck (1990) and Grebennikov & Scholtz (2004) examined larval characters for the Trogidae and the basal groups in the Scarabaeoidea respectively; d’Hotmann & Scholtz (1990) assessed the significance of male genitalia for phylogenetic inference; Nel & Scholtz (1990) compared the morphology of mouthparts of adult Scarabaeoidea; Browne *et al*. (1993) examined wing articulation and wing base characters; and Scholtz (1991, 1993) investigated the phylogenetic importance of trogid larval morphology from different geographical regions (within genera). All of these studies demonstrated that: (1) Trogidae are unquestionably a monophyletic family, within the superfamily Scarabaeoidea, and that (2) the genera and subgenera can be defined on the basis of both adult and larval autapomorphic character states.

Current classification

Despite the comprehensive treatment of the family, some authors have subsequently proposed changes to the classification established by Scholtz (1986a) and Scholtz and Peck (1990): Nikolajev (2005) revised the morphological characters of the family and split the Trogidae into two subfamilies, the nominal Troginae MacLeay, containing the genus *Trox*, and Omorginae Nikolajev, 2005 comprising the genera *Omorgus* and *Polynoncus*. Pittino (2006) elevated the subgenus *Afromorgus* Scholtz to generic status on the basis of two apomorphic characters on the male genitalia and biogeography. Zidek (2013) in his checklist of the Trogidae considered the generic status of *Afromorgus* (as proposed by Pittino) as nomenclaturally incorrect and reconsidered *Afromorgus* as a subgenus of *Omorgus* (*sensu* Scholtz 1986a). Recently Pittino (2010) described a new genus, *Madagatrox*, to accommodate a single flightless species from Madagascar. Consequently, there are currently four nomenclaturally valid extant genera in the family: *Trox* (with subgenera *Trox* and *Phoberus*), *Omorgus* (with subgenera *Omorgus*, *Afromorgus* and *Haroldomorgus*), *Polynoncus*, and *Madagatrox.* However, these changes to the classification were not based on formal phylogenetic analyses.

Geographic distribution of genera and subgenera

The Trogidae have cosmopolitan distribution and are found on all continents except Antarctica (Scholtz, 1986a). Africa and Eurasia have the richest faunas with about 100 species each, followed by Australia (57 species) and South and North America (around 50 species each) (Scholtz 1982, 1986a, 1986b, 1990b; Strümpher *et al*., 2014; Zidek, 2013). The fauna of each of the zoogeographical regions has been revised, mostly over the past 50-odd years; some of the most important revisions include those by Blackburn (1904) and Haaf (1954a; 1954b) who treated the Australian and Ethiopian/Oriental faunas, respectively; Vaurie (1955; 1962) the Nearctic and Neotropical faunas; Balthasar (1936), Pittino (1983, 1985) and Scholtz *et al*. (2007) studied the Palaearctic fauna; and Scholtz (1980, 1986b, 1990) treated the Afrotropical (Sub-Saharan Africa), Australasian and Neotropical faunas, respectively. Scholtz (1982) and Zidek (2013) catalogued the species of the world.

Genus: ***Omorgus***

This large genus contains two subgenera and about 145 described species. Members of the group are widely distributed throughout the arid regions of the southern continents extending into the southern Nearctic and Oriental regions. The genus is virtually absent from the temperate Nearctic and Palaearctic regions (Fig 2).

Subgenus: ***Omorgus***

The subgenus is distributed throughout the New World and the Australasian Region and is composed of approximately 90 species (Scholtz, 1986b, 1990b; Vaurie, 1955, 1962; Smith, 2003; Zidek, 2013). Species diversity is highest in the Australasian Region and the majority of species in the region are confined to the Australian mainland (the core distribution) with the Webster’s line in the north-west forming the distribution limit to all but three species: *Omorgus costatus* Wiedemann, *O. mollis* Arrow and *O. nanningensis* Pittino. *Omorgus costatus* occurs across the Australasian Region and its distribution extends into the western Pacific (along the Melanesian arc) and into Southeast Asia. *Omorgus mollis* occurs only in Indonesia and *O. nanningensis* is known only from China. Both of the latter two species are undoubtedly of Australian descent (Scholtz, 1986b; Pittino, 2005). The number of described *Omorgus* species in the Australasian region currently stands at 57 (Strümpher *et al*., 2014), of which one species, *O. suberosus*, was introduced from the New World and is now well established in eastern and south-western Australia and possibly even parts of southern Europe (Scholtz, 1986b).

The New World species are widespread in the southern Nearctic and the Neotropical Regions. However, species distributions show a distinct South American and North American division with a high degree of endemism; only two species are shared by both continents (*O. suberosus* Fabricius and *O. loxus* Vaurie). In South America the subgenus is represented by 14 species (Scholtz, 1990b; Pittino, 2010), most of which are confined to the southern regions of the continent with only a few species penetrating into the northern Neotropical regions. The majority of the 20 species in North America are restricted to the arid south-western and central regions (Vaurie, 1962).

Subgenus: ***Haroldomorgus***

This monotypic subgenus is endemic to South America, and its only representative, *O. batesi* Harold is fairly widespread between the northern tropical regions of Argentina and Brazil (Scholtz, 1990b; Fig 2).

Subgenus: ***Afromorgus***

The subgenus includes about 58 species and occurs throughout the Afrotropical Region extending into Arabia, the Oriental and Indo-Malayan regions. *Afromorgus* is absent from the New World and Australasian Region (Scholtz, 1980, 1986a). The majority of species (about 38) are found on the African continent, mostly in moist and arid savanna regions, but with a few species intruding into deserts of southern Africa and Arabia (Scholtz 1980, 1982; Pittino, 2005; Fig 2). The species in Asia are mainly restricted to the Indian subcontinent, with a few species extending into the Far East (Haaf, 1954a, b; Pittino, 2005; Scholtz, 1980, 1982). Interestingly, only one species, *O*. (*Afromorgus*) *chinensis* Boheman, which is widespread across China, Indo-China and Indonesia, has successfully penetrated into temperate regions of the Palaearctic.

Genus: ***Polynoncus***

This well-defined genus is endemic to South America, with the majority of the 34 species restricted to the southern one-third of the continent (Patagonian sub-region) (Scholtz, 1990b; Pittino, 2010; Zidek, 2013; Fig 2). The Patagonian sub-region is predominantly temperate and is characterized by large expanses of grassland, scrubland, dry savanna, steppes and deserts.

Genus: ***Trox***

Members of this genus, with about 148 species, are widespread throughout the temperate regions of North America, Eurasia and Africa (Scholtz, 1982; Smith, 2003; Pittino, 2006; Zidek, 2013; Fig 2). The ancestral biome of the genus was apparently temperate as this is where most of the more plesiomorphic species still occur. *Trox* are absent from the Australasian and Neotropical Regions, except for one ubiquitous species, *Trox scaber* (Linnaeus), which was undoubtedly introduced by man (Scholtz, 1986b; Scholtz, 1990b).

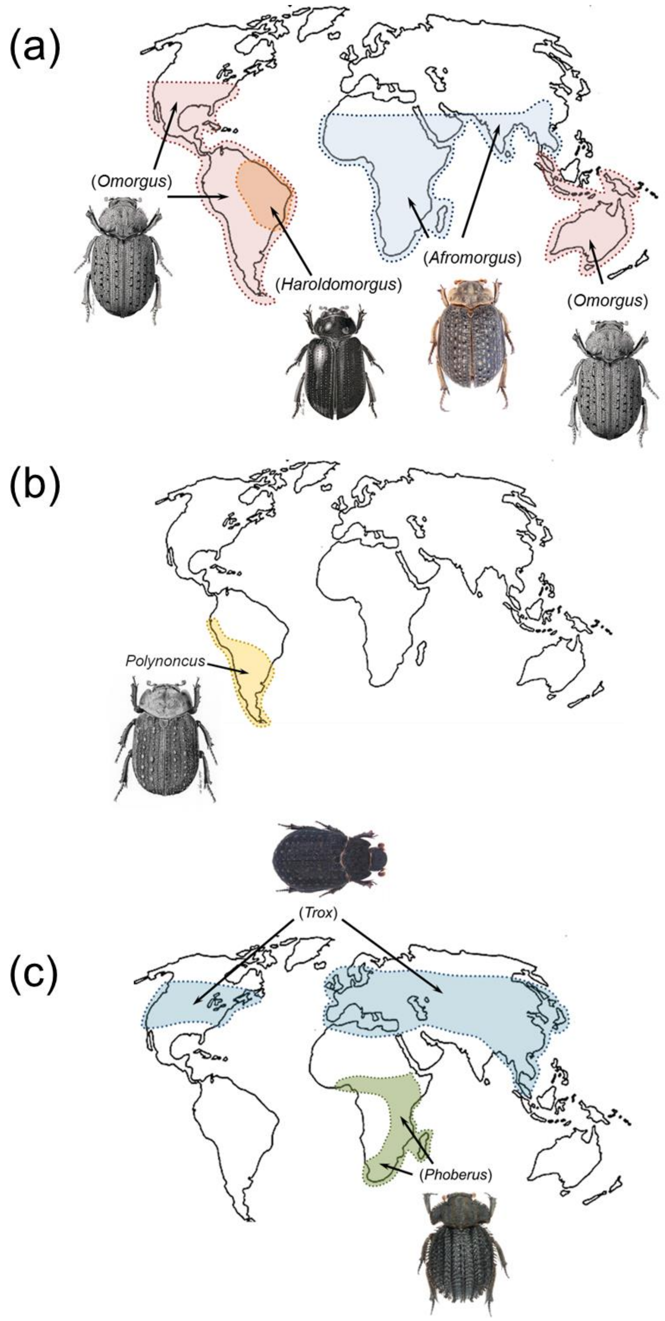

Figure 2. Distribution of extant Trogidae genera (and subgenera): (a) *Omorgus* Erichson (b) *Polynoncus* Burmeister and (C) *Trox* Fabricius.

Subgenus: ***Trox***

Trox s. str. is the larger of the two subgenera with about 108 species and its members are found in most of the major biomes of the Holarctic (Scholtz, 1982, 1986b; Pittino, 2006; Scholtz *et al*., 2007; Fig 2). A small number of species, whose natural distributions fall outside the Holarctic realm, are confined to the Afrotropical Region (Scholtz, 1986b); and one species, *T. perrieri* Fairmaire, is endemic to the island of Madagascar (Paulian, 1981) (but see chapter 2).

The Palaearctic and Nearctic species can be roughly divided into six informal species-groups (Vaurie, 1955; Pittino, 1985; Pittino & Kawai, 2006), based on external morphology and male genitalia. The largest of these groups, the "*terrestris*"-group (Pittino & Kawai, 2006), consists of mostly eastern Palaearctic species, but also includes Nearctic species. The "*hispidus*"-group is mainly distributed around the Mediterranean. The "*sabulosus*"-group contains eastern Palaearctic species. Another typical Holarctic group is the "*scaber*"-group; it is the most widespread across the Palaearctic and has related species in the Nearctic. The (now) virtually cosmopolitan species, *Trox scaber* (Linnaeus) is included in the latter. The remaining two groups, "*unistriatus*" and "*tuberculatus*" are restricted to the Nearctic (Vaurie, 1995).

Subgenus: ***Phoberus***

The majority of the species occur along the temperate eastern montane faunal exchange route (Endrödy-Younga, 1978), with a few species penetrating into the arid regions of southern Africa (Scholtz, 1980; Fig 2).

Genus: ***Madagatrox***

This monotypic genus is endemic to Madagascar, and is represented by the flightless species, *Madagatrox ranotsaraensis* Pittino (2010). It shares some overall similarities with the flightless members of *Phoberus* from the mainland (but see Chapter 3 and Zidek, 2013).

Evolution and fossil record

The Trogidae have long been considered to represent a basal group within the Scarabaeoidea, whose origin extends back to the Upper Jurassic/Lower Cretaceous (Scholtz, 1986a; Scholtz & Chown, 1995). In Scholtz's (1986a) attempt to resolve the phylogenetic relationships within the Trogidae, based on synapomorphic morphological character states and current distribution patterns, he hypothesised that they evolved in central Pangaea prior to the breakup of the supercontinent that formed Laurasia and Gondwana, where the ancestral form evolved into several lineages. Following the breakup of Pangaea, the lineage that eventually gave rise to *Trox* was restricted to Laurasia, and the ancestral *Omorgus* lineages to Gondwana. The ancestral *Omorgus* and *Polynoncus* lineages radiated on the Gondwana continents while the *Trox*-lineage radiated in the Holarctic and later another derived lineage dispersed into Africa, along the temperate eastern highland mountain faunal exchange route.

The recent discovery of Mesozoic fossils belonging to Trogidae has confirmed the long-standing view that the family represents an ancient group of beetles (Krell, 2007; Nikolajev, 2009). *Trox antiquus* Wickham 1909 from the Oligocene (~34 Mya), had until recently, been the only described trogid fossil. The number subsequently increased to 13 species belonging to five genera of which four were new for the family (Krell, 2007; Nikolajev, 2009). The extant genus *Trox*, according to the fossil evidence, was already present during the Late Jurassic-Early Cretaceous (98-144 Mya), which lends support to the proposed Laurasian origin for this genus (Scholtz, 1986a). Current estimates for the divergence of the family (McKenna & Farrell, 2009; Ahrens *et al*., 2014) are broadly consistent with the current fossil record, supporting a Late Jurassic-Early Cretaceous origin for trogids (see Chapter 2).

Molecules and phylogeny

Systematics is a biological discipline that is devoted to characterizing the diversity of life and organizing our knowledge about this diversity primarily through estimating the phylogenetic relationships between organisms (Holder & Lewis, 2003), and reveals the pattern of events that led to their distribution and diversity (Lipscomb, 1998). The goals for systematic studies are twofold, firstly to identify monophyletic groups at higher taxonomic levels, and secondly to identify lineages (i.e. species) at lower levels (Sites & Marshall, 2004). Delimiting a species is important for understanding many evolutionary mechanisms and processes. Species are also used as the fundamental units of analysis in biogeography, ecology, macro-evolution and conservation biology (Sites & Marshall, 2004). A good phylogeny is therefore of paramount importance if good phylogeographic and population studies are to follow.

Traditional classification systems were mainly focused on morphology. However, with the advent of the Polymerase Chain Reaction (PCR: Saiki *et al*., 1988), the emphasis shifted towards molecular (DNA) studies. Today, molecular systematics is an essential tool for studying evolutionary relationships among lineages and/or sequences. It uses the information that is contained in molecular data (gene sequences) to reconstruct phylogenetic relationships among taxa (San Mauro & Agorreta, 2010). Nuclear and mitochondrial ribosomal RNA genes are some of the most frequently sequenced genes for studying phylogenetic relationships. DNA sequences provide different levels of variability for specific evolutionary questions because of the high degree of variation in substitution rates among genes and gene regions (San Mauro & Agorreta, 2010). Combining molecular markers from different gene regions has become an integral part of phylogenetic studies.

The scope and aims of the study

This thesis is restricted to a study of the systematics of Trogidae, a monophyletic and biologically unique family within the Scarabaeoidea. Even though the group is taxonomically well studied, the traditional classification system (Scholtz, 1986a), which remained relatively stable for three decades, has been challenged recently. Furthermore, the existing morphological phylogeny of Trogidae does not address the ages of lineages and the associated major speciation events.

Thus, the aim and purpose of this study were to: (1) investigate the phylogenetic relationships of the world genera and subgenera to determine whether a phylogeny based on molecular data would support the existing morphological phylogeny, the first of its kind for this particular group; (2) deduce their historical biogeography by estimating the ages of major divergence events using fossil data; (3) investigate intra-generic/subgeneric relationships; (4) focus on intra-specific speciation and radiation and lastly (5) to propose taxonomic amendments based on these results.

In summary, the research questions of this project include:

1. Tracing the evolutionary history of the Trogidae
2. Dating major divergence events of different Trogidae lineages
3. Resolving phylogenetic relationships in the Afrotropical subgenus *Phoberus*
4. Investigating the loss of flight in subgenus *Phoberus*
5. Investigating speciation and radiation in the flightless *Trox capensis* species complex
6. Overview and revision of the extant genera and subgenera of the family and taxonomic amendments

Specific hypotheses are outlined within each of the following chapters.

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Chapter 2

Phylogeny of the family Trogidae (Coleoptera: Scarabaeoidea) inferred from mitochondrial and nuclear ribosomal DNA sequence data

Abstract

Trogidae constitute a monophyletic and biologically unique family within the Scarabaeoidea, being the only keratinophagous group in the superfamily. Traditionally, the family has been divided into three distinctive genera, *Polynoncus* Burmeister, *Omorgus* Erichson and *Trox* Fabricius. Although the taxonomy of the group is relatively well studied, recently changes to the existing classification have been proposed and the family as currently constituted has not been subjected to phylogenetic analyses. Here we present a molecular phylogeny for this cosmopolitan family based on three partially sequenced gene regions: 16S rRNA, 18S rRNA and 28S rRNA domain 2. Included in the analyses are representatives from the major zoogeographic regions, representing about 20% of the known trogid species diversity belonging to four of the five extant genera (and three of the four subgenera) in the family. Phylogenetic analyses performed included Parsimony and Bayesian Inference. We deduce their historical biogeography by using trogid fossils as calibration points for divergence estimates. Our analyses resolved relationships between and within genera and subgenera that are largely congruent with existing phylogeny hypotheses based on morphological data. We recovered four well-supported radiations: *Polynoncus*, *Omorgus*, Holarctic *Trox* and African *Phoberus*. On the basis of this study, it is proposed that taxonomic changes to the generic classification of the family be made. The subgenera *Trox* and *Phoberus* should to be elevated to genera to include the Holarctic and all the Afrotropical species, respectively, and *Afromorgus* returned to subgeneric rank. Estimates of divergence time are consistent with a Pangaean origin of the family in the Early Jurassic. The subsequent diversification of the major lineages is largely attributed to the breakup of Pangaea and Gondwana in the Middle Jurassic and early Late Cretaceous, respectively.

Introduction

Trogidae MacLeay (Coleoptera: Scarabaeoidea) comprise approximately 330 species worldwide (Scholtz, 1982; Smith, 2003; Pittino, 2006; Zidek, 2013). This family is well known for its remarkable feeding specialisation; all known species (adults and larvae) feed almost exclusively on keratin and are the only members of the Scarabaeoidea capable of digesting it (Scholtz, 1986a). They primarily inhabit the temperate and arid/savannah regions of the world. Africa and Eurasia have the richest fauna with about 100 species each, followed by Australia (55 species), and South and North America with around 50 species each (Pittino, 2006; Scholtz, 1982, 1986a, 1986b, 1990a; Table 1). The group is taxonomically well studied and the fauna of each of the zoogeographical regions has been revised (Balthasar, 1936; Haaf, 1954a; 1954b; Vaurie, 1955; 1962; Pittino, 1983; 1985; Scholtz, 1980; 1982; 1986b; 1990a; Scholtz *et al*. 2007; Zidek, 2013).

Table 1. Classification and biogeographical distribution of the family Trogidae.

Genus	Subgenus	Region	Distribution	# spp.
Troginae				
Trox	*Trox*	Palaearctic	Europe/Asia/Oriental	77
Trox	*Trox*	Nearctic	North America	28
Trox	*Trox-Phoberus*	Afrotropical	Sub-Saharan Africa, Madagascar	40
Madagatrox		Afrotropical	Madagascar	1
Omorginae				
Omorgus	*Omorgus*	Australasian	Australia, Indonesia, Asia	57
Omorgus	*Omorgus*	Nearctic	North America	20
Omorgus	*Omorgus*	Neotropical	South America	14
Omorgus	*Haroldomorgus*	Neotropical	South America	1
Afromorgus		Afro-Oriental	Africa, Arabia, Orient, Asia	54
Polynoncus		Neotropical	South America	34

Much of our understanding of generic-level relationships within Trogidae is the result of Scholtz's (1986a) phylogenetic study, which was the first attempt to infer relationships between and within genera based on synapomorphic characters. The resulting phylogenetic classification, which has remained relatively stable for the last three decades, divided the family into two distinct lineages, a basal *Trox* Fabricius lineage (with two subgenera, *Trox s*. str. and *Phoberus* MacLeay) and a derived lineage consisting of the genera *Polynoncus* Burmeister and *Omorgus* Erichson (with tree subgenera *Omorgus* s. str., *Haroldomorgus* Scholtz and *Afromorgus* Scholtz). Phylogenetic studies that followed Scholtz (1986a) supported these findings, and demonstrated that (1) Trogidae is a monophyletic group within the superfamily Scarabaeoidea, and (2) monophyletic genera can be defined on the basis of both adult and larval synapomorphic character states (D'Hotmann & Scholtz, 1990; Nel & Scholtz, 1990; Scholtz & Peck, 1990; Browne *et al*., 1993; Scholtz, 1991, 1993; Browne & Scholtz, 1995, 1999; Grebennikov & Scholtz, 2004; Fig 1).

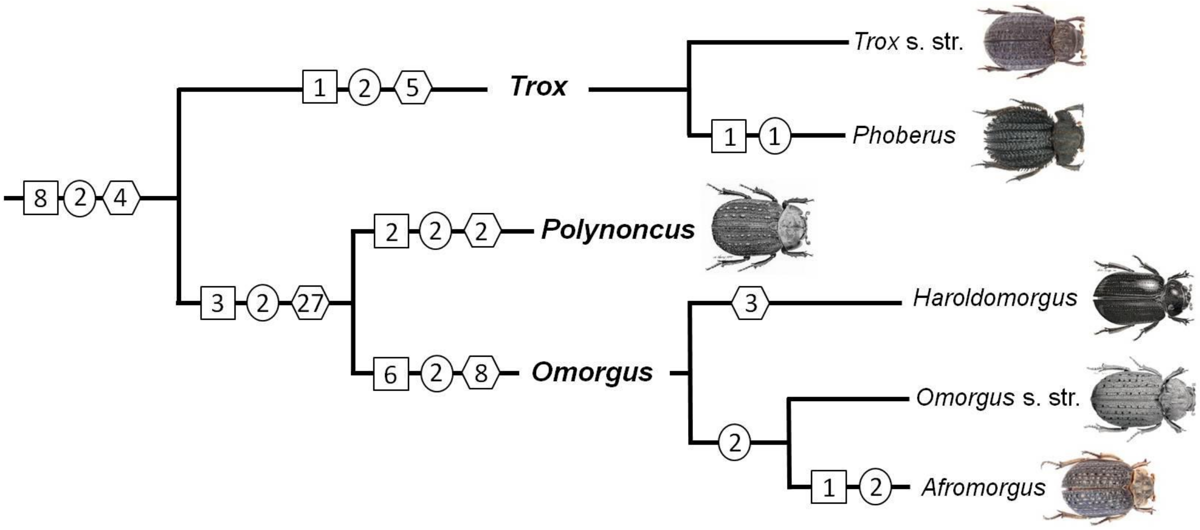

Figure 1. Phylogram of the Trogidae genera and subgenera based on larval (boxes), adult (circles) and hind wing (hexagons) synapomorphic characters. Numbers represent the number of characters which unite each node. For details on larval characters see Scholtz & Peck (1990), Scholtz (1993) and Grebennikov & Scholtz (2004); for adult (excluding hind wing) characters see Scholtz (1986a) and Browne & Scholtz (1999); for hind wing characters see Browne *et al*. (1993) and Browne & Scholtz (1995). Images depict the type species of the genus or subgenus.

Subsequent authors have proposed changes to Scholtz's (1986a) classification. Nikolajev (2005) split Trogidae into two subfamilies: Troginae MacLeay containing the genus *Trox*, and Omorginae Nikolajev comprising the genera *Omorgus* and *Polynoncus*. Pittino (2006) elevated the subgenus *Afromorgus* to generic status. A new genus from Madagascar, *Madagatrox* Pittino was recently described (Pittino, 2010). There are currently five valid extant genera in Trogidae: *Trox* (with two subgenera, *Trox* and *Phoberus*), *Omorgus* (with two subgenera, *Omorgus* and *Haroldomorgus*); *Afromorgus*, *Polynoncus*, and *Madagatrox*. However, the family as currently constituted have yet to receive any formal phylogenetic analyses.

Trogidae have long been considered to represent a basal group within the Scarabaeoidea, whose origin extends back to the Upper Jurassic/Lower Cretaceous (Scholtz, 1986a; Scholtz & Chown, 1995). In Scholtz's (1986a) attempt to resolve the phylogenetic relationships within the family, based on shared derived morphological characters and current distribution patterns, he hypothesised that trogids evolved in central Pangaea, prior to the breakup of the supercontinent that formed Laurasia and Gondwana. Following the breakup of Pangaea, the lineage that eventually gave rise to *Trox* was restricted to Laurasia, and the ancestral *Omorgus* lineages to Gondwana. The ancestral *Omorgus* and *Polynoncus* lineages radiated on the Gondwana continents while the *Trox*-lineage radiated in the Holarctic and later another derived lineage (*Phoberus*) dispersed into Africa, along the temperate eastern highland mountain faunal exchange route. The recent discovery of Mesozoic fossils of Trogidae has confirmed the long-standing view that the family represents an ancient group of beetles within the Scarabaeoidea (Browne & Scholtz, 1999; see Krell, 2007; Nikolajev, 2009a for fossil data). These fossil records also pre-date the only current (molecular) estimate for the divergence of the family of 121.4 Mya (McKenna & Farrell, 2009). Furthermore, Cretaceous fossils (98-144Mya) ascribed to *Trox* from modern northeast Asia (Nikolajev, 2007, 2009a) lends support to the proposed Laurasian origin for this lineage (Scholtz, 1986a).

The aim of this study is to investigate the phylogenetic relationships of the world genera and subgenera based on partial DNA sequences of three ribosomal gene regions (two nuclear and one mitochondrial). Secondly, we deduce their historical biogeography by estimating the ages of major divergence events using fossil data.

Materials and methods

Sampling, amplification, sequencing and processing of sequences

Specimens preserved in ethanol were obtained from all the major zoogeographic regions (Table S1), representing approximately 20% of the known trogid species diversity. Four of the five extant genera (and three of the four subgenera) in the family are represented in the ingroup sample. Only the rare, monotypic subgenus *Haroldomorgus* Scholtz and the monotypic genus *Madagatrox* are not included in our phylogeny, as specimens suitable for DNA extraction could not be obtained. Studies on the higher classification of Coleoptera and Scarabaeoidea indicate that Bolboceratidae Mulsant, Lucanidae Latreille and Trogidae are closely related clades within the superfamily Scarabaeoidea (Browne & Scholtz 1999; Smith *et al*., 2006; Hunt *et al*., 2007; Lawrence *et al*., 2011). Based on these results, representative species belonging to the families Bolboceratidae and Lucanidae were selected as suitable outgroups. Collected individuals were identified, catalogued and stored for further data analysis. Specimens are deposited at the Department of Zoology and Entomology, University of Pretoria, South Africa (UPSA).

Total genomic DNA was extracted from all individuals using the Roche High Pure PCR Template Preparation Kit (Roche Diagnostics, Penzberg, Germany). We amplified the nuclear genes 18S rDNA (18S) and 28S rDNA domain 2 (28S) and the mitochondrial gene 16S rDNA (16S) using previously reported primer sequences and a newly designed primer for 28S (Table S2). The 16S and 18S genes were amplified using the polymerase chain reactions (PCR) performed in a final volume of 50µl. PCR reaction mixtures contained 2.5mM $MgCl_2$, 20pmol of each primer, 10mM dNTPs, 1 x PCR Buffer, in the presence 1 unit of TaqDNA polymerase (Super-Therm® DNA polymerase, Separation Scientific SA (PTY) LTD, South Africa) and 50-100ng of genomic DNA template. For 28S, Emerald Amp®MAX HS PCRMastermix (TAKARA BIO INC., Otsu, Shiga, Japan) was used. Different cycling parameters were implemented for each gene (Table S3). Successful amplifications were purified using the Roche High Pure Product Purification Kit (Roche Diagnostics, Penzberg, Germany) following the manufacturer's instructions.

To obtain DNA sequences, the cycle sequencing reactions were carried out in both directions using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, USA). Cycle sequencing products were precipitated using a standard sodium acetate/ethanol precipitation protocol. All sequences generated were viewed, assembled and edited in CLC Bio main Workbench Version 5.5 (Developed by CLC Bio, http://www.clcbio.com). New sequences (Table S1) were submitted to GenBank. Partial 16S RNA sequences of two more *Trox* species, *Trox uenoi* (Accession no.AB178316) and one unidentified species (Accession no.EF487978) retrieved from GenBank, were included in the analysis.

Alignment

Processed sequences for each of the different gene regions (16S, 28S domain 2, 18S) were aligned based on secondary structure using RNAsalsa version 0.8.1.win32 (Stocsits *et al*., 2009). The program uses both secondary structure information for adjusting and refining sequences alignments and sequence information contained in the alignments to refine predictions of structure (Stocsits *et al*., 2009). To initialise the analytical process, RNAsalsa requires both an initial alignment of homologous RNA sequences and structural constraints (secondary structure predictions) for a single sequence to guide the alignment process.

The input alignments, for each gene region, were produced using MAFFT (Katoh & Toh, 2008). For 28S, structural alignment was performed based on the secondary structure of the 28S sequence of *Apis mellifera* (Gillespie *et al*., 2006). The structural constraints for 18S (*T. rudebecki* – accession number AY745581), 16S (*T. uenoi* – accession number AB178316) were predicted using the RNAfold server (Gruber *et al*., 2008; Hofacker, 2003). The server predicts minimum free energy (MFE) structures and base pair probabilities of single-stranded RNA or DNA sequences. Runs were initiated with default parameter settings for substitution cost, gap penalties and base-pairing occurrence stringencies, except for two stringency settings (-s1, -s3) which were set to 0.51. RNAsalsa constructs a final multiple sequence alignment together with a consensus structure. For a detailed discussion of RNAsalsa, refer to Stocsits *et al*. (2009). The alignments for the three partial sequences, including information on their consensus secondary structures, were concatenated into a supermatrix using the program FASconCAT version 1.0 (Kück & Meusemann, 2010a, b).

Evaluating the general structure and potential conflict in alignments

We constructed a neighbour-network of the individual and the concatenated alignments using the program SplitsTree 4 (Huson & Bryant, 2006). Neighbour-net analyses can be used to visualise conflicting signal (ambiguities) in the data or alternative phylogenetic histories through a splits graph, and help to assess the tree-likeness (congruence) of the data, independently of tree reconstruction techniques (Huson & Bryant, 2006; Wägele & Mayer, 2007). Networks were based on uncorrected *p*-distances. Parsimony uninformative sites were excluded. Bootstrap support values calculated were based on 1000 replicates.

Phylogenetic Analysis

Analyses were conducted using Maximum Parsimony and Bayesian inference for the combined dataset. The parsimony analysis was implemented in PAUP*4.010b (Swofford, 2003) with the following heuristic search setting: all characters were equally weighted and unordered, gaps were treated as missing data and uninformative sites excluded; starting tree obtained via stepwise addition with random addition of sequences with 10 replicates; branch-swapping = tree-bisection-reconnection; initial ‘maxtrees’ set to 200 with automatic increase by 100 and with ‘MulTrees’ option in effect. Where more than one most parsimonious tree was obtained, a strict consensus tree was calculated. Bootstrap support values (Felsenstein 1985) calculated were based on 1000 replicates.

Bayesian analyses were performed in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) and in PHASE v2.0 (Jow *et al*., 2002; Gowri-Shankar & Jow, 2006). Both programs can simultaneously analyse partitioned datasets using both conventional DNA models and mixed RNA/DNA substitution models.

Analysis in MrBayes was performed using GTR+G model for 16S and 28S and K80+I+G for 18S. The appropriate model of nucleotide evolution was estimated in jModel Test (Posada, 2008) under the Akaike information criterion (model parameters are summarised in Table S4). The data set was partitioned into three gene regions. Parameters for the different partitions were unlinked to obtain separate parameter estimates for each gene and the rate prior was set to variable. Flat Drichlet priors were used in all analyses. Bayesian analyses were conducted by simultaneously running two Monte Carlo Markov chains for 15 million iterations. Trees were sampled every 200th iteration. The first 15000 trees (20%) sampled were discarded as burn-in.

Analysis in PHASE was performed using the RNA7D+dG6+I model (Tillier & Collins, 1998) for paired nucleotides. Although applied here empirically, as the testing of RNA models is beyond the scope of this study, the RNA7D model is a biologically plausible model for the evolution of paired nucleotide sites (Voigt *et al*., 2008; Elven *et al*., 2010; Gazave *et al.*, 2010). For the unpaired regions (loops) we used the general time reversible model (REV+dG6+I). For further details of RNA-specific models see Savill *et al*., (2001), and Jow *et al*., (2002). Phylogenetic trees were constructed using the *mcmcphase* module from the PHASE package with default values for priors and other *mcmc* parameters. The final analysis was run for 30 million iterations, and trees were sampled every 200 iterations. The first three million iterations were discarded as burn-in, which was a sufficient period for log-likelihood values and model parameters to reach stabilisation. The module *mcmcsummarize* generated the consensus trees and all of the relevant statistics including branch lengths and clade support for each node. Optimal branch lengths were calculated (as suggested in the PHASE manual) by running an additional mcmcphase analysis (5 million generations) under the same models as the 30 million generation *mcmcphase* run. We used the consensus tree from the final analyses (30 million generations) to fix the tree topology (with topology proposals turned off) and all other parameters left unchanged. To determine if all runs from the *mcmcphase* analyses reached the same stationary distribution, output files from the *mcmcphase* analyses were formatted, using a modified version of the Perl script phase2tracer.pl (original file available from http://hymenoptera.tamu.edu/rna/download.php) to create readable input files for Tracer 1.5 (Rambaut & Drummond, 2007). We repeated the analysis three times from random starting seeds, and compared the resulting topologies and posterior probabilities.

For comparative purposes we tested a 16-state RNA model in MrBayes, which implements a 16 state RNA stem substitution model (Schöniger & von Haeseler, 1994). This 16 state RNA model considers all possible base-pairs as characters and assumes that compensatory base exchanges result from at least two substitution events (Voigt *et al*., 2008). The concatenated dataset was partitioned into loop and stem regions for 16S, 18S and 28S. The covarian model was implemented for the paired nucleotides (stems). For the loop regions a GTR+I+G (a six-state single-nucleotide model) was implemented. Parameters for the different partitions were unlinked to obtain separate parameter estimates for each gene and the rate prior was set to variable. Flat Drichlet priors were used in all analyses. Posterior probabilities were calculated by simultaneously running two Markov chains for 5 million iterations. Trees were sampled every 200 iterations, and the first 5000 trees (20%) sampled were discarded as burn-in.

To evaluate the performance of the Markov chains, from MrBayes analyses, we used the program AWTY (are we there yet?) (Nylander *et al*., 2008) to assess the convergence rates of posterior probabilities and branch lengths from sampled trees. The program Tracer was used to monitor parameter stabilisation (via inspection of estimated sample size (ESS) and graphical plots of parameter sampling). *FigTree v1.3.1* (Rambaut, 2009) was used to view all tree topologies. All Bayesian analyses were carried out on the Bioportal facility (http://www.bioportal.uio.no) at the University of Oslo, Norway.

Divergence analysis

We estimated the node ages for the major lineage-splitting events within Trogidae using the program BEAST v.1.6.2 (Drummond & Rambaut, 2007). We used a Bayesian relaxed molecular clock approach under the uncorrelated lognormal model, and the Yule speciation process was selected, for all data combined. The combined dataset was partitioned by gene. As models implemented in BEAST are not designed to take covariation into account, a separate GTR+G+I substitution was applied to each partition. Model and clock parameters were unlinked across partitions. The topology with optimised branch lengths, previously estimated using PHASE, was specified as a starting tree for the programme BEAST. Duplicate terminals were pruned from the tree in TreeEdit (Rambaut & Charleston, 2002) so that each species was only represented by a single individual.

Trogidae fossils from the Early Cretaceous serve as useful calibration points for divergence estimates (Krell, 2007; Nikolajev, 2009a). We constrained the minimum age of the Omorginae (*sensu* Nikolajev, 2005) to the late Early Cretaceous (112Mya) based on the fossil *Cretomorgus ikhbogdensis* Nikolajev (112.2–121Mya). The potential minimum age for the Holarctic *Trox* was constrained to the Mid Cretaceous (98.9Mya) based on the fossil *Trox minutus* Nikolajev (98.9–144Mya). Calibrations were modelled under a lognormal distribution with an offset, mean and standard deviation so that 95% of the prior distributions fall within the minimum and maximum ages of the fossils. Parameters of the lognormal distributions were: for *T. minutus*, offset 98.9, mean 3.075, and standard deviation 0.42; for *C. ikhbogdensis*, offset 112.2, mean 1.52, and standard deviation 0.42. Two independent MCMC analyses were run for 60 million generations with parameters sampled every 2000 generation, discarding the first 6000 (20%) trees sampled from each run as burn-in. We kept default settings for all other priors and operators. The application *LogCombiner v1.6.2* (Drummond & Rambaut, 2007) was used to combine the log and tree output files from the two independent runs. Tracer was used to assess the convergence between runs. The module *TreeAnnotator v1.6.2* (Drummond & Rambaut, 2007) generated the maximum clade credibility tree and calculated the mean ages (and 95% confidence intervals).

Results

The final combined molecular dataset consisted of 107 taxa and 1568 base pairs (bp): 16S = 405bp; 18S = 562bp and 28S (D2) = 601bp. Of the 1568 base pairs, 730 (46.6%) were paired characters, 838 (53.4%) were unpaired and 421 characters were parsimony-informative. Refer to Table S4 in the supplementary information for data characteristics and model parameters. The heuristic search from the parsimony analysis produced 49 most parsimonious trees, with tree length = 1779, consistency index (CI) = 0.381 and retention index (RI) = 0.840. The strict consensus tree, with nodal support, is presented in Fig 2. Neighbor-Net analysis of the individual and the concatenated alignment are shown in Fig 3. The consensus trees for the Bayesian analyses are shown in Fig 4 and the Supporting Information (Figs S1, S2). Summaries of the posterior probabilities (PP) and bootstrap support (BS) from the Neighbor-Net, Parsimony and Bayesian analyses, for nodes representing subfamilies, genera and subgenera recovered can be seen in Table 2.

Phylogenetic relationships

Four well-supported monophyletic lineages, *Polynoncus* (node D), *Omorgus* (node E), *Trox* (node H) and *Phoberus* (node I) were recovered across all methods of analyses (Figs 2, 4). However, tree topologies from the Bayesian analyses using mixed DNA/RNA nucleotide substitution models, were overall better resolved (Fig. 4). The latter consistently resolved the deeper phylogenetic relationships within the family, and recovered the two subfamilies, Omorginae (node B) and Troginae (node C) (*sensu* Nikolajev, 2005). The phylogenetic relationships hypothesised below were based on the results from the mixed DNA/RNA model analysis (Figs 4, S2), and largely congruent with the existing morphological phylogeny (Fig. 1).

Phylogenetic relationships within Omorginae (node B) were well resolved (Fig. 4). Two monophyletic sister groups deemed equivalent to genera were recovered; *Polynoncus* (node D) and *Omorgus* (node E). The genus *Omorgus* comprised two poorly supported sister clades; *Omorgus* (node F) representing the New World and Australasian radiations, and *Afromorgus* (node G) comprising an Afro-Oriental radiation. The only Asian representative of *Afromorgus*, *A. pauliani* (Haaf) included in the study lay in the well-supported (node O) clade containing *A. squalidus* (Olivier), the type species of the genus.

Table 2. Support values for nodes recovered representing subfamilies, genera and subgenera for the Parsimony, Bayesian and splits graphs.

		BI (DNA)	BI (RNA/DNA model)		MP	NN
		MrBayes	MrBayes	PHASE	PAUP	SplitsTree
Node	Taxonomic group	(PP)	(PP)	(PP)	(BS)	(BS)
A	Trogidae	0.69	1.00	1.00	74	-
B	Omorginae (*sensu* Nikolajev 2005)	1.00	1.00	1.00	100	97
C	Troginae (*sensu* Nikolajev 2005)	0.69	0.80	0.81	-	-
D	*Polynoncus*	1.00	1.00	1.00	100	100
E	*Omorgus*	1.00	1.00	1.00	100	99
F	*(Omorgus)*	0.83	0.71	0.71	-	-
G	*Afromorgus*	0.96	0.93	0.84	-	42
H	*Trox s. str.*	1.00	1.00	1.00	96	95
I	*Phoberus*	1.00	1.00	1.00	91	94
J	*Omorgus* (Neotropical+Nearctic group)	1.00	1.00	1.00	87	93
K	*Omorgus* (Neotropical+Australian group)	1.00	1.00	1.00	80	51
L	*Omorgus* (Australian group)	1.00	1.00	1.00	95	97
M	*Omorgus* (Neotropical group)	0.98	0.90	0.89	92	77
N	*Omorgus* (Australian group)	1.00	1.00	1.00	72	76
O	*Afromorgus* Asian representative	1.00	1.00	1.00	-	97

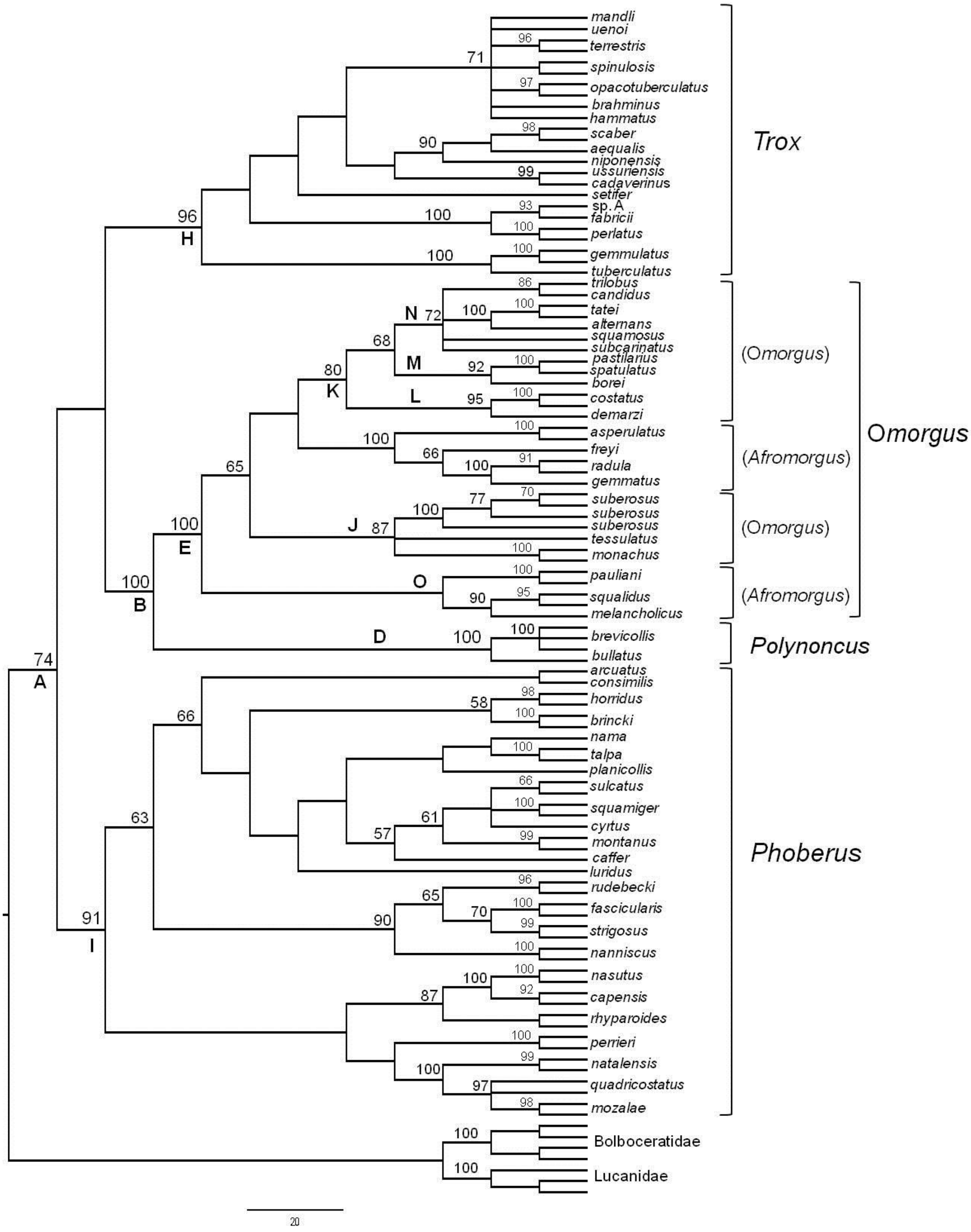

Figure 2. Strict consensus phylogram for the Trogidae of 49 most parsimonious trees for the combined dataset with bootstrap support. Only bootstrap support ≥ 50 are shown.

Within *Omorgus* there were two distinct lineages, one representing a Neotropical+Nearctic group (node J) containing *Omorgus suberosus* (Fabricius), the type species of the genus and therefore also of the subgenus *Omorgus* and a second, Neotropical+Australian group (node K). This latter clade included three ambiguously resolved clades that were biogeographically coherent but with sparse taxon sampling. One of these clades (node M) contains *Omorgus pastillarius* (Blanchard), the type species of a formerly recognised subgenus *Chesas* Burmeister. The Australian species *Omorgus gigas* Harold is the type species of the former subgenus *Megalotrox* Preudhomme De Borre, and is a relative of *Omorgus tatei* (Blackburn), found in the well-supported (node N) sister clade containing *O. pastillarius* (Fig. 1). The third well-supported clade (node L) was also Australian.

Troginae (node C) is composed of two major monophyletic radiations; the Holarctic *Trox* (node H) and the Afrotropical *Phoberus* (node I). Nearctic, Palaearctic and Oriental *Trox* species were interspersed on the tree despite obvious geographical separation among species, and although taxon sampling was only 16 species of about 100 in the region, the posterior probabilities were generally reassuring. All of the Afrotropical species were grouped together, contradicting the independent morphological data (Scholtz, 1986a). Taxon sampling of the Afrotropical group was more than adequate; 24 out of 40 known species were included in the analyses and the monophyletic status of *Phoberus* is not expected to change with increased taxon sampling. The Madagascan species, *T*. *perrieri* Fairmaire, currently placed in the subgenus *Trox*, was nested on a long branch within the Afrotropical *Phoberus* lineage.

Neighbor-Net Graph: General Structure and Conflict of Alignments

The neighbour-network analysis for the individual and the combined dataset (Fig. 3) revealed the presence of four major clusters (*Polynoncus*, *Omorgus*, *Trox* and *Phoberus*). These were largely congruent with the topologies recovered from the

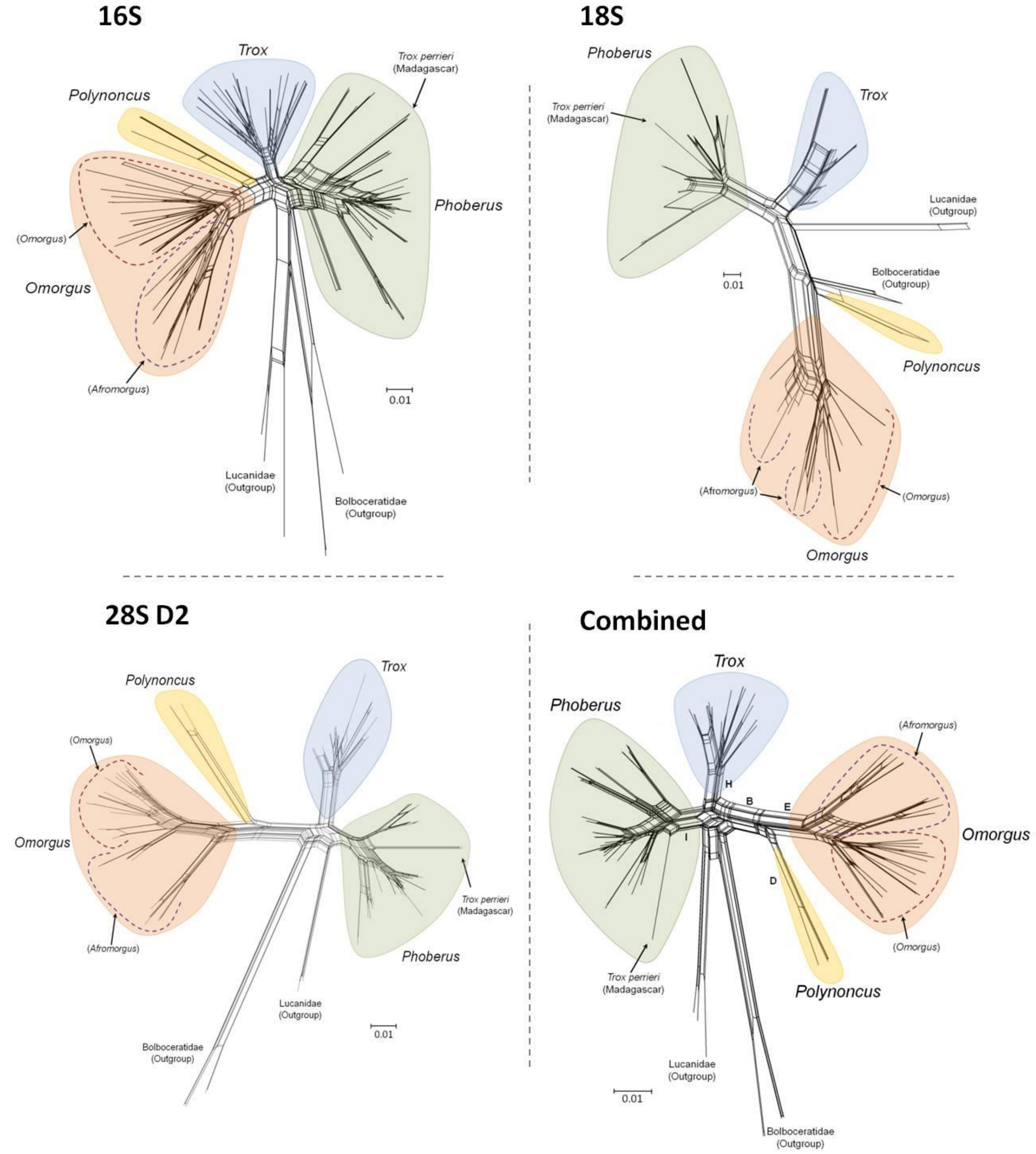

Figure 3. Neighbour-Net graph, for the individual and combined molecular datasets, based on uncorrected *p*-distances.

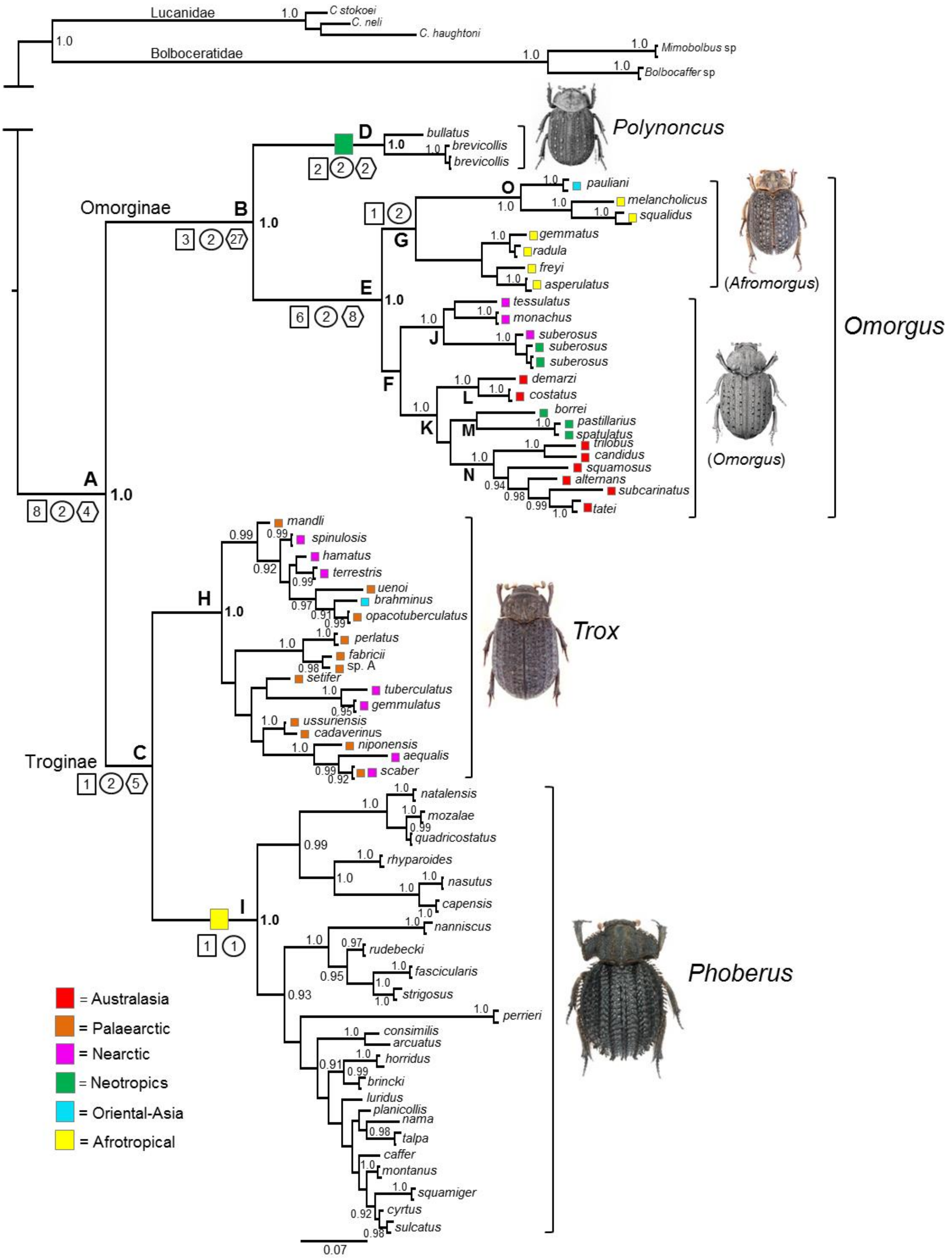

Figure 4. Bayesian topology from the PHASE analysis. Numbers next to each node are the posterior probability support. Only nodes with posterior probabilities ≥ 90% are shown. Images depict the type species of the genus or subgenus. Synapomorphic characters sets (see Fig 1) which unite each node are plotted on the phylogram.

parsimony and Bayesian analysis (Figs 3, 4). Combining the individual datasets in a single analysis improved the split support for a large number of nodes in the splits graph. There was clear split support (long parallel edges) for *Polynoncus* being the sister group to *Omorgus*. The generic separation of *Afromorgus* and *Omorgus* was not as well supported by the analysis, and *Afromorgus* showed evidence of encompassing disparate lineages. The networks showed conflicting splits between *Phoberus* and *Trox*, but the Madagascan endemic, *T. perrieri*, remained closely related to the African *Phoberus*. The support for the major splits is summarised in Table 2.

Divergence time estimates

BEAST analysis (Fig 5) dated the origin of Trogidae during the early Jurassic (194 Mya; 95% confidence interval: 152.7 – 250.1 Mya). The subfamilies Omorginae (node B) and Troginae (node C) diverged well before the Cretaceous (174 Mya; 139.7 – 210.4 Mya - well before the Cretaceous node A). The time of divergence between the Holarctic *Trox* (node H) and the Afrotropical *Phoberus* (node I) was estimated to have occurred during the Late Jurassic (156 Mya; 124.54 – 189.1Mya - node C). *Omorgus* and *Polynoncus* diverged from their last common ancestor at 117 Mya (113.63 – 122.78 Mya), also during the mid-Lower Cretaceous (node B). The estimated time of divergence between the subgenera *Omorgus* (node F) and *Afromorgus* (node G) was around 90 Mya (73.82 – 104.56 Mya - node E). The Australian and New World sister groups within *Omorgus* separated about 81 Mya (65.53 – 97.75 Mya - node F). The three ambiguously supported lineages subtending the Australian and Neotropical radiations occurred around the Cretaceous / Tertiary boundary (node K). The probable existence of ancestors of the Nearctic *Omorgus* (node J) at 46 Mya (25.97 – 67.79 Mya) predates the existing hypothesis of a Pliocene-Pleistocene colonisation of North America (~2 Mya) from South America for this group (Scholtz, 1986a).

Discussion

Molecular phylogeny and the systematics of Trogidae

Our molecular phylogeny supports the morphological phylogeny proposed by Scholtz (1986a) and Scholtz & Peck (1990) in most aspects, and the subfamily divisions

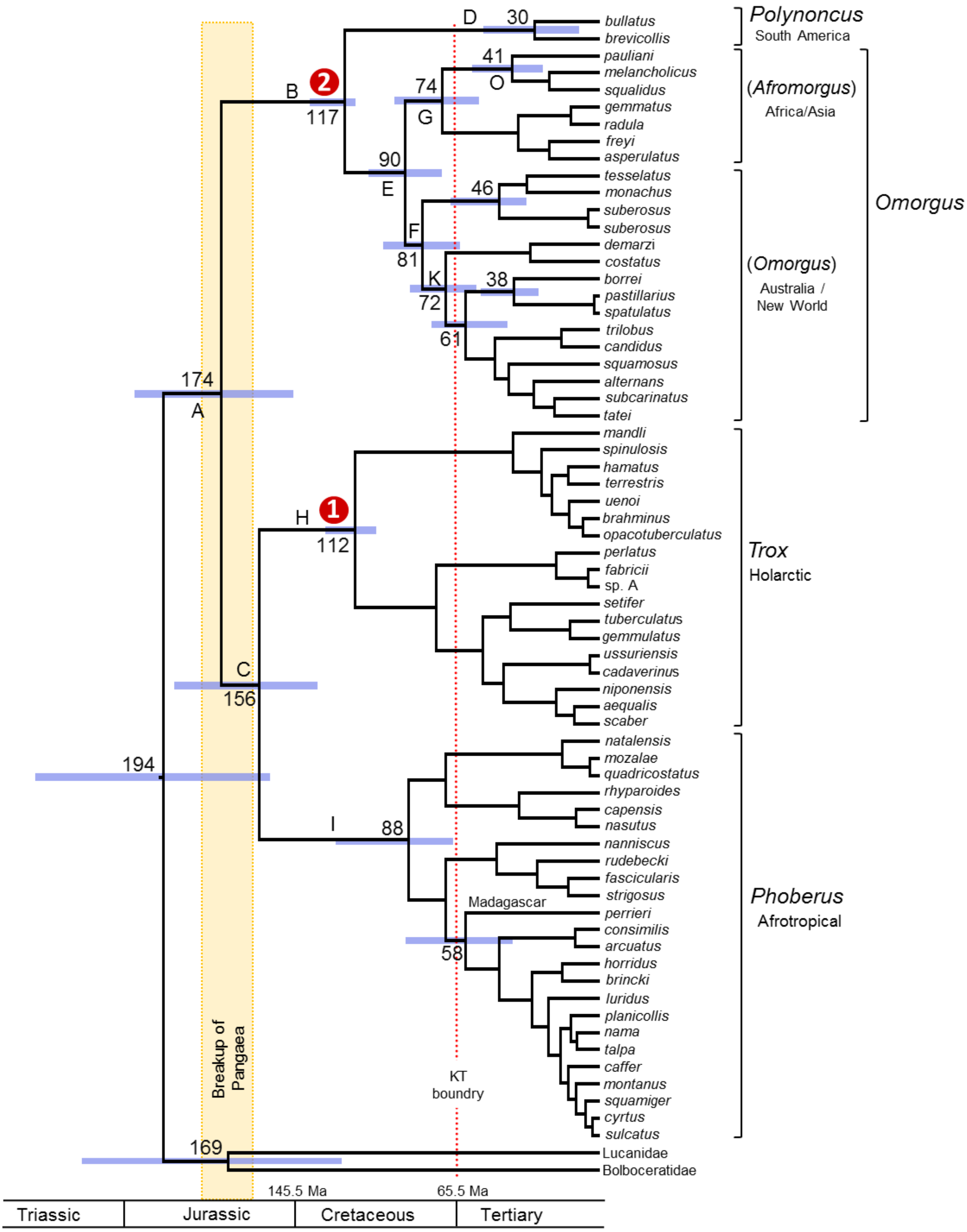

Figure 5. Maximum clade credibility cladogram from the BEAST analysis for Trogidae. Age estimates for the major divergence events in million years with 95% highest posterior density intervals depicted by node bars. Node labels 1 and 2 indicate fossil constraints.

(Trogidae-Omorginae) of Trogidae proposed by Nikolajev (2005). Omorginae was the only subfamily recovered unambiguously. Although there was not particularly good branch support for the monophyly of Troginae, there are convincing (larval and adult) morphological synapomorphic characters supporting the monophyly of Troginae and similarly for Omorginae (Scholtz, 1986a; Scholtz & Peck, 1990; Browne *et al.*, 1993; Nikolajev, 2005; Figs 1, 4).

Within Omorginae, the genera *Omorgus* and *Polynoncus* are recovered as monophyletic and the sister-group relationship is clearly supported by both morphological and molecular data (Figs 1, 4). The genus *Omorgus* is divided into two groups: (1) *Omorgus* representing the Australasian and New World species and (2) *Afromorgus*, containing all the Afrotropical and Asian species. *Afromorgus* is morphologically easily separated from *Omorgus* on the basis of two apomorphic characters on the male genitalia: (1) pars basalis fused dorsally and (2) median lobe complex, not simple (Scholtz, 1986a). Pittino (2006) considered the aforementioned characters important enough to elevate *Afromorgus* to generic status. However, our results clearly indicate that *Afromorgus* is a well-defined subgroup within the genus *Omorgus* and the generic separation of *Afromorgus* and *Omorgus s. str*. is not supported. We intuitively follow the classification system for the genus *Omorgus* and its subgenera, as proposed by Scholtz (1986a).

The subgenus *Omorgus* may well comprise several lineages equivalent to subgenera, but evidence for their monophyly is equivocal. Our results tentatively suggest resurrection of two former subgeneric names, *Megalotrox* and *Chesas*. The former is assignable to all Australian *Omorgus* species and the latter to some Neotropical representatives. Scholtz (1986a) was unable to provide any support for these species representing a monophyletic group in the morphological phylogenetic reconstruction of the family and synonymised it with *Omorgus*. Burmeister (1876) considered *O. pastillarius* unique among the Argentine species, on account of it being flightless, and placed it in the *Chesas*-group which was later treated as a subgenus by Preudhomme de Borre (1886). *Chesas* was subsequently synonymised with *Omorgus* (Vaurie, 1962; Baker, 1968; Scholtz, 1986b). At present we prefer not to recognise these groups as formal without unequivocal evidence.

The subfamily Troginae consists of two monophyletic groups, the Holarctic *Trox* and the Afrotropical *Phoberus*. *Trox* are found in most of the major biomes of the Holarctic (Scholtz, 1982, 1986b; Pittino, 2006; Scholtz *et al*., 2007). However, Palaearctic, Nearctic and Oriental groups show little phylogenetic separation, indicating probable repeated lineage-mixing across Beringia in various pulses, in response to glacial and interglacial episodes.

All the Afrotropical species group naturally together in *Phoberus*, contrary to morphological evidence that the subgenus may be polyphyletic (Scholtz, 1986a). The close relationship of the Madagascan *T. perrieri* to the African *Phoberus*, points unequivocally to dispersal from Africa, across the Mozambique Channel, to Madagascar (Nazari *et al*., 2011; Sole *et al*., 2011; Samonds *et al*., 2012). Haaf (1953) considered *T. perrieri* related to other members on the mainland, which is supported by molecular evidence.

MacLeay (1819), in addition to proposing the family name, described the genus *Phoberus* to accommodate the large flightless southern African species, *T. horridus* Fabricius. However, *Phoberus* was not generally recognised as a genus; Burmeister (1876) and Preudhomme De Borre (1886) considered *Phoberus* to represent a subgenus, including only the type species. Harold (1872) and Scholtz (1979) considered *Phoberus* a synonym of the genus *Trox*, whereas Haaf (1953) treated *Phoberus* only as a species group. Péringuey (1900), Arrow (1912) and, later, Scholtz (1980, 1982) treated *Phoberus* as a subgenus of *Trox*, but they differed in their views regarding inclusion of taxa in the subgenus. The results of the molecular phylogeny justify reinstating the genus *Phoberus* MacLeay to accommodate all Afrotropical (Sub-Saharan) species.

The contemporary classification of this family has been based exclusively on morphological characters. Our molecular phylogeny, the first for the family, provides strong support for the relationships between morphologically described genera and subgenera. On the basis of this study, it is now proposed that certain taxonomic changes to the generic classification of the family be made. The phylogenetic classification proposed closely follows the classification of Scholtz (1986a), except that we support Nikolajev's division of Trogidae into two subfamilies, Omorginae and

Troginae, and suggest that the subgenera *Trox* and *Phoberus* be elevated to genera, with *Afromorgus* being returned to subgeneric rank. The Madagascan species, *T.* (*Trox*) *perrieri*, is transferred to *Phoberus*. The taxonomic changes we propose here will be formalised in a separate publication.

Historical biogeography

The divergence dating analysis proposed a Jurassic origin for extant Trogidae. Although it is difficult to identify the exact area of origin of Trogidae, we consider modern northeast Asia as an important candidate centre for their evolution. Mesozoic fossils assignable to Troginae and Omorginae (Krell, 2007, Nikolajev, 2009a) and various basal Scarabaeoidea (Nikolajev, 2008a-d, 2010; Nikolajev & Ren, 2010; Bai *et al*., 2010, 2012, and 2013) support this view.

The north-south split of Pangaea (180-160 Mya) is considered a key geological event in the diversification of the extant Trogidae (Scholtz, 1986a). The divergence between the two subfamilies Omorginae and Troginae coincides with the initial fragmentation of the supercontinent during the Middle Jurassic, whereas the divergence of Holarctic *Trox* and African *Phoberus* corresponds to the final opening of the Tethyan seaway that eventually separated the two landmasses in the Late Jurassic (Stanley, 1986).

The evolution of *Omorgus* and *Polynoncus* proposed by Scholtz (1986a) remains plausible under our results. Ancestral Omorginae radiated extensively on West Gondwana following the fragmentation of the supercontinent. Scholtz (1986a) hypothesised that the ancestor of *Omorgus*+*Afromorgus* diverged vicariantly when Africa and South America separated. This implies that the two lineages would have diverged in the Late Cretaceous. Our estimate for divergence between the two subgenera (~ 90 Mya), although it post-dated the presumed vicariant event (at about 100 Mya), is compatible with early Late Cretaceous separation of Africa from South America (Van Bocxlaer *et al*., 2006; Sereno *et al*., 2004). The alternative scenario is that speciation occurred more recently following long distance dispersal from South America to Africa. The credibility interval (73.82 – 104.56 Mya) is wide enough to allow for both scenarios. Although long distance dispersal is possible (e.g. Price *et al.*, 2011), the morphological differences between *Afromorgus* and *Omorgus* are substantial, reflecting a long period of isolation between the two groups. We tentatively conclude that *Afromorgus* and *Omorgus* diverged vicariantly when Africa and South America finally separated during the Late Cretaceous. The radiation of *Afromorgus*, estimated at 74 (90-54) Mya, occurred after the separation of Africa from South America, considering that the subgenus is absent from the New World and Australasian Region. *Afromorgus* occurs throughout the Afrotropics and extends into Arabia, the Oriental and Indo-Malayan regions (Scholtz, 1980, 1986a).

The biogeography of the New World and Australasian *Omorgus* is complex and appears to be the result of post-Gondwana dispersal. The dispersal of ancestral *Omorgus* from South America into North America probably occurred during the Late Cretaceous to the Early Eocene when the two continents were connected across the proto-Caribbean archipelago, which formed when South America rifted from Africa (Sanmartín & Ronquist, 2004). There is evidence that the archipelago served as an important biotic exchange route (i.e. the first invasion of marsupial and placental mammals) since the mid-Cretaceous (Feller & Hedges, 1998; Sanmartín & Ronquist, 2004; Ortiz-Jaureguizar & Pascual, 2011).

A post-Gondwanan Antarctic connection between southern landmasses probably facilitated dispersal of South American *Omorgus* into Australia (Trewick, 2000; Almeida *et al.*, 2012). Divergence estimates are congruent with the time period when Australia, Antarctica and South America remained connected. The trans-Antarctic corridor served as an important dispersal route for the exchange of southern temperate biota between South America and Australia, since the Late Cretaceous and until the Early Palaeogene (Lawver *et al.*, 1992; Woodburne & Case, 1996; Sanmartín & Ronquist, 2004; Almeida *et al.*, 2012; see also Ali & Aitchison, 2009). Biotic exchange between southern landmasses was eventually disrupted by global cooling and the physical separation of the continents (Upchurch, 2008; Almeida *et al.*, 2012).

Polynoncus forms a well-defined group that evolved from an *Omorgus*-like ancestor in South America. The group is endemic to the continent and radiation into the temperate regions probably occurred only after continental fragmentation. The formation of the Andes mountain range probably provided the temperate biome preferred by members of this genus, the majority of which are restricted to the southern one-third of the continent (Patagonian sub-region) (Scholtz, 1990).

Our results suggest a different evolution for the *Trox* and *Phoberus* to that proposed by Scholtz (1986a). Troginae may have diverged vicariantly when the opening of the Tethyan seaway finally separated Laurasia and Gondwana. The lineage confined to Africa gave rise to *Phoberus*, and the Laurasian lineage to *Trox*. According to the fossil evidence, modern East Asia was most likely the ancestral area of origin for extant *Trox*, which lends support to the proposed Laurasian origin for this genus (Scholtz, 1986a; Krell, 2007; Nikolajev, 2008a, 2009a). *Phoberus* is endemic to continental Africa which is probably a direct result of the geographic isolation of the African continent, from the Middle Cretaceous to the Early Miocene, following the fragmentation of Gondwana (Gheerbrant & Rage, 2006). The presence of *Phoberus* in Madagascar is best explained by sweepstakes dispersal (Rabinowitz & Woods, 2006; Ali & Huber, 2010). Out-of Africa dispersal to the island, during the Palaeogene (60 to 20 Mya) period was largely facilitated by ocean currents that flowed from mainland towards Madagascar (Rabinowitz & Woods, 2006; Ali & Huber, 2010, Samonds *et al*., 2012). Trans-oceanic dispersal from Africa to Madagascar has been successfully invoked for various groups of mammals, amphibians, reptiles, insects (including dung beetles) and plants (Yoder & Nowak, 2006; Sole *et al*., 2011; Townsend *et al*., 2011; Zhou *et al*., 2012).

Conclusion

In this study we have provided the first molecular phylogeny for this group and successfully resolved relationships between and within genera and subgenera for the family. One of the more surprising results was the monophyly of *Phoberus*. Based on the evidence we propose reinstating the genus *Phoberus* to accommodate all Afrotropical (including Madagascan endemic) species. The results reemphasise the validity of the classification system for the genus *Omorgus* and its subgenera, as proposed by Scholtz (1986a). The study provides evidence of Madagascan trogid fauna having an African origin. Colonisation of the island probably occurred via dispersal from the mainland as opposed to ancient vicariant events. Divergence analysis was able to date the major events for the origin of extant lineages of Trogidae. The subsequent diversification of the major lineages is largely attributed to

Pangaea and Gondwana vicariance events in the Mid-Jurassic and early Late Cretaceous, respectively. The separation of the landmasses resulted in the evolution of four distinct groups: *Omorgus*, *Polynoncus*, *Trox* and *Phoberus*. Phylogenetic subdivision within the family indicates a complex evolutionary history for the four major groups, and further studies should investigate the regional biogeography of the major lineages. Trogidae constitute a monophyletic and biologically unique family within Scarabaeoidea. It is clear that the switch to keratin feeding very early in scarabaeoid evolution, provided trogids with a readily available food source that is mostly free of competitors and this has remained so since their initial radiation. To this day they are among the very last of the succession of insects that colonise and feed on animal remains, an adaptation that has endured and stood them in good stead since the Jurassic.

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Supporting Information

Table S1. List of taxa used in this study along with their GenBank accession numbers and locality data. X - Indicates PCR amplification failed.

Genus Species	Locality	ID	GenBank Accession Numbers 16S	 28S (D2)	 18S
Bolboceratidae (Outgroup)					
Bolbocaffer sp.	Botswana: Kgalagadi Transfrontier Park, January 2005, C. Deschodt & P. Tshikae	B3	EF570410	KC801330	KC801187
Bolbocaffer sp.	Botswana: Kgalagadi Transfrontier Park, January 2005, C. Deschodt & P. Tshikae	B4	EF570406	KC801331	KC801186
Mimobolbus sp.	Botswana: Kgalagadi Transfrontier Park, January 2005, C. Deschodt & P. Tshikae	B1	EF570408	KC801328	KC801189
Mimobolbus sp.	Botswana: Kgalagadi Transfrontier Park, January 2005, C. Deschodt & P. Tshikae	B2	EF570409	KC801329	KC801188
Lucanidae (Outgroup)					
Colphon haughtoni	South Africa: Western Cape Prov., Hexriver Mountains, January, 2008, W.P. Strümpher & C. Deschodt	C2	KC763256	KC810333	KC801233

Genus			GenBank Accession Numbers		
Species	Locality	ID	16S	28S (D2)	18S
C. neli	South Africa: Western Cape Prov., Swartberg Mt. Range, January 2008, W.P. Strümpher & C. Deschodt	C1	KC763267	KC801332	KC801232
C. stokoei	South Africa: Western Cape Prov., Hottentots-Holand Mt. Range, December 2007,W.P. Strümpher & C. Deschodt	C3	KC763248	KC801334	KC801234
Polynoncus					
P. brevicollis	Chile: San Antonio; Aguas Buenas; 3.IX.1999; Leg. V. Manuel Dieguez M.	3A	EF570366	KC801325	KC801221
P. brevicollis	Chile: San Antonio; Aguas Buenas; 3.IX.1999; Leg. V. Manuel Dieguez M.	3B	EF570373	KC801326	KC801220
P. brevicollis	Chile: San Antonio; Aguas Buenas; 3.IX.1999; Leg. V. Manuel Dieguez M.	3C	EF570385	X	X
P. bullatus	Chile: Curico; Rauco-fundo La Pancora; 31.VII.1999; Leg. V. Manuel Dieguez M.	1	EF570378	KC801327	KC801222
Omorgus (Omorgus)					
O. alternans	Australia: 31°33'33"S, 115°38'21"E, Yanchep, West. Aus., 6.XI.2010, M. Keady	OA2	KC801122	KC801297	KC801217
O. borrei	Argentina: Formosa, Laguna Yema. S24°19'54"	FE2	KC801118	KC801302	KC801202

Genus Species	Locality	ID	GenBank Accession Numbers 16S	 28S (D2)	 18S
	W61°17'73", 11.XII.2008, F.C. Ocampo, G. SanBlas, F. Campon.				
O. candidus	Australia: Yarramulla HS.; Undara Nat. Park, Qld.; 9.ii.2003; G. Monteith	12B	EF570379	KC801296	KC801206
O. costatus	Australia: Qld: 26°23'Sx146°12'E; Charleville, 5km NW; 3-5. Mar.2003, 310m, G. Monteith, C. Burwell, Mulga 51123	19A	EF570390	KC801304	KC801219
O. costatus	Australia: N Qld, Gilbert River nr. Georgetown; 4.ii.2003; J. Hasenpusch	10	EF570391	KC801303	KC801218
O. demarzi	Australia: Qld: 26°23'Sx146°12'E; Charleville, 5km NW; 3-5 Mar 2003, 310m; G. Monteith, C. Burwell; Mulga 51123	17	EF570380	KC801307	KC801203
O. monachus	USA: Alabama, Madison County, Huntsville, Monte Sano State Park, 19.V.2005, Paul K. Lago	91	KC801116	KC801311	KC801198
O. monachus	USA: Mississippi, Coahoma County, Mississippi River; 10mi WNW, Clarkesdale, 10.V.2005, J. King	93C	KC801115	KC801312	KC801199
O. pastillarius	Argentina: Mendoza, Molargile; 1049m; S35°53'21" W68°37'34"; F. Escobar	FE1	KC801133	KC801306	KC801205
O. spatulatus	Argentina: Salinas de Nihuil, 7.III.2002	4	KC801132	KC801305	KC801204

Genus			GenBank Accession Numbers		
Species	Locality	ID	16S	28S (D2)	18S
O. squamosus	Australia: NEQ: 17°16'S 145°52'E, Bellenden Ker Tap Stn. 17-18.Apr.1997. Monteith, Russel & Ovendan, 1560m	OA1	KC801123	KC801301	KC801211
O. subcarinatus	Australia: Carnarvon, W.A., 21.III.2011, P. Hutchinson, A. Sundholm	OA3	KC801124	KC801298	KC801201
O. suberosus	Argentina: La Roja; Aimogasta; Ruto 60; S28°35' W66°44'; dead horse; 25.II.2002; Medina & Scholtz	5A	EF570389	KC801309	KC801197
O. suberosus	Argentina: La Roja; Aimogasta; Ruto 60; S28°35' W66°44'; dead horse; 25.II.2002; Medina & Scholtz	5B	EF570386	KC801308	KC801195
O. suberosus	Argentina: La Roja; Aimogasta; Ruto 60; S28°35' W66°44'; dead horse; 25.II.2002; Medina & Scholtz	5C	EF570393	X	KC801194
O. suberosus	USA: Arizona, Santa Cruz County; Pena Blanca Canyon; 17.VIII.2002; W. Moore	8	EF570370	KC801310	KC801196
O. tatei	Australia: Wadana Nature Reserve, ca 90km NE Geraldon, WA, S28°07' 30" E115°11'11", 16-17.IX.2009, T.F. Houston	4W	KC801120	KC801299	KC801200
O. tatei	Australia: Wadana Nature Reserve, ca 90km NE Geraldon, WA, S28°07' 30" E115°11'11", 16-17.IX.2009, T.F. Houston	5W	KC801121	KC801300	X

Genus			GenBank Accession Numbers		
Species	Locality	ID	16S	28S (D2)	18S
O. tesselatus	USA: Coll. S. Whipple, 2009	Otes01	KC801117	KC801313	KC801193
O. trilobus	Australia: Qld, Charleville, Ward River Crossing, 4.III.2003, G. Monteith	16	KC801119	KC801295	KC801207
Afromorgus					
A. asperulatus	Botswana: Nossob Camp; Kalahari Gemsbok Park; 25.I.2003	31	EF570412	KC801315	KC801190
A. asperulatus	Botswana: Kgalagadi Transfrontier Park; S26°24' 29.4" E20°42'32.7"; 913m; P. Tshikae	102A	EF570411	KC801314	KC801191
A. freyi	Botswana: Kalahari Gemsbok Park, Nossob Camp, 25.i.2003	27	KC801129	KC801316	KC801192
A. gemmatus	Senegal: Mbour, VIII.1995	Ogem01	KC801131	KC801319	KC801209
A. melancholicus	South Africa: KwaZulu-Natal, Ndumu Game Reserve, XI. 2002, J. Harrison	49A	KC801126	KC801322	KC801212
A. pauliani	Laos: Vientiane Nat. University Campus, 18°2,534''N 102°37.768''E, 2-5.VI.2008, leg. S. Tarasov	1W	KC801127	KC801323	KC801214
A. pauliani	Laos: Vientiane Nat. University Campus, 18°2,534''N 102°37.768''E, 2-5.VI.2008, leg. S. Tarasov	2W	KC801128	KC801324	KC801213
A. radula	South Africa: Kruger National Park, Skukuza Camp, 12.II.2003	38A	EF570387	KC801318	KC801210

Genus Species	Locality	ID	GenBank Accession Numbers 16S	 28S (D2)	 18S
A. radula	Botswana: North Central Kalahari, P. Tshikae	105	KC801130	KC801317	KC801208
A. squalidus	South Africa: Kgalagadi Transfrontier Park; S26°24'29.4" E20°42'32.7", 913m; P. Tshikae	73A	EF570407	KC801320	KC801215
A. squalidus	South Africa: Freestate Prov., Ladybrand, 1700m, 24.X.2007, S29.14971° E27.40688°, WP Strümpher	Omsq01	KC801125	KC801321	KC801216
Trox (Trox)					
T. aequalis	USA: : Alabama; Madison County; Huntsville; Monte Sano State Park; 21.V.2005; Paul K. Lago	90	EF570375	X	KC801181
T. brahminus	Laos: Bolaven Plateau, Bam. Thongray, 15°14.054'N 106°31.867'E, 1000m, 8-16.VI.2008, S. Tarasov	TbA1	KC801106	KC801275	KC801167
T. cadaverinus	China: Mt.Zheng-nan-gou, Wuchang City, Heilongjiang, 1-11.VI.2011, Li Jingke	Tcad01	KC801108	KC801285	KC801178
T. fabricii	Greece: Naroc - Nassa, 15.III.2011, O. Boilly	Tfab	KC801110	KC801293	KC801184
T. gemmulatus	Mexico: Chihauhau; Creel, 11.VII.2002; W. Moore	9A	EF570363	KC801290	KC801175
T. gemmulatus	Mexico: Chihauhau; Creel, 11.VII.2002; W. Moore	9B	EF570359	KC801291	KC801174
T. hamatus	USA: New Jersey; Somerset Co.; Hutcheson Memorial Forest; 3-5.VIII.2004; pitfall w/ dog faeces	83B	EF570381	KB801278	KC801169
T. mandli	China: Mt.Zheng-nan-gou, Wuchang City, Heilongjiang, 1-11.VI.2011, Li Jingke	Tdo	KC801107	KC801281	X

Genus			GenBank Accession Numbers		
Species	Locality	ID	16S	28S (D2)	18S
T. niponensis	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.VI.2005; Satoru Nu leg.	100	EF570413	KC801289	KC801180
T. opacotuberculatus	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.VI.2005; Satoru Nu leg.	101A	EF570382	KC801277	KC801165
T. opacotuberculatus	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.VI.2005; Satoru Nu leg.	101B	EF570368	KC801276	KC801166
T. perlatus	Spain: Andalusia Camping Torre del Oro (ca 25km SE Huelva); 37°05'41.9"N 06°43'44.1"W; 18.V.2006; D. Ahrens & S. Fabrizi	TpE1	KC801111	KC801294	KC801185
T. perlatus	Spain: Andalusia Camping Torre del Oro (ca 25km SE Huelva); 37°05'41.9"N 06°43'44.1"W;18.V.2006; D. Ahrens & S. Fabrizi	TpE2	KC801112	X	KC801183
T. perrieri	Madagascar: La Mandraka, 16.I.2007, Olivier Boilly	Tpr02	KC801098	KC801273	KC801223
T. perrieri	Madagascar: La Mandraka, 16.I.2007, Olivier Boilly	Tpr03	KC801099	KC801274	X
T. scaber	UK: Great Britain, Oxford	TSc01	KC801114	KC801288	KC801179
T. scaber	UK: Great Britain, Oxford	TSc02	KC801113	KC801287	KC801182
T. setifer setifer	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.VI.2005; Satoru Nu leg.	99	EF570365	KC801286	KC801176
T. spinulosis	USA: Mississippi; Coahoma County; Mississippi	96A	EF570362	KC801279	KC801170

Genus Species	Locality	ID	GenBank Accession Numbers 16S	 28S (D2)	 18S
	River; 10mi WNW Clarksdale; 10.V.2005; Jonas King				
T. spinulosis	USA: Mississippi; Coahoma County; Mississippi River; 10mi WNW Clarksdale; 10.V.2005; Jonas King	96B	EF570376	KC801280	KC801172
T. terrestris	USA: Florida, Archbold, Biol. Stat., nr L. Placid; 22-23.xi.2002; V. Grebennikov	7A	EF570383	KC801283	KC801171
T. terrestris	USA: Florida, Archbold, Biol. Stat., nr L. Placid; 22-23.xi.2002; V. Grebennikov	7B	EF570394	KC801282	KC801168
T. tuberculatus	USA: Mississippi; Coahoma County; Mississippi River; 10mi WNW Clarksdale; 10.V.2005; Jonas King	95	EF570364	KC801292	KC801173
T. ussuriensis	China: Mt.Zheng-nan-gou, Wuchang City, Heilongjiang, 1-11.VI.2011, Li Jingke	Tuss01	KC801109	KC801284	KC801177
Trox (Phoberus)					
T. arcuatus	South Africa: Western Cape Prov., Kweekkraal farm; 9km west of Riversdale; U. Kryger; 22.X-8.XI.2003	69	EF570395	KC801249	KC801148
T. brincki	Lesotho: Mokhotlong District, 19.X.2007, S29°31'6.60" E29°11'12.99", 3089m, WP. Strümpher	TbL02	KC801079	KC801241	KC801140
T. brincki	Lesotho: Mokhotlong District, 19.X.2007, S29°31'6.60" E29°11'12.99", 3089m, WP. Strümpher	TbL04	KC801080	KC801242	KC801141
T. caffer	South Africa: Northern Cape Prov., nr. Hanover,	Tcaf01	KC801076	KC801240	KC801137

Genus Species	Locality	ID	GenBank Accession Numbers 16S	 28S (D2)	 18S
	S30.99228° E24.50198°, 1396m, 10.III.2008. WP. Strümpher				
T. capensis	South Africa: Western Cape Prov., Moordenaarskop, 940m, 15.XII.2007, S34.09809° E18.96465°, WP Strümpher	CMK05	KC801095	KC801271	KC801224
T. capensis	South Africa: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher	CSn03	KC801094	KC801272	KC801225
T. consimilis	South Africa: Northern Cape Prov., S31°12'26.74'' Calvinia district, E19°40'47.14'', 838m, 29.VIII.2009, CH. Scholtz	Tcon01	KC801091	KC801250	KC801149
T. cyrtus	South Africa: Northern Cape Prov., Kamieskroon, A. Frolov,C. Deschodt, 1-13.IX.2003; S30°15'58'' E 17°55'30''	67B	EF570384	KC801236	KC801161
T. fascicularis	South Africa: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher	ffSn03	KC801087	KC801256	KC801156
T. fascicularis	South Africa: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP.	ffSn04	KC801088	KC801257	KC801154

Genus			GenBank Accession Numbers		
Species	Locality	ID	16S	28S (D2)	18S
	Strümpher				
T. horridus	South Africa: Western Cape Prov., Gansekraal, 05.IX.2008, S33.52562° E18.32054°; 20m, C. Deschodt	ThGk02	KC801083	KC801243	KC801164
T. horridus	South Africa: Western Cape Prov., Gansekraal, 05.IX.2008, S33.52562° E18.32054°; 20m, C. Deschodt	ThGk06	KC801084	KC801244	KC801163
T. luridus	South Africa: Western Cape Prov., Vanrhynsdorp, S31°32'0" E18°42'12", 05.VIII.2008, CH. Scholtz & WP Strümpher	Tlur01	KC801082	KC801245	KC801136
T. montanus	Kenya: Aberdare N.P.; 30.X.2002-03.XI.2002; 3100m; Between Kiandongoro & Mutobio gates, V. Grebennikov	22A	EF570377	KC801238	KC801152
T. montanus	Kenya: Aberdare N.P.; 30.X.2002-03.XI.2002; 3100m; Between Kiandongoro & Mutobio gates, V. Grebennikov	22B	EF570371	KC801239	KC801153
T. mozalae	Mozambique: Maputo Elephant Reserve, 27.XI.2007, S26.51006° E32.91270°, WP. Strümpher	mmer01	KC801104	KC801265	KC801142
T. mozalae	Mozambique: Maputo Elephant Reserve, 27.XI.2007,	mmer02	KC801105	KC801266	KC801145

Genus Species	Locality	ID	GenBank Accession Numbers 16S	 28S (D2)	 18S
	S26.51006° E32.91270°, WP. Strümpher				
T. nama	Namibia; Boom River, canyon ca.10km of estuary; A. Frolov leg.; 30-31.III.2003; S27°55'28.3" E17°01'14.6"; 590m	25A	EF570403	KC801253	KC801139
T. nanniscus	South Africa: Eastern Cape Prov., Grahamstown, 635m, S33°16'14.79" E26°28'46.03", 14-16.XI.2008, WP. Strümpher	Tnan05	KC801093	KC801260	KC801230
T. nanniscus	South Africa: Eastern Cape Prov., Grahamstown, 635m, S33°16'14.79" E26°28'46.03", 14-16.XI.2008, WP. Strümpher	Tnan06	KC801092	KC801259	KC801231
T. nasutus	South Africa: Western Cape Prov., near Simonstown, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt	TnST01	KC801097	KC801270	KC801228
T. nasutus	South Africa: Western Cape Prov., near Simonstown, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt	TnST02	KC801096	KC801269	KC801229
T. natalensis	South Africa: KZN, Durban, 16.XI.2008, S29°54'56.13" E30°56'9.13", WP. Strümpher	TnD01	KC801101	KC801263	KC801147
T. natalensis	South Africa: KZN, Durban, 16.XI.2008,	TnD04	KC801100	KC801264	KC801146

Genus			GenBank Accession Numbers		
Species	Locality	ID	16S	28S (D2)	18S
	S29°54'56.13" E30°56'9.13", WP. Strümpher				
T. planicollis	South Africa: Northern Cape Prov., S31°12'26.74'' E19°40'47.14'', 29.VIII.2009. CH. Scholtz	Tplan01	KC801075	KC801246	KC801138
T. quadricostatus	South Africa: KZN, Ndumu Game Reserve, 2002.11.01, J. Harrison	Tq01	KC801103	KC801261	KC801143
T. quadricostatus	South Africa: KZN, Ndumu Game Reserve, 2002.11.01, J. Harrison	Tq02	KC801102	KC801262	KC801144
T. rhyparoides	South Africa: Eastern Cape Prov. Baviaanskloof, Poortjies (Poort); U. Kryger; 22.X-8.XI.2003	70A	EF570372	KC801267	KC801226
T. rhyparoides	South Africa: KZN, Ngome , A. Frolov, 2003	54A	EF570402	KC801268	KC801227
T. rudebecki	Lesotho: Sani Top, 2880m, 19.X.2007, 29°34'43.12"S 29°16'50.75''E, WP. Strümpher	RL01	KC801089	KC801258	KC801158
T. rudebecki	Lesotho: Sani Top, 2880m, 19.X.2007, 29°34'43.12"S 29°16'50.75''E, WP. Strümpher	RL25	KC801090	X	KB801159
T. squamiger	South Africa: Freestate Prov., Ladybrand, 1841m, 30.X.2007, S29.14971° E27.40688°, WP. Strümpher	SqL08	KC801078	KC801252	KC801151
T. squamiger	South Africa: Freestate Prov., Ladybrand, 1841m, 30.X.2007, S29.14971° E27.40688°, WP. Strümpher	SqL02	KC801077	KC801251	KC801150
T. strigosus	South Africa: Western Cape Prov., S31.48519°	Tstrg01	KC801086	KC801255	KC801157

Genus Species	Locality	ID	GenBank Accession Numbers 16S	 28S (D2)	 18S
	E23.73770°, 19.I.2008, WP Strümpher				
T. strigosus	South Africa: Western Cape Prov., S31.48519° E23.73770°, 19.I.2008, WP Strümpher	Tstrg02	KC801085	KC801254	KC801155
T. sulcatus	South Africa: Freestate Prov., Parys, 13.X.2003, C.H. Scholtz	62	EF570397	KC801237	KC801160
T. sulcatus	South Africa: Eastern Cape Prov., Willowmore; Timbi 2; U. Kryger; 22.X-8.XI.2003	66	EF570398	KC801235	KC801162
T. talpa	South Africa: Western Cape Prov., Kweekkraal farm; 9km west of Riversdale; U. Kryger; 22.X-8.XI.2003	68	EF570400	KC801248	KC801135
T. talpa	South Africa: Freestate Prov., Ladybrand, 1700m, 24.X.2007, S29.14971° E27.40688°, WP. Strümpher	Tt02	KC801081	KC801247	KC801134

Table S2. Summary of oligonucleotide primers used in this study.

Gene	Primer	Direction	Primer sequence (5'→3')	Reference
16S	16sf (luisa)	forward	atgtctttttgakwataatwtaaag	Orsini *et al*. (2007)
	16sr (luisa)	reverse	acgctgttatccctaaggtaattt	Orsini *et al*. (2007)
18S	18s-intfw-st12	forward	atcaagaacgaaagttagag	Haring & Aspöck (2004)
	18s-rev1	reverse	atggggaacaattgcaagc	Haring & Aspöck (2004)
28S(D2)	D2-3551	forward	cgtgttgcttgatagtgcagc	Gillespie *et al*. (2005)
	D2-4057	reverse	tcaagacgggtcctgaaagt	Gillespie *et al*. (2005)
	Wer-F1	forward	taagygggtggtaaactc	This study
	Wer-R1	reverse	tatagcgtcgcygacgggcg	This study

References (Table S2)

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 and Evolution*, **22**, 1593–1608.

Haring, E. & Aspöck, U. (2004) Phylogeny of the Neuropteridae: a first molecular approach. *Systematic Entomology*, **29**, 415–430.

Orsini, L., Koivulehto, H. & Hanski, I. (2007) Molecular evolution and radiation of dung beetles in Madagascar. *Cladistics*, **23**, 145–168.

Table S3. PCR thermal cycling profiles.

	Stage 1	Stage 2 – Thermal cycling				Stage 3
Gene	Initial denaturation	# Cycles	Denaturation	Annealing	Elongation	Final elongation
16S	94°C-90sec	35	(94°C-60sec,	48°C-90sec,	72°C-90sec)	72°C-1min
18S	95°C-2min	30	(95°C-10sec,	48°C-10sec,	72°C-90sec)	72°C-5min
28S(D2)	96°C-20sec	30	(96°C-15sec,	60°C-20sec,	72°C-60sec)	72°C-1min

Table S4. Data characteristics and estimated model parameters for 16S, 28S domain 2, 18S and combined dataset.

	16S	28S D2	18S	Combined
Number of samples	107	100	101	107
Aligned positions	405	601	562	1568
Paired characters (%)	112 (27.7)	268 (46.6)	350 (62.3)	730 (46.6)
Unpaired characters (%)	293 (72.3)	333 (55.4)	212 (37.7)	838 (53.4)
Parsimony informative sites	168	215	38	421
Tree length (MP)	918	676*	79	1779
Number of tree (MP)	10951	889*	208147	49
CI/RI (MP)	0.300/0.770	0.491/0.862*	0.620/0.942	0.381/0.840
Best fit model (AIC)	GTR+G	GTR+G	K80+I+G	GTR+G
A frequency	0.3555	0.2083	0.2134	0.7826
C frequency	0.814	0.3105	0.2622	0.2265
G frequency	0.1346	0.3144	0.3037	0.2555
T frequency	0.4284	0.1669	0.2206	0.2635
Gamma (G)	0.2040	0.2610	0.1460	0.1500
Invariable sites (I)	0	0	0.5600	0

CI = consistency index, RI = retention index, MP = Maximum Parsimony, ***** = MP analysis performed with reduced data set due to time and computational constraints.

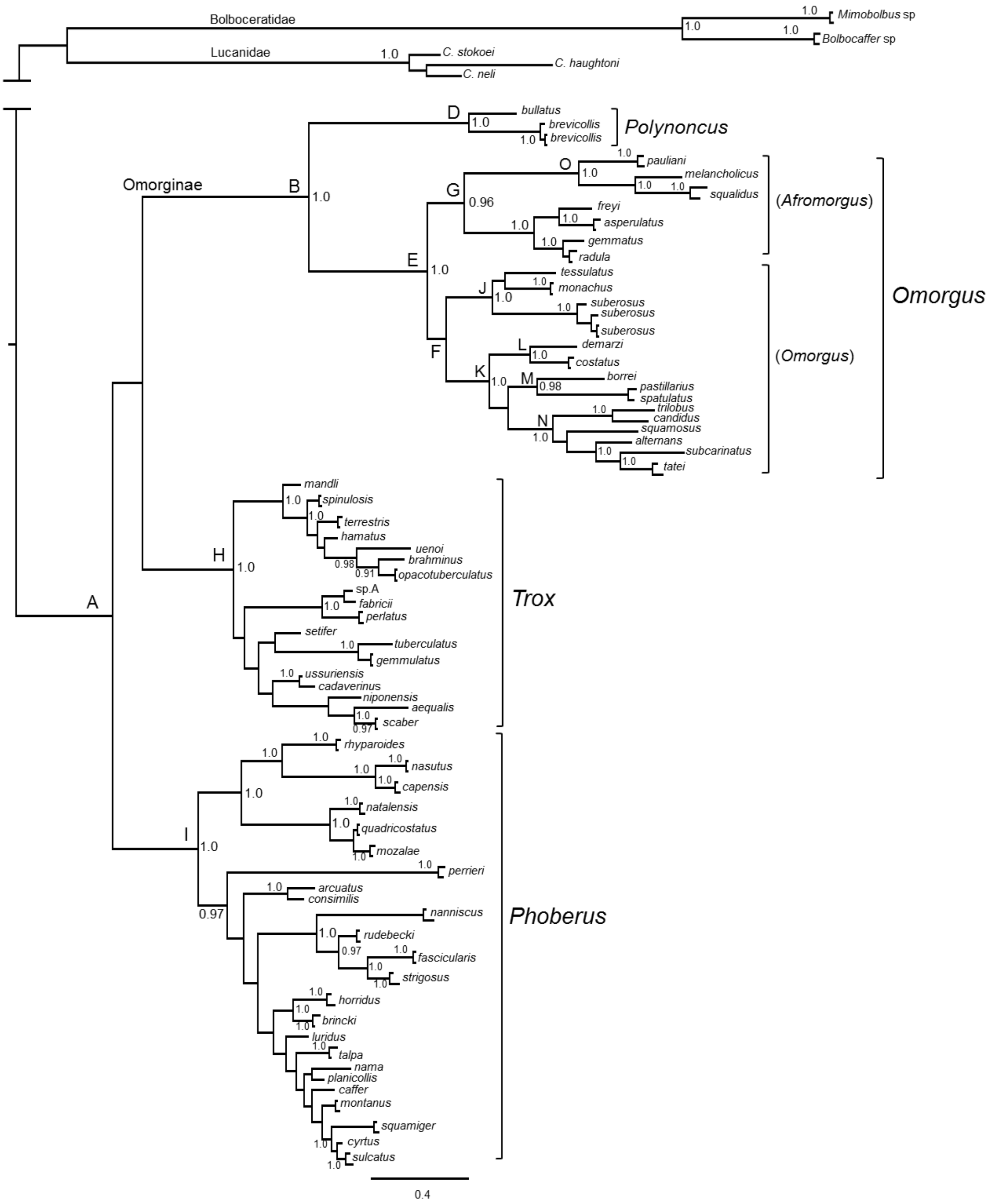

Figure S1. Phylogeny of Trogidae inferred from the combined 16S, 28S domain 2 and 18S (sequence-based alignment) dataset and implementing a conventional DNA model setup using MrBayes.

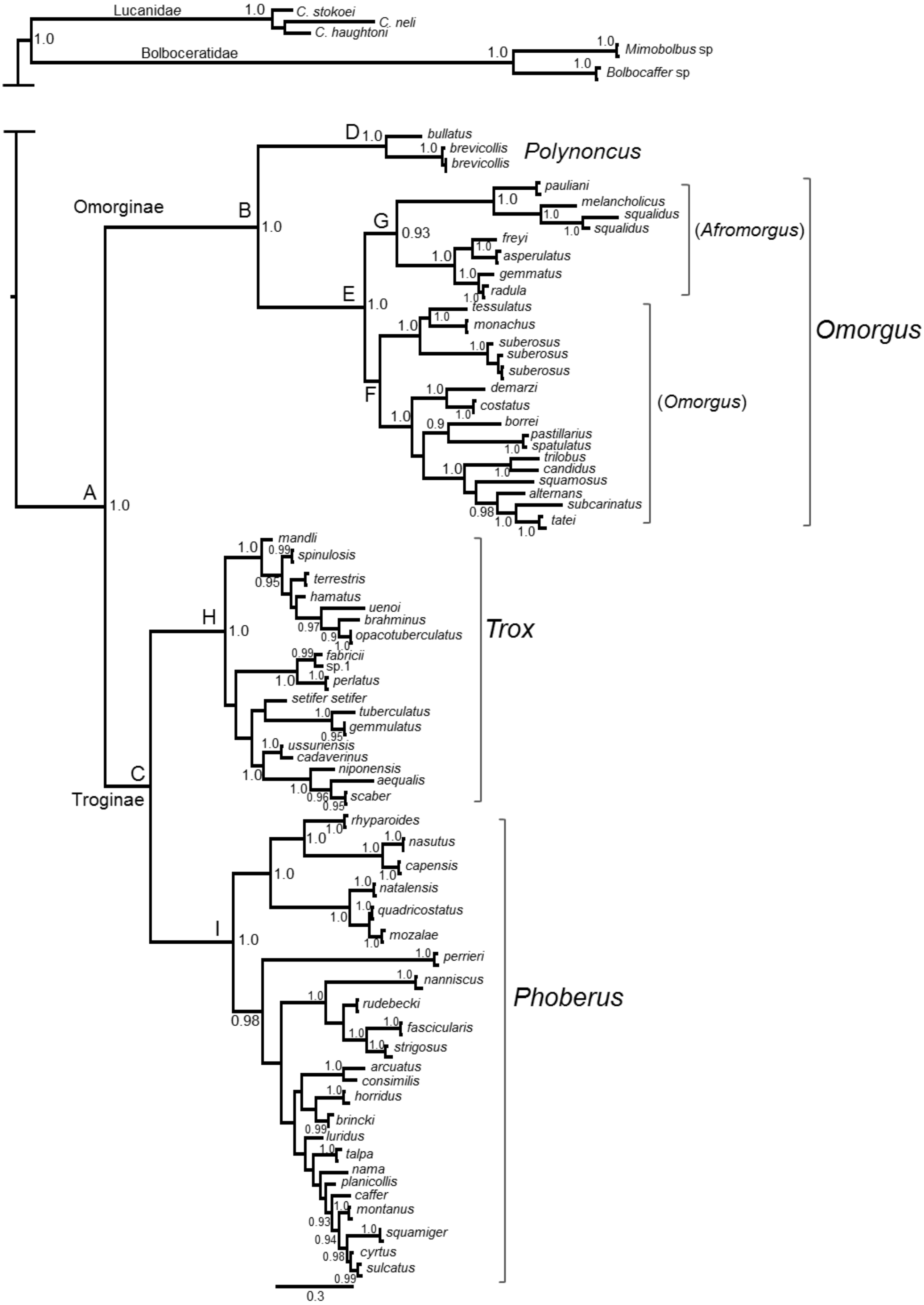

Figure S2. Phylogeny of Trogidae inferred from the combined 16S, 28S domain 2 and 18S (sequence-based alignment) dataset and implementing a mixed RNA/DNA (16-state RNA) model setup using MrBayes.

Chapter 3

Evolution of flightlessness in the Afrotropical *Trox* (*Phoberus*) (Coleoptera: Trogidae)

Abstract

Southern Africa has a high incidence of flightlessness among relict groups of various Scarabaeoidea (Coleoptera). One of these, the monophyletic subgenus *Phoberus* MacLeay (Scarabaeoidea: Trogidae) is of particular interest; about one-third (14 of 40) of the species in the Afrotropical region have lost the ability to fly. To gain insights into the evolution of flightlessness in this small Afrotropical subgenus, phylogenetic relationships among species were inferred using molecular sequence data. Four partial genes (COI, 16S, 18S and 28S domain 2) were sequenced for 27 species, covering all of the recognised species-groups. Analyses performed included parsimony, Maximum Likelihood and Bayesian inference. Estimated times of divergence were based on published mutation rates of 0.012 and 0.0075 substitution per site per million years for COI. Our results recovered eight monophyletic lineages, supporting the morphology-based subdivisions of the subgenus. Flightlessness evolved at least five times within the subgenus. There is no strong support for the reacquisition of functional wings once they are lost. Diversification, and the present relictual distributions, in the African *Phoberus* can be linked to climate and geological events associated with the Miocene and Pliocene Climate Optimum in southern Africa. The evolution of flightlessness in *Phoberus* is consistent with the habitat stability hypothesis. All flightless species occupy refugial areas with relatively long environmental stability.

Introduction

Flightless taxa are generally regarded as ideal indicators of evolutionary and biogeographical change due to their low vagility and restricted distributions (Bruhl, 1997; Davis et al., 2001; but see also Trewick, 2000; Fattorini, 2007). In addition, the loss of flight also makes them vulnerable to extinction during changes in the environment but this makes them good indicators of stable and persistent habitats too (Slater, 1977; Erwin, 1979; Bruhl, 1997; Fattorini, 2007). In the southern African region, it appears that most flightless scarabaeoid beetle species occur in refugial areas (mountains, relict forests, and deserts), which by definition have a relatively long history of environmental stability (Scholtz, 2000). This makes them ideal for answering questions regarding speciation events and possible factors that led to the loss of flight.

The Trogidae MacLeay are unique among the Scarabaeoidea being the only family of keratinophages. Adults and larvae feed on a variety of keratinous matter, such as skins, hair of mammalian carcasses, carnivore excrement, owl pellets and feathers (Scholtz, 1986, Scholtz and Chown, 1995; Sugiura and Ikeda, 2014). They are extremely well adapted (morphologically, behaviourally and physiologically) to exploit the ephemeral and patchy distribution of animal remains in nature and have evolved several strategies that contributed to their survival, especially during adverse environmental conditions (Baker, 1968; Scholtz, 1980, 2000; Friedländer and Scholtz, 1993; Scholtz and Caveney, 1988, 1992). They are usually the last in a succession of decomposers to utilise a carcass and remain at the food source as long as it remains viable, sometimes for many successive generations (Scholtz, 1986; Scholtz and Caveney, 1988).

The traditional morphology-based phylogenetic classification (Scholtz, 1986) divided this relatively small (± 330 spp.) cosmopolitan family into two distinct lineages, the *Trox* Fabricius lineage (with two subgenera, *Trox s.s*. and *Phoberus* MacLeay) and a lineage consisting of the genera *Polynoncus* Burmeister and *Omorgus* Erichson (with three subgenera *Omorgus s.s*., *Haroldomorgus* Scholtz and *Afromorgus* Scholtz). *Omorgus* is mainly restricted to the Gondwanan continents and *Polynoncus* is endemic to South America. The genus *Trox*, with about 140 species, is widely distributed throughout the Holarctic, with about 40 species occurring in the Afrotropical region (Scholtz, 1980). Evidence from a molecular phylogeny of the Trogidae (Strümpher et al., 2014) indicates that all Afrotropical (including Madagascan) *Trox* species are supported as monophyletic, grouping naturally in the subgenus *Phoberus*. This contradicts the previous hypothesis that the Afrotropical *Trox* species are polyphyletic (Scholtz, 1986). The monophyletic subgenus *Trox* (*Trox*) comprises all of the Holarctic species (Strümpher et al., 2014).

Most extant *Phoberus* species occur in the Afrotemperate region, with the greatest speciation in southern Africa (Scholtz, 1980). The Afrotemperate region contains a disjunct, temperate montane forest biome, which extends from the Cape region of South Africa in the south to the Ethiopian highlands in the north-east, and to the mountains of Sierra Leone and Guinea in the west (White, 1983; Griswold, 1991; Galley, 2007). The southern African *Phoberus* species typically show a mainly temperate eastern montane and coastal forest distribution but with apparent subsequent radiation into south-west coastal areas, and a small number of species appearing in savanna and arid regions. However, they have retained "temperate" biological features, being most active during cool periods (Scholtz, 1980).

The Afrotropical species of *Phoberus* can roughly be divided into ten species-groups, based on external morphology and male genitalia (Scholtz, 1979, 1980; Strümpher and Scholtz, 2011). The group is furthermore characterised by a high incidence of flightless species; about one-third (14 of 40) of *Phoberus* species in the Afrotropical region having lost the ability to fly. Their distribution is restricted to the southern African greater escarpment and the coastal plain of southern Africa (Fig 2). This narrow area of land, extending around the rim of South Africa, is characterised by a high incidence of flightlessness among relict groups of various Scarabaeoidea (Coleoptera) (Scarabaeidae: Medina and Scholtz, 2005; Sole et al., 2005; Deschodt et al., 2007, 2011; Deschodt and Scholtz, 2008; Sole and Scholtz, 2012; Lucanidae: Endrödy-Younga, 1988, Switala et al., 2014; Trogidae: Scholtz, 1979, 1980; van der Merwe and Scholtz, 2005; Strümpher and Scholtz, 2009, 2011). The Mediterranean region is the only other area where flightless species of *Trox* (*Trox*) occur (Pittino, 2006, 2011). The ten Mediterranean species are considered brachypterous (Pittino, 2011), whereas all flightless species in southern Africa are micropterous (Scholtz, 1981, 2000). Unlike their Mediterranean counterparts, all southern African flightless species (and both sexes) exhibit extreme morphological changes that are associated with species that have been flightless for a long time or have evolved from a flightless ancestor ("old aptery") (Scholtz, 1981, 2000; Fig 1).

Figure 1. Photograph showing morphological differences in alate vs. micropterous species of Afrotropical *Trox* (*Phoberus*) species. Alate form are typically characterised by a sharp humeral angle, large scutellum, raised shoulder knobs (humeral calli) and an elongate body shape; compared to micropterous species which have a rounded humeral angle, small scutellum, reduced or no humeral calli, and a rounded body shape (see Scholtz, 1981, 2000).

The endemic, relictual, flightless species of *Phoberus* are of particular interest. Flightlessness is a counterintuitive evolutionary development, given the many advantages of flight. The molecular phylogeny of the Trogidae (Strümpher et al., 2014) indicates that the character state of flightlessness is homoplasious in the Afrotropical species, implying that several independent events would have triggered this phenomenon. This makes the question of why loss of flight occurred multiple times only in southern Africa, but nowhere else on the continent, all the more intriguing. Flightless southern African species of *Phoberus* appear to be relicts from historically temperate climates. They exhibit a typical Cape-to-Drakensberg distribution pattern (Fig 2), occurring mainly in two geographically distinct areas; the Cape Centre (*sensu* Stuckenberg, 1962) which is dominated by the Cape Fold Mountains and along the eastern greater escarpment and coastal edge of South Africa which is dominated by the Drakensberg Regional Mountain System (*sensu*

White, 1978). Both are geologically and climatically stable regions of southern Africa that have persisted throughout the Tertiary. The flightless species generally occupy the summits, forested palaeo-refugia on the sea-facing slopes and the coastal plains; no flightless *Phoberus* species occur in the more arid interior of southern Africa. This distribution pattern is very similar to distribution patterns recorded for other palaeogenic invertebrate groups (Stuckenberg, 1962; Picker and Samways, 1996; Daniels et al., 2013; Switala et al., 2014).

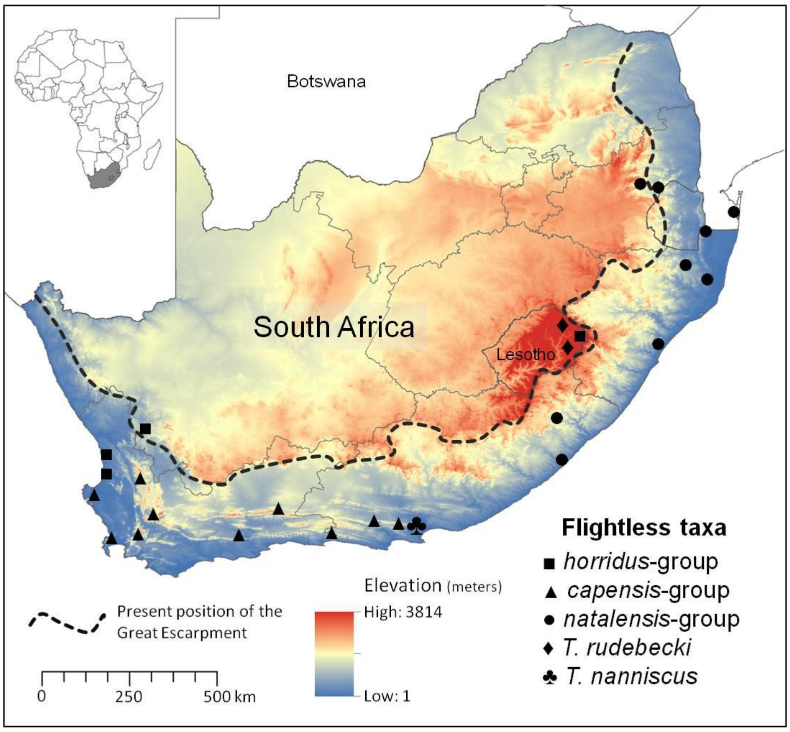

Figure 2. Map showing the distribution of flightless relict *Trox* (*Phoberus*) species in southern Africa.

From a geological standpoint, the southern African (interior) landscape is ancient (McCarthy and Rubidge, 2005), and the geomorphic processes and events that shaped the modern landscape of the subcontinent can be traced back to the breakup of Gondwana in the Middle to Late Jurassic (Lewis, 2008). Since then, the physiographic features of southern Africa have consisted of an elevated interior plateau, separated from a narrow coastal plain by a distinct, horse-shoe-shaped escarpment (McCarthy and Rubidge, 2005; Moore et al., 2009; Clarke et al., 2011). The latter may represents a passive erosional remnant of the continental margin that has eroded back from the original zone of rifting, by means of parallel retreat to its present position, about 60 Mya (McCarthy and Rubidge, 2005; McCarthy, 2009; Clarke et al., 2011; see also Green et al., 2013) and has remained a prominent topographical feature of the southern African landscape since then. In the pre-Miocene, the greater escarpment would have provided a high and continuous temperate region, extending around the rim of southern Africa. The region is of great biological significance because much of the subcontinent's floristic diversity and endemism is centred on it and the adjacent coastal foreland, while the interior plateau remains relatively impoverished (Cowling and Hilton-Taylor, 1994; Van Wyk and Smith, 2001; Steenkamp et al., 2005).

The Miocene and Pliocene were some of the most important periods of Africa's climatic and geological history, and the extant biotic composition of Africa was largely determined by the geomorphic and climate-induced evolutionary filtering events at the time (Plana, 2004; Cowling et al., 2005). Three major phenomena in particular had a profound influence on the evolution of southern Africa's biota: 1) tectonic uplift, 2) the decline in global temperatures and 3) establishment of C4 grass-dominated ecosystems (Scheiter et al., 2012). The onset of global cooling in the Mid-Miocene caused increased aridity across the African continent. The widespread aridification resulted in contraction of sub-tropical forests and the expansion of open savanna woodlands and grasslands. Tectonic uplift, during the early Miocene (18 Mya) and later, during the Pliocene (5 Mya), further accentuated the escarpment, especially the eastern section. The accentuated escarpment reduced rainfall in the eastern interior by limiting the inflow of moist air from the Indian Ocean, which further increased the east-to-west rainfall gradient. The west coast became very arid, due to the contemporaneous development of the Benguela current and the onset of the Antarctic circumpolar current, which created the deserts in the west of South Africa, while the east coast remained moist and humid. Consequently, there was an increase in the seasonality of rainfall, and an expansion of grasslands across the interior of southern Africa (McCarthy and Rubidge, 2005). The latter was most likely mediated by fire due to increased aridity across the sub-continent (Scheiter et al., 2012).

The correct interpretation of the evolution of flightlessness depends on an understanding of the historical relationship between species. Phylogenetic analysis of the clade will allow us to trace the original loss(es) of flight and examine the effect of flightlessness on speciation. Consequently, the objectives of this study were to: (1) hypothesise phylogenetic relationships for the flightless and volant Afrotropical *Phoberus* species based on molecular data; (2) estimate the divergence times and ages of the flightless taxa in the clade, and relate them to past climatic and geological events; and (3) to gain further insights into the evolution into flightlessness we performed an ancestral-character state reconstruction of wing loss.

Although analyses of molecular data (Strümpher et al., 2014) clearly indicate that *Phoberus* merits higher taxonomic rank, for the purpose of this study, it will be treated as a subgenus of *Trox* that includes all Afrotropical species.

Materials and Methods

Sampling, amplification, sequencing and processing of sequences

All ten species-groups are represented in the in-group sample (Table 1), which consisted of 29 individuals representing 27 (out of 40) Afrotropical species. Flightless *Phoberus* are rare and difficult to collect and the majority of the species are known only from their type localities. Eleven (out of 14) flightless species are included in the in-group. Following Strümpher et al. (2014) members of the Holarctic subgenus *Trox* (*Trox*) were chosen to represent the out-groups (Table 1). Collected individuals were identified, catalogued and stored for further analysis. Specimens are deposited at the Department of Zoology and Entomology, University of Pretoria, South Africa (UPSA).

DNA extraction, PCR amplification and cycle sequencing were performed following the protocol described in Strümpher et al. (2014). Two nuclear genes (18S rRNA and 28S rRNA domain 2) and two ribosomal mitochondrial genes (16S rRNA and Cytochrome Oxidase I) were amplified using previously reported primer sequences

Table 1. List of taxa, collection data and sequence accession numbers of trogid beetles used in this study; DC = Database code; X = indicates PCR amplification failed. Groups follow Scholtz (1980).

			GenBank Accession Numbers			
Species	Locality data	DC	16S	28S(D2)	18S	CO1
Holarctic *Trox* (Outgroup)						
T. opacotuberculatus	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.VI.2005, Satoru Nu	101A	EF570382	KC801277	KC801165	KM371721
T. scaber	Great Britain, Oxford	TSc01	KC801114	KC801288	KC801179	KM371722
T. terrestris	USA: Florida, Archbold, Biol. Stat., near L. Placid, 22-23.XI.2002, V. Grebennikov	7A	EF570383	KC801283	KC801171	KM371720
'*horridus*'- group (9/12)						
T. brincki	Lesotho: Mokhotlong District, 19.X.2007, 29°31'6.60" E 29°11'12.99", 3089m, WP. Strümpher	TbL04	KC801080	KC801242	KC801141	KM371710
T. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher	CSn03	KC801094	KC801272	KC801225	KM371717
T. capensis	RSA: Western Cape Prov., Outeniqua Pass, S33.90000° E22.40107°, 668m, 25.XII.2007, C Deschodt	COP01	KM371690	KM371681	KM371686	KM371719

Species	Locality data	DC	GenBank Accession Numbers 16S	 28S(D2)	 18S	 CO1
T. horridus	RSA: Western Cape Prov., Gansekraal, 05.IX.2008, S33.52562° E18.32054°, 20m, C. Deschodt	ThGK02	KC801083	KC801243	KC801164	KM371708
T. mozalae	Mozambique: Maputo Elephant Reserve. 27.XI.2007, S26.51006° E32.91270°, WP. Strümpher	MMER01	KC801104	KC801265	KC801142	KM371712
T. nasutus	RSA: Western Cape Prov., near Simonstown, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt	TnST01	KC801097	KC801270	KC801228	KM371718
T. natalensis	RSA: KZN, Durban, 16.XI.2008, S29°54'56.13" E30°56'9.13", WP. Strümpher	TnD01	KC801101	KC801263	KC801147	KM371714
T. quadricostatus	RSA: KZN, Ndumu Game Reserve, 2002.11.01, J. Harrison	Tq01	KC801103	KC801261	KC801143	KM371711
T. youngai	RSA: Mpumalanga, near Barberton, 15.III.2012, WP. Strümpher	TY01	KM371691	KM371680	KM371682	KM371713
T.aculeatus	RSA: Western Cape Prov., Robertson district, 07.VII.2012, R. Muller	Tac01	KM371689	KM371678	KM371687	KM371709
'*rhyparoides*'- group (1/1)						

			GenBank Accession Numbers			
Species	Locality data	DC	16S	28S(D2)	18S	CO1
T. rhyparoides	RSA: Eastern Cape Prov., Baviaanskloof, Poortjies (Poort), 22.X-8.XI.2003, U. Kryger	70A	EF570372	KC801267	KC801226	KM371716
'*pusillus*'- group (1/1)						
T. pusillus	DRC: Niari Province, 25km SE of Mayoko Village, 02°25'46"S 12°59'17°E, 754m, 24.XI.2013, Leg. L. Niemand & C. Ngoulou	Tpus01	KM371692	X	KM371683	KM371715
'*montanus*'- group (1/1)						
T. montanus	Kenya: Aberdare N.P.; 30.X.2002 - 03.XI.2002, 3100m; Between Kiandongoro & Mutobio gates, V. Grebennikov	22A	EF570377	KC801238	KC801152	KM371705
'*fascicularis*'- group (5/5)						
T. f. fascicularis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher	ffSn03	KC801087	KC801256	KC801156	KM371694
T. fascicularis rowei	RSA: KZN, Sani Pass, 29°35'57.3"S 29°18'25.3"E, 6.VIII.2003, C. Deschodt	57A	EF570361	KM371679	KM371685	KM371693
T. nanniscus	RSA: Eastern Cape Prov., Grahamstown, 635m, S33°16'14.79" E26°28'46.03", 14-16. XI. 2008, WP. Strümpher	Tnan05	KC801093	KC801260	KC801230	KM371723

			GenBank Accession Numbers			
Species	Locality data	DC	16S	28S(D2)	18S	CO1
T. rudebecki	Lesotho: Sani Top, 2880m, 19. X. 2007, 29°34'43.12"S 29°16'50.75"E, WP. Strümpher	RL01	KC801089	KC801258	KC801158	KM371696
T. strigosus	RSA: Western Cape Prov., S31.48519° E23.73770°, 19.I.2008, WP Strümpher	Tstrg01	KC801086	KC801255	KC801157	KM371695
'*pennicilatus*'- group (2/6)						
T. arcuatus	RSA: Western Cape Prov., Kweekkraal Farm, 9km west of Riversdale, 22.X-8.XI.2003, U. Kryger	69	EF570395	KC801249	KC801148	KM371697
T. consimilis	RSA: Northern Cape Prov., S31°12'26" E19°40'49", 838m, 29.VII.2009, CH. Scholtz	Tcon01	KC801091	KC801250	KC801149	KM371698
'*talpa*'- group (1/1)						
T. talpa	RSA: Western Cape Prov., Kweekkraal farm; 9km west of Riversdale; U. Kryger; 22.X-8.XI.2003	68	EF570400	KC801248	KC801135	KM371707
'*sulcatus*'- group (7/±15)						
T. caffer	Lesotho: Mokhotlong District, 19.X.2007, S 29°31'6.60" E 29°11'12.99", 3089m, WP.	Tcaf02	KM371688	KM371677	KM371684	KM371704

			GenBank Accession Numbers			
Species	Locality data	DC	16S	28S(D2)	18S	CO1
	Strümpher					
T. cyrtus	RSA: Northern Cape Prov.; 6km S of Kamieskroon; 1-13.IX.2003; S30°15'58.15" E 17°55'30.19"; A. Frolov & C. Deschodt	67B	EF570384	KC801236	KC801161	KM371702
T. luridus	RSA: Western Cape Prov., Vanrhynsdorp, S31°32'00" E18°42'12", 05.VII.2008, CH. Scholtz & WP. Strümpher	Tlur01	KC801082	KC801245	KC801136	KM371706
T. nama	Namibia: Boom River, canyon ca. 10km of estuary, 30-31.III.2003, S27°55'28.3" E17°01'14.6", alt 590m, A. Frolov	25A	EF570403	KC801253	KC801139	KM371700
T. planicollis	RSA: Northern Cape Prov., S31°12'26" E19°40'49", 838m, 29.VII.2009, CH. Scholtz	Tplan01	KC801075	KC801246	KC801138	KM371699
T. squamiger	RSA: Freestate Prov., Ladybrand, 1841m, 30.X.2007, S29.14971° E27.40688°, WP. Strümpher	SqL02	KC801077	KC801251	KC801150	KM371703
T. sulcatus	RSA: Eastern Cape, Willowmore, 22.X-8.XI.2003, U. Kryger	66	EF570398	KC801235	KC801162	KM371701

Species	Locality data	DC	GenBank Accession Numbers			
			16S	28S(D2)	18S	CO1
Madagascar endemic						
T. perrieri	Madagascar: La Mandraka, 16.I.2007, Olivier Boilly	Tpr02	KC801098	KC801273	KC801223	X

that are summarised in Table S1. Different cycling parameters (Table S2) were implemented to amplify the desired gene regions. Cycle sequencing products were precipitated using a standard sodium acetate/ethanol precipitation protocol and sequenced bidirectionally. Sequences generated were viewed and edited in CLC Bio main Workbench Version 5.5 (Developed by CLC Bio, http://www.clcbio.com/). New sequences were submitted to GenBank (see Table 1). We included 16S, 18S and 28S domain 2 sequences generated by Strümpher et al*.* (2014).

Alignment

RNAsalsa version 0.8.1.win32 (Stocsits et al., 2009) was used for all of the structure-guided alignments for 16S, 18S and 28S domain 2 (refer to Strümpher et al., 2014 for rRNA alignment procedures; for a detailed discussion on RNAsalsa refer to Stocsits et al*.*, 2009). The partial sequences for COI were aligned using the program package MAFFT (Katoh and Toh, 2008) with default settings. The alignments for the four gene regions (COI, 16S, 18S and 28S domain 2) were concatenated into a super-matrix using the program FASconCAT version 1.0 (Kück and Meusemann, 2010).

Phylogeny

Phylogenetic relationships were inferred by the following methods described below:
Maximum Parsimony Analysis (*MP*): A parsimony analysis was implemented in PAUP*4.010b (Swofford, 2003) with the following heuristic search setting: all characters were equally weighted and unordered, gaps were treated as missing data and uninformative sites were excluded; starting tree obtained via stepwise addition with random addition of sequences with 10 replicates; branch-swapping = tree-bisection-reconnection; initial ‘maxtrees’ set to 200 with automatic increase by 100 and with ‘MulTrees’ option in effect. Bootstrap values were calculated based on 1000 replicates. A strict consensus tree was calculated from the most parsimonious trees obtained.

Bayesian Inference Analysis (BI): Bayesian analysis was performed with MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). The data set was partitioned into four gene regions. jModeltest (Posada, 2008) was used to select the appropriate model of nucleotide evolution under the Akaike information criterion (Akaike, 1974) (model parameters are summarised in Table 2). Analysis was performed using the GTR+I+G model for COI, 16S and 28S, and HKY+I+G for 18S. Parameters for the different partitions were unlinked to obtain separate parameter estimates for each gene and the rate prior was set to variable. Flat Drichlet priors were used in all analyses. Bayesian analyses were conducted by simultaneously running two Monte Carlo Markov chains, with one cold and three heated chains, for 15 million iterations. Trees were sampled every 200 iterations. Tracer v1.5 (Rambaut and Drummond, 2007) was used to monitor parameter stabilisation (via inspection of estimated sample size (ESS) and graphical plots of parameter sampling). The first 20% of trees sampled were discarded as burn-in. FigTree v1.3.1 (Rambaut, 2009) was used to view all tree topologies.

Maximum Likelihood Analysis (ML): Analysis was conducted in RAxML (Randomized Axelerated Maximum Likelihood) version 8.20 (Stamatakis, 2014) on the concatenated dataset under a GTR+G model. A single run was conducted with 1000 bootstrap inferences, followed by a thorough ML search. Each inference was initiated with a random starting tree, and employed the rapid hill-climbing algorithm (Stamatakis et al., 2007).

Neighbor-Network Analysis: We constructed a neighbour-network (based on uncorrected *p*-distances) of the individual and the concatenated alignments using the program Splitstree 4 (Huson and Bryant, 2006). The latter can be used to visualize conflicting signal (ambiguities) in the data or alternative phylogenetic histories through a splits graph, and help to assess the tree-likeness (congruence) of the data, independently of tree reconstruction techniques (Huson and Bryant, 2006; Wägele and Mayer, 2007). Parsimony-uninformative sites were excluded, and bootstrap support of splits was calculated based on 1000 replicates.

Congruence between genes

To estimate the contribution of each dataset towards a combined phylogeny, Partitioned Bremmer Support (PBS) (Baker et al., 1998) was calculated at each node from the strict consensus tree of the combined parsimony analysis using TreeRot version 3 (Sorenson and Franzosa, 2007) and PAUP. The Bremmer support (BrS) is the sum of the PBS values at each node (Baker et al., 1998). Partition Congruence

Indices (PCI) was calculated to estimate the degree of congruence between the four gene regions (Brower, 2006). The PCI and BS values are equal when there is no conflict between partitions, and the PCI value decreases until it becomes negative (Brower, 2006) as the conflict increases.

Divergence analysis

The node ages for the major lineage-splitting events were estimated using the software BEAST v.1.6.2 (Drummond et al., 2006; Drummond and Rambaut, 2007). Cytochrome Oxidase I (COI) data for those individuals successfully sequenced was used. The Madagascan endemic, *Trox perrieri*, is not included in the dating analysis as the PCR amplification for COI failed. As no fossil evidence is available for this group with which to estimate time of origin for lineages, we opted to use published mutation rates of 0.0075 and 0.012 mutational changes per base per million years, respectively, to cover the range of rates reported for COI mtDNA (Wirta et al., 2008; Sole and Scholtz, 2010). For the analyses we used a Bayesian relaxed molecular clock approach under the uncorrelated lognormal model and a Yule speciation process for all data combined. The model and clock parameters were unlinked across partitions. The in-group was constrained to be monophyletic. The topology estimated using MrBayes was specified as a starting tree for BEAST. Two independent MCMC analyses were run for 10 million generations with parameters sampled every 1000 generations. Default settings were kept for all other priors and operators. Tracer was used to monitor parameter stabilisation and to assess the convergence between runs. LogCombiner v1.6.2 from the BEAST package was used to combine the tree and log output files from the two independent runs. The first 20% of trees sampled from each run was discarded as burn-in. The TreeAnnotator v1.6.2 application from the BEAST package was used to generate a maximum clade credibility tree and calculate the mean ages. Tree topologies were viewed in FigTree. The divergence analysis was run twice to validate consistency of the time estimates between runs.

Rates of diversification within Afrotropical Phoberus

To visualise the temporal variation in diversification rates within the subgenus, a lineage-through-time (LTT) plot ($\log_{10}$ lineages versus time) was constructed using Tracer.

Character trait mapping

To explore whether there was evidence for the re-evolution of wings in the subgenus *Phoberus*, we reconstructed the ancestral-character evolution of wing loss using parsimony, likelihood and Bayesian approaches.

Parsimony and likelihood analyses were implemented in the software package Mesquite v2.75 (Maddison and Maddison, 2011) and based on the phylogenetic tree derived from Bayesian inference analysis. Flight-capable and flightless forms were coded as 0 and 1, respectively. For the parsimony analysis, characters were traced with the states unordered. For the likelihood reconstruction we compared two models: (1) an irreversible model (MK1) with one rate for all transitions between states, and (2) an asymmetrical model (Asymm. 2-param.) with separate rates for forward and reverse transitions. To test whether the asymmetric two-parameter model offers a better fit than the one-parameter model, we compared the resulting likelihood scores in a likelihood-ratio test (degrees of freedom (d.f.) = 1)

Bayesian analysis was performed using the *Multistate* module of Bayestraits v2 (Pagel et al., 2004; available at http://www.evolution.rdg.ac.uk). One advantage of the program is that it can account for phylogenetic uncertainty in its ancestral state reconstruction by sampling the posterior distribution of a set phylogenetic tree. We used 2000 randomly selected post-burn-in trees from the Bayesian (MrBayes) analysis and the character states of the extant taxa. Bayestraits estimate ancestral states for the most recent common ancestor (MRCA) of selected taxa regardless of whether they form a monophyletic group in a particular topology, which allows the program to incorporate information about trait evolution from all trees in the posterior distribution (Yoder et al., 2010). Character states were estimated for every node by defining a clade with the *AddMRCA* command. We implemented a Reversible-Jump Markov Chain Monte Carlo (RJMCMC) analysis that was run for 5 million iterations, sampling every 200th iterations and discarding the first 100 000 samples as burn-in. An exponential (0.00, 0.10) hyperprior was used for the rate parameters. We calculated the mean values of the proportional likelihoods for each node. Bayesian analysis was run twice. Ancestral state at several nodes of particular interest, and nodes with ambiguous support for either state, were reanalysed by constraining them to a given state (0 and 1) using the *fossil* command. By comparing the resulting harmonic means of the two states, by means of a Bayes factor (BF = 2 x (log [harmonic mean (better model)] - log [harmonic mean (worse model)], we evaluated the support for each state. A positive Bayes factor favours the better fitting model, a value of < 2 indicates weak support, 2-5 indicates positive evidence of the better fitting model, and values of greater than five or greater than 10 can be interpreted as “strong” and “very strong”, respectively.

Results

The final combined molecular dataset consisted of 32 taxa and 2622 base pairs (bp): COI = 1095, 16S = 389 bp; 18S = 562 bp and 28S (D2) = 576 bp. Data characteristics and estimated model parameters for individual and combined datasets are shown in Table 2. The resulting neighbour-net splits graph (Fig 3) had a recomputed fit = 97.74, and LS fit = 99.91. The heuristic search from the parsimony analysis produced one parsimonious tree (length = 2679 steps; CI = 0.322; RI = 0.497; Fig 2). The best ML tree found, and the consensus tree produced from Bayesian Inference, are shown in Fig S3 and Fig 4, respectively.

Congruence between genes

Partitioned Bremmer support values of the combined dataset indicated a high level of congruence between the four gene regions for most of the nodes (Tables S3). The partition with the most conflict came from the mitochondrial ribosomal gene 16S, in which eight nodes out of 29 were in conflict with the combined tree. This was followed, in descending order, by 28S domain 2, 18S and COI, with 5, 4 and 1 conflicting nodes, respectively (Table S3).

Phylogenetic analysis

The tree topologies from the MP, ML and BI were broadly congruent with respect to species-level relationships, but the deeper phylogenetic relationships were overall better-resolved in the ML and Bayesian analysis. The resulting network splits tree

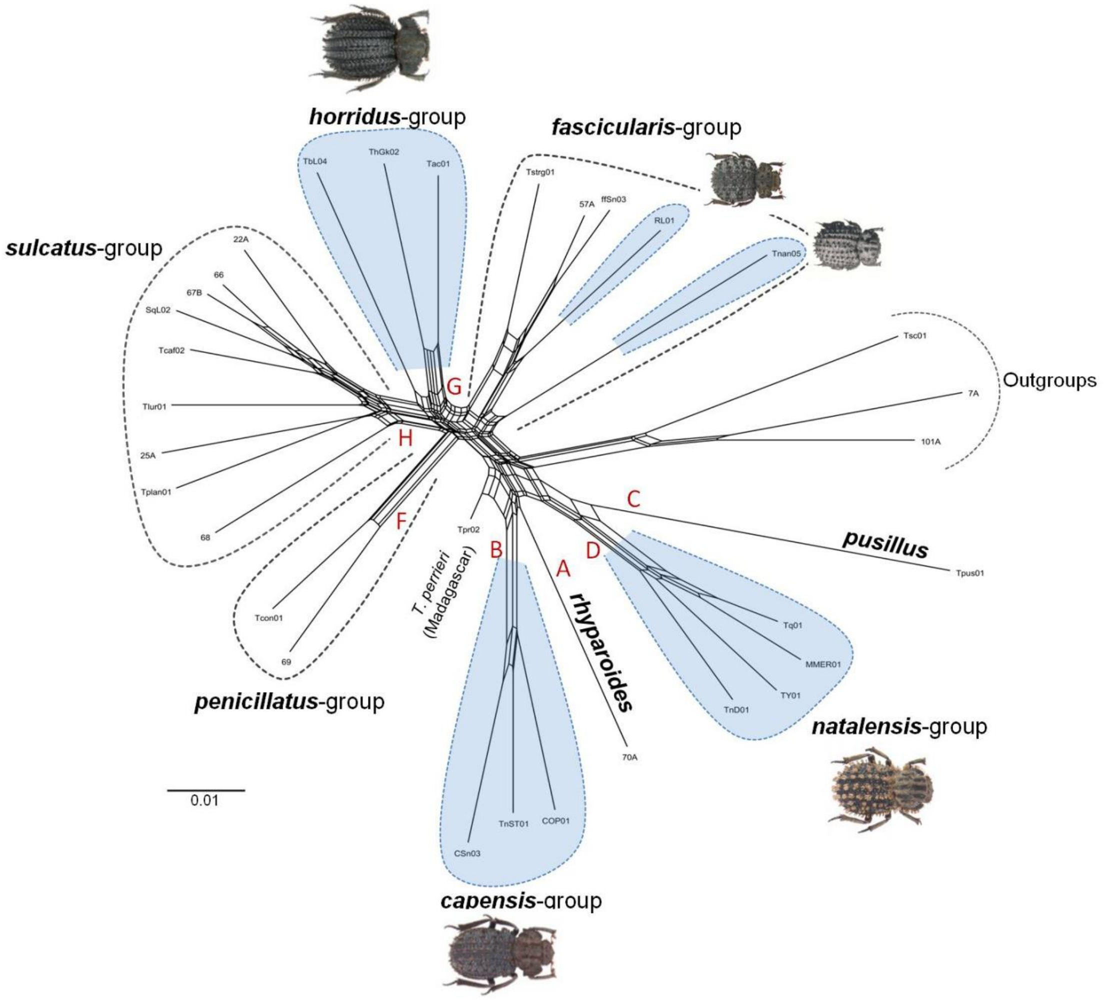

Figure 3. Neighbour-net graph for the combined molecular dataset, based on uncorrected *p*-distances. Flightless clades/taxa are highlighted in blue blocks.

was similar to the topologies recovered from the MP, ML and BI analyses and combining the individual datasets in a single analysis improved the split support for a large number of nodes in the splits graph. Overall, eight well-supported lineages/groups (nodes A-G, Table 3) were recovered across all methods of analysis. Summaries of the bootstrap support (BS) and posterior probabilities (PP), from the different approaches, for the major lineages recovered can be seen in Table 3.

Table 2. Data characteristics and estimated model parameters for 16S, 18S, 28S domain 2 and COI, and combined dataset; CI = consistency index, RI = retention index, MP = Maximum Parsimony, AIC = Akaike Information Criterion, ti/tv = transition/transvertion ratio.

	16S	COI	28S(D2)	18S	Combined
Number of samples	32	31	31	32	32
Aligned positions	389	1095	576	562	2622
Parsimony informative sites	82	369	74	15	540
Tree length (MP)	274	2148	188	25	2679
Number of tree (MP)	1269	2	16	325	1
CI/RI	0.438/0.641	0.290/0.434	0.543/0.797	0.760/0.900	0.322/0.497
Best fit model (AIC)	GTR+I+G	GTR+I+G	GTR+G	HKY+I	GTR+I+G
A frequency	0.3969	0.3433	0.2029	0.2109	0.1788
C frequency	0.0829	0.1207	0.3197	0.2608	0.1860
G frequency	0.1058	0.1292	0.3354	0.3049	0.2246
T frequency	0.4145	0.4068	0.1421	0.2234	0.3308
Gamma (G)	0.7910	0.5880	0.6080	-	0.8250
Invariable sites (I)	0.6130	0.4950	0.5920	0.7170	0.6600
Kappa	-	-	-	2.5876	-
(ti/tv)	-	-	-	1.2699	-

Table 3. Support values for nodes recovered representing the major groups for the parsimony (MP), Maximum Likelihood (ML), splits graph (NN) and Bayesian inference (BI); BS = Bootstrap support, PP = Posterior probability; Nodes/clades not recovered/supported are marked with (-); * = *sensu* Strümpher & Scholtz (2011).

Node	Species group	MP (BS)	ML (BS)	NN (BS)	BI (PP)
A	'*rhyparoides*' - group	100	100	100	1.00
B	'*capensis*' - group*	100	100	100	1.00
C	'*pusillus*' - group	100	100	100	1.00
D	'*natalensis*' - group*	100	99	100	1.00
E	'*fascicularis*' - group	61	96	-	1.00
F	'*penicillatus*' - group	100	100	100	1.00
G	'*horridus*' - group	53	70	55	0.95
H	'*sulcatus*' - group	97	94	99	1.00

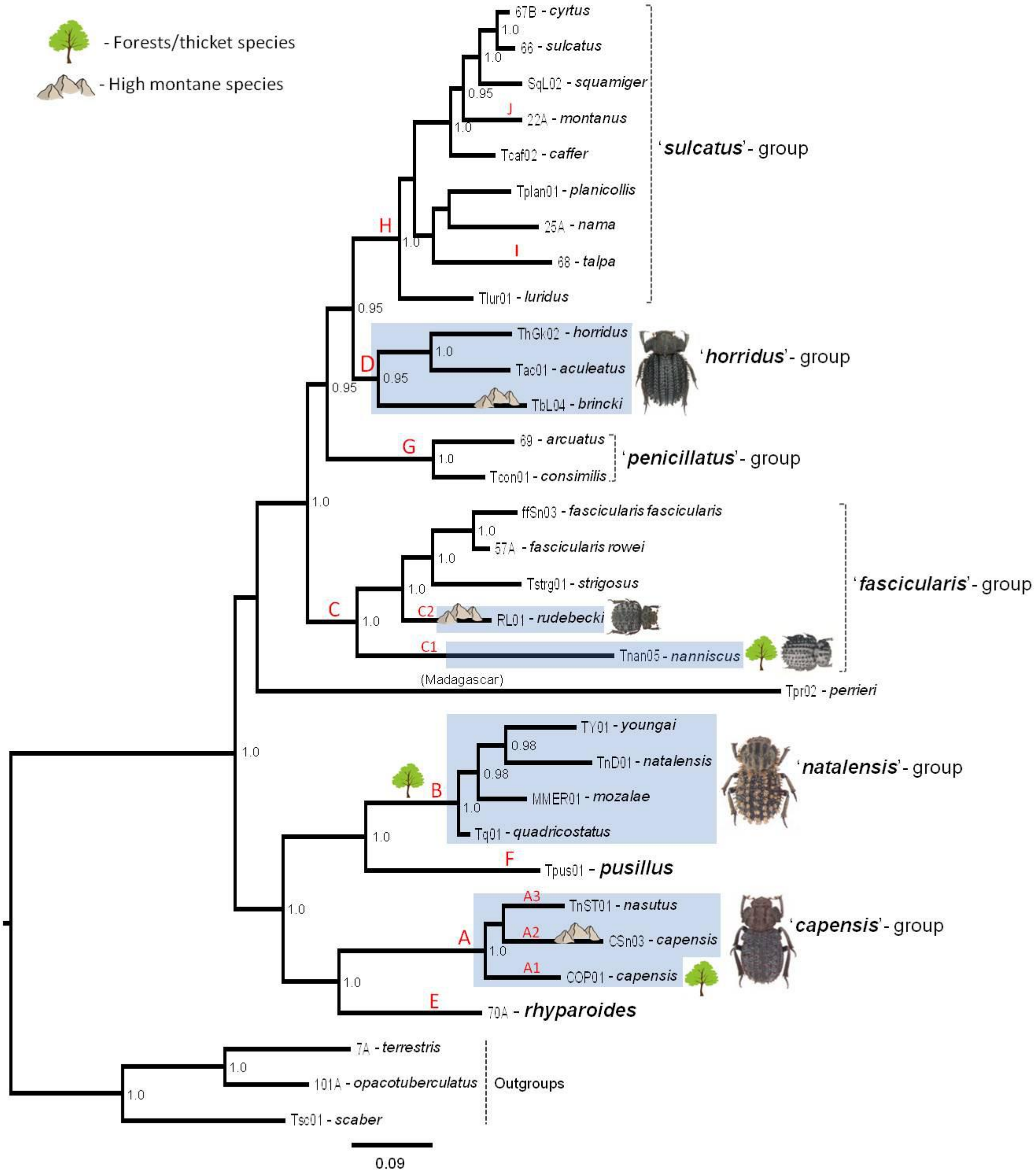

Figure 4. Bayesian topology from the MrBayes analysis for the combined dataset (two mitochondrial and two nuclear genes). Numbers next to each node are posterior probabilities. Only posterior probabilities ≥ 90% are shown. Flightless clades/taxa are highlighted in blue blocks.

The analyses recovered three monophyletic flightless species-group lineages (node A, B and D), each with a number of species. The first lineage (node A) comprises two morphologically similar species, *T. capensis* Scholtz and *T. nasutus* Harold, representing the *capensis*-group. *Trox capensis* splits into two genetically distinct sub-lineages (node A1 and A2). The second lineage (node B), the *natalensis*-group, consists of four species, *T. natalensis* Haaf, *T. quadricostatus* Scholtz, *T. mozalae* Strümpher and Scholtz and *T. youngai* Strümpher and Scholtz. The *horridus*-group (node D), with three species, *T. horridus* MacLeay, *T. aculeatus* Harold and *T. brincki* Haaf, represents the third flightless lineage.

All four species in the *fascicularis*-clade (node C), as defined by Scholtz (1980), group together. The group comprises two winged species, *T. strigosus* Haaf and *T. fascicularis* Wiedemann and two flightless species, *T. nanniscus* Péringuey (node C1) and *T. rudebecki* Haaf (node C2). *Trox rhyparoides* Harold and *T. pusillus* Péringuey, two distinct winged species, both are sister to flightless groups, the *capensis*- and *natalensis*-groups, respectively. *Trox arcuatus* Haaf and *T. consimilis* Haaf, two (of six) members of the *penicillatus*-group (node G), formed a well-supported, monophyletic clade and is sister to the *horridus*-group + *sulcatus*-group. The *sulcatus*-group (node H) comprises many winged taxa. Two groups, the *talpa*- and *montanus*-groups (nodes I and J respectively) (Scholtz, 1980), are nested within the *sulcatus*-clade. The Madagascan endemic, *T. perrieri* Fairmaire, did not group with any of the other Afrotropical species groups, but is nested on a long branch within the Afrotropical *Phoberus* lineage.

Dating Analysis

Node ages derived from the COI dataset analysed with a mutation rate of 0.012 substitution per site per million years (s/s/Mya) yielded much younger dates compared to the node ages obtained from the 0.075 s/s/Mya rate (Fig 5b, Table 4).

The mean estimates for the earliest split in the subgenus probably occurred 39 (29.0/52.0) to 63 (46.2/80) Mya, for 0.012 and 0.0075 s/s/Mya, respectively (upper and lower credibility limits in parenthesis). The ancestor of the *horridus*-group diverged from its winged ancestor at around 27.3 (20.0/36.2) to 43.5 (31.6/56.8) Mya, The *capensis*-group diverged from its closest relatives at 28.9 (20/39.7) to 46.2 (31.6/61.9) Mya. The *natalensis*-group diverged from a winged ancestor 25.6 (17.3/36) or 40.9 (28.1/56.7) Mya. The two flightless members of the fascicularis group, *T. nanniscus* and *T. rudebecki* diverged from their nearest ancestors 30 (21.6/40.5) or 48.3 (34.5, 63.4) Mya, and 17 (10.9/24.5) or 27 (17/38) Mya, respectively. Divergence within the extant *natalensis*- and *capensis*-groups occurred

13 or 23 Mya. The divergence within the large *sulcatus* clade occurred 19.1 (13.6/25.7) or 30.4 (21.5/40.1) Mya. The node ages derived from the faster mutation rate (0.012 s/s/Mya) were comparable to divergence time estimates from the molecular phylogeny for the Trogidae (Strümpher et al., 2014, see Table 4).

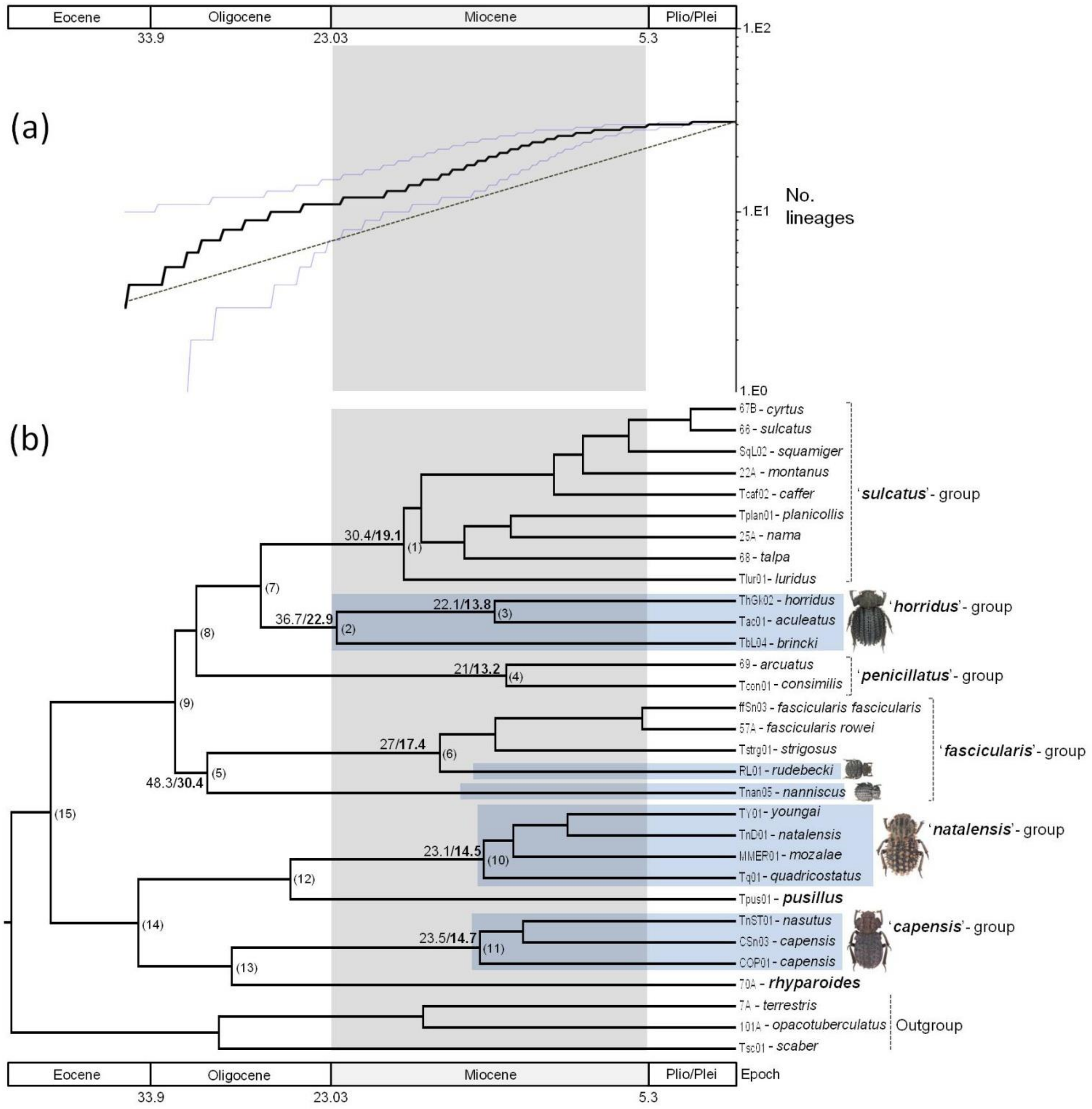

Figure 5. (a) Lineage-through-time plot showing the rate of diversification (solid line); dotted line shows a theoretical constant rate. (b) Maximum clade credibility cladogram from the BEAST analysis. Numbers above nodes are estimates of divergence times (in millions of years for 0.0075 and 0.012 s/s/Mya rates respectively), whereas the numbers in brackets correspond to node numbers in Table S4 and represent the divergence times for nodes recovered representing the various lineages/taxa. Flightless clades/taxa are highlighted in blue blocks.

Rates of diversification

The lineage-through-time plot (Fig 5a) for the Afrotropical *Phoberus* show a high initial diversification rate throughout the early- to mid-Oligocene (24-36 Mya), followed by a decrease in the diversification rate across the Oligocene-Miocene boundary (18-35 Mya). For the greater part of the Miocene (~7-17 Mya), the rate of accumulation of lineages appears constant. The rate of lineage diversification decreases from the Pliocene onwards.

Character trait mapping

The parsimony analysis (Fig S3a) indicates five independent transitions (steps) to the flightless condition (*capensis*-, *natalensis*-, *horridus*-group, *T. nanniscus* and *T. rudebecki*) with no reversals. Characters mapped using the Mk-1 and the Asymm. 2 (Fig 6, S3b) parameter models on the Bayesian topology produced largely similar results (Mk-1: $-\ln L = -15.3781$, Asymm. 2: $-\ln L = -15.3225$). There was no significant difference ($P = 0.9\text{-}0.1$) between the two models based on the test statistic for the Chi-squared test with one degree of freedom. Bayesian ancestral state reconstruction for the most recent common ancestors of selected clades is shown in supporting information (Fig S4, Tables S4). Hypothesis testing using the *fossil* command found no significant support for a given state for nodes reanalysed (all BF values < 2, not shown). Character trait optimisation shows that the volant state is the most likely ancestral character state for all of the flightless lineages. All methods support five independent transitions to flightlessness. There is no strong support for the reacquisition of functional wings once it is lost, regardless of models tested.

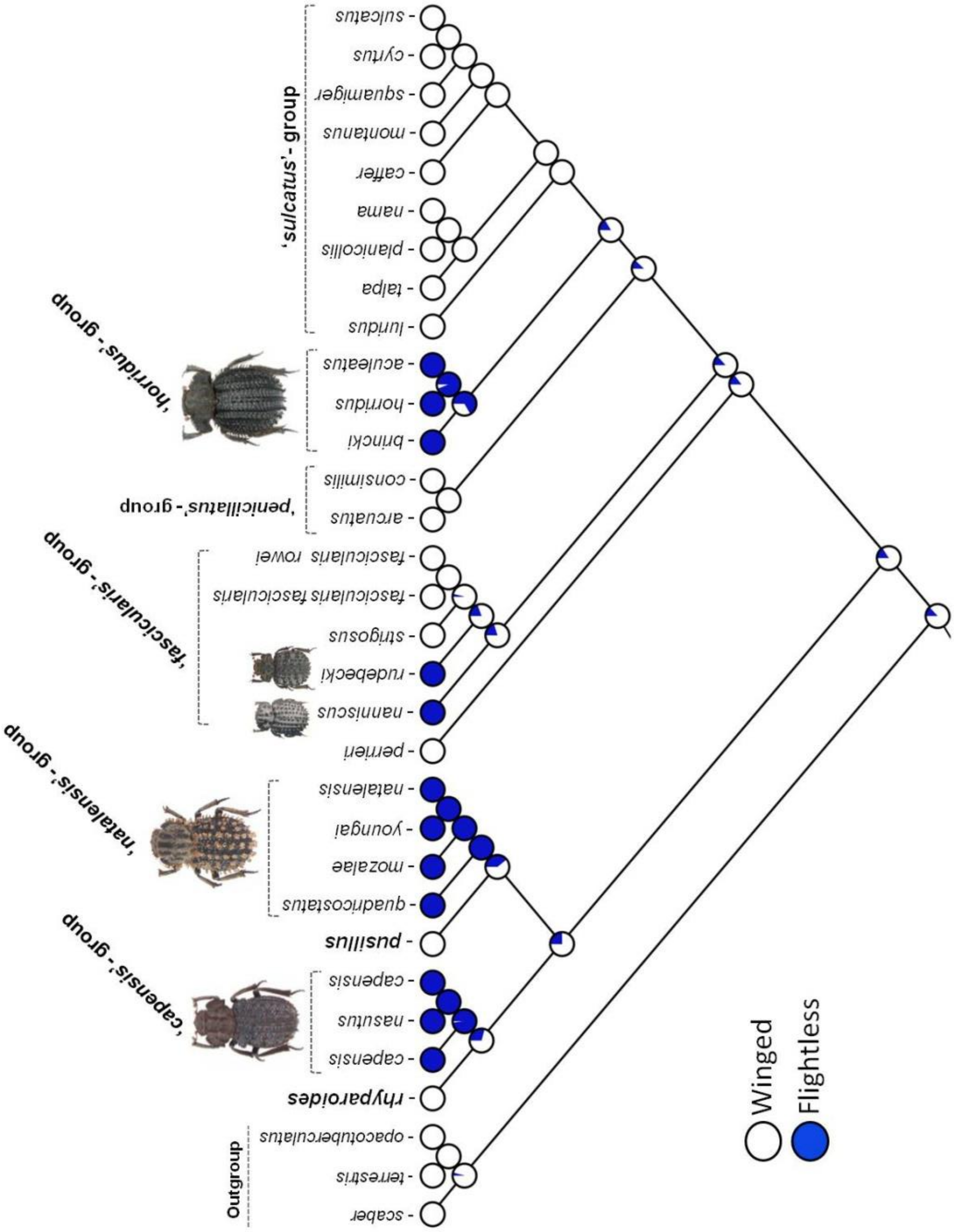

Figure 6. Maximum Likelihood ancestral-character evolution of wing loss in the subgenus *Phoberus* is displayed at the respective nodes. Pie diagrams represent the likelihood proportion of each ancestral state.

Table 4. Divergence estimates in millions of years (Mya) from the BEAST analysis, with 95% upper and lower credibility intervals. Node numbers follow figure 5.

		Cytochrome oxidase I (COI)					
		0.0075 s/s/My		0.012 s/s/My		(Strümpher *et al*. 2014)	
Node	Taxa/Species-groups	Divergence (Mya)	95% CI (Mya)	Divergence (Mya)	95% CI (Mya)	Divergence (Mya)	95% CI (Mya)
1	*'sulcatus'*-group	30.5	21.5, 40.1	19.1	13.6, 25.7	23.3	14.3, 34.6
2	*'horridus'*-group	36.7	24.9, 49.1	22.9	15.8, 31.3	17.2	6.0, 31.2
3	(*T. horridus* + *T. aculeatus*)	22	13.5, 32.5	13.8	8.5, 20.4	X	X
4	*'penicillatus'*-group	21	11.7, 32.0	13.2	7.6, 20.5	11.2	3.0, 23.2
5	*'fascicularis'*-group	48.3	34.5, 63.4	30.4	21.6, 40.5	47.3	27.6, 69.6
6	*T. rudebecki* + (*T. strigosus* + *T. fascicularis*)	27	17.0, 38.0	17.0	10.9, 24.5	28.6	13.3, 47.1
7	('*sulcatus'*-group) + ('*horridus'*-group)	43.5	31.6, 56.8	27.3	20.0, 36.2	30.8	18.7, 46.2
8	('*sulcatus'*-group) + ('*horridus'*-group) + ('*penicillatus'*-group)	49.5	36.2, 63.6	31.0	23.2, 41.3	46.0	27.9, 67.8
9	('*sulcatus'*-group) + ('*horridus'*-group) + ('*penicillatus'*-group)+('*fascicularis'*-group)	51.5	37.8, 66.3	32.3	24.2, 42.9	70.8	49.2, 96.4
10	*'natalensis'*-group	23.1	15.5, 32.0	14.5	9.8, 20.6	17.0	6.6, 32.1

11	*'capensis'*-group	23.5	15.2, 33.1	14.7	9.3, 20.7	12.0	4.0, 20.0
12	*T. pusillus* + (*'natalensis'*-group)	40.9	28.1, 56.7	25.6	17.3, 36.0	X	X
13	*T. rhyparoides* + (*'capensis'*-group)	46.2	31.6, 61.9	28.9	20.0, 39.7	37.1	19.3, 59.5
14	(*'capensis'*-group + *T. rhyparoides*) + (*'natalensis'*-group + *T. pusillus*)	54.8	39.5, 71.6	34.4	25.1, 46.1	70.7	46.8, 98.8
15	*Phoberus*	62.9	46.2, 80.8	39.0	29.5, 52.0	87.7	62.3, 116.7

Discussion

Phylogeny

Relationships within *Phoberus* were resolved well and the results of our analyses largely support eight monophyletic lineages (Fig 4; Table 3). Taxon sampling was comprehensive, with two-thirds (27/40) of Afrotropical species included in the study. Although they need formal testing, the molecular subdivisions for the most part match the informal morphological subdivisions of the subgenus (Scholtz, 1980; Strümpher and Scholtz, 2011). Broader taxon sampling could possibly better resolve the phylogenetic relationships of *T. perrieri* within the subgenus and relationships within the *sulcatus*-group. Our analyses obtained three well-supported monophyletic flightless lineages in the subgenus: *natalensis*-, *capensis*- and the *horridus*-group. Previously, all flightless taxa in the region were considered to represent a species group, the *horridus*-group, on the basis of their extreme morphological changes as a result of "old aptery", and a typical Cape-to-Drakensberg distribution pattern (Scholtz, 1979, 1980). Based on the results from this study, only three species (*T. horridus*, *T. brincki* and *T. aculeatus*) are regarded as members of the *horridus*-group. Members of the group are characteristically large (13-17mm) compared to all other flightless species, which are very small (4-8mm). Flightless taxa collectively form a polyphyletic group and the morphological similarities that they share are due to convergence as a result of the independent loss of flight.

The close relationship, for example, between *T. brincki* and its geographically-distant relatives suggests a historical link between these species. It is evident from the present disjunct distribution of the members of the *horridus*-group that they occur at opposite extremes of the escarpment (Fig 2). *Trox brincki* is a high-montane species found only at altitudes above 2800 m above sea level on the eastern Escarpment (Drakensberg Range). In contrast, its closest relatives, *T. aculeatus* and *T. horridus*, are found along the south-western section of the greater escarpment and the West Coast, about 1000 km south-west of the high Drakensberg Mountains. Although the current elevation and varied nature of the topography of the escarpment are the result of two periods of uplift during the Miocene and Pliocene, and subsequent erosion cycles (McCarthy and Rubidge, 2005), the dating analysis indicates that the *horridus*-ancestor diverged prior to these events. It is plausible that ancestral *Phoberus* lineages (winged and wingless forms) were once widely distributed along this cool (ancient) temperate biome, suggesting a continuously distributed and persistent habitat.

The forest-associated *natalensis*-group comprises half (7/14) of all flightless *Phoberus* in southern Africa and our results confirm the close relationships among these taxa that is suggested by their general morphology (Strümpher and Scholtz, 2011). All members in this group have localised distributions (between 25°S and 35°S (outside the Cape zone)) and are confined to Afrotemperate forest fragments along the eastern greater escarpment and coastal edge of South Africa (Fig 2).

The *capensis*-group, which appears basal in the phylogeny, comprises two morphologically similar species, *Trox nasutus* Harold restricted to the Cape Peninsula, and *T. capensis* which is widely distributed across the Cape Fold Mountains and the southern Cape. The populations are now spatially discrete and restricted to forest and montane refugia along the mountains. Our results indicate that *T. capensis* exhibits a more complex diversification history, splitting into two genetically distinct sub-lineages; one inhabiting relict forests of the southern Cape and the other occupying currently disjunct high montane refugia of the Cape Fold Mountains.

The evolution of flightlessness

The evolution of the conditions that led to flightlessness happened independently at least five times in *Phoberus* (Fig. 6). The multiple evolutionary transitions to flightlessness in this small subgenus suggest that the group was predisposed for flightlessness to evolve. The predisposing evolutionary events most likely involved the occupation of environments/habitats where favourable biotic and abiotic conditions (e.g. space, food, mates, temperature and humidity) were sufficient to support the long-term persistence of populations without the need for substantial dispersal (Ward, 1981; Roff, 1990; Wagner and Liebherr, 1992; Oswald, 1996; Brühl, 1997). Under such conditions, selection will favour those individuals that are able to reduce the biological cost associated with maintaining the flight apparatus (e.g. wings and musculature); whereas taxa occurring in more heterogeneous (unstable) environments cannot typically shed these costs because of their dependence on the flight apparatus for dispersal to new favourable habitat patches (Ward, 1981, 1983; Oswald, 1996).

Geological and climatic (historical) stability, although not always apparent, appear to be the overriding factors that contribute to flightlessness in many scarabaeoids (Scholtz, 2000). Historically-stable local habitats acted as refugia in the past, providing suitable conditions for the survival of relict species or for speciation events (Scholtz, 2000; Cunha et al., 2011). Geologically and climatically stable areas, especially of the southern continents, tend to have higher incidences of flightless species compared to areas of less stability (Slater, 1975, 1977; Scholtz, 2000). This is evident, for example, among the relict Australian and southern African flightless canthonine dung beetles (Scholtz, 2000; Medina and Scholtz, 2005; Deschodt and Scholtz, 2008; Mlambo et al., 2011; Sole and Scholtz, 2012), which occur in refugial areas with a long history of environmental stability. On the southern African subcontinent, they are found on the Cape Fold Mountains, in relict Afrotemperate forest patches, in disjunct temperate forests of the eastern escarpment and coastal edge, and along the arid west coast (Scholtz, 2000; Frolov and Scholtz, 2003; Sole et al., 2005; Deschodt and Scholtz, 2008; Deschodt et al., 2007, 2011; Sole and Scholtz, 2010). The situation in southern Africa appears to be similar for flightless *Phoberus*. Flightless species have restricted distributions that overlap with those of flightless canthonines; in some instances, isolated relict forest patches harbour both endemic *Phoberus* and canthonine taxa (Scholtz and Howden, 1987; Medina and Scholtz, 2005; van der Merwe and Scholtz, 2005). The southern African greater escarpment provided the stable temperate ancestral biome (spatially extensive and temporally persistent) necessary for the evolution of flightless taxa. They have managed to survive in a range of refugia there.

Aptery in the subgenus Phoberus and its relationship to habitat

Flightless *Phoberus* in the region are presently found in a variety of habitat types but all of them have persistence and stability in common. Temperate forests at low latitudes encouraged flightlessness in southern African scarabaeoids because the forest habitat provided stable and persistent microhabitats (Scholtz, 2000). Southern Africa is the only region where flightless trogids are found in forests. The presence of Afrotemperate forest in southern Africa extends back to the Late Cretaceous

(Stuckenberg, 1962; Griswold, 1991; Eeley et al., 1999; Laws et al., 2007). The persistence of an ancestral forest biome, since the Late Cretaceous and into the Mid-Tertiary (Griswold, 1991), provided a homogeneous habitat with very stable environmental conditions. Habitat stability associated with the ancestral forest biome may have promoted the loss of flight in the ancestors of the *natalensis* and *capensis* lineages.

One species, *T. nanniscus* is restricted to disjunct patches of the thicket biome or Albany Thicket in the south-eastern Cape (Mussina and Rutherford, 2006). The southern African thicket biome, derived largely from forest elements, has been a major component of southern African vegetation since the Eocene (Cowling et al., 2005; Schrire et al., 2005). It has undergone marked contraction since the Pliocene due to major climate changes across Africa (Cowling et al., 2005). The biome provided stable and persistent microhabitats that are conducive to flight loss (Sole and Scholtz, 2012) much like those found in forest habitats and may, therefore, have contributed to the loss of flight in *T. nanniscus*.

Trox rudebecki and *T. brincki*, confined to the alpine habitats of the high Drakensberg Range. Although their flightless condition may be attributed to environmental pressures associated with high mountains, such as isolation, low temperatures or high winds, the overriding factor that contributed towards flightlessness appears to be historical environmental stability. The Drakensberg escarpment is a geologically and climatically stable region of southern Africa where these conditions have persisted throughout the Tertiary.

Trox horridus and *T. aculeatus* are confined to the arid west coast and to parts of the arid western escarpment of southern Africa. The arid west coast of southern Africa is another historically stable region with a high incidence of flightlessness among many groups of scarabaeoid beetles (Scholtz, 2000; Sole et al., 2005; Deschodt and Scholtz, 2008; Deschodt et al., 2007, 2011). As for the other flightless species, the overriding factor that contributed towards flightlessness in this group appears to be historical environmental stability. Even though the loss of flight is assumed to be a physiological adaptation to arid conditions, the adaptation may have facilitated their survival by increasing the species' desiccation tolerance (Duncan and Byrne, 2002, 2005). Like all of the other flightless species, they qualify as cool temperate species.

Diversification of flightless lineages

The most significant period of diversification of flightless (and flight capable) *Phoberus* was between the Miocene and Pliocene epochs (23-2.6 Mya).

Winged species are currently mainly confined to more open habitats. The aridification and expansion of savanna and grassland habitats could have promoted diversification in the groups inhabiting the dry regions. Members of the *penicillatus*- and *sulcatus*- groups have probably evolved/radiated in the arid Karoo and Kalahari, while some species still occupy the temperate (probably ancestral) montane biome (Scholtz, 1980). Divergence estimates indicate that the origins of flightlessness in *Phoberus* are old and pre-date the Miocene Epoch (> 23 Mya), before the aridification of the sub-continent. Pre-Miocene evolution of flightlessness has also been recorded in several southern African endemic canthonine lineages (Mlambo, 2011; Sole and Scholtz, 2010, 2012). The majority of flightless *Trox* species (8 out of 14 species) are either confined to, or closely associated with, disjunct relictual Afrotemperate forest fragments (van der Merwe and Scholtz, 2005, Strümpher and Scholtz, 2009). Forest vegetation dominated southern Africa until the early Eocene (Cowling et al., 2005; Aduse-Poku et al., 2009), which appears to have contracted since the Mid-Tertiary (~32.5 Mya) due to climate changes in southern Africa as well as tectonic uplift events in the Miocene (and Pliocene). Climate shifts and resulting habitat changes have been suggested as possible causes for a number of faunal lineage diversification events in the Oligocene and Miocene periods in southern Africa (Schaefer and Renner, 2008; Tolley et al., 2008; Aduse-Puko et al., 2009; Willows-Munro and Matthee, 2009; Sole and Scholtz, 2010).

Increased environmental homogeneity and millennia of stability may have been the primary adaptive causes of flightless lineages in Afrotropical *Phoberus* (Scholtz, 2000), but this cannot account entirely for the high diversity of flightless species in the subgenus. Once flight is lost in a particular lineage, factors other than those associated with homogenous habitat and stable environment promote speciation. Climate-induced fragmentation of historically stable and persistent habitats probably drove speciation in the flightless lineages through genetic drift associated with reduced population sizes. Consequently, diversification, as well as the present relictual distributions, in the African *Phoberus*, can be linked to climate changes in southern Africa as well as geological events associated with the Miocene (and Pliocene) Climate Optimum.

Multiple losses of flight versus reversals to the flying phenotype

Our results show that it is unlikely that flight ability re-evolved in this group once it was lost. Besides, the body shape in trogids and even apparently unrelated structures such as eyes, change dramatically when trogids cease to fly (Scholtz, 1981; see also Browne et al., 1993; Scholtz, 2000). All flightless *Phoberus* species (males and females) exhibit extreme morphological change. The re-evolution of wings (and other complex morphological structures) lost in the course of evolution is generally considered unfeasible, or even impossible (Dollo's law) (Simpson, 1953; Porter and Crandal, 2003). The premise is that without selective pressure, the genetic and/or developmental pathways responsible for the expression of that character will, over time, degenerate through the accumulation of mutations (Marshall et al., 1994). In trogids, any reversal to the flying phenotype would require substantial changes to an already highly modified body and the process would most likely be a gradual, rather than an abrupt one. For the time being at least, the independent (multiple) losses of flight rather than reversals to the flying phenotype is the most parsimonious hypothesis (see also Snäll et al., 2007).

Conclusion

The evolution of flightlessness in *Phoberus* is consistent with the habitat stability hypothesis. All flightless species occupy refugial areas with relatively long environmental stability (Scholtz, 2000). Flightlessness evolved at least five times within the subgenus, providing additional support for the occurrence of the phenomenon of the independent evolution of flight loss which has occurred multiple times in various African Scarabaeoidea groups (Scholtz, 2000; Harrison and Phillips, 2003; Forgie et al., 2005; Mlambo et al., 2011; Sole and Scholtz, 2010). Diversification, and the present relictual distributions, in African *Phoberus* can be linked to climate and geological events associated with the Miocene and Pliocene Climate Optimum in southern Africa. Flightless *Phoberus* species (males and females) exhibit extreme morphological changes because of “old aptery” and our results support the independent (multiple) losses of flight rather than reversals to the flying phenotype in *Phoberus. Phoberus* is a promising model group for studying evolutionary trends relating to flightlessness, speciation and biogeography.

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Supporting information

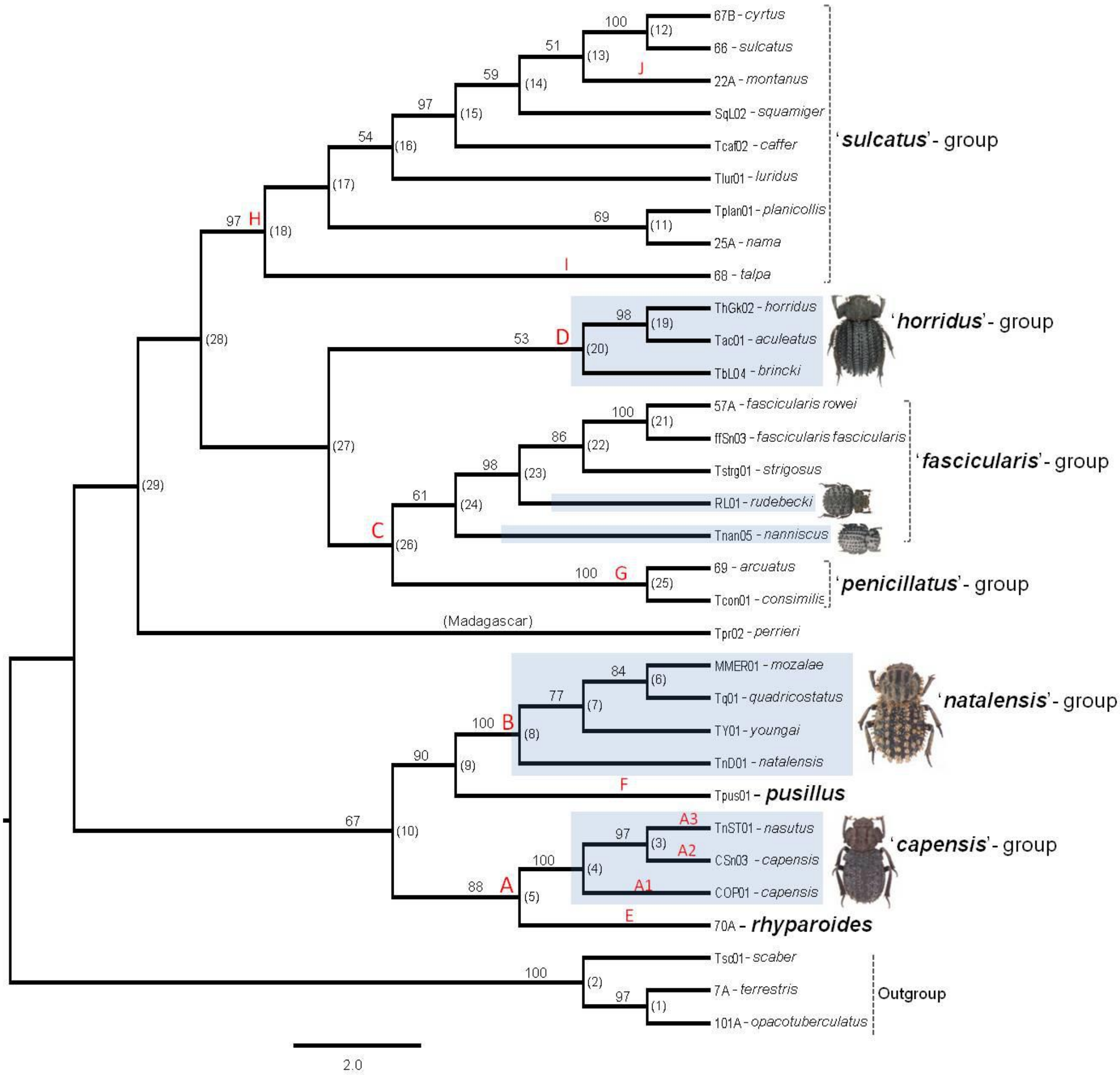

Figure S1. Strict consensus phylogram for the combined dataset (two mitochondrial and two nuclear genes) with bootstrap support values. Numbers in brackets correspond to node numbers in Table S3 for partitioned Bremer support. Only bootstrap support ≥ 50 are shown. Flightless clades/taxa are highlighted in blue blocks.

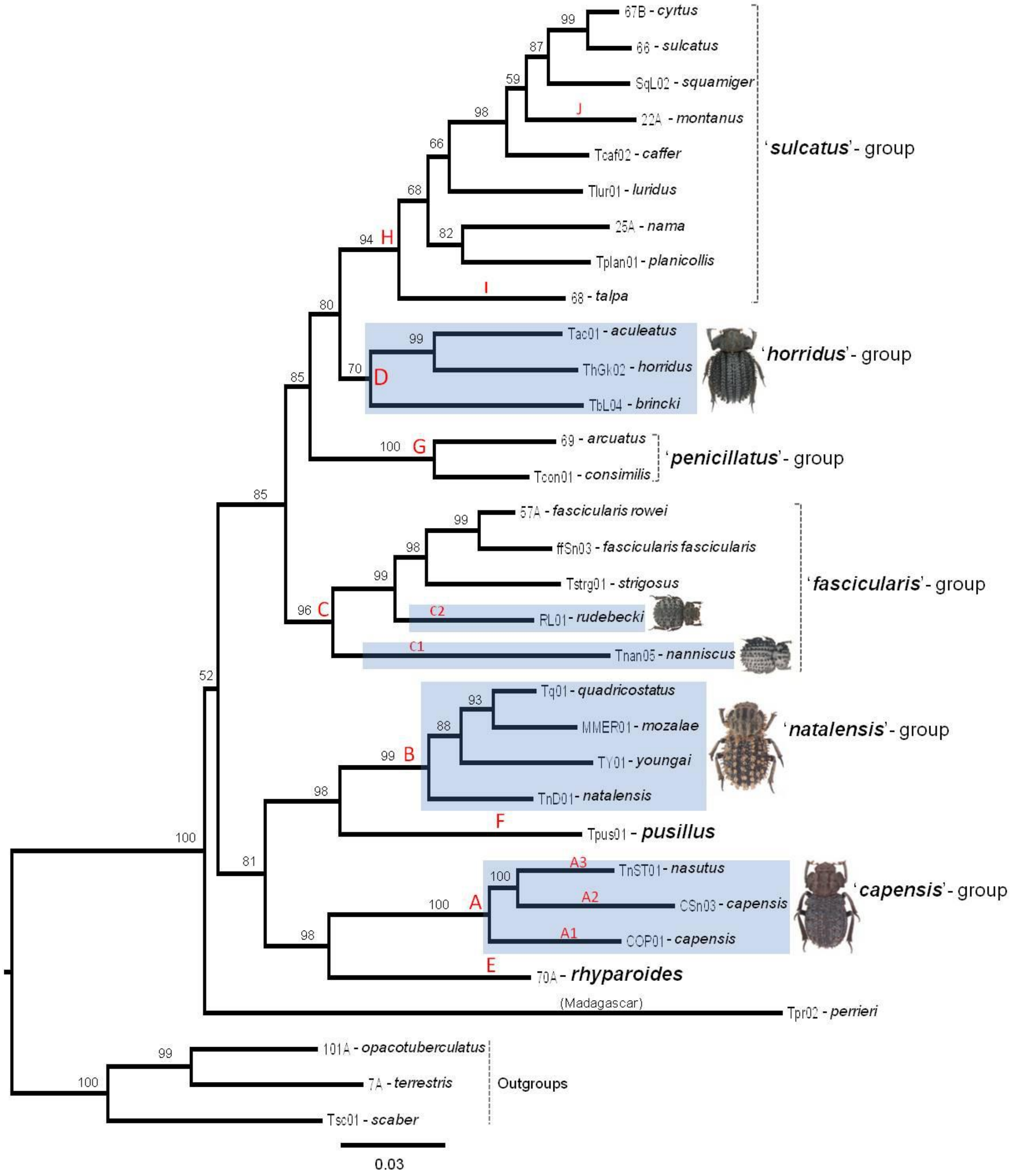

Figure S2. Maximum Likelihood phylogram from the RAxML analyses with bootstrap support. Only bootstrap support ≥50 are shown. Flightless clades/taxa are highlighted in blue blocks.

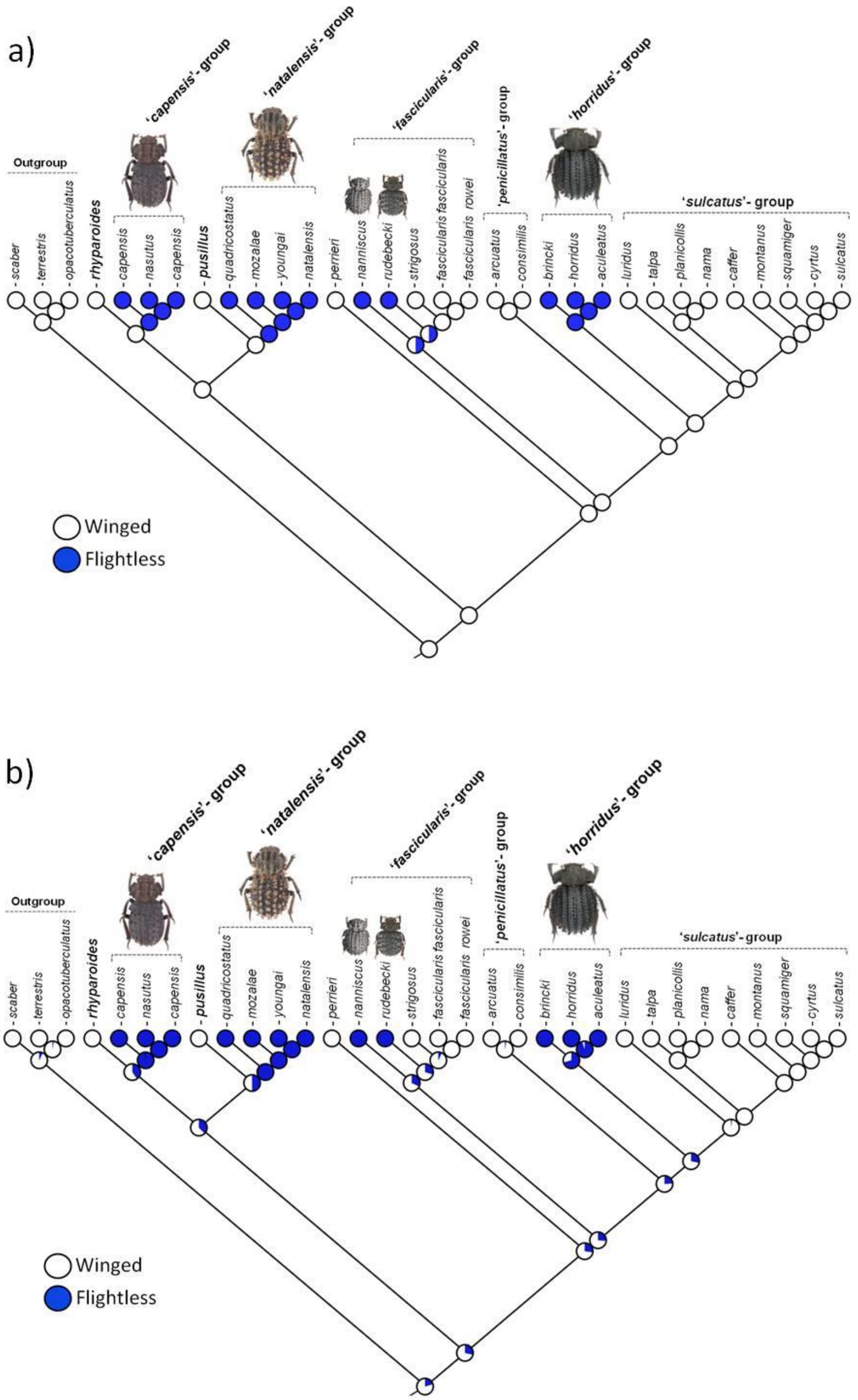

Figure S3: Character trait reconstruction, (a) Parsimony and (b) Likelihood approach.

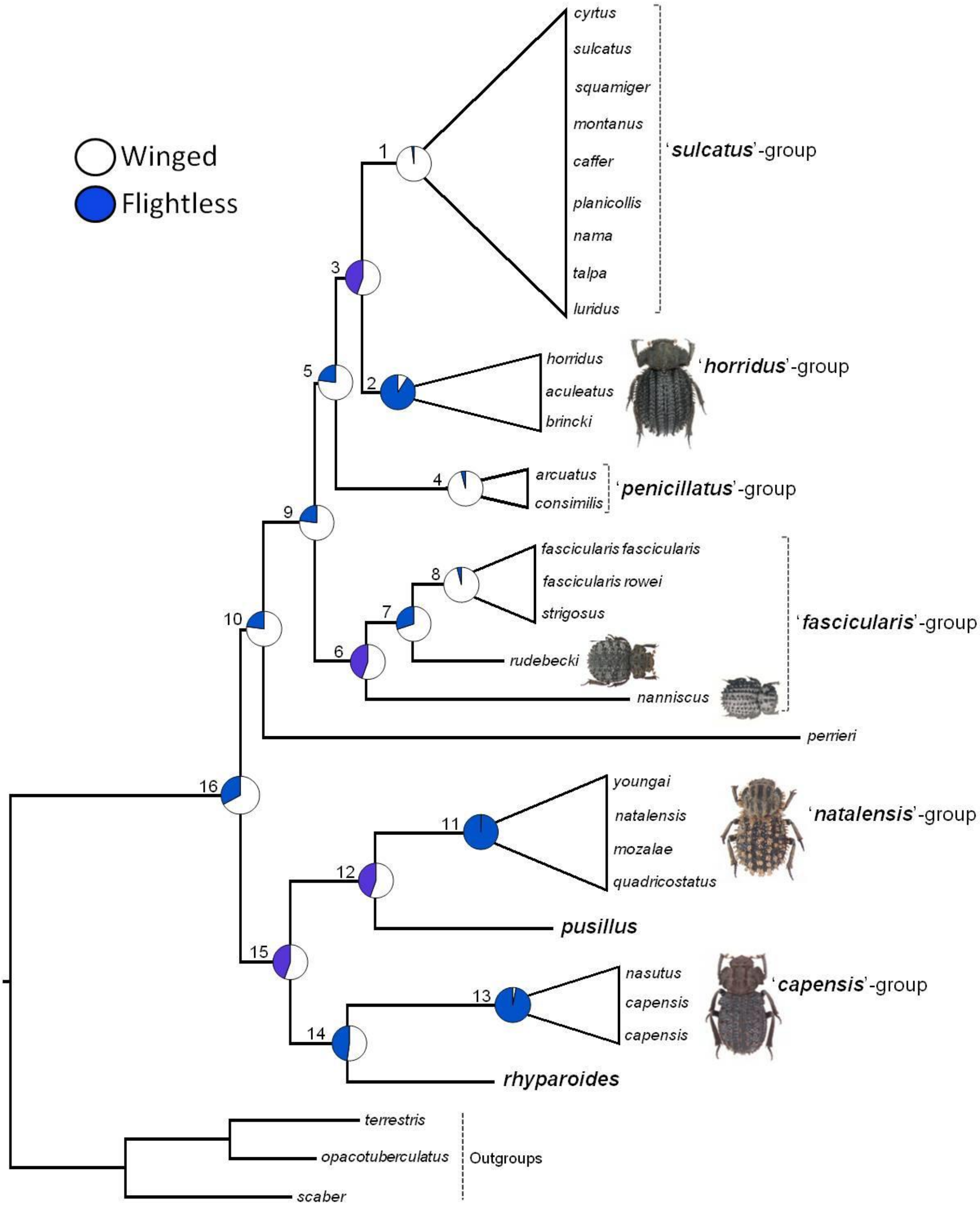

Figure S4. Simplified phylogeny showing the character states for the most recent common ancestor estimated by BAYESTRAITS.

Table S1. Summary of oligonucleotide primers used in this study.

Gene	Primer	Direction	Primer sequence (5'→3')	Reference
COI	CI-J-1718	forward	ggaggatttggaaattgattagttcc	Simon *et al*. (1994)
	TL2-N-3014	reverse	tccaatgcactaatctgccatatta	Simon *et al*. (1994)
16S	16sf (luisa)	forward	atgtctttttgakwataatwtaaag	Orsini *et al*. (2007)
	16sr (luisa)	reverse	acgctgttatccctaaggtaattt	Orsini *et al*. (2007)
18S	18s-intfw-st12	forward	atcaagaacgaaagttagag	Haring & Aspöck (2004)
	18s-rev1	reverse	atggggaacaattgcaagc	Haring & Aspöck (2004)
28S(D2)	D2-3551	forward	cgtgttgcttgatagtgcagc	Gillespie *et al*. (2005)
	D2-4057	reverse	tcaagacgggtcctgaaagt	Gillespie *et al*. (2005)

Table S2. PCR thermal cycling profiles.

	Stage 1	Stage 2 – Thermal cycling				Stage 3
Gene	Initial denaturation	Cycles	Denaturation	Annealing	Elongation	Final elongation
COI	94°C-90sec	35	(94°C-22sec,	50°C-30sec,	72°C-90sec)	72°C-1min
16S	94°C-90sec	35	(94°C-60sec,	48°C-90sec,	72°C-90sec)	72°C-1min
18S	95°C-2min	30	(95°C-10sec,	48°C-10sec,	72°C-90sec)	72°C-5min
28S(D2)	96°C-20sec	30	(96°C-15sec,	60°C-20sec,	72°C-60sec)	72°C-1min

Table S3. Bremmer support indices calculated from the parsimony analysis. Node numbers correspond those on the parsimony phylogram (Fig 4). BS, Bootstrap support; BrS, Bremmer support; PCI, partition congruence indices; PBS, partitioned Bremmer support.

					PBS values			
Node	Groupings	BS	BrS	PCI	16S	18S	28S	COI
1		97	16	18.5	3.5	-1	-3	16.5
2	Outgroup	100	30	33.0	12	3	15	0
3		97	11	13.8	-1	0	1	11
4	'*capensis*'	100	18	21.0	8	3	7	0
5	('*capensis*')+('*rhyparoides*')	88	11	14.0	1	3	7	0
6		84	8	11.0	2	0	2	4
7		77	5	8.0	2	0	2	1
8	'*natalensis*'	100	20	23.0	9	2	9	0
9	(*natalensis*)+(*pusillus*)	90	9	12.0	2	2	5	0
10		67	5	7.1	-2.25	0	1.25	6
11		69	3	6.0	0	0	0	3
12		100	12	14.8	1	0	-1	12
13		51	1	0.0	-1	-1	0	3
14		59	2	3.0	-1	-1	1	3
15		97	11	13.8	-1	0	2	10
16		54	1	4.0	0	0	1	0

					PBS values			
Node	Groupings	BS	BrS	PCI	16S	18S	28S	COI
17		-	4	6.0	-1	0	-1	6
18	'*sulcatus*'	97	10	13.0	0	1	0	9
19		98	14.09	17.1	2.76	2	1.33	8
20	'*horridus*'	53	3	6.0	1	0	1	1
21		100	19	22.0	5	0	0	14
22		86	10	12.9	4.67	0.33	5.33	-0.33
23		98	14	17.0	1	0	2	11
24	'*fascicularis*'	61	6	9.0	0	0	2	4
25	'*penicillatus*'	100	20	23.0	5	2	13	0
26		-	3	5.0	0	0	-1.5	4.5
27		-	3	2.7	-1	0	-4	8
28		78	6	8.7	4	-1	3	0
29		-	1	2.0	-1	0	2	0

Table S4. Posterior probability of ancestral character state from Bayestraits analysis. Node numbers follows Fig S4.

Node	Winged (0)	Flightless (1)
1	0.98	0.02
2	0.08	0.92
3	0.55	0.45
4	0.97	0.03
5	0.77	0.23
6	0.55	0.45
7	0.7	0.3
8	0.97	0.03
9	0.74	0.26
10	0.71	0.29
11	0.01	0.99
12	0.56	0.44
13	0.03	0.97
14	0.56	0.43
15	0.52	0.48
16	0.69	0.31

References (Table S1)

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Chapter 4

Allopatric speciation in the flightless *Phoberus capensis* (Coleoptera: Trogidae) group, with description of two new species

Abstract

The name *Phoberus capensis* (Scholtz) is applied to a small flightless, keratinophagous beetle endemic to the Cape Floristic Region of South Africa. Its gross distribution stretches from roughly 1000 km from the Cederberg (S32°24'22" E19°04'50") to Grahamstown (S33°20'07" E26°32'50"). The populations are spatially discrete, restricted to relict forests of the southern Cape and disjunct high montane refugia of the Cape Fold Mountains. We test the hypothesis that there is more than one distinct species nested within the name *P*. *capensis*. Phylogenetic relationships among populations were inferred using molecular sequence data. The results support three distinct evolutionary lineages, which were also supported by morphological characters. Divergence time estimates suggest Pliocene-Pleistocene diversification. Based on these results, it is suggested that the *P*. *capensis* lineage experienced climatically-driven allopatric speciation with sheltered Afrotemperate forests and high mountain peaks serving as important refugia in response to climatic ameliorations. The *P. capensis* complex thus represents a speciation process in which flight-restricted populations evolved in close allopatry, possibly as recently as the Pleistocene. Two divergent and geographically distinct lineages are described as novel species: The new species, *P*. *disjunctus* sp. n. and *P*. *herminae* sp. n., are illustrated by photographs of habitus and male aedeagi.

Introduction

The Cape Floristic Region (CFR) of the southern-western Cape region of South Africa is recognised as one of the world's six Floral Kingdoms (Myers et al. 2000; Mucina & Rutherford 2006; Cowling 2009). The region exhibits exceptional floral diversity and high levels of biotic endemism (Linder 2003, 2005). It is characterised by a Mediterranean (temperate) type climate and a unique sclerophyllous, fire-prone shrubland known as Cape 'fynbos' (Linder 2003, 2005; Galley & Linder 2006; Mucina

& Rutherford 2006). The development of the drought-resistant (pyrophytic) vegetation of the CFR is attributed to dramatic climate shifts from warm, tropical conditions to drier, more seasonal conditions during the Miocene/Pliocene (Cowling et al. 2009; Dupont et al. 2011; McDonald & Daniels 2012). Various factors, including the development of the cold Benguela Current along the west coast of southern Africa during the late Miocene (Siesser 1980; Pickford & Senut 1999), and tectonic uplift during the early Miocene (18 Mya) and the Pliocene (5 Mya), caused progressive aridification of the sub-continent. The combination of climate fluctuations, tectonic uplift and marine transgression and regression associated with the Pleistocene glacial cycle resulted in considerable habitat fragmentation, extinction and expansion events within the biome, thus providing an important stimulus for speciation and diversification (Partridge et al. 1999; Linder 2005; Tolley et al. 2006; Cowling et al. 2009). Climatically-driven speciation has been shown for a variety of taxa in the CFR (Tolley et al. 2006; Price et al. 2007, 2010; Swart et al. 2009; Linder et al. 2010; McDonald & Daniels 2012; Sole et al. 2013).

The CFR is dominated by the ancient Cape Fold Mountains (CFM) which can be divided into several mountain blocks: (1) a single western mountain range running parallel to the Atlantic Ocean coast, and (2) a double mountain range running parallel to the Indian Ocean coast (Linder 2003). The mountain ranges are separated by arid, low-lying valleys and plains, sheltered ravines and deeply incised gorges (Cowling et al. 2009). The CFM consists of highly erosion-resistant sandstone and most of these mountains reach altitudes higher than 1500 m above sea level, contributing to the heterogeneity of the region. These high mountains act as barriers to maritime moisture moving inland from the Atlantic and Indian Oceans, which has a profound effect on the local climate because seaward-facing slopes are moister and inland plains in the rain shadow of these mountains are very arid (Linder 2003).

The CFR and the CFM specifically provide suitable habitat and refugia for a number of relict flightless Scarabaeoidea (Coleoptera) (Scarabaeidae: Medina & Scholtz 2005; Sole et al. 2005; Deschodt et al. 2007, 2011; Deschodt & Scholtz 2008; Sole & Scholtz 2013; Lucanidae: Endrödy-Younga 1988, Switala et al. 2014; Trogidae: Scholtz 1979, 1980). The flightless species generally occupy the summits, forested palaeo-refugia on the seaward-facing slopes and the coastal plains. These cool, temperate environments may have acted as buffer zones and long-term refugia for many organisms in response to the effects of changing climate (Stuckenberg 1962; Endrödy-Younga 1978; Linder & Vlok 1991; Geldenhuys 1997; Midgley et al. 2001; Daniels et al. 2013).

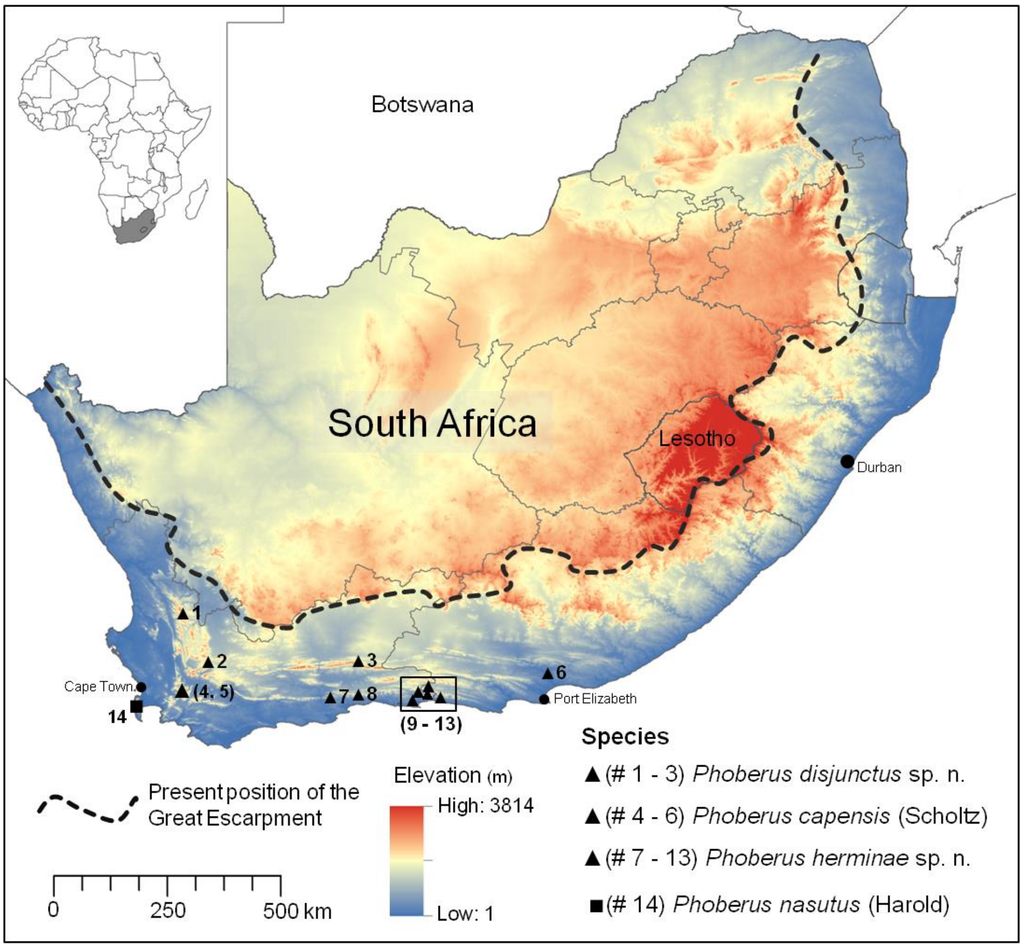

Figure 1. Map of the distribution of *Phoberus capensis* Scholtz, *P. disjunctus* sp. n., *P. herminae* sp. n. and *P nasutus* (Harold). Sample locality numbers follow Table 1.

Phoberus capensis (Scholtz) (Scarabaeoidea: Trogidae) is a small (4-7 mm) flightless, keratinophagous beetle endemic to the CFR of South Africa. Its gross distribution stretches roughly 1000 km from the Cederberg (S32°24'22" E19°04'50") to Grahamstown (S33°20'07" E26°32'50"). However, its populations are clearly spatially discrete, restricted to relict forests of the southern Cape and disjunct high montane refugia of the CFM (Fig 1). Its habitat specificity, disjunct population distributions and low vagility (due to flightlessness) make *P. capensis* an ideal species for answering questions regarding speciation and diversification events. In this study we test the hypothesis that there is more than one distinct species nested within what is currently recognised as *P. capensis*. To do this we analyse mitochondrial DNA sequences and also estimate the divergence times and ages of the populations.

Material & Methods

Sampling and laboratory protocols

Phoberus capensis was sampled from localities across its known distribution (Table 1, S1). *Phoberus nasutus* (Harold), the sister species (Strümpher et al., 2014), was used as the outgroup. The latter is restricted to the Cape Peninsula – an isolated mountainous area surrounded by the Atlantic Ocean in the west and the Cape Flats to the east (Macdonald & Daniels 2012). Collected specimens were deposited at the Department of Zoology and Entomology, University of Pretoria, South Africa (UPSA).

Total genomic DNA was extracted from a leg of each of 53 individuals and partially sequenced for the protein-coding mitochondrial Cytochrome Oxidase I subunit (COI) gene and the mitochondrial 16S small subunit ribosomal RNA (16S) gene. The primer pairs used for amplification and sequencing were COI: C1-J-1718 with TL2-N-3014 (Simon et al. 1994); and 16S: 16sf with 16sr (Orsini et al. 2007). DNA fragments for the two gene regions were amplified using the polymerase chain reaction (PCR) performed in a final volume of 25 µl made up of 20 pmol of each primer, Emerald Amp®MAX HS PCRMastermix (Takara Bio Inc., Otsu, Shiga, Japan), and 50-100 ng of genomic DNA template.

Thermal cycling parameters for COI comprised an initial denaturation for 90 s at 95°C followed by 35 cycles at 94°C for 22 s, annealing at 50°C for 30 s and 72°C for 90 s with a final elongation step at 72°C for 1 min; and for 16S an initial denaturation at 94°C for 90 s followed by 35 cycles at 94°C for 60 s, annealing at 48°C for 90 s and 72°C for 90 s with a final elongation step at 72°C for 1 min.

Successful amplifications were purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel) following the manufacturer’s instructions. To obtain DNA sequences, the cycle sequencing reactions were carried out in both directions using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, USA). Cycle sequencing products were precipitated using a standard sodium acetate/ethanol precipitation protocol. All sequences were viewed, edited and assembled in CLC Bio 5.6 (http://www.clcbio.com/). New sequences were submitted to GenBank (see Tables S2 and S3 in supporting information).

Table 1. Specimens of *Phoberus capensis* group collected from the Western Cape and Eastern Cape provinces, South Africa. *N* = population number, *n* = number of individuals collected from each locality, Alt = Altitude of collection sites above sea level in meters.

N	Sample Locality	Alt (m)	Coordinates	*n*
1	Cederberg Mountains	1547 m	S32°24'22.43" E19°04'50.37"	1
2	Hexrivier Mountains, Matroosberg	1880 m	S33°22'22.23" E19°39'34.77"	4
3	Swartberg Mountains, Swartberg Pass	1510 m	S33°20'59.27" E22°15'51.90"	6
4	Hottentots-Holland, Sneeukop	1406 m	S34°02'21.08" E18°59'24.94"	13
5	Hottentots-Holland, Moordenaarskop	940 m	S34°05'53.12" E18°57'52.74"	2
6	Grahamstown, Signal Hill	622 m	S33°20'07.54" E26°32'50.23"	2
7	Langeberge, Ruiterbos	800 m	S33°52'31.37" E22°01'22.00"	3
8	Outeniqua Mountains, Outeniqua Pass	668 m	S33°54'02.05" E22°24'04.09"	3
9	Tsitsikama Forest, Nature's Valley	38 m	S33°58'02.00" E23°33'36.80"	2
10	Knysna, Diepwalle Forest	420 m	S33°57'40.92" E23°09'22.79"	3
11	Knysna, Buffalo Valley	81 m	S33°59'29.51" E23°17'55.34"	4
12	Knysna, Harkerville Forest	276 m	S34°03'00.53" E23°12'00.34"	9
13	Stormsrivier	102 m	S33°58'18.01" E23°53'53.00"	1
14	Cape Peninsula, nr Simonstown	98 m	S34°13'19.20" E18°24'38.48"	3

Alignment

The sequences were aligned using the program package MAFFT (Katoh & Toh 2008) with default settings. The alignments for the two gene regions (COI, 16S) were concatenated into a supermatrix using the program FASconCAT version 1.0 (Kück & Meusemann 2010).

Phylogenetic analysis

jModel Test (Posada 2008) was used to select the appropriate model of sequence evolution under the Akaike information criterion (Akaike 1974). Phylogenetic trees were inferred using Maximum Parsimony (MP), Bayesian Inference (BI) and Maximum Likelihood (ML) approaches.

A parsimony analysis was implemented in PAUP*4.010b (Swofford 2003) with the following heuristic search setting: all characters were equally weighted and unordered, gaps were treated as missing data and uninformative sites were excluded; starting tree obtained via stepwise addition with random addition of sequences with 10 replicates; branch-swapping = tree-bisection-reconnection; initial ‘maxtrees’ set to 200 with automatic increase by 100 and with ‘MulTrees’ option in effect. Bootstrap values were calculated based on 1000 replicates. A strict consensus tree was calculated from all of the most parsimonious trees obtained.

Bayesian analysis was performed for individual genes and the combined gene regions using the program MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The data set was partitioned into two gene regions. Analysis was performed using GTR+G+I model for COI, a GTR+G model for 16S. Parameters for the different partitions were unlinked to obtain separate parameter estimates for each gene and the rate prior was set to variable. Flat Drichlet priors were used in all analyses. Bayesian analyses were conducted by simultaneously running two Monte Carlo Markov chains, with one cold and three heated chains, for 5 million iterations. Trees were sampled every 200 iterations. Tracer v1.5 (Rambaut & Drummond 2007) was used to monitor parameter stabilisation (via inspection of estimated sample size (ESS) and graphical plots of parameter sampling). The first 20% of trees sampled were consequently discarded as burn-in.

Maximum Likelihood analysis was conducted in RAxML (Randomized Axelerated Maximum Likelihood) version 8.20 (Stamatakis 2014) on the concatenated dataset under a GTR+G model. A single run was conducted with 1000 bootstrap inferences, followed by a thorough ML search. Each inference was initiated with a random starting tree, and employed the rapid hill-climbing algorithm (Stamatakis et al. 2007). FigTree v1.3.1 (Rambaut 2009) was used to view all tree topologies.

Haplotype phylogenetic relationships were assessed by a split-decomposition neighbour-net analysis on individual and combined dataset using SplitsTrees 4

(Huson & Bryant 2006). Pairwise genetic distances (*p*-distances) were calculated between groups using Mega 6 (Tamura et al. 2013) for COI and 16S. Groups were based on the well-supported lineages recovered within the phylogenetic analysis.

Molecular diversity, molecular structure and historical demography

Haplotype (*h*) and nucleotide diversity (π) for each gene region were assessed using DnaSP v. 5 (Librado & Rozas 2009). Analysis of molecular variance (AMOVA), calculated in Arlequin version 3.5 (Excoffier & Lischer 2010), was performed on the COI mtDNA locus to explore population structure within the genetic variation. Groupings tested within the AMOVA were based on the well-supported lineages recovered within the phylogenetic and neighbor-network analyses. A population was defined as all individuals coming from a single geographic locality.

Divergence analysis

The node ages for the lineage-splitting events were estimated using the software BEAST v. 1.6.2 (Drummond & Rambaut, 2007). Cytochrome Oxidase I (COI) data for those individuals successfully sequenced was used. As no fossil evidence with which to estimate time of origin for lineages is available for this group, we opted to use published mutation rates of 0.0075 and 0.012 (Brower 1994; Juan et al. 1995; Farrell 2001; Smith & Farrell 2005; Wirta et al. 2008) mutational substitutions per site per million years (s/s/Myr), respectively, to cover the range of rates reported for COI mtDNA. A Bayesian relaxed molecular clock approach under the uncorrelated lognormal model and a Yule speciation was used for divergence time estimation. The ingroup was constrained to be monophyletic. The ML topology was specified as a starting tree for BEAST. Two independent MCMC analyses were run for 20 million generations with parameters sampled every 1000 generations. Default settings were kept for all other priors and operators. Tracer was used to monitor parameter stabilisation and assess the convergence between runs. LogCombiner v1.6.2 from the BEAST package was used to combine the tree and log output files from the two independent runs. The first 20% of trees sampled from each run was discarded as burn-in. The TreeAnnotator v1.6.2 application from the BEAST package was used to generate a maximum clade credibility tree and calculate the mean ages. Tree topologies were viewed in FigTree. The divergence analysis was run twice to validate consistency of the time estimates between runs.

Morphology

Where possible, male and female specimens from the different sampling localities were examined and male genitalia dissected and photographed. In addition, the holotype and several paratype specimens of *P. capensis* (Scholtz) were examined using Zeiss dissecting microscopes. Images of set habitus specimens were taken with a Canon EOS 550D and 100 mm macro lens. Focus stacking was performed using the software Helicon Focus version 5.3. Male genitalia and components were photographed under a Leica M165 C microscope, using the Leica DMC 2900 digital camera. Morphological terminology follows Scholtz (1980).

Results

Phylogenetic analysis

The final combined molecular dataset consisted of 56 taxa and 1608 base pairs (bp): COI = 1152 bp, 16S ≈ 456 bp; 252 characters were parsimony informative, 1327 were constant and 280 were variable. Data characteristics and estimated model parameters for individual and combined datasets are shown in Table 2.

The heuristic search from the parsimony analysis produced 3360 most parsimonious trees, with tree length = 444, consistency index (CI) = 0.691 and retention index (RI) = 0.960. The strict consensus tree, with nodal support, is presented in Fig S1. Trees obtained from the BI and ML analyses yield very similar topologies, hence only the Bayesian phylogram is presented with Bayesian posterior probability (PP) and ML bootstrap support (BS) for nodes (Fig 3). The resulting neighbour-net splits tree (Fig 2) for the combined dataset had a recomputed fit = 93.01, and LS fit = 99.48. Individual gene trees and splitsgraphs show similar phylogenetic groupings as for the combined dataset and are thus not presented.

Three distinct evolutionary lineages (nodes A, B and C), each with strong nodal support (PP = 1.0, BS = 100; Fig 2, 3), were recovered across all methods of analysis. Clade A is divided into two well-supported (PP = 1.0, BS = 100; Fig 2, 3), geographically separated populations, Sneeukop/Moordenaarskop (node A1) and Grahamstown (node A2). Clade B split into three well-supported (PP = 1.0, BS = 100) geographically discrete populations, representing the Swartberg Pass (node

B1), Matroosberg (node B2) and Cederberg (node B3) sampling localities. Clade C (PP = 1.0, BS = 100; Fig 2, 3) comprises taxa from several localities: Knysna, Tsitsikama, the Outeniqua Pass, Ruiterbos and Stormsrivier. Phylogenetic relationships in this clade are not well-resolved and taxa are interspersed on the tree, despite obvious geographical separation among the populations.

For COI, pairwise genetic distances between defined groups (Table 3) range from 5.4% to 11.9%, and for 16S between 0.4% and 2.6%. The percentage pairwise genetic distance, for COI within clades (A,B,C) was low overall, with 3.93% for clade A, 1.4% for clade B and 0.5% for clade C, and for 16S it was 0.5% for clade A, 0.1% for clade B and 0.1% for clade C.

Table 2. Data characteristics and estimated model parameters for COI, 16S and combined datasets; CI = consistency index, RI = retention index, MP = Maximum Parsimony, AIC = Akaike Information Criterion.

	COI	16S	Combined
Number of samples	56	40	56
Aligned positions	1152	456	1608
Parsimony informative sites	227	25	252
Tree length (MP)	410	31	444
Number of tree (MP)	616	4	3360
CI/RI	0.680/0.959	0.903/0.986	0.691/0.960
Best fit model (AIC)	GTR+I+G	GTR+G	GTR+G
A frequency	0.3246	0.3899	0.3467
C frequency	0.1583	0.1451	0.1574
G frequency	0.1413	0.0959	0.1260
T frequency	0.3758	0.3691	0.3698
R(a) [AC]	4.6039	2279.2626	5.0453
R(b) [AG]	18.3256	2980.1105	22.6645
R(c) [AT]	3.8626	1718.1791	3.1252
R(d) [CG]	1.0425	1.0000	1.6269
R(e) [CT]	31.2071	11551.1508	31.6934
R(f) [GT]	1.0000	1.0000	1.0000
Gamma (G)	0.4440	0.0150	0.1080
Invariable sites (I)	0.3890	-	-

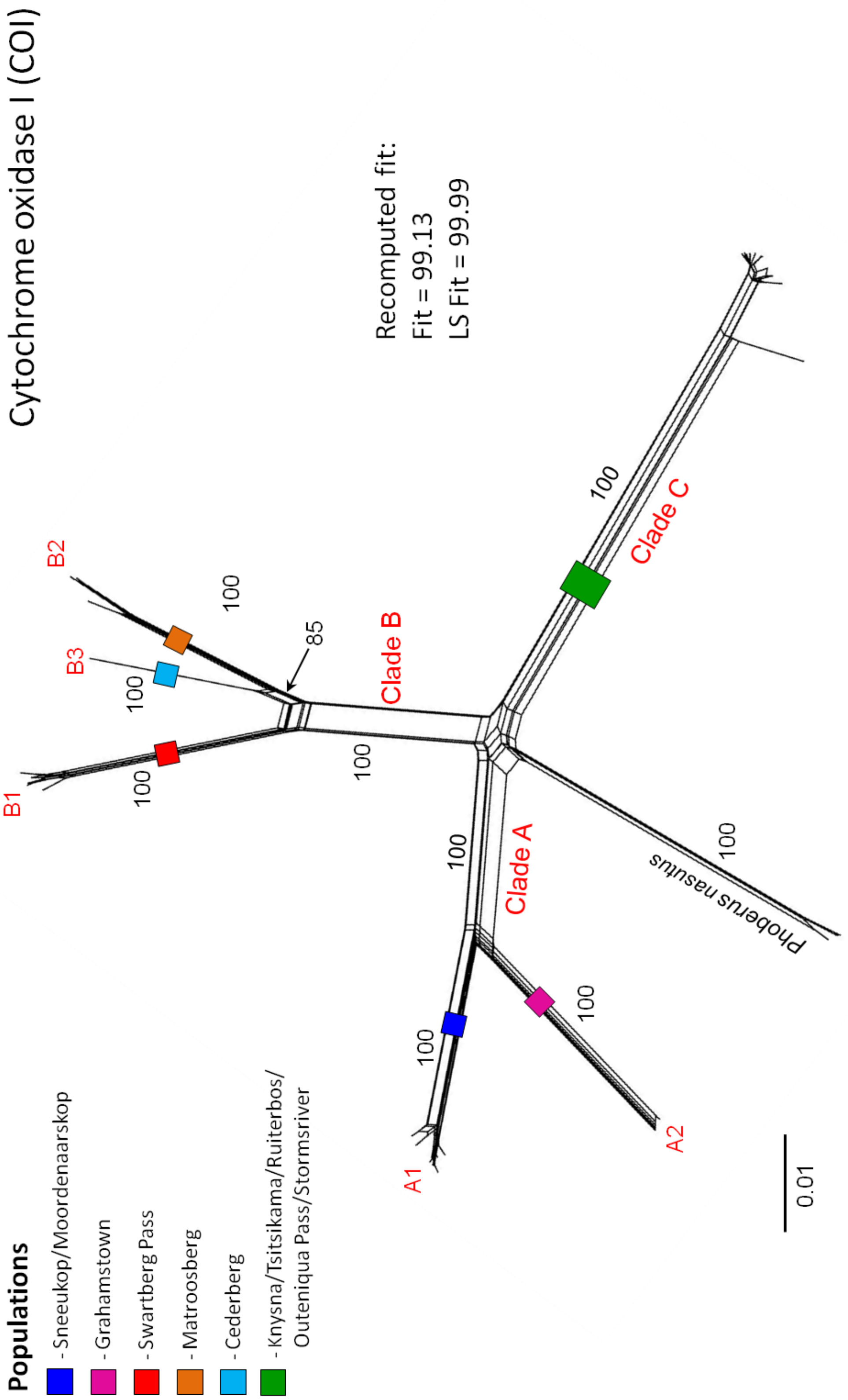

Figure 2. Neighbour-net graph for the mtDNA COI dataset with bootstrap support, based on uncorrected *p*-distances.

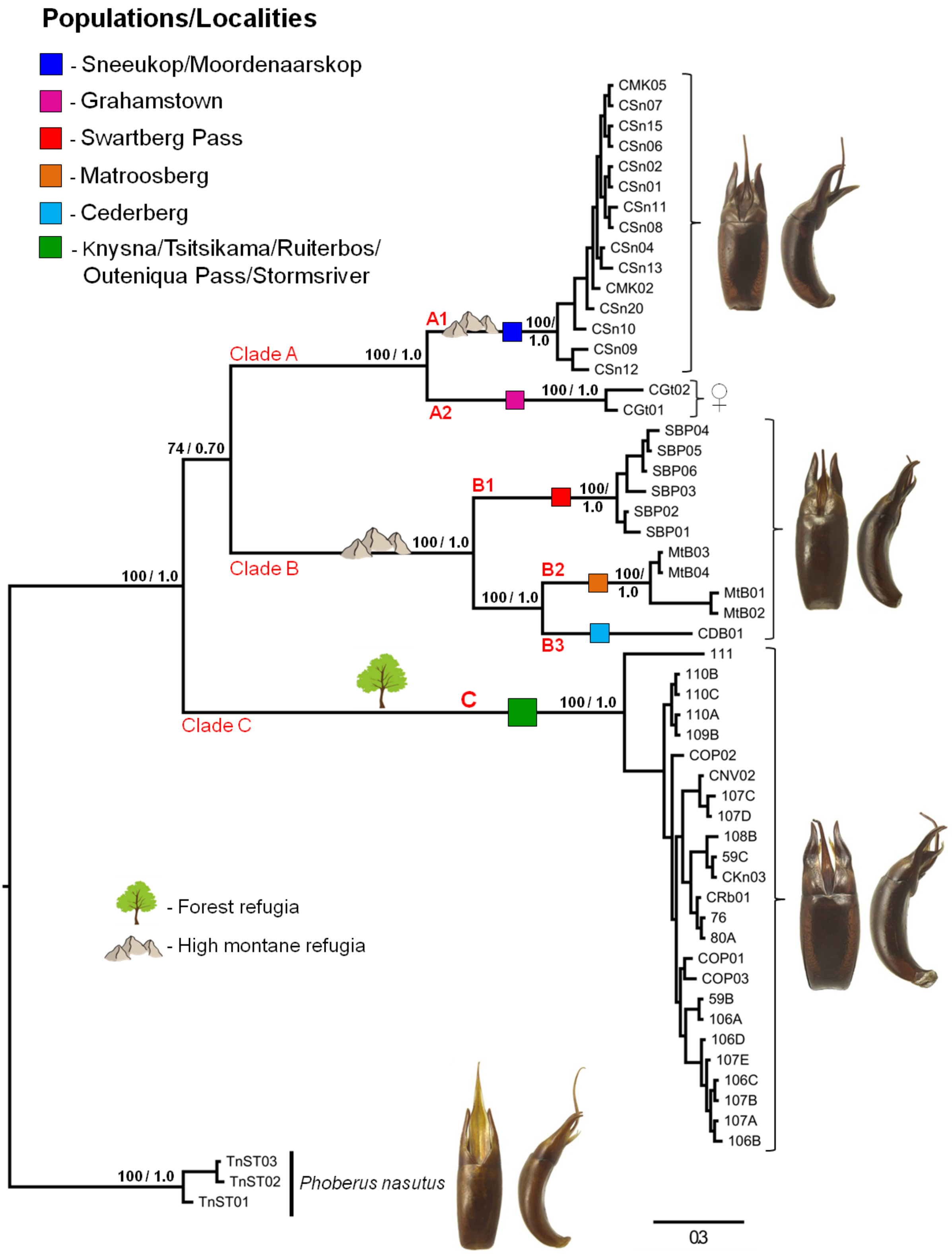

Figure 3. Bayesian topology from the MrBayes analysis for the combined dataset. Numbers next to each node are (ML) bootstrap support and the (BI) posterior probabilities. Images depict the male genitalia type for the respective lineages.

Table 3. Percentage pairwise genetic distances between and within clades for COI (below the diagonal) and 16S (above the diagonal).

	A1	A2	B1	B2	B3	C
A1		0.7%	1.9%			2.3%
A2	5.4%					
B1	10.4%			0.7%	0.4%	2.6%
B2			5.7%		0.2%	
B3			6.0%	4.9%		
C	11.7%		11.9%			

Genetic structure and population genetics

Among the 53 individuals of *P. capensis* sequenced for COI, 32 different haplotypes were identified while only 11 haplotypes were identified from the 38 individuals sequenced for 16S. Haplotype diversity for COI was high (0.962 ± 0.014) and somewhat lower but more variable for 16S (0.83 ± 0.031). Nucleotide diversity for COI was 0.069 ± 0.0035 with the average nucleotide differences being 79.827, while 16S had a nucleotide diversity of 0.01834 ± 0.00142 and an average nucleotide difference of 8.215. Statistics of general nucleotide diversity are summarised in Table 4. There were no shared haplotypes among the three clades (A, B, C) and between populations within clades, indicating no gene flow between these populations or possible sampling artefacts, e.g. small sample sizes. The fixation value (F_{st}) of 0.8570 ($p < 0.001$) from the AMOVA analysis indicated strong genetic structure between the three clades. Differences among the three groups accounted for 85.70% of the variance, while 14.30% could be attributed to variation among populations within groups.

Table 4. Summary of F_{st} statistics calculated by AMOVA (Excoffier & Lischer 2010).

Species	**Source of Variation**	**F_{st}**	**%**	**P**
Phoberus capensis	Among groups variation		85.7	< 0.001
	Within groups variation		14.3	< 0.001
	Fixation index	0.8570		< 0.001

Divergence times

The divergence time analyses indicate that the *P. capensis* complex is 3.77 or 6.03 Myr old, for 0.012 and 0.0075 s/s/Myr, respectively (Table 5). *Phoberus capensis* diverged from its closest relative, *P. nasutus* (outgroup), at 4.3 or 6.85 mya. Divergence within clade B occurred 2.06 or 3.76 mya, while divergence within clade A occurred 1.95 or 3.06 Mya. Clade C diverged around 1.33 or 2.13 mya.

Table 5. Time to most recent common ancestor (in millions of years) for the lineages identified in the phylogenetic tree (Fig 3). Upper and lower 95% confidence limits are shown in brackets.

	Substitution rate	
Clades	**0.012**	**0.0075**
Clade A	1.95 (0.98, 3.36)	3.06 (1.47, 5.29)
Sneeukop/Moordenaarskop (A1)	0.85 (0.31, 1.78)	1.34 (0.5, 2.79)
Clade A+Clade B	3.3 (1.95, 5.24)	5.26 (3.05, 8.17)
Clade B	2.06 (1.09; 3.41)	3.27 (1.68, 5.44)
Matroosberg (B2) + Cederberg (B3)	1.34 (0.56; 2.36)	2.12 (0.91, 3.80)
Matroosberg (B2)	0.53 (0.15; 1.16)	0.84 (0.23, 1.86)
Swartberg Pass (B1)	0.58 (0.17; 1.33)	0.93 (0.25, 2.18)
Clade C	1.33 (0.54, 2.70)	2.13 (0.81, 4.24)
Ingroup	3.77 (2.19, 5.96)	6.03 (3.47, 9.43)
Root	4.3 (2.46, 6.94)	6.85 (3.85, 11.01)

Morphology

Pronotal and elytral features in *P. capensis* vary greatly with size of the individual. In smaller specimens, many features tend to be more irregular or obscured (for example intercostal punctures and fovae) and/or pronounced (like tubercles and ridges), thus making it difficult to identify characters that can be used to identify clades/groups within *P. capensis*. Despite the lack of diagnostic pronotal and elytral characters, fairly consistent differences exist between specimens of clade B and clades A and C. Specimens from the Cederberg, Matroosberg and Swartberg Pass (Clade B) are generally smaller (4-7mm) and have the discal area of the pronotum more evenly rounded, with a shallow median depression and tubercles that are not distinctly raised. Members of clade A and C are larger (5-10mm) and the discal area of the pronotum is raised, with a deep median depression and distinct tubercles.

Examination of the male genitalia shows three major types corresponding to the recovered clades A1, B and C. However, variation in the male genitalia within each clade, the small number of male specimens available for study and the lack of male specimens from Grahamstown (A2), make it difficult to distinguish between all clades on the basis of male genitalia alone.

Discussion

Phylogeny and divergence time estimates

Despite the small sample sizes from some localities, the results of this study indicate there are at least three well-supported evolutionary lineages within *P. capensis* with high (10-12%) sequence divergence between them at the COI locus, which is similar to that found for other flightless scarabaeoids (Sole & Scholtz 2013; Switala et al. 2014). The high sequence divergence between the three clades justifies recognising them as distinct species using previously-cited threshold values recorded between closely related insect species (Herbert et al., 2003; Price et al. 2007; Astrin et al. 2012; for limitations of genetic distance as a criterion in delineating species see Ferguson (2002) and Brower (2006)). All three clades (A, B and C) are geographically discrete with apparently no dispersal between them (Fig 1).

The results show that clades A and B may comprise divergent and geographically distinct populations which can be interpreted as phylogenetically independent lineages. However, these populations are morphologically indiscernible and due to the small number of specimens obtained from the different localities, we refrain from drawing any conclusions regarding the (taxonomic) status of these populations and will not discuss them further here.

Molecular dating hypothesised the diversification of the *P. capensis* group during the Pliocene-Pleistocene. This time period was characterised by habitat and vegetation shifts associated with climatic change in the CFR (Cowling et al. 2009; Swart et al. 2009; Tolley et al. 2006), and the contraction of forested areas and with the establishment of 'fynbos' as the spatially dominant vegetation (~3 to 5 mya; Linder 2003; Galley & Linder 2006). Based on results of this study, it is suggested that *P. capensis* experienced climatically-driven allopatric speciation with sheltered Afrotemperate forest and high mountain peaks serving as important refugia during climatic ameliorations (Stuckenberg 1962; Endrödy-Younga 1978; Picker & Samways 1996; Voje et al. 2009; Daniels et al. 2013). *Phoberus capensis* thus represents a speciation process in which flight-restricted populations evolved in close allopatry, possibly as recently as the Pleistocene.

Taxonomic considerations

These results indicate that the samples represented at least three distinct species according with the phylogenetic species concept (Nixon & Wheeler 1990; Wheeler & Platnick 2000). The Hottentots-Holland clade (clade A) from the Sneeukop/Moordenaarskop localities has morphology identical to the type material of *Phoberus capensis* (Scholtz 1979). The type locality for *P. capensis* is the nearby Jonkershoek Mountain (Scholtz 1980). In stark contrast, situated about 600 km east of the Hottentots-Holland Mountains is the Grahamstown sister population (A2) and the most easterly distribution for *P. capensis.* This population may represent a distinct lineage, but with only two female specimens at our disposal its status remains uncertain and warrants further investigation.

The Swartberg assemblage (clade B), comprises specimens collected from three different mountain ranges, Cederberg, Matroosberg and Swartberg. They are morphologically uniform and can be distinguished from the other two lineages by differences on the pronotum and male genitalia. The different mountain populations are genetically divergent and may represent novel lineages but this requires further investigation. Extensive sampling is needed in the region to delineate distribution boundaries.

The Southern Cape clade (clade C) has a large and fragmented distribution mostly in the Afrotemperate forests along the southern coast (Knysna and Tsitsikama) and in the high-lying forest fragments in the CFM (Outeniqua, Tsitsikama and Langeberg Mountain ranges). The unresolved phylogenetic relationships within the clade, their low divergence, and close association with Afrotemperate forests, suggest that these localities/populations may have been connected in the recent past when forests were widely continuous in southern and eastern Africa (Partridge et al. 1999).

On the basis of the differences discussed above we describe two novel species. *Phoberus capensis* (Scholtz) has had an inconstant taxonomic history. In his original description Scholtz (1979), at the time, treated *P. capensis* as a member of the genus *Trox* Fabricius, and placed it in the subgenus *Phoberus* MacLeay in his revision of the Afrotropical species (Scholtz 1980), and in his revision of the Trogidae (Scholtz 1986), *T. capensis* was transferred to *Trox* s.str. However, recent molecular evidence suggests that all Afrotropical species of *Trox* form a monophyletic group, falling within *Phoberus* (Strümpher et al. 2014). Strümpher et al. (2014) were explicit that *Phoberus* merits higher taxonomic status and in their paper *Trox capensis* has already been informally placed in *Phoberus* as a genus. Strümpher et al. (2015) subsequently restored *Phoberus* to genus rank to include all of the Afrotropical (including Madagascan endemic) species. From a nomenclatural perspective we thus consider *T. capensis* and the novel species described here as members of the genus *Phoberus* MacLeay. Descriptions of the new species are limited to characters with past diagnostic value. No differences were observed in the external morphology of males and females. Members of the species complex are morphologically similar to *P. nasutus* (Harold) and can best be distinguished on the basis of the male genitalia (Scholtz 1980).

Systematics

Type specimens

Scholtz (1979) in his original generated a large type series (225 specimens) which he deposited in collections around the world. For logistical reasons not all type material was re-examine. Nonetheless, the bulk of the type series (160 specimens), including the holotype, were retained at the Ditsong Museum of Natural History (TMSA) and the Department of Zoology & Entomology, University of Pretoria (UPSA). We deemed the number of primary types available for examination sufficient for this study. All paratypes designated by Scholtz (1979) and the collections housing type material are listed within this paper in the type material section under the description of the each taxon. Type material that could not be examined is marked by an asterisk (*) next to the repository acronym. All label data were cited verbatim and information on type material examined was copied using “/” between lines, and “//” between labels.

Institutions to which new specimens or type material belongs or in which they have been deposited are abbreviated as follows:

AMNH: American Museum of Natural History, New York, NY, USA
BMNH: The Natural History Museum, London, UK
HMUG: Hunterian Museum, University of Glasgow, Glasgow, UK
HNHM: Hungarian Natural History Museum, Budapest, Hungary
ISNB: Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium
MNHN: Muséum National d'Histoire Naturelle, Paris, France
MZLU: Museum of Zoology, Lund University, Lund, Sweden
NMK: National Museums of Kenya, Nairobi, Kenya
NCSA: National Collection of Insects, Roodeplaat, Pretoria, South Africa
NHRS: Naturhistoriska Riksmuseet, Stockholm, Sweden
RMCA: The Royal Museum for Central Africa, Tervuren, Brussels, Belgium
SAM: Iziko South African Museum, Cape Town, South Africa
SMTD: Staatliches Museum für Tierkunde, Dresden, Germany
SMW: State Museum, Windhoek, Namibia
TMSA: Ditsong Museum of Natural History, Pretoria, South Africa
UPSA: Department of Zoology & Entomology, University of Pretoria, South Africa
ZMUC: Zoological Museum, University of Copenhagen, Copenhagen, Denmark
ZSBS: Zoologische Sammlung des Bayerischen Staates, Munich, Germany

Genus: *Phoberus* MacLeay, 1819
Phoberus MacLeay, 1819: 137-138; Strümpher et al. 2014: 557-558; Strümpher et al. 2015: 15.

Phoberus capensis (Scholtz) (Plate A: 1a, b; Figs 2, 3: Clade A)
Trox capensis Scholtz 1979:174
Trox (*Phoberus*) *capensis* Scholtz, 1980:71
Trox (*Trox*) *capensis* Scholtz, 1986: 361
Phoberus capensis Strümpher et al., 2014: Table S1 ff.; Strümpher et al., 2015: 27.

Diagnosis

Phoberus capensis is morphologically similar to *P disjunctus* sp. n., but can be distinguished from the latter by the raised discal area of the pronotum and deep median depression and distinct tubercles. *Phoberus disjunctus* sp. n. has the discal area of the pronotum more evenly rounded, with a shallow median depression and tubercle not distinctly raised. Male genitalia of both species are distinct (Plate A: 1b).

Redescription

Size: 7-8 mm, width: 4-5 mm (*n* = 20).

Head: Clypeus triangular; frons with two oval setose ridges; antennal scape setose, slightly longer than wide, pedicel attached to apex of scape.

Pronotum: Attenuated anteriorly, sides broadly flattened, surface pitted; lateral margins with fringes of short setae; discal area raised, median depression deep divided into two halves by low transverse ridge, anterior half of median depression broad, posterior half narrow; discal ridges high, interrupted approximately in middle; median basal tubercles fused to discal ridges; another ridge is present laterad of, and parallel to, the median ridges, ridges and tubercle setose.

Elytra: Scutellum small and oval, humeral calli absent; sides flattened; lateral margins with fringes of short setae; even numbered costae distinct, tubercles with tufts of setae, tubercles raised, single or partially fused to form distinct ridges; odd numbered costae indistinct; intercostal punctures irregular large and shallow; elytral profile convex, attaining maximum height behind middle.

Male genitalia: parameres symmetrical, attenuated towards the apex, parameres curving distinctly downwards, pars basalis thick, not distinctly c-shaped and slightly longer than parameres, ratio of length of parameres to length of pars basalis about 1:1. Median lobe exposed in dorsal view, flattened basally and attenuated sharply into long, narrow pointed projection (flagellum) extending beyond parameres; pair of blade-like projections ventrally between parameres (Plate A: 1b).

Distribution: *Phoberus capensis* is restricted to the Hottentots-Holland Mountain range in the greater Cape Fold Mountain Range and confined to the high mountain peaks (Fig 1).

Type material: Holotype, ♂: Jonkershoek Mnt. / Forest Reserve / SE 33 19 Cc / 20.XII.1978 / C.H. Scholtz (TMSA); Paratypes: S. Afr., S.W. Cape / Nuweberg, Sneeukop / 34.03S - 18.59E // 16.XI.1973, E-Y: 265 / ground traps, 130 days / leg. Endrödy-Younga (30 TMSA; 1 UPSA; 1 BMNH*; 1; MNHN*; 1 NHRS*; 1 ISNB*; 1

RMCA*; 1 ZSBS*; 1 HNHM*; 1 NCSA*; 1 SMW*; 1 SAM*); S. Afr., W. Cape / Nuweberg Camp / 34.03S 19.04E // 14.XI.1973, E-Y: 244 / groundtrap: fish / leg. Endrödy-Younga (13 TMSA; 1 UPSA; 1BMNH*; 1 ISNB*; 1 RMCA*; 1 ZSBS*); S. Afr.: W. Cape / Hawequas / 33°34'S 19°08'E // 5.XI.1973, E-T: 202 / sifted litter / leg. / Endrödy-Younga (2 TMSA); Stellenbosch / 20.7.1965 / H. Geertsema (1 UPSA); Ditto (10 UPSA*; 3 BMNH*; 3 MNHMS*; 3 NHRS*; 3 ISNB*; 3 RMCA*; 3 ZSBS*; 3 HNHM*; 3 NCSA*; 3 SMW*; 3 SAM*). Type material examined bears the new type label: HOLOTYPE [or PARATYPE] / *Phoberus capensis* (Scholtz) / Strümpher et al. 2015 (printed on white rectangle with red border).

Additional material examined: RSA: Western Cape Prov. / Caledon, Sneeukop / Hottentots-Holland Mnts. / S34.05000° E18.98333 / 16.XI.1973, E-Y: 265 / Soil trap, Coll: EY-STR (20 TMSA).

Phoberus disjunctus Strümpher **sp. n.** (Plate A: 2a, b; Figs 2, 3 Clade B)

Diagnosis

Phoberus disjunctus is similar to *P*. *capensis* but can be distinguished from the latter by an evenly rounded pronotal disc and shallow median depression; *P. capensis* has a high pronotal disc with a deep median depression. Male genitalia of both species are distinctive (Fig 3; Plate A).

Description

Size: 5-6 mm, width: 3-4 mm (*n* = 25).

Pronotum: discal area of pronotum evenly rounded, with shallow median depression, tubercle not distinctly raised.

Male genitalia: parameres symmetrical, attenuated towards the apex, in lateral profile straight and curved apically; pars basalis longer than parameres, the ratio of length of parameres to length of pars basalis about 1:1.5, pars basalis variable in shape and thickness; median lobe exposed in dorsal view, flattened basally and attenuated sharply into short narrow pointed projection (flagellum) only slightly longer than parameres; genitalia in ventral view with a pair of blade-like projections between parameres (Plate A: 2b).

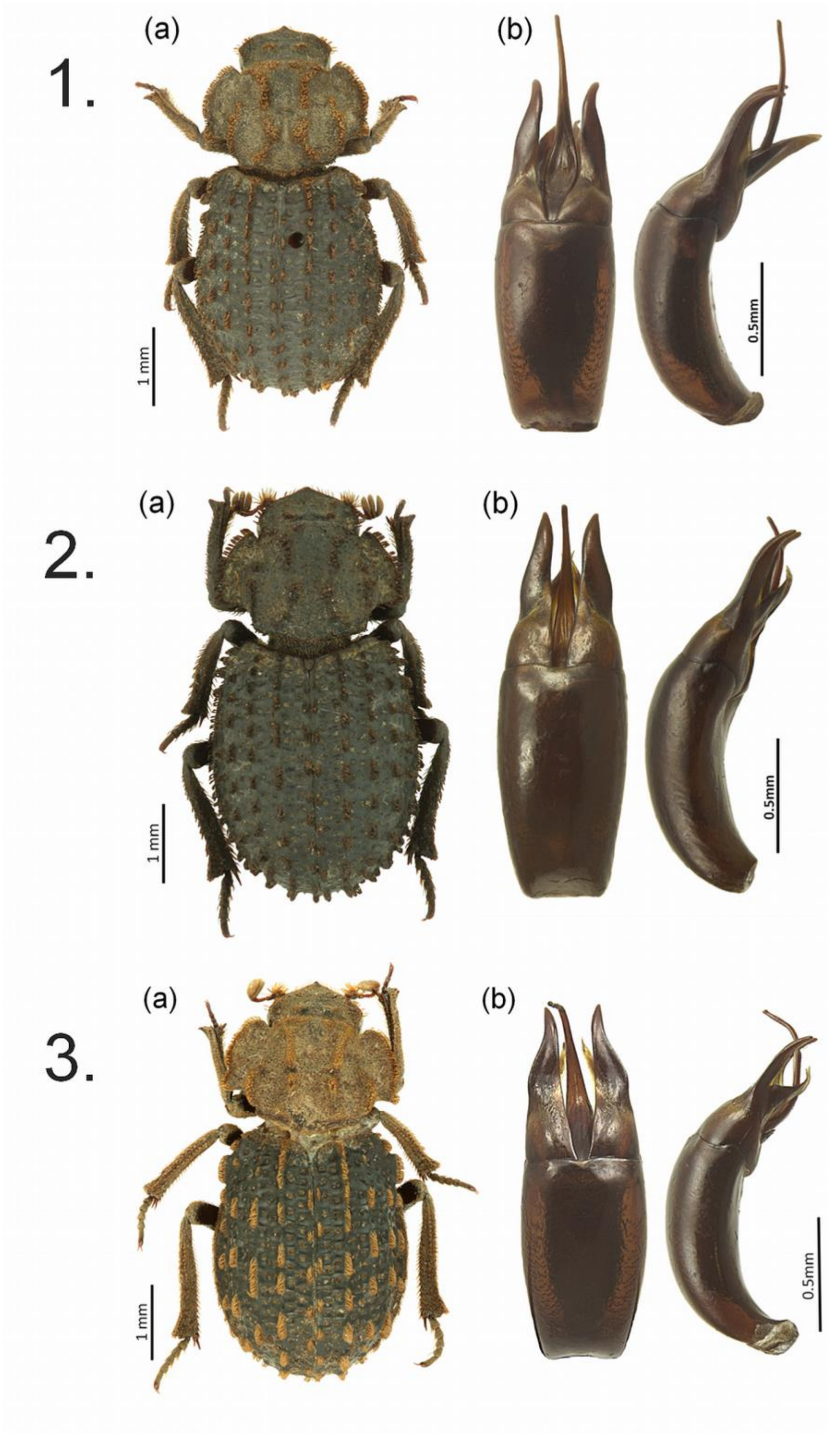

Plate A: **1(a)** Adult habitus of *Phoberus capensis* Scholtz; **1(b)** Aedeagus of *Phoberus capensis* (Scholtz): dorsal and lateral view. **2(a)** Adult habitus of *Phoberus disjunctus* sp. n.; **2(b)** Aedeagus of *Phoberus disjunctus* sp. n.: dorsal and lateral view. **3(a)** Adult habitus of *Phoberus herminae* sp. n.; **3(b)** Aedeagus of *Phoberus herminae* sp. n.: dorsal and lateral view.

Distribution: This species is distributed (disjunctly) along the Swartberg, Matroosberg and the Cederberg Mountains in the greater Cape Fold Mountain Range and confined to the high mountain peaks (Fig 1).

Type material: Holotype ♂: S. Afr.: Cape, Cederberg / Jeep track, 1380 m / 32.24S - 19.10E // 01.IX.1981, E-Y: 1878 / ground traps, 63 days / leg. Endrödy-Younga // ground traps with meat bait // *Trox capensis* Scholtz / det. C.H. Scholtz (white rectangle printed) (TMSA); Paratypes: S. Afr.: Cape, Cederberg / Jeep track, 1380 m / 32.24S - 19.10E // 01.IX.1981, E-Y: 1878 / ground traps, 63 days / leg. Endrödy-Younga // ground traps with meat bait // *Trox capensis* Scholtz / det. C.H. Scholtz (white rectangle printed) (2 TMSA); S. Afr: Swartberge / Blesberg - E[ast], 2000 m / 23[33].25S - 22.41E // 06.XI.1978, E-Y: 1508 / ground traps, 40 days / leg. Endrödy-Younga // ground traps with faeces bait (9 TMSA); S. Afr: Swartberge / Blesberg - E[ast], 2000 m / 23[33].25S - 22.41E // 05.XI.1978, E-Y: 1505 / ground traps, 41 days / leg. Endrödy-Younga // ground traps with faeces bait (10 TMSA); S. Afr: Swartberge / Blesberg - W[est], 1820 m / 33.25S - 22.40E // 06.XI.1978, E-Y: 1512 / ground traps, 41 days / leg. Endrödy-Younga // ground traps with meat bait (1 TMSA); S. Afr: Swartberge / Blesberg - W[est], 1820 m / 33.25S - 22.40E // 06.XI.1978, E-Y: 1513 / on flowers / leg. Endrödy-Younga (1 TMSA); S. Afr: Swartberge / Blesberg - W[est], 1850 m / 33.24S - 22.40E // 07.XI.1978, E-Y: 1514 / ground traps, 40 days / leg. Endrödy-Younga // ground traps with meat bait (4 TMSA); RSA: Western Cape Province / Swartberg Range, Blesberg / 33.25S 22.41E, 1820 m // 16.XII.1987, E-Y: 1532B / leg. Endrödy-Younga, (1 NCSA; 4 TMSA; 2 UPSA); RSA: Western Cape Prov. / Swartberg Pass, nr. Oudtshoorn / 13.III.1979, ground traps (meat) / Coll: Breytenbach (3 UPSA). Type material examined bears the new type label: HOLOTYPE [or PARATYPE] / *Phoberus disjunctus* sp.n. / Strümpher et al. 2015 (printed on white rectangle with red border).

Etymology: From the Latin *disjunctus*, meaning separated or disconnected, in reference to the disjunct montane distribution of this species.

Phoberus herminae Strümpher **sp. n.** (Plate A: 3a, b; Figs 2, 3 Clade C)

Diagnosis

Phoberus herminae has very similar external morphology to *P. capensis,* and the only reliable means of identification involves the male genitalia. Constant differences exist in the shape of the parameres and the median lobe (Fig 3; Plate A).

Description

Size: 7-10 mm, width: 4-6 mm (*n* = 28).

External morphology as for *P. capensis*.

Male genitalia: parameres symmetrical, attenuated towards the apex, parameres curved, pars basalis thick, c-shaped and longer than parameres, ratio of length of parameres to length of pars basalis about 1:1.5. Median lobe exposed in dorsal view, flattened basally and attenuated broadly into narrow pointed projection (flagellum) extending beyond parameres, often with knob-like structure on apex; genitalia with a single pair of ventral blade-like projections between parameres (Plate A: 3b).

Distribution: This species is distributed (disjunctly) along coastal edge and the Tsitsikama, Outeniqua and Langeberg Mountain Ranges of the south Western Cape Province (Fig 1).

Type material: Holotype ♂: S. Afr., Cape Prov. / Knysna forest / 22km N Knysna // 08-12.III.1992 / leg. J. Klimaszweski (TMSA). Paratypes: S. Afr., Cape Prov. / Knysna Forest / 22km N Knysna // 08-12.III.1992 / leg. J. Klimaszweski (TMSA 2; UPSA 2); Baviaanskloof Mnts. / Studtis, SE 33 23 Db / 1627m, 09.VIII.1978 / E. Breytenbach (3 UPSA; 1 BMNH*; 1 MNHN*; 1 NHRS*); S. Afr., S. Cape / Harkerville Forest / 34.04.S - 23.10E // 13.12.1976 / E-Y: 1311 / ground traps, 4 days / leg. Endrödy-Younga // ground traps with / faeces bait (6 TMSA; 2 UPSA; 1 MNHN*); Bloukrans Forest / Knysna, SE 34 23 Ab / Jan.1978 / C.H. Scholtz (1 UPSA); Goudveld Forest / Knysna, SE 33 22 Dd / Jan. 1978 / C.H. Scholtz (1 UPSA; 1 SMTD*); Kafferkop Forest / Knysna, SE 34 23 Aa / Jan.1978 / C.H. Scholtz (1 UPSA); Tsitsikama Forest / SE 33 23 Dc, Jan.1978 / C.H. Scholtz (2 UPSA); S. Afr., S. Cape / Keurboomstrand / 34.00S – 23.27.E // 18.12.1976, E-Y: 1301 / ground traps 8 days / leg. Endrödy-Younga (4 TMSA; 1 UPSA); Bergplaas Forest / Knysna, SE 33 22 Dc / Jan.1979 / J.H. Botha (2 UPSA; 2 AMNH*; 2 ZMUC*; 2 HMUG*; 1 ISNB*; 2 NMK*; 1 RMCA*); S. Afr.: S. CapeMt. / Grootberg, W., 1450 m / 33.55S 20.51E // 2.XI.1978, E-Y: 1501 / groundtraps, 35 days / leg. Endrödy-Younga // ground traps with faeces bait (49 TMSA). Ysternek Forest / Knysna, SE 33 23 Cc / Jan.1978 / C.H Scholtz (1 UPSA; 1 RMCA*; 1 ZSBS*); Diepwalle Forest / Knysna, 33 23 Cc / Jan.1978 / C.H. Scholtz (1 MZLU*). All type material examined bears the new type label: HOLOTYPE [or PARATYPE] / *Phoberus herminae* sp. n. / Strümpher et al. 2015 (printed on white rectangle with red border).

Additional material examined: RSA: Western Cape Province / Outeniqua Pass, 25.XII.2007 / S33.90000° E22.40107°, 668m / Baited pitfall trap, C Deschodt (5 UPSA); S. Afr: Cape Prov. / Lily Vlei Nat. Res. / 33.56S 23.02E // Gouna State Forest, IV.1983, , J. Koen (12 TMSA; 4 UPSA); S. Afr.: Cape Prov. / Diepwalle St. Forest / 33.56S 23.09E // January1984 / dry forest litter / leg. J. Koen (2 UPSA); Diepwalle St. Forest / 33.56S 23.09E // February 1984 / dry forest litter / leg. J. Koen (1 TMSA); Diepwalle St. Forest / 33.56S 23.09E // 02. 1984 / wet forest / leg. J. Koen (1 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // January 1985 / pitfall trap / leg. J. Koen (2 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // February 1985 / pitfall trap / leg. J. Koen (2 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // 7. 1985 / pitfall trap / leg. J. Koen (3 TMSA); S. Afr.: Cape Prov. / Groenkop / 33.57S 22.33E // 10. 1985 / pitfall trap / leg. J. Koen (3 TMSA); S. Afr.: SE Cape Mts / Baviaanskloofberg / 33.36S - 24.23E // 15.XI.1982, 1035 m. / groundtraps, 30 days / leg. W. Breytenbach // ground traps with meat bait (8 TMSA).

Etymology: It is with great pleasure that I name this species after my wife Hermien Viljoen for her support of my entomological pursuits.

Acknowledgements

We are grateful to members of the Scarab Research Group, at the University of Pretoria, for field assistance. We thank Ruth Müller of the Ditsong Museum of Natural History, Pretoria, for lending us their valuable specimens for this study, and two anonymous reviewers for their helpful comments. This project was supported by funding to Clarke H. Scholtz from the National Research Foundation of South Africa (NRF). We acknowledge and thank Cape Nature (Western Cape Province) for granting permits for field collections.

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Supporting Information

Table S1. List of taxa and collection data of trogid beetles used in this study; Y = indicates PCR amplification successful, X = indicates PCR amplification failed. * = Sequences generated by Strümpher et al. (2014); (UPSA) = depository for molecular voucher.

Species	Locality data	Code	16S	CO1
Outgroup				
Phoberus nasutus	RSA: Western Cape Prov., near Simonstown, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt (UPSA)	TnST01	KC801097*	Y
P. nasutus	RSA: Western Cape Prov., near Simons Town, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt (UPSA)	TnST02	KC801096*	Y
P. nasutus	RSA: Western Cape Prov., near Simons Town, 07.IX.2008, S34.22200° E18.41069°, 80m, C. Deschodt (UPSA)	TnST03	Y	Y
Ingroup				
Phoberus capensis	RSA: Western Cape Prov., Moordenaarskop, 940m, 15.XII.2007, S34.09809° E18.96465°, WP Strümpher (UPSA)	CMK02	Y	Y
P. capensis	RSA: Western Cape Prov., Moordenaarskop, 940m, 15.XII.2007, S34.09809° E18.96465°, WP Strümpher (UPSA)	CMK05	X	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn01	Y	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn02	Y	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn04	Y	Y

Species	Locality data	Code	16S	CO1
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn06	X	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn07	X	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn08	X	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn09	Y	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn10	Y	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn11	X	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn12	Y	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn13	Y	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn15	X	Y
P. capensis	RSA: Western Cape Prov., Sneeukop, S34.03919° E18.99026°, 1300m, 18.XII.2007, WP. Strümpher (UPSA)	CSn20	Y	Y
P. capensis	RSA: Eastern Cape Prov., Grahamstown, Signal Hill, 620m, S33°20'07" E26°32'50", 03.IX.2008, WP. Strümpher (UPSA)	CGt01	X	Y
P. capensis	RSA: Eastern Cape Prov., Grahamstown, Signal Hill, 620m, S33°20'07" E26°32'50", 03.IX.2008, WP. Strümpher (UPSA)	CGt02	Y	Y
Phoberus disjunctus sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB01	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB02	Y	Y

Species	Locality data	Code	16S	CO1
P. disjunctus sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB03	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Matroosberg, 1880m, S33°22'22" E19°39'34", 22.XI.2010, CH. Scholtz (UPSA)	MtB04	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP01	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP02	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP03	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP04	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP05	Y	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Swartberg Pass, 1510m, S33°20'59" E22°15'52", 15.XI.2011, WP. Strümpher & CH. Scholtz (UPSA)	SBP06	X	Y
P. disjunctus sp. n.	RSA: Western Cape Prov., Cederberg Conservancy, 1547m, S32°24'22" E19°04'50", 12.XII.2011, WP. Strümpher & CH. Scholtz (UPSA)	CDB01	Y	Y
Phoberus herminae sp. n.	RSA: Western Cape Prov., Outeniqua Mountains, Outeniqua Pass, 668m, S33.90000° E22.40107°, 25.XII.2007, C Deschodt (UPSA)	COP01	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Outeniqua Mountains, Outeniqua Pass, 668m, S33.90000° E22.40107°, 25.XII.2007, C Deschodt (UPSA)	COP02	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Outeniqua Mountains, Outeniqua Pass, 668m, S33.90000° E22.40107°, 25.XII.2007, C Deschodt (UPSA)	COP03	Y	Y
P. herminae sp. n.	RSA: Eastern Cape Prov., Langeberge, Ruiterbos, 800m, S33°52'31" E22°01'22", 15.XI.2004, C. Deschodt (UPSA)	CRb01	Y	Y
P. herminae sp. n.	RSA: Eastern Cape Prov., Langeberge, Ruiterbos, 800m, S33°52'31" E22°01'22", 15.XI.2004, C. Deschodt (UPSA)	76	Y	Y

Species	Locality data	Code	16S	CO1
P. herminae sp. n.	RSA: Eastern Cape Prov., Langeberge, Ruiterbos, 800m, S33°52'31" E22°01'22", 15.XI.2004, C. Deschodt (UPSA)	80A	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Natures Valley, 38m, S33°57'55" E23°33'36", 07.VIII.2009, WP. Strümpher (UPSA)	CNV02	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Natures Valley, 40m, 33°58'02" E23°33'36", 02.VII.2004, C. Deschodt (UPSA)	108B	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Diepwalle Forest, 4 30m, S33°57'40" E23°09'22", 15.VI.2003, C. Deschodt (UPSA)	CKn03	X	Y
P. herminae sp. n.	RSA: Western Cape Prov., Diepwalle Forest, 4 30m, S33°57'40" E23°09'22", 15.VI.2003, C. Deschodt (UPSA)	59B	X	Y
P. herminae sp. n.	RSA: Western Cape Prov., Diepwalle Forest, 4 30m, S33°57'40" E23°09'22", 15.VI.2003, C. Deschodt (UPSA)	59C	X	Y
P. herminae sp. n.	RSA: Western Cape Prov., Buffalo Valley, 81m, S33°59'29" E 23°17'55", 02.VII.2004, C. Deschodt (UPSA)	109B	X	Y
P. herminae sp. n.	RSA: Western Cape Prov., Buffalo Valley, 118m, S33°58'56" E 23°18'06", 02.VII.2004, C. Deschodt (UPSA)	110A	X	Y
P. herminae sp. n.	RSA: Western Cape Prov., Buffalo Valley, 118m, S33°58'56" E 23°18'06", 02.VII.2004, C. Deschodt (UPSA)	110B	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Buffalo Valley, 118m, S33°59'29" E 23°18'06", 02.VII.2004, C. Deschodt (UPSA)	110C	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106A	X	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106B	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106C	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	106D	X	Y

Species	Locality data	Code	16S	CO1
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107A	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107B	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107C	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107D	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Harkerville Forest, 276m, S34°03'00" E23°12'00", 27.VI.2004, C. Deschodt (UPSA)	107E	Y	Y
P. herminae sp. n.	RSA: Western Cape Prov., Stormsrivier. December, 2005 (UPSA)	111	Y	Y

Table S2. GenBank accession numbers for the COI haplotypes of *Phoberus capensis*, *P*. *disjunctus* **sp. n.**, *P*. *herminae* **sp. n.** and *P*. *nasutus.*

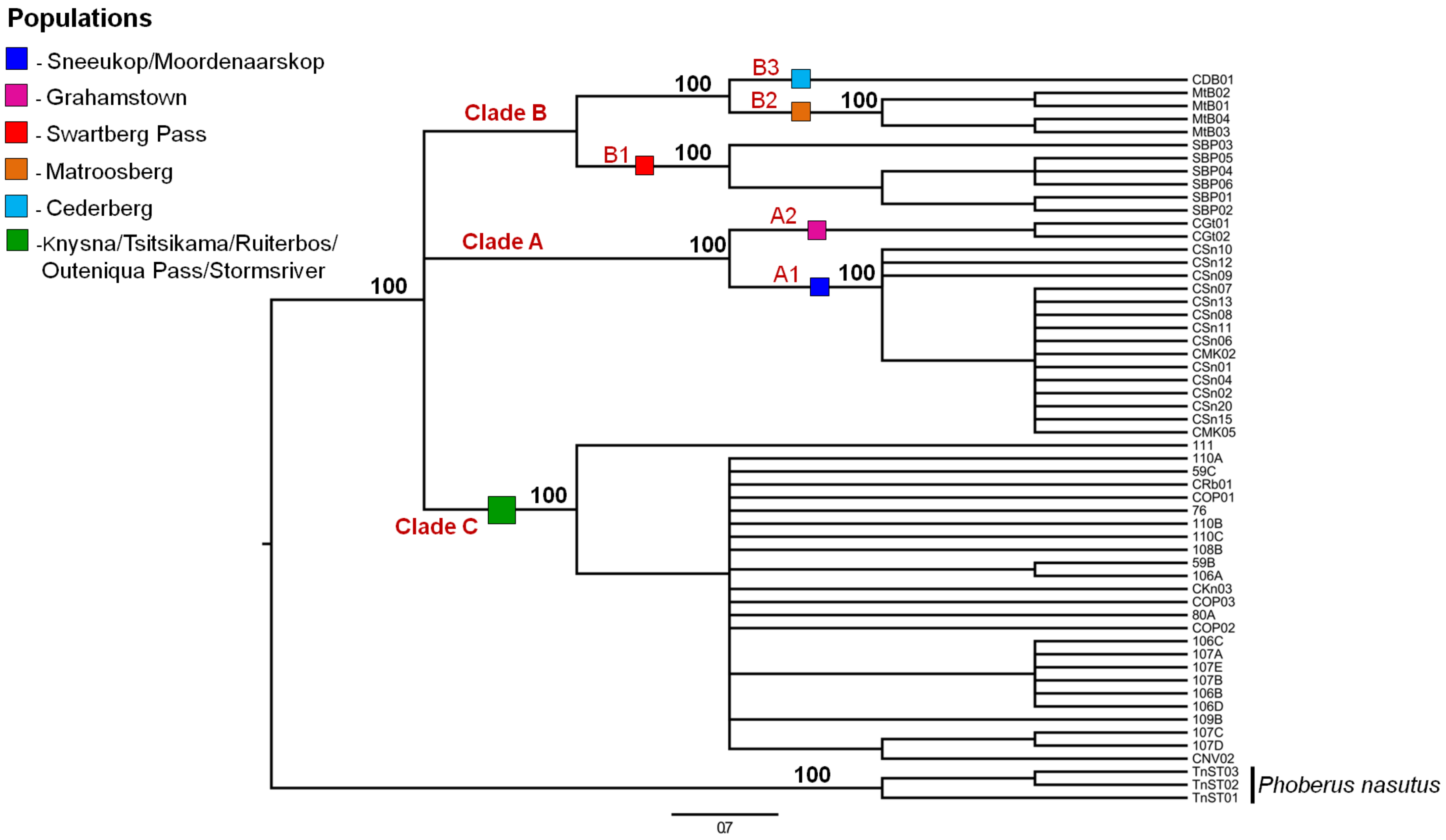
Species	**Population/Locality**	**Haplotype**	**Individuals**	**GenBank accession #**
Phoberus capensis	Sneeukop / Moordenaarskop	TCAA01	CSn01, CSn02, CSn04, CSn06, CSn07, CSn08, CSn15, CMK05	KT075105
	Moordenaarskop	TCAA02	CMK02	KT075106
	Sneeukop	TCAA03	CSn20	KT075107
	Sneeukop	TCAA04	CSn11	KT075108
	Sneeukop	TCAA05	CSn13	KT075109
	Sneeukop	TCAA06	CSn10	KT075110
	Sneeukop	TCAA07	CSn09	KT075111
	Sneeukop	TCAA08	CSn12	KT075112
	Grahamstown	TCAA09	CGt02	KT075113
	Grahamstown	TCAA10	CGt01	KT075114
Phoberus disjunctus **sp. n.**	Cederberg	TCBA01	CDB01	KT075115
	Matroosberg	TCBA02	MtB01	KT075116
	Matroosberg	TCBA03	MtB02	KT075117
	Matroosberg	TCBA04	MtB03, MtB04	KT075118
	Swartberg Pass	TCBA05	SBP01	KT075119
	Swartberg Pass	TCBA06	SBP02	KT075120
	Swartberg Pass	TCBA07	SBP04	KT075121
	Swartberg Pass	TCBA08	SBP05, SBP06	KT075122
	Swartberg Pass	TCBA09	SBP03	KT075123

Species	Population/Locality	Haplotype	Individuals	GenBank accession #
Phoberus herminae **sp. n.**	Outeniqua Pass	TCCA01	COP01	KT075124
	Ruiterbos	TCCA02	76, 80A, CRb01	KT075125
	Knysna	TCCA03	109B, 110A, 110B, 110C	KT075126
	Outeniqua Pass	TCCA04	COP02	KT075127
	Outeniqua Pass	TCCA05	COP03	KT075128
	Knysna	TCCA06	107A, 107B, 107E, 106C, 106D	KT075129
	Knysna	TCCA07	106B	KT075130
	Knysna	TCCA08	108B	KT075131
	Knysna	TCCA09	59C, CKn03	KT075132
	Knysna	TCCA10	106A, 59B	KT075133
	Knysna	TCCA11	107C, 107D	KT075134
	Tsitsikama	TCCA12	CNV02	KT075135
	Stormsrivier	TCCA12	111	KT075136
Phoberus nasutus	Cape Peninsula	TNA01	TnST01	KT075102
	Cape Peninsula	TNA02	TnST02	KT075103
	Cape Peninsula	TNA03	TnST03	KT075104

Table S3. GenBank accession number for the 16S haplotypes of *Phoberus capensis*, *P*. *disjunctus* **sp. n.** and *P*. *herminae* **sp. n.**
* = Sequences generated by Strümpher et al. (2014).

Species	**Population/Locality**	**Haplotype**	**Individuals**	**GenBank accession #**
Phoberus capensis	Sneeukop / Moordenaarskop	TCAB01	CSn01, CSn02, CSn04, CSn10, CSn12, CSn13, CSn20	KT008923
	Grahamstown	TCAB02	CGt02	KT008924
Phoberus disjunctus **sp. n.**	Cederberg / Matroosberg	TCBB05	CDB01, MtB03, MtB04	KT008929
	Matroosberg	TCBB04	MtB01, MtB02	KT008928
	Swartberg Pass	TCBB01	SBP01, SBP02	KT008925
	Swartberg Pass	TCBB02	SBP03	KT008926
	Swartberg Pass	TCBB03	SBP04, SBP05	KT008927
Phoberus herminae **sp. n.**	Knysna	TCCB01	107A, 107B, 107C, 107E, 106B, 106C	KT008930
	Outeniqua Pass / Tsitsikama/ Ruiterbos / Knysna	TCCB02	107D, 80A, CRb01, 76, COP01, COP02, COP03, 110B, 110C, 108B, CNV02	KT008931
	Stormsrivier	TCCB03	111	KT008932
Phoberus nasutus	Cape Peninsula		TnST01	KC801097*
	Cape Peninsula		TnST02	KC801096*

Figure S1. The strict consensus Parsimony tree.



Chapter 5

Overview and revision of the extant genera and subgenera of Trogidae (Coleoptera: Scarabaeoidea)

Abstract

Extant genera and subgenera of the Trogidae (Coleoptera: Scarabaeoidea) are reviewed. Contemporary classifications of this family have been based exclusively on morphological characters. The first molecular phylogeny for the family recently provided strong support for the relationships between morphologically defined genera and subgenera. On the basis of morphological, molecular and biogeographical evidence, certain taxonomic changes to the genus-level classification of the family are now proposed. Trogidae is confirmed as being divided into two subfamilies, Omorginae Nikolajev and Troginae MacLeay, the former with two genera, *Omorgus* Erichson and *Polynoncus* Burmeister; and the latter with two genera, *Trox* Fabricius and *Phoberus* MacLeay **stat. rev.** *Phoberus* is restored to generic rank to include all of the Afrotropical (including Madagascan endemic) species; *Afromorgus* is confirmed at subgeneric rank within the genus *Omorgus*; and the monotypic Madagascan genus *Madagatrox* **syn. n.** is synonymised with *Phoberus.* The current synonymies of *Pseudotrox* Robinson (with *Trox*), *Chesas* Burmeister, *Lagopelus* Burmeister and *Megalotrox* Preudhomme de Borre (all with *Omorgus*) are all accepted to avoid creating speculative synonyms before definitive phylogenetic evidence is available. New combinations resulting from restoring *Phoberus* to a monophyletic genus are listed as an Appendix.

Introduction

Trogidae represents a small family within the very large and diverse superfamily Scarabaeoidea (Browne and Scholtz 1999). Trogids are relatively secretive and elusive beetles, which can be attributed to their remarkable feeding specialisation. Adults and larvae of all known species are considered truly keratinophagous (keratin-digesting) beetles. Of the insects, only some clothes moth (Tineidae) larvae, bird lice (Mallophaga), and adult and larval hide and museum beetles (Dermestidae) are adapted to digesting keratin. Trogids are the only members of the Scarabaeoidea with this adaptation which is undoubtedly of major evolutionary significance (Scholtz 1980, 1986a; Scholtz and Caveney 1988; Hughes and Vogler 2006).

The family comprises some 330 species that primarily inhabit the temperate and arid savanna regions of the world (Scholtz 1982, 1986a; Smith 2003; Pittino 2006; Zidek 2013). Africa and Eurasia have the richest faunas with about 100 species each, followed by Australia (55 species) and South and North America (around 50 species each) (Scholtz 1982, 1986a, 1986b, 1990; Zidek 2013). The fauna of each of the zoogeographical regions has been revised, mostly over the past 60-odd years; some of the most important revisions include those by Blackburn (1904) and Haaf (1954a; 1954b), who treated the Australian and Afrotropical-Oriental faunas, respectively; Vaurie (1955; 1962) the Nearctic and Neotropical faunas; Balthasar (1936), Pittino (1983, 1985) and Scholtz et al. (2007) the Palaearctic fauna; and Scholtz (1980, 1986b, 1990) the Afrotropical (Sub-Saharan Africa), Australasian and Neotropical faunas, respectively. Scholtz (1982) and Zidek (2013) catalogued the species of the world.

Taxonomic history

The taxonomic history of the Trogidae extends back 257 years to when the first ‘trogid’ species, *Scarabaeus sabulosus* Linnaeus, was described. Numerous systematists subsequently contributed and by the end of the nineteenth century all of the major taxonomic groups had been established. As the extant genera and subgenera are taxonomically well-defined by their morphological characters, only a brief overview of their taxonomic history is presented here. For a more detailed account refer to Baker (1968), Vaurie (1955) and Scholtz (1980, 1986a, 1986b, 1990).

Fabricius (1775) described the genus *Trox*, (from the Greek *trog*, which means to gnaw). MacLeay (1819) proposed the family name Trogidae, and described the genus *Phoberus*. The latter, was however, not generally recognised as a genus; it was either considered to be a monotypic subgenus (Burmeister 1876; Preudhomme de Borre 1886), a synonym of *Trox* (Harold 1872; Scholtz 1979a), a species group

(Haaf 1953) or a subgenus of *Trox* (Péringuey 1900; Arrow 1912; Scholtz 1980, 1982).

In 1847 Erichson erected the genus *Omorgus* for two American species originally placed in *Trox*, separating the North American species into two genera, *Trox* and *Omorgus*. Other authors, however, considered *Omorgus* either as synonymous with *Trox* (Lacordaire 1856; Harold 1872; Horn 1874; Loomis 1922); as a subgenus of *Trox* (Burmeister 1876; Péringuey 1900, 1908; Gerstaecker 1873; Arrow 1912; Balthasar 1936; Robinson 1940; Scholtz 1980, 1982) or several species groups (Vaurie 1955, 1962; Haaf 1953, 1954a; Scholtz 1979b). Baker (1968) restored *Omorgus* to a valid genus based on differences in larval and adult morphology between *Trox* and *Omorgus*.

Arrow (1912) placed three well-defined genera in the family: *Trox*, *Glaresis* Erichson and *Cryptogenius* Westwood. Petrovitz (1968) described the genus *Afroglaresis* in the Trogidae, to be later synonymised with *Glaresis* (Scholtz et al. 1987). Robinson (1948) proposed the genus *Pseudotrox* for one North American species, *T. laticollis* LeConte, but it was subsequently synonymised with *Trox* (Vaurie 1955).

Several other subgenera have been proposed for various species or species groups. Burmeister (1876) reviewed trogids of Argentina and split them into different groups, *Omorgus*, *Chesas*, *Lagopelus* and *Polynoncus*, which he treated as subgenera of the genus *Trox*. Preudhomme de Borre (1886) established the subgenus, *Megalotrox* (of *Trox* Fabricius) for some of the very large flightless Australian species. *Chesas*, *Lagopelus* and *Megalotrox* were later synonymised with *Omorgus* (Vaurie 1962). *Polynoncus* has remained a well-defined group. Other authors followed Burmeister and treated *Polynoncus* as a subgenus of *Trox* (Preudhomme de Borre 1886; Arrow 1912; Scholtz 1982).

Until the mid-nineteen eighties Trogidae classification was based mainly on overall physical similarity of species and limited character sets and none of the earlier revisions addressed evolutionary patterns or relationships in the family. The problem was whether to classify Trogidae (1) as a large, variable genus (*Trox*) with numerous species groups (for example Vaurie 1955), (2) as a single genus (*Trox*) with several distinct subgenera (for example Burmeister 1876; Scholtz 1982); or (3) two genera (*Trox* and *Omorgus*) with unspecified internal relationships (for example Erichson 1847; LeConte 1854).

Morphological phylogeny

The first comprehensive phylogenetic approach to classification for this group was made by Scholtz (1986a), who was the first to infer relationships among and within genera based on synapomorphic character states. The resulting phylogenetic classification, which has remained relatively stable for the last three decades, divided the family into two distinct lineages, a morphologically plesiomorphic *Trox* lineage (with two subgenera, *Trox* and *Phoberus*) and a relatively apomorphic lineage consisting of *Polynoncu*s and *Omorgus* (with three subgenera, *Omorgus*, *Haroldomorgus* Scholtz and *Afromorgus* Scholtz).

Scholtz (1986a) found there was no phylogenetic justification for the retention of *Glaresis* and *Cryptogenius* within Trogidae because they shared no synapomorphies with *Trox*. Scholtz et al. (1987a, b) subsequently placed *Glaresis* in a monotypic family, Glaresidae Kolbe and transferred *Cryptogenius* to Hybosoridae Erichson. Glaresidae until recently was still treated, by some authors, as a subfamily of Trogidae (Smith et al. 2006; Ratcliffe and Paulsen 2008), however recent studies have clearly demonstrated their phylogenetic independence (Bai et al. 2012a, b; Ahrens et al. 2014).

A series of studies that followed Scholtz (1986a) supported these findings: Scholtz and Peck (1990) and Grebennikov and Scholtz (2004) examined larval characters for the Trogidae and the basal groups in the Scarabaeoidea respectively; d'Hotmann and Scholtz (1990) assessed the phylogenetic significance of male genitalia; Nel and Scholtz (1990) compared the morphology of mouthparts of adult Scarabaeoidea; Browne et al. (1993) examined wing articulation and wing base characters; and Scholtz (1991, 1993) investigated the phylogenetic importance of larval morphology of congeneric trogids from different geographical regions. All of these studies demonstrated that: (1) Trogidae is a monophyletic group within the superfamily Scarabaeoidea; and (2) monophyletic genera can be defined on the basis of synapomorphic character states of the morphology of both adults and larvae.

Current classification

Despite these comprehensive treatments of the family, some subsequent authors have proposed changes to the classification established by Scholtz (1986a) and Scholtz and Peck (1990): Nikolajev (2005) revised the morphological characters of the family and split the Trogidae into two subfamilies, Troginae MacLeay, containing only the genus *Trox*, and Omorginae Nikolajev, comprising the genera *Omorgus* and *Polynoncus*. Pittino (2006) elevated the subgenus *Afromorgus* to genus status on the basis of apomorphic character states on the male genitalia and biogeography. Zidek (2013) in his checklist of the Trogidae considered the generic status of *Afromorgus* (as proposed by Pittino) as nomenclaturally incorrect and reconsidered *Afromorgus* as a subgenus of *Omorgus* (*sensu* Scholtz 1986a). Recently Pittino (2010) described a new genus, *Madagatrox*, to accommodate a single flightless species from Madagascar.

Consequently, there are currently four nomenclaturally valid extant genera in the family: *Trox* (with subgenera *Trox* and *Phoberus*), *Omorgus* (with subgenera *Omorgus*, *Afromorgus* and *Haroldomorgus*), *Polynoncus*, and *Madagatrox.* However, these changes to the classification were not based on formal phylogenetic analyses.

Molecular phylogeny

My recent molecular phylogeny (Strümpher et al. 2014b; Fig 1), based on partial DNA sequences of three ribosomal gene regions (two nuclear and one mitochondrial), is the first for the family and provides robust support for the relationships of genera and subgenera, cross-validating the morphological phylogeny (Scholtz 1986a; Scholtz and Peck 1990) in most aspects. On the basis of this study and the morphological evidence, certain taxonomic changes to the genus-level classification of the family are proposed here. The arguments supporting the classification of the extant crown group genera and subgenera are assessed.

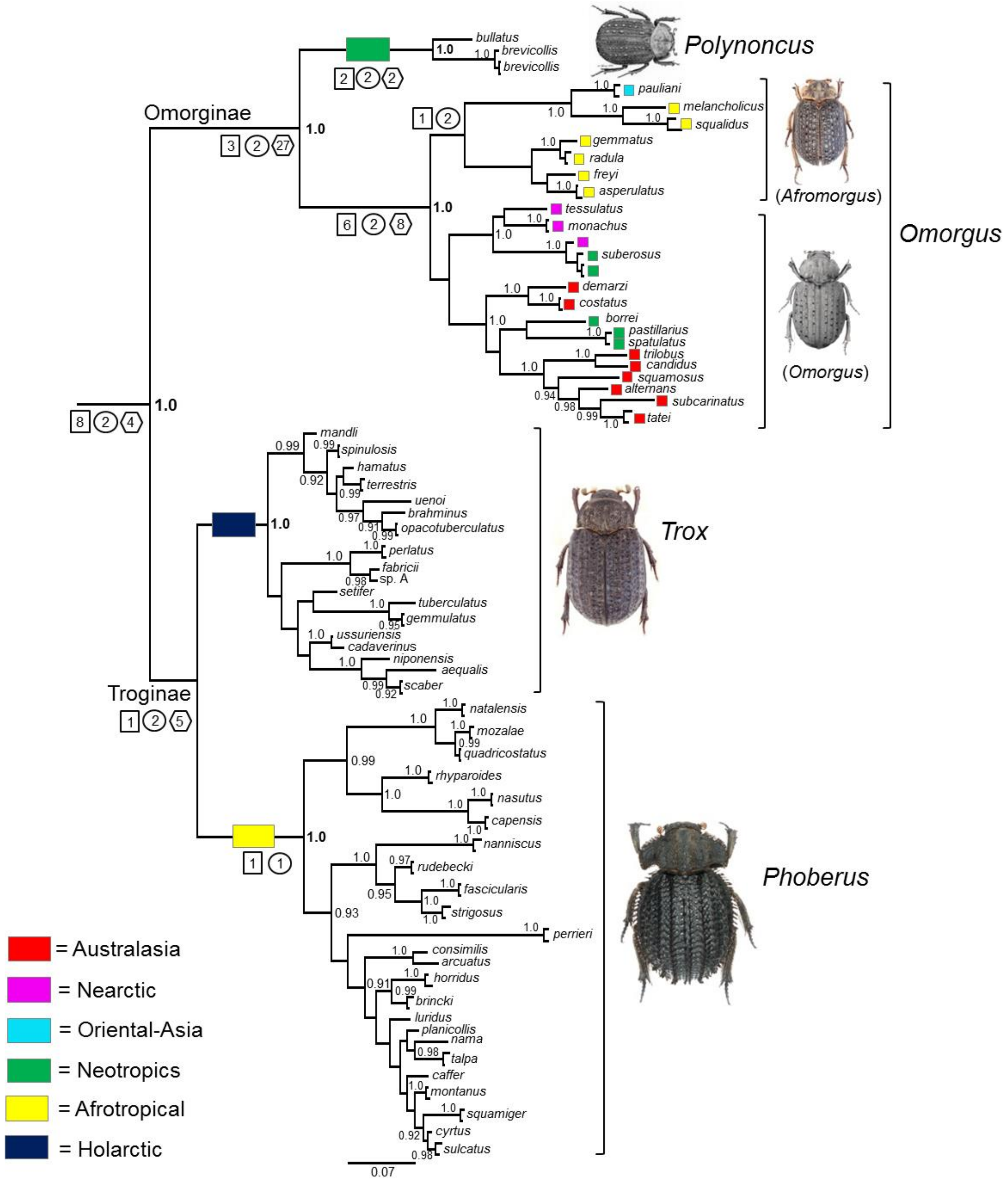


Figure 1. Molecular phylogeny of the Trogidae (redrawn from Strümpher et al. 2014b). Numbers next to each node are the posterior probability support. Only nodes with posterior probabilities ≥ 90% are shown. Images depict the type species of the genus or subgenus. Synapomorphic characters sets (see Strümpher et al. 2014b) which unite each node are plotted on the phylogram. Branches colour coded for geographic distributions.

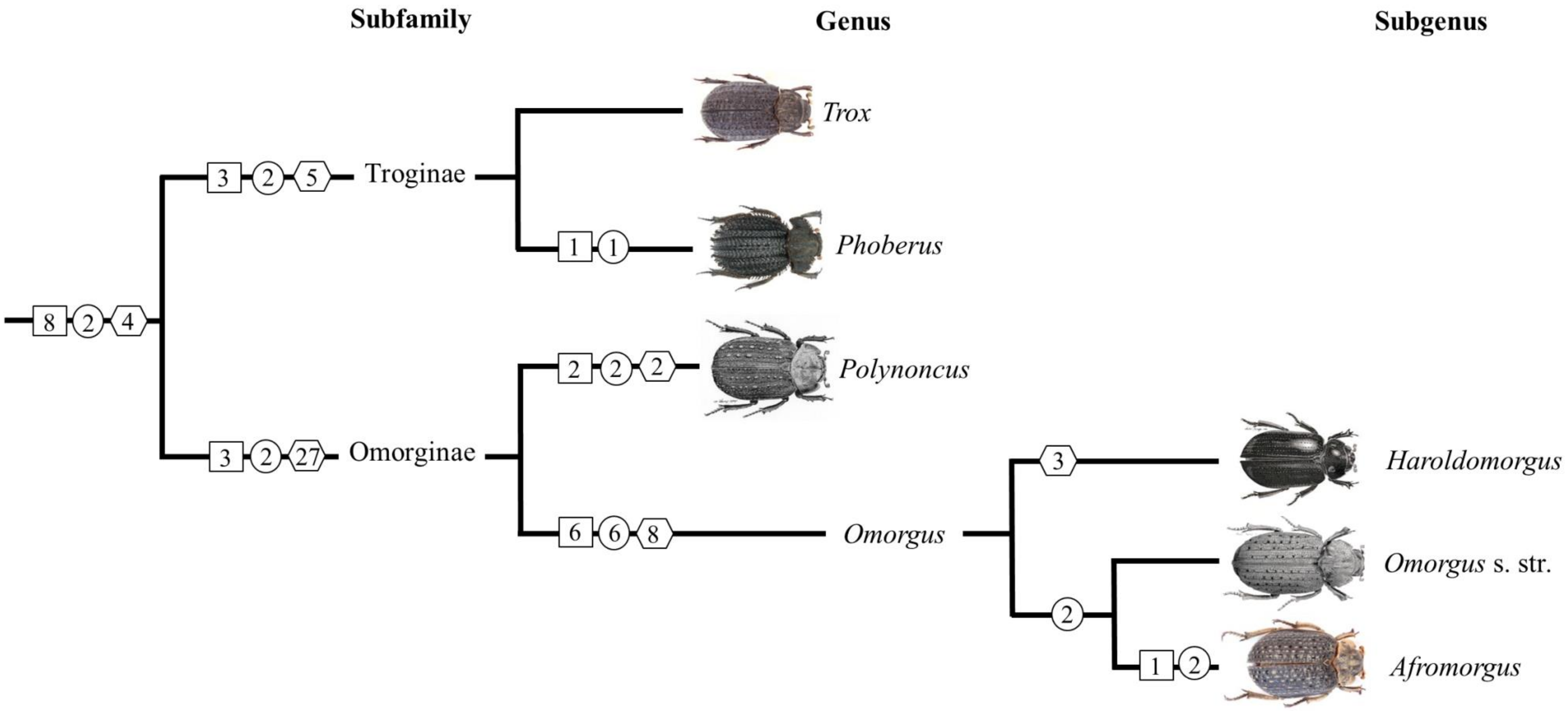


Figure 2. Proposed classification of the Trogidae, based on the phylogenetically important morphological characters and the molecular phylogeny presented by Strümpher et al. (2014b). The larval (boxes), adult (circles) and hind wing (hexagons) synapomorphic characters which unite each node are plotted on the phylogram. Numbers represent the number of characters that unite each clade. For details on larval characters, see Scholtz and Peck (1990), Scholtz (1993) and Grebennikov and Scholtz (2004); for adult (excluding hind wing) characters, see Scholtz (1986a) and Browne and Scholtz (1999); for hind wing characters, see Browne et al. (1993) and Browne and Scholtz (1995). Images depict the type species of the genus or subgenus.

Family **Trogidae**

Molecular phylogenetic characters (Strümpher et al. 2014b; Fig 1) clearly support the subfamily division of Trogidae MacLeay proposed by Nikolajev (2005). Both subfamilies (Omorginae Nikolajev and Troginae MacLeay) are monophyletic and diagnosable by adult and larval synapomorphies (Fig 2; Appendix 2). Troginae is characterised by their round antennal scape, a metatibial spur longer than first two tarsal segments, elytra and pronotum with complex body setae (plumose, spatulate or squamose) as well as several wing characters; the larvae are characterised by a having biforous spiracles; an indistinct fronto-clypeal suture and the setae absent on the second mesothoracic dorsal lobe. Omorginae on the other hand are defined by an elongated antennal scape, a metatibial spur as long as first two tarsal segments, dense metatarsal setae and a large number of synapomorphic wing characters; the larvae have cribriform spiracles; a distinct fronto-clypeal suture and setae present on the second mesothoracic dorsal lobe.

The phylogenetic relationships within the two subfamilies were well resolved (Figs 1). Two monophyletic lineages within each subfamily were clearly diagnosable by synapomorphies (Figs 2, Appendix 2), and are regarded here as distinct genera: the Holarctic *Trox* Fabricius and African *Phoberus* MacLeay within Troginae; and *Omorgus* Erichson and *Polynoncus* Burmeister within Omorginae. I maintain the status of both subfamilies as currently defined by Nikolajev (2005).

Trox

The genus *Trox* was described by Fabricius (1775) for the Palaearctic species *T. sabulosus* (Linnaeus). The defining adult and larval characteristics of this genus (in the strictest sense), which essentially distinguished Holarctic (and a small number of Afrotropical) trogid species from that of other zoogeographic regions, were: [for adults] the oval scutellum, round antennal scape; apical attachment of the pedicel to the scape; reticulated eyes; base of pronotum not restricted; quadrangular serrated hind legs; metatibial spur longer than the first tarsal segment; adults usually small (4-12mm) (Balthasar 1936; Haaf 1953; Vaurie, 1955); [for larvae] biforous spiracles; indistinct fronto-clypeal suture; and second antennal segment with sensory cone (Baker 1968).

From this nomenclatural basis, a phylogenetic analysis of a larger suite of morphological characters led Scholtz (1986a) to conclude that the genus was paraphyletic and divided the genus in two subgenera, *Trox* s. str. and *Phoberus*. *Phoberus* was originally described as a genus by MacLeay (1819) for the flightless Afrotropical species *P. horridus* (Fabricius). However, assigning species to *Phoberus* remained problematic with some Afrotropical species not fitting clearly into either *Trox* or *Phoberus* (Péringuey 1900; Haaf 1953; Scholtz 1979b, 1980). The lack of defining morphological characters can to some extent be attributed to the high incidence of flightlessness among the Afrotropical taxa making the group morphologically diverse. Scholtz (1986a) transferred all, but nine, of the Afrotropical *Trox* species described at the time to the subgenus *Phoberus*. The remaining nine Afrotropical species (*Trox nasutus* Harold, *T. capensis* Scholtz and *T. natalensis* Haaf, *T. rhyparoides* Harold, *T. pusillus* Péringuey, *T. fascicularis* Wiedemann, *T. strigosus* Haaf, *T. nanniscus* Péringuey, *T. rudebecki* Haaf) were placed together with all the Holarctic species in the subgenus *Trox*.

However, molecular phylogenetic analysis (Strümpher et al. 2014b) clearly demonstrated the existence of two geographically distinct monophyletic lineages within this genus (Fig 1); the Holarctic *Trox* and Afrotropical *Phoberus*. All nine Afrotropical species previously placed in the subgenus *Trox* s. str. group naturally within the Afrotropical *Phoberus*. Even the Madagascan species, *T*. *perrieri* Fairmaire, currently placed in the subgenus *Trox*, is phylogenetically nested within the Afrotropical *Phoberus* lineage (Strümpher et al. 2014; Fig 1). Fairmaire (1899) and Haaf (1953) considered *T. perrieri* related to other members on the mainland, which is supported by molecular evidence (Strümpher et al. 2014b; Fig 1). The close relationship of *T. perrieri* to the Afrotropical *Phoberus* points to an African origin for the Madagascan trogid fauna (Strümpher et al. 2014b). The clade into which *P. horridus* falls is confined to Africa and Madagascar and can be defined by synapomorphies of adult and larval morphology that are not part of the original definition of *Phoberus* (Fig 2, Appendix 2). The Holarctic clade containing the type species of *Trox* can also be characterised in this way. *Phoberus* and *Trox* can therefore be made reciprocally monophyletic. Therefore, all Afrotropical species of *Trox* can be reassigned to *Phoberus*.

I consider the molecular and morphological monophyly, geographic distributions and defining morphological synapomorphies sufficient to rank them equivalent to genera. Dispensing with the subgeneric divisions would simplify the classification of the family by removing an uninformative level (subgenus *Trox*) in the nomenclatural hierarchy, since Troginae and *Trox* would otherwise be defined by the same synapomorphies. Robinson's (1948) monotypic Nearctic genus *Pseudotrox* was synonymised with *Trox* (Vaurie 1955), a decision that is not at odds with my proposals. Similarly, based on these results I also reinstate the genus *Phoberus* **stat. rev.** to accommodate all the Afrotropical species (Scholtz 1980; 1982; 1986b; 1993; van der Merwe and Scholtz 2005; Strümpher and Scholtz 2009, 2011). This also has the advantage of removing an uninformative level (subgenus *Phoberus*) in the nomenclatural hierarchy. The list of species, including new combinations, belonging to *Phoberus* is presented in Appendix 1.

Madagatrox

Pittino (2010) described the new genus *Madagatrox* from a single, incomplete female specimen. This flightless representative of the genus is morphologically very similar to other flightless species on the mainland, especially those species belonging to the *natalensis*-group in *Phoberus* (Strümpher and Scholtz 2009, 2011), and is based on problematic diagnostic characteristics.

Pittino (2010) listed several pronotal and elytral characters (autapomorphies) that distinguish this taxon from other trogids (Pittino 2010) but these characters should be treated with caution. Some of these characters (autapomorphies), apart from not having any phylogenetic value in recognizing *Madagatrox*, are characteristic of flightless southern African and other trogid species with extreme morphological changes as a result of "old aptery" (see Scholtz 1980, 2000). Moreover, in small flightless trogids, external characters vary greatly with size of the individual. In very small specimens, for example, many features tend to become irregular or obscured (for example intercostal punctures and fovae) and/or pronounced (like tubercles and ridges), thus making it generally difficult to identify taxonomically relevant characters (personal observations WPS). These observations mean that such characters are of dubious value in diagnosing *Madagatrox*. Similarly, in some of the flightless species on the mainland, and especially in very small individuals, the fifth segment on the protarsus is deeply recessed in the apex of the fore tibia, giving the appearance of a four-segmented protarsus or a pseudotetramerous condition (personal observations WPS). The single specimen of *Madagatrox* differs from all other trogids in having a four-segmented, rather than a five-segmented protarsus on its only intact protibia (Pittino 2010), but the latter condition is likely pseudotetramery, because of the small size of the representative specimen and its flightless condition. Until such time as additional (male and female) specimens become available for study, I consider the condition pseudotetramerous (see also Zidek 2013) and therefore a dubious defining autapomorphy. This leaves *Madagatrox* effectively undiagnosed.

Although the genus was not included in the molecular analysis (Strümpher et al. 2014b), but consideration of its morphological description suggests very strongly that phylogenetic analysis would place it in or very near the *natalensis*-group within the *Phoberus* clade (Strümpher and Scholtz 2009, 2011). This would render the *Phoberus* clade (and *Trox* as currently defined) paraphyletic and several phylogenetically and biologically insignificant taxa would have to be described to preserve *Madagatrox* under the principle that (crown) taxa should be monophyletic.

Consequently, I propose that the genus *Madagatrox* be synonymised with *Phoberus* **stat. rev.** This also has the advantage of removing a redundant monotypic taxon from the classification.

Omorgus

Omorgus is a monophyletic group supported by a large number of adult and larval morphological synapomorphies (Scholtz 1986a; Scholtz and Peck 1990; Browne et al. 1993) and molecular evidence (Strümpher et al. 2014b, Fig 2). Because of the strong support for the monophyly for this genus and its clear morphological diagnosability (Scholtz 1986a, 1993; Scholtz and Peck 1990; Browne et al. 1993), I maintain its status as delineated by Scholtz (1986a).

Scholtz (1986a) divided *Omorgus* into three phylogenetically (and geographically) distinct subgenera; *Haroldomorgus*, *Omorgus* s. str. and *Afromorgus*. The monotypic subgenus *Haroldomorgus* occurs in South America, *Omorgus* in the southern Nearctic, Neotropical and Australasian Regions, and *Afromorgus* occurs in the Afrotropical and Oriental Regions. The molecular phylogeny of Strümpher et al. (2014), which did not include *Haroldomorgus*, shows that *Omorgus* comprise two well-supported sister lineages, one (the *Omorgus*-lineage) containing the New World and Australasian species, and the other (the *Afromorgus*-lineage) comprising the

Afrotropical and Oriental species, which to a large extent supports the morphological subdivisions of Scholtz (1986a). Because of the strong support for the monophyly for the subgenera and their clear morphological diagnosability (Scholtz 1986a, 1993; Scholtz and Peck 1990; Browne et al. 1993), I maintain them as delineated by Scholtz (1986a). Even though one can equally argue that the subgenera can be treated as genera based on the morphological diagnosability and unique geographical distributions of these taxa (as for *Phoberus* and *Trox*); maintaining the subgeneric classification provides a better indication of the phylogenetic relationships within *Omorgus*.

Omorgus (***Omorgus***)

All of the Australasian and New World *Omorgus* species group phylogenetically in this taxon (Scholtz 1986a; Strümpher et al. 2014b). In the past, the New World and Australasian *Omorgus* has been the subject of many attempts at species groupings. Apart from *Omorgus*, other subgenera (of *Trox* Fabricius) proposed for species included, *Megalotrox* Preudhomme de Borre (1886), *Lagopelus* Burmeister (1876) and *Chesas* Burmeister (1876). Preudhomme de Borre (1886) established the genus *Megalotrox* for one of the very large, flightless Australian species, *T. gigas* Harold, and six species are currently placed in the taxon (Arrow 1912; Haaf 1954b). Similarly, Burmeister (1876) considered *O. pastillarius* Blanchard unique among the Argentine species, on account of it being flightless, and placed it in the monotypic subgenus *Chesas*, to which no other species have been added. *Lagopelus* was established for a single, winged species, *Trox ciliatus* Blanchard, and the genus remains monotypic. *Chesas*, *Lagopelus* and *Megalotrox* were later synonymised with *Omorgus* (Vaurie 1962; Baker 1968; Scholtz 1986b).

Results from the molecular phylogeny of the Trogidae indicated the subgenus *Omorgus* may well comprise several lineages equivalent to subgenera (Strümpher et al. 2014b). Strümpher et al. (2014) discussed the resurrection of *Megalotrox* and *Chesas*. The former is assignable to some Australian *Omorgus* species and the latter to some Neotropical representatives. However, evidence for their monophyly is inadequate to draw any concrete conclusions (Strümpher et al. 2014b) and I prefer to not risk creating unnecessary synonyms and to wait for unequivocal evidence of their membership. Therefore, *Omorgus* s. str. is retained as the nominal subgenus of

Omorgus and the current synonymies *Chesas*, *Lagopelus* and *Megalotrox* (all with *Omorgus* s. str.) are all accepted.

Omorgus (***Haroldomorgus***)

Exemplars of this rare monotypic subgenus were not included in the molecular phylogeny of the Trogidae and without molecular data the placement of this subgenus remains uncertain. The morphology suggests that *Haroldomorgus* is the sister clade to *Omorgus* s. str. (Scholtz 1986a; Browne et al. 1993; see also Vaurie 1962). For the time being I consider *Haroldomorgus* a close relative of *Omorgus*. Therefore, *Haroldomorgus* retains its status as a subgenus within the genus *Omorgus*.

Omorgus (***Afromorgus***)

Afromorgus has had an inconstant taxonomic history. It was originally described as a subgenus of *Omorgus* (Scholtz 1986a), elevated to genus rank (Pittino 2006) and recently treated again as a subgenus of *Omorgus* (Zidek 2013). Molecular evidence clearly indicates that *Afromorgus* is a well-defined clade containing all the Afrotropical and Asian species within the genus *Omorgus* (Strümpher et al. 2014b; Fig 1) and can be defined by synapomorphies of adult and larval morphology (Fig 2, Appendix 2).

I intuitively follow the classification system for the genus *Omorgus* and its subgenera, as proposed by Scholtz (1986a; see also Zidek 2013). *Afromorgus* is, consequently retained as a subgenus of the genus *Omorgus*.

Polynoncus

Polynoncus remains a well-defined group and is the sister-group to the genus *Omorgus* (see also Scholtz 1986a; Scholtz and Peck 1990; Browne et al. 1993). Molecular and morphological data support the monophyly of the group. Its generic status is maintained since recognising *Omorgus* as a genus obliges according *Polynoncus* the same status in phylogenetic systematics.

Fossils: The fossilised taxa are not dealt with in this paper. Details regarding the fossilised taxa are provided by Krell (2007) and Nikolajev (2007; 2009).

In conclusion, the phylogenetic classification proposed here basically follows the system proposed by Scholtz (1986a), except that the Trogidae are accepted as consisting of two subfamilies, Omorginae and Troginae following Nikolajev (2005). The subgenus *Phoberus* is restored to genus and the subgenus *Trox* falls away because of the promotion of *Phoberus*. The Afrotropical species of *Trox* are transferred to *Phoberus*; and the Madagascan genus *Madagatrox* is synonymised with *Phoberus* and its only species is transferred to *Phoberus*. The classification of the genus *Omorgus* and its subgenera, as proposed by Scholtz (1986a), is maintained with *Afromorgus* confirmed as a subgenus. The current synonymies of *Pseudotrox* Robinson (= *Trox*), *Chesas* Burmeister (= *Omorgus*), *Lagopelus* Burmeister (= *Omorgus*) and *Megalotrox* Preudhomme de Borre (= *Omorgus*) are all accepted to avoid creating speculative synonyms before definitive phylogenetic evidence is available. I are confident that the proposed changes produce a balanced and stable classification for this unique beetle family (Fig 2). New combinations resulting from restoring *Phoberus* to a monophyletic genus are listed in Appendix 1.

Taxonomy

FAMILY: Trogidae MacLeay

Trogidae MacLeay, 1819: 36

Type genus: *Trox* Fabricius, 1775: 31

Lists of references to the family are provided in Scholtz (1982), see additional sources cited within Zidek (2013)

Diagnosis

Length: 4–30mm.

Colour: Flavescent, reddish brown, grey or black.

Head: Eyes not divided by genae; mandibles vertical, robust; frons smooth, bi- or quadrituberculate or ridged; 10-segmented antennae with basal segment robust, three-segmented club, free, setose; antennal scape may be virtually round with apical pedical attachment, elongate with apical pedicel attachment, or elongate with subapical pedicel attachment; clypeus usually triangular or broadly rounded, it may be horizontal (straight) or slightly bent down; with the apex distinctly deflexed at right angles to the clypeal disc, or slightly reflexed.

Pronotum: Convex; usually wider than long, pronotal margins attenuated anteriorly; the sides may be broad and flat, obsolete or intermediate between them; the total pronotal width may be narrower than the elytra, as wide or wider; pronotal lateral margin may be straight, attenuated anteriorly or posteriorly, smooth, notched posteriorly, dentate or with one or two distinct incisions; pronotal disc may be prominently sculpted with distinct median ridges and sub-median ridges and tubercles, smooth or setose.

Scutellum: Hastate or oval.

Elytra: Convex or flat; elongated with sharp humeral angle and distinct humeral calli in winged individuals or rounded with rounded humeral angle and no humeral calli in flightless individuals; elytra striate, convex, declivous behind, usually tuberculate or setose or both, seldom smooth; epipleurae distinct.

Hind wing: Wings present or absent, where present, with M-Cu loop and one or two apical detached veins, RP3+4 vein lost; wing articulation characterised by 2Ax subalare tendon attachment point short, narrow and apically rounded; 2BP with transverse crimps on the medial vein, mesal of the bridge and distal of 2BP, BMP-CuA brace either reduced or modified (for comprehensive overview on hind wing morphology in Trogidae see Browne et al. 1993).

Abdomen: Completely covered by hind wings; with five distinct fused ventral sternites; pygidium concealed by elytra; with seven or eight functional spiracles.

Legs: Profemora enlarged; coxae virtually contiguous; claws simple; tarsi 5-5-5.

Male genitalia: Typically trilobite type but varies from simple to complex and asymmetrical; the basal piece may be longer or shorter than the parameres, membranous or fused dorsally; parameres usually simple and symmetrical; median lobe may be hollow, simple or asymmetrical and varies from complex to highly complex; internal sac small without armature, large and armed or unarmed or large and armed with sclerites; temones present or absent, when present it can be long or short; genital segment is a well-developed genital capsule or a well-developed u-shaped spiculum gastrale.

Larvae: Typically scarabaeiform, white or cream in colour, heavily sclerotized cranium and prominent sclerotized shields on either side of the prothorax; 3-segmented antenna; with well-developed lateral ocelli; distinct or faint fronto-clypeal suture; galea and lacinia distinctly separated; galea 2-segemented; 4-segmented maxillary palpi; presence of maxillary and mandibular stridulatory apparatus;

epipharyngeal tormae symmetrical or asymmetrical, fused or divided; left mandible with 2-3 mandibular teeth; spiracles biforous or cribriform, the latter have a closing apparatus; legs four-segmented and well developed, with prominent claw; larvae lack a stridulatory apparatus.

Distribution: Cosmopolitan. Species primarily inhabit the temperate and arid/savanna regions of the world.

Natural history: Adult trogids exhibit thanatosis when alarmed or disturbed, retracting their legs and head and remaining motionless, which probably helps them to escape potential predators (Ratcliffe 1991). Adults are able to stridulate by rubbing a coarse plate (plectrum) on the outer surface of the first ventral segment of the abdomen against a file on the inside margin of their elytra (Vaurie 1955; Lawrence and Britton 1991). Adults of some species are attracted to light. Adults and larvae feed primarily on keratin. Trogids are among the last insects to visit the dried remains of dead animals where adults and larvae may be found feeding on various sources of keratinous matter (e.g. hair, skin, hooves, nails and feathers). Keratin is an important structural component (a fibrous protein) of hair, fur, hooves and feathers. Even though there are records of opportunistic feeding on insects (Young and Hamm 1985; Van Emden 1948) some reports and field observations from Australia indicate that a group of large flightless species appear to feeds on various insects (or insect remains), mostly ants and termites, rather than carrion (Houston et al. 2009). Surprisingly, none of these large, flightless species appear to have ever been found at carrion, and attempts at feeding them on carcasses were unsuccessful (Houston et al. 2009).

Key to the extant subfamilies, genera and subgenera of adult Trogidae

1. Antennal scape round .... Troginae … **2**

\- Antennal scape elongate .... Omorginae … **3**

2. Aedeagus with simple, apically divided median lobe, restricted to the Holarctic Region .... *Trox*

\- Aedeagus with complex median lobe, restricted to the Afrotropical Region .... *Phoberus* **stat. rev.**

3. Scutellum oval, clypeus deflexed, pedicel attached apically to scape *Polynoncus*

\- Scutellum hastate, clypeus reflexed or straight, pedicel attached sub-apically to scape.... .... *Omorgus* … **4**

4. Clypeus straight .... *Omorgus* (*Haroldomorgus*)
- Clypeus reflexed **5**
5. Aedeagus with pars basalis fused dorsally................... *Omorgus* (*Afromorgus*)
- Aedeagus with pars basalis membranous dorsally .... *Omorgus* (*Omorgus*)

SUBFAMILY: **Troginae** MacLeay

Troginae MacLeay 1819: 59; Nikolajev 2005: 322

Type genus: *Trox* Fabricius, 1775: 31

See also Scholtz (1982) for a list of references for the subfamily

Diagnosis

Adults

Length: 4–17mm

Head: Antennal scape round, pedicel apically attached, clypeus straight, triangular or broadly rounded; prostheca on mandibles well-developed.

Pronotum and elytra: setae complex or specialized, usually plumose, spatulate or squamose.

Scutellum: Round or oval, not restricted at base.

Hind wing: With two apically detached veins between the cubitus and first complete anal vein in winged species (for comprehensive overview on hind wing morphology of Trogidae see Browne et al. 1993).

Abdomen: with seven functional spiracles.

Legs: metatibial spur longer than first two tarsal segments, metatarsal setae isolated or sparsely distributed.

Male genitalia: Aedeagus elongate and characteristically arched; basal piece longer than the parameres, evenly sclerotized; parameres simple; median lobe hollow, and simple (in *Trox*) to complex (in *Phoberus*); internal sac small, without armature, and does not extend beyond the median lobe; temones long and thin and but may form a spoon-shaped expansion proximally; genital segment may be a well-developed genital capsule (Holarctic taxa) or a spiculum gastrale (Afrotropical taxa).

Larvae: The larvae of the subfamily can easily be distinguished from members of the other subfamily by their biforous spiracles; an indistinct fronto-clypeal suture; second antennal segment with sensory cone; prothorax with two small lateral lobes; setae on prothoracic margin absent; setae present on the second dorsal lobe on the abdomen (in the dorsal view).

Distribution: The Holarctic Region and the Afrotropical Region. Troginae are absent from Australasian and Neotropical Regions, except for one ubiquitous species, *Trox scaber* (Linnaeus), which was undoubtedly introduced by man (Scholtz 1986b; 1990).

Natural history: Many of the species are often found on carnivore faeces and owl pellets, in birds' nests or in burrows.

Composition: Contains two extant genera, the type genus *Trox* Fabricius and *Phoberus* MacLeay **stat. rev.**, and about 150 species.

GENUS: ***Trox*** Fabricius

Trox Fabricius 1775: 31.

Type species: *Scarabaeus sabulosus* Linnaeus, 1758 (by subsequent designation, Latreille 1810) (Fig 3a).

List of synonyms and references to the genus (and as subgenus) is provided in Scholtz (1982), see also Zidek (2013).

Diagnosis

Length: 4–12mm.

Colour: Flavescent to black, habitus relatively setose.

Head: Frons bituberculate or quadrituberculate, or flat, with setose ridges or smooth; clypeus horizontal, broadly rounded or triangular; antennal scape rounded, attached apically, first segment of club (in lateral view) flat and same width throughout.

Pronotum: Usually not constricted, with base applied closely to elytra; pronotal margins densely setose.

Scutellum: Oval, not restricted at base.

Legs: Hind tibia with one or more teeth or spines externally; metatarsal segment with isolated setae, longest spur on metatibia longer than first two tarsal segments; hind tarsal segment ventrally with only scattered setae, claw with one seta.

Male genitalia: Aedeagus slender, with simple median lobe, usually with apex divided, pars basalis fused dorsally; genital segment in the form of a primitive genital capsule.

Larvae: Members of the genus has the phobae on proximal region of hypopharynx united basally. For diagnosis of Holarctic taxa see Baker (1968: 21), Scholtz and Lumaret (1991) and Shabalin (2013).

Distribution: Widespread throughout the Nearctic and Palaearctic Regions, with a small number of species occurring in the Oriental Region (Fig 3b).

Comments: Although formal testing is required, the Palaearctic and Nearctic species can be roughly divided into six groups based on external morphology and male genitalia (Vaurie 1955; Pittino 1985; Pittino and Kawai 2006; Scholtz et al. 2007). The largest of these groups, the "*terrestris*"-group (Pittino and Kawai 2006), consists of mostly eastern Palaearctic species, but also comprises species from the Nearctic. The second largest group "*hispidus*" is mainly distributed in the Mediterranean. The "*sabulosus*" group consists of eastern Palaearctic species. Another typical Holarctic group is the "*scaber*"-group; it is the most widespread across the Palaearctic and has related species in the Nearctic. The (now) virtually cosmopolitan species, *Trox scaber* (Linnaeus) is included in the latter. The remaining two groups, "*unistriatus*" and "*tuberculatus*" are restricted to the Nearctic (Vaurie 1955).

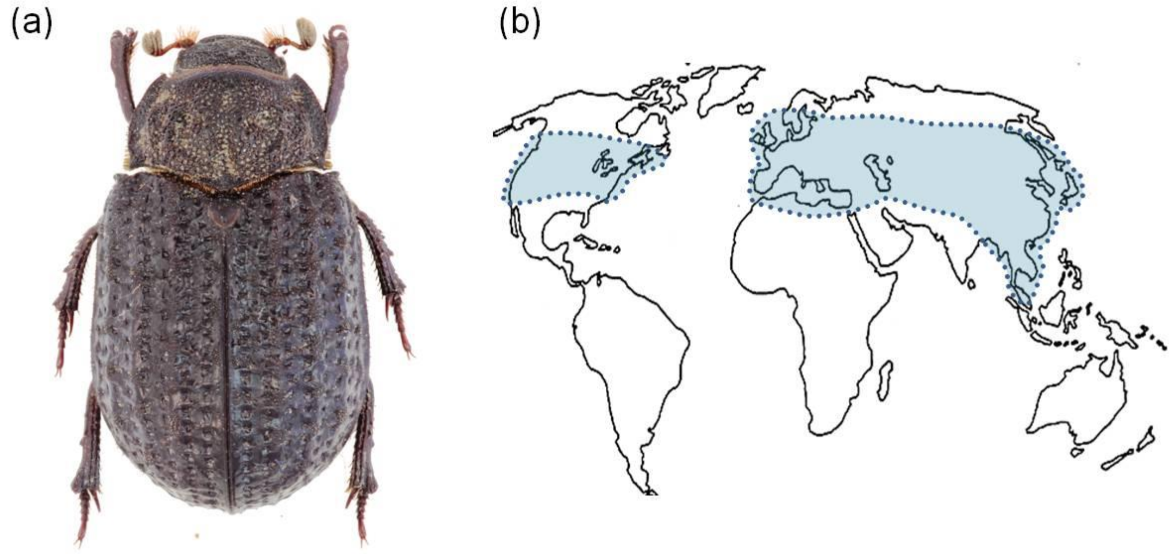


Figure 3. (a) The type species of the genus *Trox* Fabricius, *Trox sabulosus* Linnaeus (b) Distribution of the genus *Trox.*

GENUS: *Phoberus* MacLeay **stat. rev.**

Phoberus MacLeay, 1819: 137-138.

Type species: *Trox horridus* Fabricius, 1775, by monotypy (Fig 4a).

Trox (*Phoberus*): Burmeister, 1876: 264; Preudhomme de Borre, 1886:59; Péringuey, 1900: 453 (*pars*); Arrow, 1912: 53 (*pars*); Scholtz, 1980:17; Scholtz, 1982: 15; Scholtz, 1986a: 361; Scholtz, 1993: 6 [larvae]; Zidek, 2013: 6.
Madagatrox Pittino, 2010: 75. **syn. n.**

Diagnosis

Length: 4–17mm.

Colour: Grey or reddish brown to black.

Head: Clypeus triangular; apex pointed with apical portion bent down at right angles to disk or straight; frons bituberculate, rounded or ridged, setose ridges or smooth; surface of frons and clypeus punctuate; antennal scape rounded or slightly elongate, and pedicel attached to apex of scape (except for *P. brincki* Haaf, where it is attached sub-apically) with setae; eyes large and rounded (diameter of eye approximately half the distance between the eyes) in flight capable species, eye small (diameter approximately one-third the distance between the eyes) in flightless species.

Pronotum: Pronotal margins attenuated anteriorly, smooth or irregular; pronotal sides narrow to broadly flattened; margins densely setose or with fringes of stiff setae or with irregular setae (as for *P. brincki*), setae spatulate and squamose or pubescent; pronotal surface tomentose to smooth; pronotal length at least half or more the elytral length in flightless species or small relative to abdomen in winged species; pronotal discal area varies from smoothly rounded with ridges and tubercle vestigial to ridges and tubercles distinctly raised and depressions well-defined.

Scutellum: Oval, distinct in winged forms and small in flightless forms.

Elytra: Elongated with sharp humeral angle and distinct humeral calli in winged individuals or rounded with rounded humeral angle and no humeral calli in flightless individuals; sides flattened; lateral margins usually with fringes of setae; even numbered costae prominent with tubercles, or with fused tubercles to form distinct costal ridges; ridges and tubercles usually with tufts of setae; profile convex attaining maximum height in the middle or behind the middle.

Legs: Metatarsal segments ventrally with isolated setae or sparsely setose; longest metatibial spur longer than first two tarsal segments or scarcely longer or not than first tarsal segment; five segmented protarsus, but four-segmented in *Madagatrox* **syn. n.** (the latter is probably a pseudotetramerous condition).

Male genitalia: Aedeagus slender, symmetrical; simple to complex median lobe usually consisting of various blades, plates and hooks; pars basalis fused dorsally, usually as long or longer than parameres; genital segment in a form of a spiculum gastrale.

Larvae: Members of the genus has the phobae on proximal region of hypopharynx not united basally. For diagnosis of Afrotropical taxa see Scholtz (1993: 6).

Distribution: Restricted to Africa, mainly southern Africa and Madagascar (Fig 4b). The majority of the species are distributed along the temperate eastern montane faunal exchange route (Endrödy-Younga 1978), with a few species penetrating into the arid regions of southern Africa (Scholtz 1979a, 1980).

Comments: *Phoberus* MacLeay is restored to genus. Afrotropical (including Madagascan) species are transferred to *Phoberus* (see Appendix 1). *Phoberus* is the genus with the highest incidence of flightless species (about 35 % of the genus), making the group morphologically diverse. The genus can be roughly divided into ten informal species-groups based on external morphology, male genital anatomy (Scholtz 1980; Strümpher and Scholtz 2011).

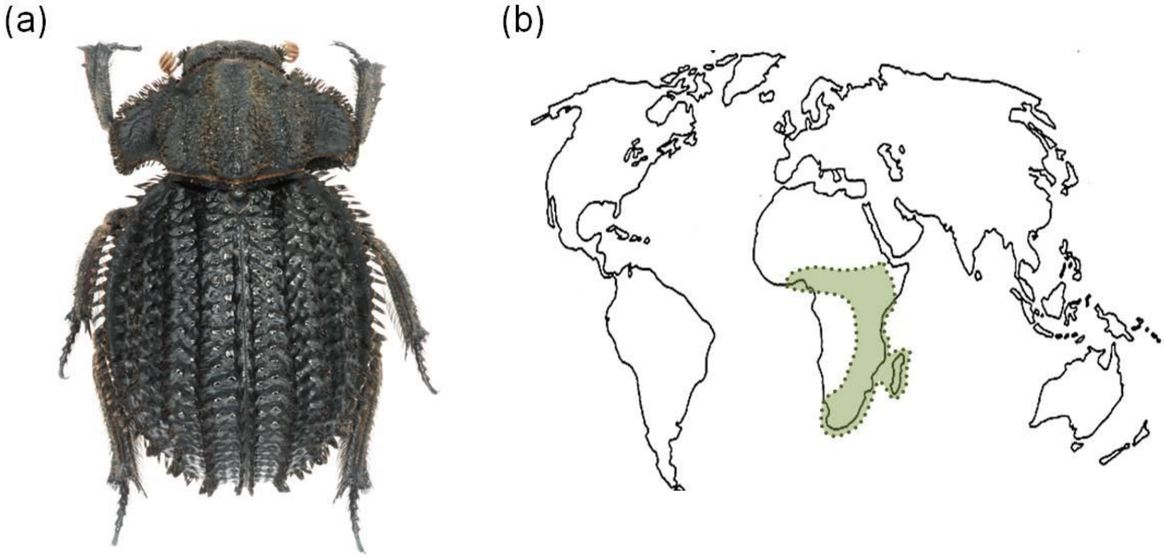


Figure 4. (a) The type species of the genus *Phoberus* MacLeay, *Phoberus horridus* (Fabricius) **comb. nov.** (b) Distribution of the genus *Phoberus*

SUBFAMILY: **Omorginae** Nikolajev

Original spelling and citation: Omorgini Nikolajev 2005: 322

Type genus: *Omorgus* Erichson, 1847: 107

Diagnosis

Length: 6–30mm.

Head: Antennal scape elongate; pedicel attached apically or subapically; clypeus straight, reflexed or deflexed.

Scutellum: Hastate or oval.

Pronotum and elytra: setae simple, straight, elongated and pointed.

Hind wing: With one or two apically detached veins between the cubitus and first complete anal vein (for comprehensive overview on hind wing morphology in Trogidae see Browne et al. 1993).

Abdomen: with eight functional spiracles.

Legs: metatibial spur as long as the first tarsal segments; metatarsal setae dense.

Male genitalia: Aedeagus robust; basal piece shorter than the parameres; pars basalis can be dorsally fused or open; parameres simple, symmetrical, may or may not be fused basally; median lobe may be simple or specialised; internal sac large, armed or unarmed; temones present or absent; genital segment a well-developed u-shaped spiculum gastrale.

Larvae: The larvae of the subfamily can easily be distinguished from members of the other subfamily by their cribriform spiracles; a distinct fronto-clypeal suture; second antennal joint with sensory disc, or small cone and sensory area; prothorax with large single lateral large lobe; setae present on prothoracic margins; setae present on the second dorsal lobe on the abdomen (in the dorsal view); glossa with eight or more setae; two rows of parallel hypopharyngeal phobae present; anterior angle of frons with more than three setae.

Distribution: Occur throughout the arid regions of the southern continents, extending into the southern Nearctic and Oriental Regions.

Composition: Contains two genera, the type genus *Omorgus* Erichson and *Polynoncus* Burmeister.

GENUS: ***Omorgus*** Erichson

Omorgus Erichson, 1847: 111; LeConte, 1854: 211; Baker, 1968: 1; Scholtz, 1986a: 361; Scholtz, 1993:2 [larvae]; Zidek, 2013: 6

A list of references to the genus [as *Trox* (*Omorgus*)] is provided in Scholtz (1982, 1986a, 1986b, 1990).

Type species: *Trox suberosus* Fabricius (by subsequent designation, Lacordaire, 1856).

Diagnosis

Length: 5–30mm.

Colour: Flavescent to black.

Head: Frons bituberculate or smooth; clypeus with rim reflexed; antennal scape elongate, pedicel attached apically; well-developed prostheca on mandibles.

Pronotum: Usually constricted at base; pronotal margins with isolated, pointed, simple setae.

Scutellum: Hastate, constricted at base.

Hind wing: With one apically detached vein (for comprehensive overview on hind wing morphology in Trogidae see Browne et al. 1993).

Legs: Metatarsal segments ventrally with dense setae; longest spur on hind tibia not or scarcely longer than the first tarsal segment.

Male genitalia: Aedeagus varies considerably between continental groups; basal piece membranous (New World/Australasian taxa) or sclerotized (Afro-Oriental taxa) dorsally; parameres usually simple and symmetrical; median lobe simple (New World/Australasian groups) or complex (Afro-Oriental groups); internal sac large, usually armed with sclerites, spines or setae but the armature varies between taxa; sclerites may be present (Afro-Oriental taxa) or absent (New World/Australasian taxa); temones present (in Afro-Oriental taxa) and absent in (New World/ Australasian taxa).

Larvae: *Omorgus* larvae from different geographical regions can be distinguished from each other by phylogenetically important characters (see Scholtz 1991, 1993)

Distribution: Southern Nearctic, Neotropical, Afrotropical, Oriental and Australasian Regions (Fig 8).

SUBGENUS: *Omorgus* (*Omorgus*) Erichson

Omorgus (*Omorgus*) Erichson: Scholtz, 1986a: 362; Scholtz, 1990: 1404; Scholtz, 1993: 2 [larvae]; Zidek, 2013: 1

Type species: *Trox suberosus* Fabricius (by subsequent designation, Lacordaire, 1856) (Fig 5).

Trox (*Chesas*) Burmeister, 1876: 264; Preudhomme de Borre, 1886: 59; Arrow, 1912: 53

Type species: *Trox pastillarius* Blanchard, 1846, original designation. Syn. by Vaurie (1962: 109, 115).

Trox (*Lagopelus*) Burmeister, 1876: 265; Preudhomme de Borre, 1886: 59; Arrow, 1912: 53

Type species: *Trox ciliatus* Blanchard, 1846, original designation. Syn. by Vaurie (1962: 109, 115).

Trox (*Megalotrox*) Preudhomme de Borre, 1886: 59; Arrow, 1912: 53

Type species: *Trox gigas* Harold, subsequent designation by Scholtz (1982).

Diagnosis

Length: 5–30mm.

Male genitalia: Aedeagus robust; median lobe simple; pars basalis small, membranous dorsally.

Larvae: New World and Australasian taxa are characterised by the concave sensory disc on the second antennal segment (see also Scholtz 1993). For diagnosis of New World and Australasian taxa see Baker (1968: 39) and Scholtz (1991, 1993).

Distribution: Southern Nearctic, Neotropical and Australasian Regions (Fig 8).

Comments: All New World and Australasian species belong to this subgenus.

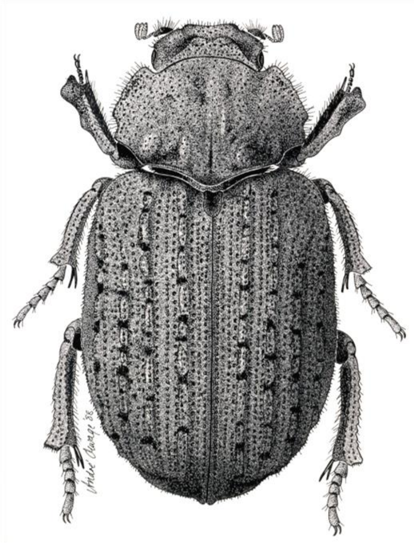


Figure 5. The type species of the subgenus *Omorgus* (*Omorgus*) Erichson, *O*. (*Omorgus*) *suberosus* Fabricius. Image reproduced from Scholtz (1990).

SUBGENUS: *Omorgus* (*Haroldomorgus*) Scholtz

Omorgus (*Haroldomorgus*) Scholtz, 1986a: 362; Scholtz, 1990: 1418; Zidek, 2013: 6

Type species: *Trox batesi* Harold 1872: 126, by original designation and monotypy (Fig 6).

Diagnosis

Length: 9–10 mm.

Colour: Flavescent.

Head: Frons bituberculate, clypeus horizontal; antennal scape elongate, pedicel attached sub-apically.

Pronotum: Glabrous.

Scutellum: Oval.

Elytra: Smooth, glabrous.

Hind wing: Reduced (for comprehensive overview on hind wing morphology in Trogidae see Browne et al. 1993).

Legs: Metatarsi ventrally with dense setae; longest spur on metatibia not or scarcely longer than first tarsal segment.

Male genitalia: Aedeagus robust, simple, median lobe pointed; pars basalis membranous dorsally.

Larvae: Unknown

Distribution**:** Central to eastern South America (Fig 8).

Comments: *Haroldomorgus* is monotypic. Members of this rare species display incipient winglessness (Browne et al. 1993).

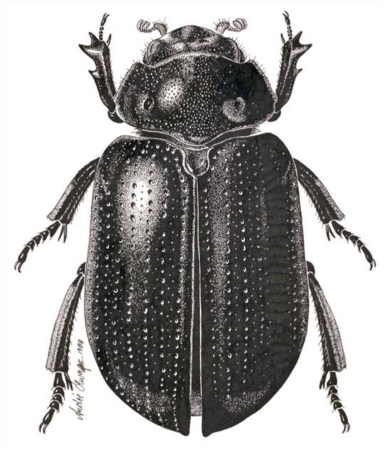


Figure 6. The type species of the subgenus *Omorgus* (*Haroldomorgus*) Scholtz, *O*. (*Haroldomorgus*) *batesi* Harold. Image reproduced from Scholtz (1990).

SUBGENUS: *Omorgus* (*Afromorgus*) Scholtz

Omorgus (*Afromorgus*) Scholtz, 1986a: 362; Scholtz, 1993: 2 [larvae]; Zidek, 2013: 6

Type species: *Trox squalidus* Olivier, 1789: 12 (by subsequent designation, Scholtz, 1986a: 362) (Fig 7).

Afromorgus: Pittino, 2006: 26

Diagnosis

Length: 8–21mm.

Colour: Grey, brown to black.

Head: Clypeus with rim reflexed; antennal scape elongate and pedicel attached sub-apically.

Male genitalia: Aedeagus robust; median lobe complex, with ridges, knobs, foveae; pars basalis large, fused dorsally.

Larvae: Larvae from Oriental-Asia unknown, but African members of the subgenus are characterised by a convex sensory cone on distal end of segment two. For diagnosis of African taxa see Scholtz (1993: 2).

Distribution: Afro-Oriental Regions (Fig 8).

Comments: All the African, Arabian and Oriental species belong to this subgenus.

Figure 7. The type species of the subgenus *Omorgus* (*Afromorgus*) Scholtz, *O*. (*Afromorgus*) *squalidus* Olivier

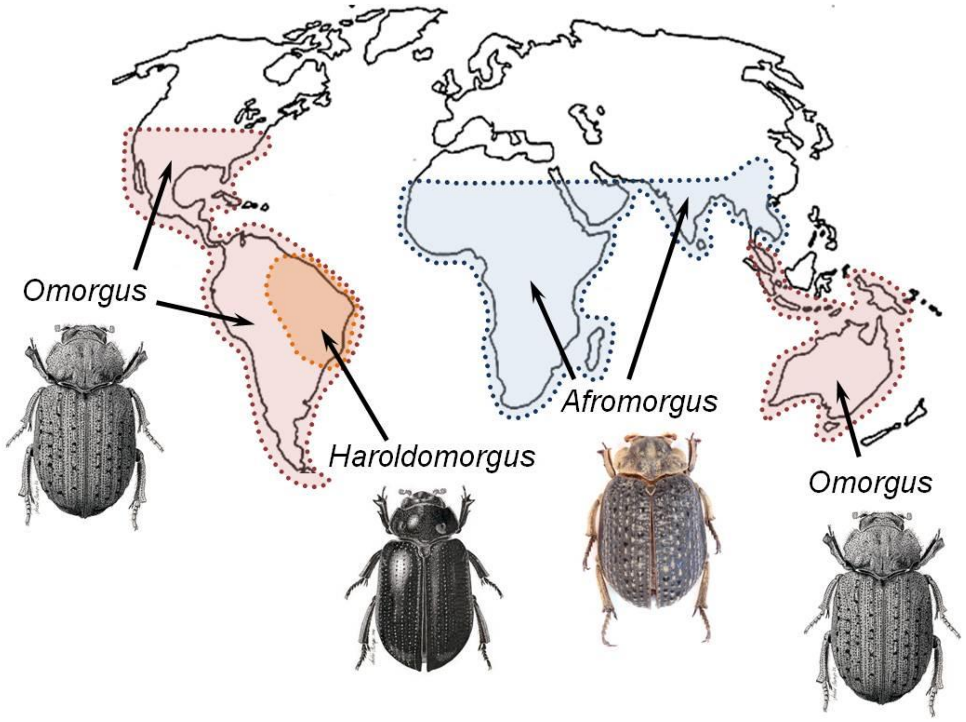


Figure 8. Distribution of the subgenera of *Omorgus* Erichson: *O*. (*Omorgus*), *O*. (*Afromorgus*) and *O*. (*Haroldomorgus*)

GENUS: ***Polynoncus*** Burmeister

Trox (*Polynoncus*) Burmeister, 1876:264 (*pro. parte*); Preudhomme de Borre, 1886:59 (*pro. parte*); Arrow, 1912:53; Scholtz, 1982: 15.

Type species: *Trox pedestris* Harold, 1872:128 (by subsequent designation, Scholtz, 1986a: 362) (Fig 9a).

Polynoncus: Scholtz, 1986a: 362; Scholtz, 1990: 1419; Zidek, 2013: 6.

Diagnosis

Length: 10–20 mm.

Colour: Varies from grey to black.

Head: Frons bituberculate; clypeus with apical portion bent down abruptly, at right angles to disc of clypeus; antennal scape elongate, pedicel attached apically; prostheca on mandibles reduced.

Pronotum: Margins with isolated, pointed, simple setae.

Scutellum: Oval.

Hind wing: With two apically detached veins (for comprehensive overview on hind wing morphology in Trogidae see Browne et al. 1993).

Legs: Metatarsi ventrally with dense setae; longest spur on metatibia, not or scarcely longer than first tarsal segment.

Male genitalia: Aedeagus robust, complex; with the basal piece shorter than the parameres; pars basalis membranous dorsally; parameres are simple and symmetrical and not fused basally; median lobe specialised and varies from complex to highly complex, narrow, ligulate, sometimes asymmetrical; temones short; genital segment a u-shaped spiculum gastrale; internal sac large, armed or unarmed.

Larvae: For diagnosis of the genus see Scholtz and Peck (1990).

Distribution**:** Neotropical Region. Members of the genus are endemic to South America (Fig 9b).

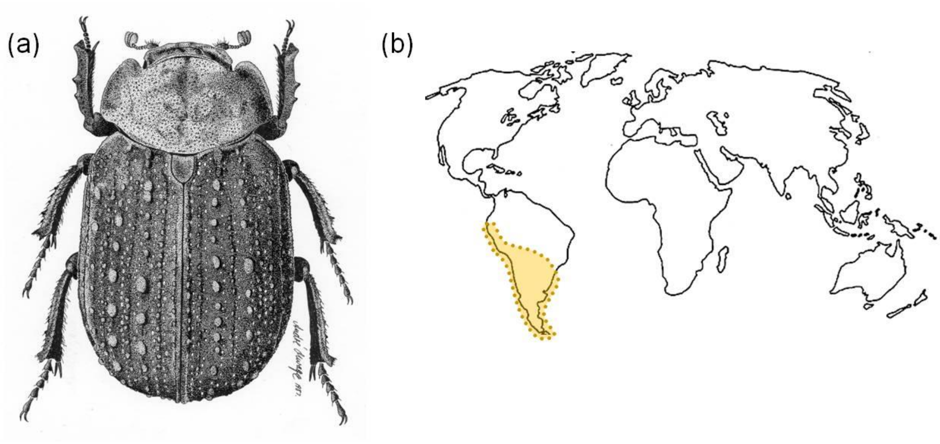


Figure 9. (a) The type species of the genus *Polynoncus* Burmeister, *Polynoncus pedestris* (Harold) (b) Distribution of the genus *Polynoncus.* Image of type species reproduced from Scholtz (1990).

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Appendix 1

List of Afrotropical (including Madagascar) species belonging to the genus *Phoberus* MacLeay **stat. rev.** (in alphabetical order). All new combinations resulting from restoring *Phoberus* to a monophyletic genus are indicted below. * = species previously in the genus *Trox* (Fabricius). ** = species previously in the genus *Madagatrox* Pittino. Synonyms not listed: for details on synonyms see Scholtz (1982) and Zidek (2013).

Genus: *Phoberus* MacLeay, 1819: 137. **stat. rev.**
Type species: *Trox horridus* (Fabricius 1775: 818), by monotypy.
Trox (*Phoberus*): Burmeister 1876: 264; Preudhomme de Borre 1886: 59; Péringuey 1900: 453 (*pars*); Arrow 1912: 53 (*pars*); Scholtz 1980: 17; Scholtz 1982: 15; Scholtz 1986a: 361; Zidek 2013: 6.
Madagatrox Pittino 2010: 75. **syn. n.**

aculeatus (Harold 1872: 37) **comb. nov.**
arcuatus (Haaf 1953: 323) **comb. nov.**
braacki (Scholtz 1980: 87) **comb. nov.**
brincki (Haaf 1958a: 475) **comb. nov.**
caffer caffer (Harold 1872: 41) **comb. nov.**
caffer lilianae, (Scholtz 1980: 96) **comb. nov.**
* *capensis* (Scholtz 1979: 174) **comb. nov.**
consimilis (Haaf 1953: 324) **comb. nov.**
cyrtus (Haaf 1953: 333) **comb. nov.**
elmariae (van der Merwe and Scholtz 2005: 184) **comb. nov.**
* *fascicularis fascicularis* (Wiedemann 1821: 129) **comb. nov.**
* *fascicularis rowei* (Scholtz 1980: 78) **comb. nov.**
gunki (Scholtz 1980: 86) **comb. nov.**
horridus (Fabricius, 1775: 818) **comb. nov.**
levis (Haaf 1953: 325) **comb. nov.**
luridus (Fabricius 1781: 496) **comb. nov.**
miliarius (Gmelin 1790: 1587) **comb. nov.** (*Incertae sedis* - see Scholtz, 1980: 99)

montanus (Kolbe 1891: 22) **comb. nov.**

mozalae (Strümpher and Scholtz 2009: 73) **comb. nov.**

nama (Kolbe 1908: 125) **comb. nov.**

* *nanniscus* (Péringuey 1900: 458) **comb. nov.**

* *nasutus* (Harold 1872: 34) **comb. nov.**

* *natalensis* (Haaf 1954: 97) **comb. nov.**

necopinus (Scholtz 1986b: 29) **comb. nov.**

ngomensis (van der Merwe and Scholtz 2005: 182) **comb. nov.**

nigrociliatus nigrociliatus (Kolbe 1904: 292) **comb. nov.**

nigrociliatus nyansanus (Haaf 1953: 330) **comb. nov.**

nigrociliatus nyassicus (Haaf 1953: 330) **comb. nov.**

penicillatus (Fahraeus 1857: 383) **comb. nov.**

* *perrieri* (Fairmaire 1899: 519) **comb. nov.**

planicollis (Haaf 1953: 337) **comb. nov.**

puncticollis (Haaf 1953: 330) **comb. nov.**

pusillus (Péringuey 1908: 634) **comb. nov.**

quadricostatus (Strümpher and Scholtz 2009: 76) **comb. nov.**

** *ranotsaraensis* (Pittino 2010: 77) **comb. nov.**

* *rhyparoides* (Harold 1872: 32) **comb. nov.**

* *rudebecki* (Haaf 1958a: 474) **comb. nov.**

squamiger (Roth 1851: 133) **comb. nov.**

sternbergi (van der Merwe and Scholtz 2005: 183) **comb. nov.**

* *strigosus* (Haaf 1953: 319) **comb. nov.**

sulcatus (Thunberg 1787: 38) **comb. nov.**

talpa (Fahraeus 1857: 380) **comb. nov.**

youngai (Strümpher and Scholtz 2011: 340) **comb. nov.**

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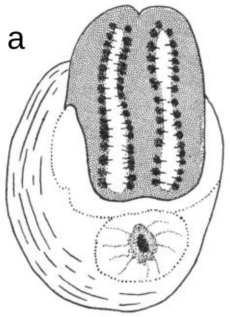
Appendix 2

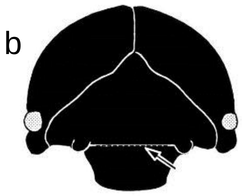
Phylogenetically important larval and adult characters of subfamilies, genera and subgenera.

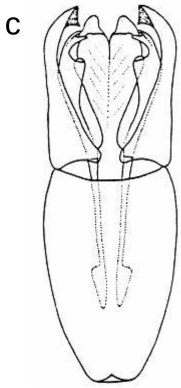
Subfamily: Troginae
° Antennal scape round
° Metatibial spur longer than first two tarsal segments
° With complex body setae (plumose, spatulate or squamose)
° With five autapomorphic wing characters (Browne et al. 1993)
† Spiracle Biforous (a)
† Fronto-clypeal suture indistinct (b)
† Setae absent on the second mesothoracic dorsal lobe

Genus: *Trox*
° Median lobe simple
° Genital segment in the form of a primitive genital capsule
† Phobae on proximal region of hypopharynx united basally

Genus: *Phoberus* stat. rev.
° Median lobe exhibiting complex structure with hooks, blades and spines (c)
° Genital segment in the form of a spiculum gastrale
† Phobae on proximal region of hypopharynx not united basally







Subfamily: Omorginae

° Antennal scape elongate

° Metatibial spur as long as first two tarsal segments

° Metatarsal setae dense

° With 27 synapomorphic wing characters (Browne et al. 1993)

† Spiracle Cribriform (d)

† Fronto-clypeal suture distinct

† Setae present on the second mesothoracic dorsal lobe

Genus: *Omorgus*

° Pedicel attachment – sub-apical (e)

° Wing venation – one apical detached vein

° With 8 autapomorphic wing characters (Browne et al. 1993)

† 2nd antennal joint with sensory disc (f)

† Left mandible with three mandibular teeth (g)

† Haptolachus with sensory cone

† Epicranial stem long

† Epipharynx shape, complexity – rounded , bilobed

† Setae on prothoracic disk – present

Subgenus: *Afromorgus*

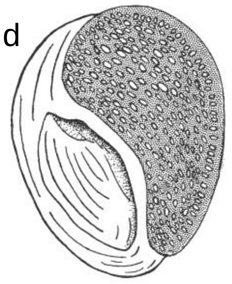
° Median lobe complex usually characterised by dorsal knobs, fovea and or ridges, frequently with erect lamina on either side of median lobe (features absent in *Omorgus* s.s.)

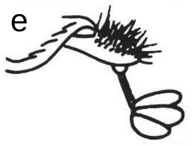
° Pars basalis forms an unbroken sclerified segment (h) (in *Omorgus* s.s. divided median membranous lamina)

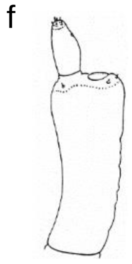
† Antenna with convex sensory cone on distal end of segment two

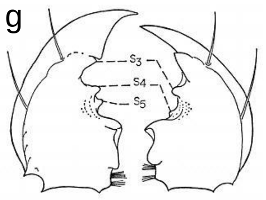
Subgenus: *Haroldomorgus*

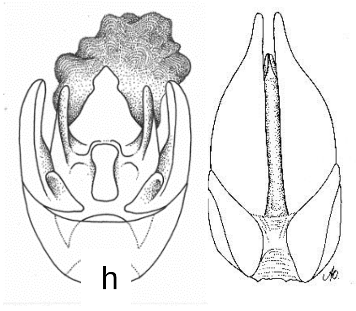
° With three autapomorphic wing characters (= reductions) which may be indicative of incipient flightlessness (Browne et al. 1993)







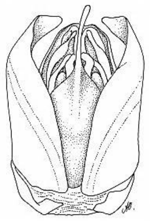


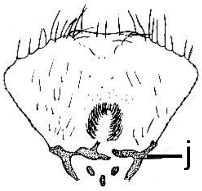


(Afromorgus (Omorgus)

Genus: *Polynoncus*

° Median lobe complex, specialised, even asymmetrically (i)
° Clypeus deflexed
° With two autapomorphic wing characters (Browne et al. 1993)
† 2nd antennal joint – sensory cone and area
† Epipharyngeal tormae – asymmetrical, divided (j)


i



○ = Adult characters, † = Larval characters

Images reproduced from: (Baker 1968; Scholtz 1980, 1990, 1993; Scholtz and Peck 1990; Browne and Scholtz 1999; Grebennikov and Scholtz 2004; Ratcliffe and Paulsen 2008)

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Chapter 6

General conclusion

One of the major aims of the study was to produce a molecular phylogeny for the Trogidae. In this study I: (1) demonstrated that deeper relationships for the Trogidae can be successfully resolved with the incorporation of RNA secondary structure and RNA nucleotide substitution models during alignment and phylogenetic reconstruction processes; (2) provided the first comprehensive molecular phylogeny for this group; (3) successfully resolved relationships between and within genera and subgenera for the family; (4) was able to date the major speciation events for the genera and subgenera of the Trogidae and (5) proposed necessary amendments to Trogidae classification based on the molecular phylogeny.

In addition to addressing the key hypotheses of Trogidae evolution, this study provides valuable insights in to the evolution of flightlessness in African *Phoberus*. Flightlessness evolved at least five times in this group. Diversification, and the present relictual distributions in the group can be linked to climate and geological events associated with the Miocene and Pliocene Climate Optimum in southern Africa. The African *Phoberus* is a promising model genus for studying evolutionary trends relating to flightlessness, speciation and biogeography.

A number of possibilities for future research can be proposed from this study. I have identified the following research study that may be of particular interest.

Biogeography of the genus *Omorgus* Erichson

Phylogenetic sub-division within the genus *Omorgus* indicates a complex evolutionary history for the Trogidae. From my results it is clear that the North American, South American, Australian and African lineages diverged early and radiated independently. It would be interesting to extend taxon sampling to more *Omorgus* species and to investigate the regional biogeography of the (1) New World and Australasian *Omorgus* lineages and (2) the Afrotropical-Oriental *Afromorgus* lineages.