Systematics of Trogidae (Coleoptera): new South African species, and a molecular phylogeny of the family.

by

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I, Yolandi van der Merwe, declare that the thesis/dissertation, which I hereby submit for the degree MSc. Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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SUMMARY

At its inception, taxonomy simply provided guidelines for nomenclature. It has since developed into a science applied to problems relating to economics, conservation and even law. Taxonomy is arguably one of the most important pillars of the biological sciences, providing the framework from which all other studies are conducted. We expand this essential foundation by describing four new species of Trox in Chapter 2 of this dissertation - the first to be recorded in South Africa since Scholtz's comprehensive revision of the family in 1980. All four new species are flightless and are restricted to densely vegetated areas. Based on their morphological characteristics, we conclude that the new species probably belong to the endemic South African *"horridus"*-group of Trox. At present, morphological studies suggest that Trogidae consists of only three genera – Polynoncus, Omorgus and Trox. The current consensus on the zoogeography of trogids, based on their current distribution patterns, is that they evolved in Central Pangaea, prior to the split that formed Laurasia and Gondwanaland. The Trox lineage is thought to have speciated in temperate Laurasia, invading Africa via a temperate faunal exchange route only after Gondwanaland had separated into the southern landmasses. However, in Chapter 3, our work, based on a molecular dataset, challenges these ideas. We investigated the phylogenetic relationships within the family by analysing the 16S ribosomal subunit gene on the mitochondrial genome, which has proved useful in investigating sub-familial relationships due to its fairly conserved nature. We performed both

phenetic (Neighbor-Joining and Minimum Evolution) and likelihood (Parsimony, Maximum Likelihood and Bayesian Inference) analyses on the resulting molecular dataset. We found only partial support for the theories suggested by the morphological dataset. In all analyses, we find four major groups - Polynoncus, Omorgus, Holarctic Trox (Trox s. str.) and African Trox (Phoberus) - not three as suggested by previous morphological studies. We strongly support the promotion of Phoberus to full generic status as it is represented by a monophyletic group in all analyses. Given the evolutionary divergence suggested by our molecular phylogeny, we still propose a Pangaean origin for Trox sensu lato, but suggest that this lineage might have had its origin in what would become North Africa. It is likely to have inhabited the globe-spanning metamorphic geological features known as the Pan-African Belts, which would have offered the ancestral temperate biome preferred by this genus. We theorize that the formation of the Tethys Sea would have separated Trox s. str. from the Phoberus lineage.

CHAPTER 1

Introduction

Y. van der Merwe, C.H. Scholtz & A.D.S. Bastos

It is said of a famous lecture, held on the 7th of April 1951, that when biologist J.B.S. Haldane was asked to give his opinion on the possibility of life being discovered on distant planets and what form this life would take, he didn't dismiss outright the theological aspect of such an event. Instead he remarked: "The Creator, if He exists, has a special preference for beetles" (Slater 1951). The aforementioned is thought to be an incorrect quotation of one of Haldane's favourite phrases, which is "an inordinate fondness for beetles" (Gould 1995), but the essence of the statement holds true.

Although it is impossible to know exactly how many undescribed species are alive today, attempts have been made to estimate this number. The most well known of these were the experiments conducted by Terry Erwin (Rosenzweig 1995 & Thompson 1994).

Conducting his study in the Neotropics, Erwin used insecticide to fog the canopies of nineteen specimens of Luehea seemannii, a leguminous tree, over the course of three years and in different seasons. The falls of dead insects were collected using tarpaulins stretched underneath the canopy and then sorted according to order and species. He reportedly collected 9000 beetles belonging to approximately 1200 species.

Erwin then assumed Luehea seemannii was the exclusive host to approximately 13.5%, or 162 species, of the total number of beetle species he had collected. Further, he assumed that there were 50,000 species of trees in tropical rainforests globally. On these assumptions, over eight million beetle species existed that would specialize on a single tree species given that Luehea seemannii was an example of the average tropical tree. Add to this Erwin's estimate of just under three million beetle species that live on more than one tree species and we come to a rough estimate of eleven million beetle species that reside in the tropical canopies. With the addition of species that do not live in the canopy, temperate species, and non-terrestrial species, the figure would be even larger: Erwin excluded such species from his estimate (Erwin 1988, 1997).

Beetles are believed to make up approximately 40% of total arthropod diversity. If the above rough estimate is then used, we can extrapolate the total arthropod diversity to anything between 30 to 40 million species (Rosenzweig 1995 & Thompson 1994), while some sources even cite estimates as high as 80 million species (Gullan & Cranston 2005). The widely accepted estimate falls between five and 30 million species globally (Bartlett et al 1999).

The higher estimates might very well be true, as Erwin saw no evidence of the number of novel species declining with the number of sampling events. If the rarefaction curve for scientific sampling is considered, we find an initial exponential incline where continued high returns per

sampling event should encourage further sampling, followed ultimately by a plateau where no new data will be added to the database with continued sampling (Magurran 2003). Erwin's experiment showed no signs of reaching such a "point of diminishing returns".

Understandably, Erwin's estimates have sparked much debate. Similar studies were conducted for Pimpline wasps in the Neotropics (Bartlett et al 1999), Diptera in temperate regions such as Britain and Canada, and Hymenoptera and Diptera that inhabit the tropical canopies of Borneo – each study claiming to have found the "true most diverse insect fauna" (Gullan & Cranston 2005).

Whatever the true number, the point of Erwin's experiments was primarily to encourage further research, expeditions and explorations to either support or refute his estimates - a thrown gauntlet in scientific terms.

The challenge is one to be taken up by taxonomists working out of museums and other collections to describe and catalogue new species. If we consider the number of natural history museums in the world (over 650 according to the University of Washington Libraries' website) and generously assume a team of five taxonomists for each, we conclude that there are far too few to deal with the veritable mountain of undescribed species; bearing in mind that to date, only about 1.5 million species in total have been described. If these 3250 taxonomists were dedicated solely to the task of describing the eleven million beetles species as estimated by Erwin and they managed to consistently describe one species per week each, it would take just over 65

years of non-stop work to achieve. Given that funding is hard to come by at the best of times, and that species are rarely discovered at the high rate proposed, the sheer magnitude of the task becomes even clearer.

One of the greatest challenges taxonomists face is encapsulated by a very simple question: “What is a species?” Most people have an intuitive understanding of "species". To the layperson, a species is a group of organisms that look the same, identifiable by simple observation of some key features that determine its inherent type (Gould 1979). This “instant recognition of kind” has been taken further by some groups into pseudosciences such as baraminology, which attempt to explain biological diversity in a religious framework. These theories however do not hold to many scientific principles and are generally rejected by the scientific community.

Although they are hardly more based in science than baraminology, the roots of modern taxonomy lie in the theories of Aristotle (Mader 1998). Aristotle proposed that in order to describe any living being, one must first specify its summum genus (“largest possible group of things of the same type”) and then specify its infima species (“smallest possible category”). In practical terms, this required one to identify the type of organism and then identify a way to distinguish it from all other examples of that type.

Well into the eighteenth century, long Latin phrases composed of a generic name and differentia specifica were often used to achieve this. For example, in order to differentiate bees from all other insects, Gessner’s

description read as follows: "Apis est animal insectum, volatile, quadripenne, sepes, exangue, mellis artificio solum pollens" ("A bee is an insect animal, flying, four-winged, common, without claws, the only one capable of making honey") (Gessner 1634).

A "shorthand" version of these phrases was required to simplify scientific correspondence and thus the binomial system of nomenclature was adopted. First introduced by Carolus Linnaeus in the mid-eighteenth century, the system prescribes the identification of each organism by a combination of two names, genus and species, of which the species name may not be used for any other organism within the genus and may not be changed once assigned. This two-part combination is unique to an organism and may not be used to describe any other organism (Mader 1998).

It is important to remember that the Linnaean system laid down rules for nomenclature and not taxonomy. In other words, the Linnaean system was essentially devised to ease the identification of species and did not necessarily reflect the evolutionary relatedness between organisms.

Linnaeus assumed that organisms should be grouped based solely on observable characteristics. Using these observations, he compiled his initial classification of organisms. As time passed and the science advanced, what qualified as "observable characteristics" has changed; so it followed that the hierarchical ranks proposed by Linnaeus for the purposes of nomenclature have been adapted to taxonomic ranks to describe the interrelatedness of organisms. Whereas Linnaeus only prescribed six ranks in his original system

(Kingdom, Class, Order, Genus, species and variety in the case of plants) these ranks have been subdivided to accommodate the greater administrative requirements necessitated by the expansion of knowledge with regards to the natural world. Entomology in particular, which as a discipline is brimming with species requiring classification, has driven the creation of many of these extra hierarchical levels.

The application of these ranks is governed by formal codes of biological nomenclature. The rules governing the nomenclature and classification of animals, including insects, are contained in the International Code of Zoological Nomenclature, which was last revised in 1999, and maintained by the International Commission on Zoological Nomenclature.

Initially, descriptions focused mainly on morphology. However, as it is believed that purely morphological classifications can frequently be complicated or obscured because of convergent evolution – a phenomenon where organisms with similar trophic functions have evolved similar morphology to cope with their lifestyles – emphasis has since shifted to molecular and, in particular, DNA studies. Taxonomy now takes its cue from evolution. Whereas taxonomy alone refers strictly to classification, the field of systematics applies taxonomy in an evolutionary framework.

Although it is widely accepted that molecular or DNA studies reflect the “true” evolutionary relationship between organisms, these types of studies are not without their shortfalls. In the case of DNA, each nucleotide position constitutes an observable characteristic that theoretically leads to millions of

possible observations per organism (Page & Holmes 1998). The limitation inherent in the DNA code is that each of these nucleotide positions can only take on one of four possible states, which lends itself to saturation – that is, a succession of changes may result in a nucleotide that is the same as and therefore indistinguishable from the ancestral one.

The real driver behind taxonomy is not necessarily how it is done, but rather why it is done. There are probably many reasons why one would venture into this field.

It may be a purely academic pursuit, the simple expansion of human knowledge. Darwin's On The Origin of Species had a single illustration – a simple tree-like diagram that Darwin used to explain how he envisioned evolution and ecosystems. Since Darwin's writings, the elucidation of the "Tree of Life" has been a goal of biological science. Biologists from all over the world are collaborating on projects such as the well-known Tree Of Life web project. The project's website endeavours to be a depository of easily accessible information, providing information about biological diversity and phylogenetics to cater to all levels of interest. When one considers the difficulty in obtaining funding, "academic pursuit" is usually a by-product rather than the focus of any study.

Another motivator may be economic gain. Once we understand the evolutionary relationships between organisms, they may reveal biologically interesting patterns of variation and may aid in the identification of closely related economically useful species. Take, for example, a study done on the

mint family, Lamiaceae. Although all of the genera in this family produce essential oils that may be harvested, some of the genera appear to be oil-rich while others are oil-poor (El-Gazzar & Watson 1970). Susceptibility to Puccinia menthae, a fungal infection that affects mint plants, is prevalent in the oil-rich group. However, it would seem that some groupings in this assemblage may well be resistant to the fungus. The study suggests that these oil-rich yet resistant genera be investigated for their commercial potential.

In the case of insects, it is usually identifying the economically harmful species that is of greater import. The Bactrocera dorsalis complex together constitutes the Oriental fruit fly, one of the most important groups of Southeast Asian agricultural pests (Adsavakulchai et al 1998). Some of the species of the Bactrocera subgenus attack soft fruits and flowers of plants. The fourteen closely related species of the aforementioned complex present with very similar morphological characteristics that have lead to a preponderance of synonyms, homonyms, misidentifications and the use of questionable morphological characters to establish supra-specific groups. It is in cases such as this that the usefulness of molecular techniques becomes clear.

The buzzword since the late eighties, however, has been conservation. The website of the International Union for the Conservation of Nature and Natural Resources (IUCN) currently lists over 1200 insect species as endangered for 2007, of which roughly 70 are beetle species. This may not

seem like a significant percentage of the total estimated diversity of insects, but the fear is that we do not have enough information on most of this diversity to assess their conservation status.

Insects are essential for proper ecosystem functioning, more so than any of the other animal groups. Insects are responsible for nutrient recycling, plant propagation, the maintenance of plant community composition and structure via feeding, serving as a food source for insectivorous organisms and the maintenance of animal community structure by spreading diseases or feeding on smaller animals. Because of these vital functions, the loss of a single insect species may be more catastrophic than the loss of a charismatic vertebrate species. These so-called keystone species include groups such as termites. Termites are believed to be responsible for tropical soil structuring by converting the cellulose of fallen trees into usable nutrients in tropical environments (Gullan & Cranston 2005).

Very few insects enjoy individual legal protection. More often than not, governments offer blanket protection to insect groups, although some legislation does exist to protect conspicuous, often geographically restricted, threatened species. An example of such an insect is the now famous Addo flightless dung beetle. Circellium bacchus is a large, flightless dung beetle confined to sandy regions in the Eastern Cape Province of South Africa. Conservation plans for this beetle included proposed translocations between areas to bolster faltering populations. A molecular study undertaken by Kryger et al (unpubl.) suggested that the Addo Elephant National Park

population was a distinct genetic entity from the other populations investigated. The amount of genetic divergence between the Addo Elephant National Park population and all the other populations were in fact akin to the amount of divergence one would expect to find between sister species for this group. The study concluded that translocations would therefore be ill-advised, as it would be detrimental to the genetic diversity of the species as a whole.

The combination of taxonomy and conservation has lead to new legal concerns for the biological community. The Wielangta landmark trial, in the Federal Court of Australia, revolved around the possible effects that Forestry Tasmania's proposed logging activity would have on an endangered beetle species, the Wielangta stag beetle. Senator Bob Brown, leader of the Australian Greens, argued that the proposals would be in violation of conservation law (Marshall 2006). The court held that this was the case, as there would be a "significant impact" on the habitat of the beetles, and that Forestry Tasmania's proposals were in violation of their official forestry management agreement with the State of Tasmania.

In this case, taxonomy was of vital importance, as it could not be established whether a population of similar beetles from Maria Island was genetically distinct from the Wielangta population. It was on this point that Forestry Tasmania argued that their logging activity was not having a significant impact on the beetle's usual habitat but the plaintiff's expert had found beetles within the type of habitat that was proposed for logging. It was

therefore important for the plaintiff's case that these beetles had been correctly identified.

It is unlikely that Linnaeus would have realised that his simple system of naming organisms would be the subject of debate in the courts of law rather than the halls of science, but this trial has highlighted that monitoring programs are legally important, and require accurate taxonomy. Accurate taxonomy was used to remove a major plank of the defence's case.

Another lesson learnt was that biologists should be prepared for the competitive environment of the legal world, where pressure exists for objectivity to be compromised by commercial matters. One biologist who gave evidence revealed that the defendant had persuaded him to remove statements from his report that weakened the case. The objectivity of the science presented in legal matters should never be in question, especially in a case, such as this, which has great environmental import.

Even though taxonomists are presented with a seemingly insurmountable challenge, the historical and contemporary importance of taxonomy should not be subject to dispute. With this in mind, this dissertation, although not dealing with economically important or endangered species, will further extend the range of this particular discipline in taking a small step along the 65 year (or so we estimate) road to taxonomy's ultimate goal.

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CHAPTER 2

New species of flightless Trogidae from relict South African forest fragments.

Y. van der Merwe & C.H. Scholtz

The Trogidae represent an ancient, monophyletic lineage of keratin-feeding Scarabaeoidea, the only group in the superfamily to do so and one of few insect groups capable of digesting keratin (Scholtz & Chown 1995). Trogids are a relatively small (300 species) cosmopolitan family comprising three genera, Trox Fabricius, 1775, Omorgus Erichson, 1847 and Polynoncus Burmeister, 1876. Both Trox and Omorgus have relatively wide distributions, the former occurring in the Holarctic and Afrotropical regions and the latter occurring mostly in arid areas of the southern continents. Polynoncus is restricted to South America (Scholtz 1986b).

Adults and larvae of all known species feed almost exclusively on various sources of keratin although they may opportunistically feed on living insect eggs and sessile larvae (Roffey 1958; Scholtz 1986b; van Emden 1948). Trogids tend to be the last insects to invade carcasses (Braack 1986) and in arid areas may remain with mummified remains for two years and longer (Scholtz & Caveney 1988)

In this paper we describe four new species of Trox, the first to be recorded in South Africa since Scholtz's (1980) revision of the family. It brings

to seven the number of new species for the continent since then (Scholtz 1983, 1986a, 1991). All four new species are flightless and are restricted to densely vegetated areas namely, highland forest, lowland hills and highland grassveld surrounded by dense bush. All these areas are very small by insect distribution norms.

The South African forest biome is highly fragmented and most fragments are represented by patches of less than $1km^2$ (Eeley et al. 1999). Midgley et al. (1997) speculated that southern African forests may be naturally patchily distributed and may therefore not pose a great conservation concern. Eeley et al. (1999) proposed that Pleistocene hyperthermal periods characterized by warmer, wetter conditions may have caused an expansion of the forest biome whereas cooler drier hypothermal periods may have caused a reduction in the extent of forests. This expansion and contraction of the forest biome may have resulted in formerly contiguous populations of taxa being isolated in forest refugia during the cooler, drier periods, possibly facilitating divergence.

All the species we describe in this paper are flightless. Scholtz (1981) suggested that some of the morphological changes associated with flightlessness may occur over a relatively short period of time, since alate and apterous individuals can occur at either extreme of a species' range. Flightlessness in trogids is mostly driven by environmental pressures. Flightless trogids are most often found in deserts, on mountains, on islands and forests. Southern Africa is the only region where flightless trogid species

occur in forests (Scholtz 2000). One of the major conditions thought to favour the secondary loss of wings is increased environmental homogeneity (Scholtz 2000). Scholtz (2000) hypothesized that temperate forests at low latitudes in the southern hemisphere encouraged flightlessness in southern African trogids because the forest habitat provides stable and persistent microhabitats. The relatively stable patches of relict forest could have promoted the stability of populations as well as increasing the population density (Scholtz 1981). Flight for the sake of population maintenance became redundant.

The terminology used in this paper is based on that of Scholtz (1980). The institutions in which studied material and types are housed are listed below along with the curators in charge of the collections: BMNH: British Museum (Natural History), London, United Kingdom, M. Kerley; MNHU: Institute of Systematic Zoology, Museum für Naturkunde Humboldt-Universität zu Berlin, Germany, M. Uhlig; SANC: National Collection of Insects, Plant Protection Research Institute, Pretoria, South Africa, R. Stals; SAMC: South African Museum, Cape Town, South Africa, M. Cochrane; TMSA: Transvaal Museum, Pretoria, South Africa: R.Müller, J. Harrison; UPSA: Department of Zoology and Entomology, University of Pretoria, South Africa, C.H. Scholtz.

Genus **Trox** Fabricius, 1775

Subgenus **Trox (Phoberus)** M'leay, 1819

Trox (Phoberus) ngomensis sp. n., Figs 2.1, 2.2

Diagnosis

This species is similar to T. natalensis Haaf, T. elizabethae sp. n., T. elmariae sp. n. and T. sternbergi sp. n. T. ngomensis has setae on all the ridges and tubercles of the pronotum, whereas T. natalensis, T. elizabethae and T. elmariae do not. T.ngomensis and T. sternbergi can only be reliably distinguished by examining the male genitalia (see Fig. 2.2 for T. ngomensis and Fig. 2.4 for T. sternbergi).

Description

Size. Length 5.5–6.5 mm, width 3–3.5 mm (n = 20).

Head. Clypeus triangular, apex pointed, frons present with two oval setose ridges, antennal scape longer than wide, pedicel attached apically to scape.

Pronotum. Attenuated anteriorly, lateral margins with fringes of short setae, lateral margins smooth but notched posteriorly, sides broadly flattened, median depression shallow with raised tubercles, discal area raised, discal ridges pronounced, median and laterad discal ridges pronounced and easily

distinguishable, median basal tubercle pronounced, anterolateral and lateral basal tubercles pronounced, all ridges and tubercles setose, anterior edge of pronotum sparsely setose.

Scutellum. Very small, oval.

Elytra. Sides flattened, lateral margin with fringe of short setae, sutural margin with tufts of setae, four costae visible with irregular tufts of setae, intercostal area characterized by shallow regular diameter foveae, humeral callus completely reduced, profile convex attaining maximum height behind the middle.

Male genitalia. Slender, parameres symmetrical, anterior edge of median lobe with shallow “u”-shaped notch, parameres approximately one quarter of total length of aedeagus in profile (see Fig. 2.2a,b).

Type material examined. Holotype (♂ TMSA) with the following data: South Africa, KwaZulu-Natal, Ngome State Forest, 27° 50’ S 31° 25,3’ E, dense forest, 1000 m, unbaited pitfall trap: 17.iii.1992 – 17.iv.1992, leg. M. van der Merwe, Ngome project grid 3D, University of Pretoria; and 166 paratypes (100 SANC; 14 UPSA; 13 BMNH; 13 MNHU; 13 SAMC; 13 TMSA) with same data but with altitude varying from 1000–1140 m and collection dates ranging throughout the year, leg. M. van der Merwe or R. Stals.

Etymology. The species is named for the type locality, a fragment of beautiful relict montane forest.

Distribution. Recorded only from the type locality.

Fig. 2.1 Adult habitus of Trox ngomensis. Scale bar = 1mm.

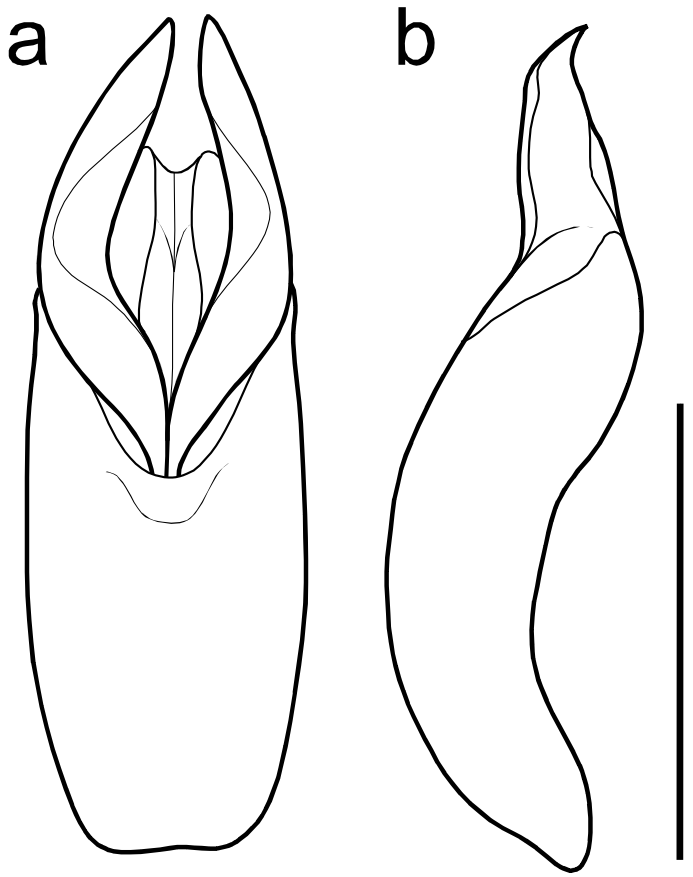

Fig. 2.2 Aedeagus of Trox ngomensis a) dorsal view, b) profile view. Scale bar = 1mm.

Trox (Phoberus) sternbergi, sp.n., Figs 2.3, 2.4

Diagnosis

This species is close to T. natalensis Haaf, T. elizabethae sp. n., T. elmariae sp. n. and T. ngomensis sp. n. T. sternbergi has setae on all the ridges and tubercles of the pronotum, whereas T. natalensis, T. elizabethae and T. elmariae do not. T. ngomensis and T. sternbergi can only be reliably distinguished by examining the male genitalia (see Fig. 2.2 for T. ngomensis and Fig. 2.4 for T. sternbergi).

T. sternbergi shares the same basic structure as described in detail for T. ngomensis above, with the exception of the following characteristics:

Description

Size. Length 5.5–6.5 mm, width 3.2–3.6 mm (n = 8).

Pronotum. Discal ridges not as pronounced, median and laterad discal ridges easily distinguishable, median basal tubercle clearly visible, anterolateral and lateral basal tubercles clearly visible.

Male genitalia. Slender, parameres symmetrical, anterior edge of median lobe “m”-shaped, parameres approximately one third of total length of aedeagus in profile (see Fig. 2.4a,b).

Type material examined. Holotype (♂ TMSA) and 7 paratypes (2♂, 2♀ TMSA; 1♂, 1♀ UPSA; 1♂ BMNH) with the following data: South Africa, Zululand, Hluhluwe Game Reserve, 28° 05’ S 32° 04’ E, 18/11/1992-

20/11/1992, E-Y: 2828, groundtraps, 10days, leg. Endrödy-Younga, groundtrap with meat bait.

Etymology. “Sternberg” is a family name of the first author. This species is named after Willem Sternberg Johannes van der Merwe with the utmost appreciation for his guidance and support to Y.vd.M.

Distribution. Known only from the type locality.

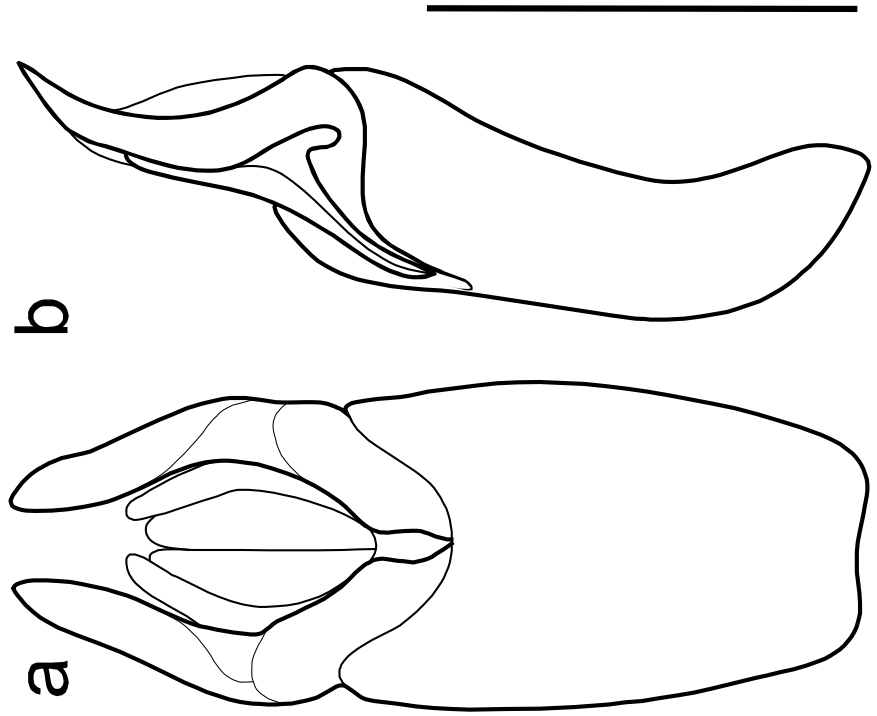

Fig. 2.4 Aedeagus of Trox sternbergi a) dorsal view, b) profile view. Scale bar = 1mm.

Fig. 2.3 Adult habitus of Trox sternbergi. Scale bar = 1mm.

Trox (Phoberus) elizabethae, sp.n., Figs 2.5, 2.6

Diagnosis

This species is similar to T. natalensis Haaf, T. sternbergi sp. n., T. ngomensis sp. n. and T. elmariae sp. n. T. sternbergi and T.ngomensis have setae on all the ridges and tubercles of the pronotum, whereas T. elizabethae does not. T.elizabethae can be distinguished from T. natalensis and T. elmariae by virtue of the "v"-shaped ridge on its pronotum and by examining the male genitalia (see Fig. 2.6 for T.elizabethae, Fig. 2.8 for T. elmariae and Fig. 2.9 for T. natalensis).

Description

Size. Length 6–7 mm, width 3–3.2 mm (n = 9).

Head. Clypeus triangular, apex pointed, frons present with two oval ridges, antennal scape longer than wide, pedicel attached apically to scape.

Pronotum. Base narrower than apex, sides broad anteriorly, lateral margins with sparse fringes of short setae, lateral margins smooth but notched posteriorly, median depression deep and interrupted in the middle, median discal ridges high and except for interruption in the middle stretch over the whole length of the pronotum, posterior half of median discal ridge after the interruption is "v"-shaped, another disc stretches at an angle from this interruption to the anterolateral corner of the disc, an indistinct lateral disc

approximately parallel to the posterior half of the median discal ridge is also present.

Scutellum. Very small, oval.

Elytra. Sides flattened, lateral margins with sparse fringes of short setae, sutural margins with tufts of setae, four costae visible with irregular tufts of short setae, second costa a ridge on the anterior two-thirds of the elytra and isolated tubercles on the posterior third, other costae with rows of isolated tubercles, intercostal area characterized by shallow regular diameter foveae, humeral callus completely reduced, profile convex attaining maximum height behind the middle.

Male genitalia. Slender, parameres symmetrical, inner parameres with squared off ends (see Fig. 2.6a,b).

Type material examined. Holotype (♂ TMSA) and 8 paratypes (6 TMSA; 1♂ BMNH; 1♂ UPSA) with the following data: South Africa, KwaZulu-Natal, Ndumu Nature Reserve, 26.54 S – 32.17 E, 21.11.2002, E-Y: 3552, Hyena dung, leg. J. Harrison.

Etymology. This species is named for Elizabeth van der Merwe with greatest affection.

Distribution. Recorded only from the type locality.

Fig. 2.5 Adult habitus of Trox elizabethae. Scale bar = 1mm.

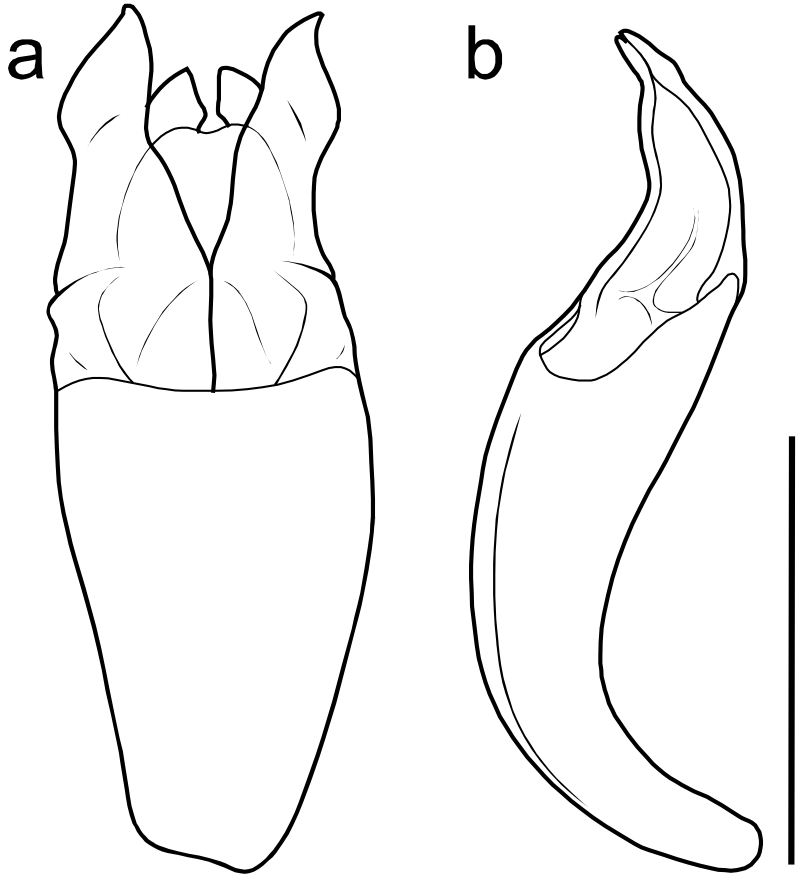

Fig. 2.6 Aedeagus of Trox elizabethae a) dorsal view, b) profile view. Scale bar = 1mm.

Trox (Phoberus) elmariae, sp.n., Figs 2.7, 2.8

Diagnosis

This species is similar to T. natalensis Haaf, T. sternbergi sp. n., T. ngomensis sp. n. and T. elizabethae sp. n. T. sternbergi and T.ngomensis have setae on all the ridges and tubercles of the pronotum, whereas T. elmariae does not. T. elmariae can be distinguished from T. natalensis and T. elizabethae only by examining the male genitalia (see Fig. 2.6 for T. elizabethae, Fig. 2.8 for T. elmariae and Fig. 2.9 for T. natalensis).

T. elmariae shares the same basic structure as described in detail for T. elizabethae above, with the exception of the following characteristics:

Description

Size. Length 5.3–7.5 mm, width 2.8–4 mm (n = 16).

Pronotum. Lateral margins with fringes of short setae, posterior half of median discal ridge after the interruption is not forked.

Elytra. Lateral margins with fringes of short setae.

Male genitalia. Slender, parameres symmetrical, inner parameres with rounded ends (see Fig. 2.8a,b).

Type material examined. Holotype (♂ TMSA) and 15 paratypes (13 TMSA; 1♂ BMNH; 1♂ UPSA) with the following data: South Africa, the former S Natal Weza [present KwaZulu-Natal], Impetyene grassveld, 30.37 S –

29.42 E, 16.11.1989, E-Y: 2678, groundtrap, 12days, Endrödy & Klimaszewski, groundtrap with faeces bait.

Etymology. This species is named for Elmari van der Merwe for her unending enthusiasm for Yolandi van der Merwe's studies.

Distribution. Recorded only from the type locality.

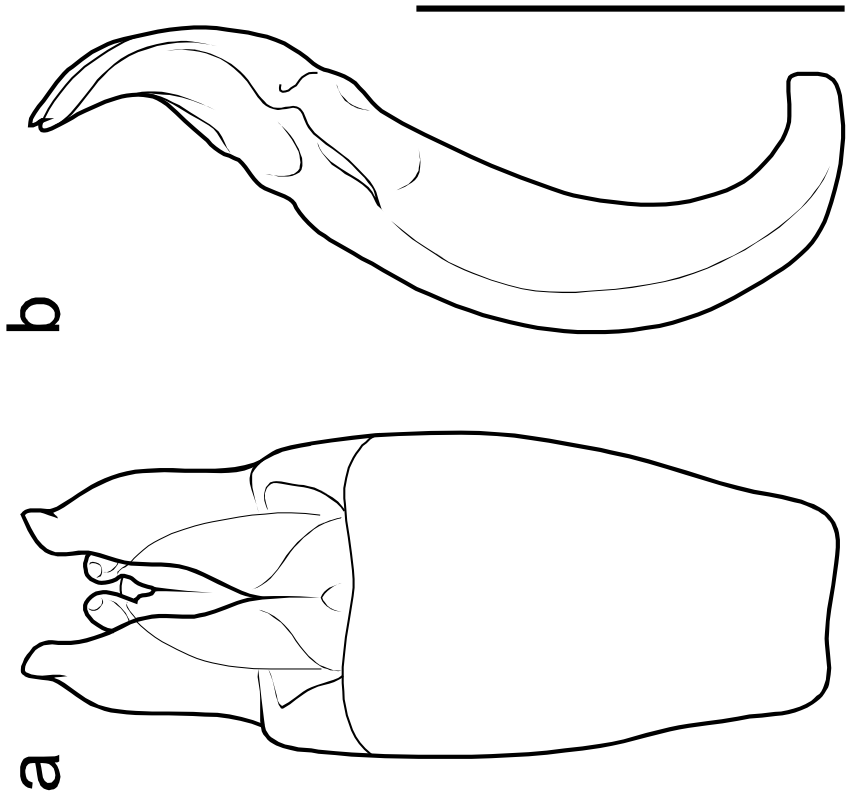

Fig. 2.8 Aedeagus of Trox elmariae a) dorsal view, b) profile view. Scale bar = 1mm.

Fig. 2.7 Adult habitus of Trox elmariae. Scale bar = 1mm.

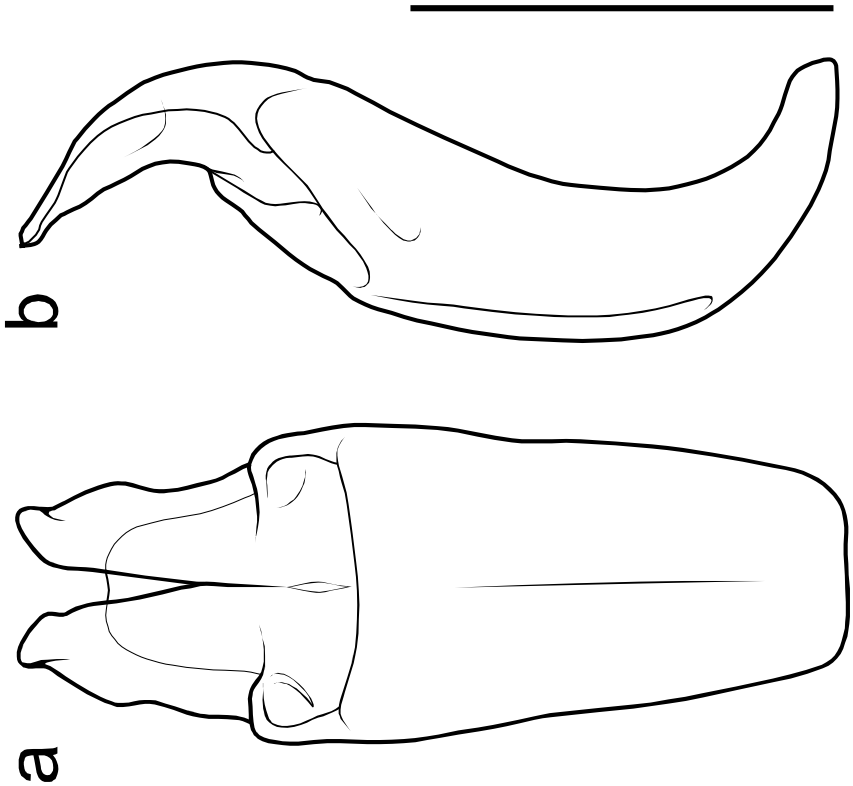

Fig. 2.9 Aedeagus of Trox natalensis a) dorsal view, b) profile view. Scale bar = 1mm.

DISCUSSION

The new species probably belong to the South African endemic *"horridus"*-group of Trox species (Scholtz 1979) since morphologically they are most similar to other members of the group, most noticeably T. natalensis. However, the group was defined on characters that can probably be attributed to loss of flight capabilities so there is some doubt whether the group is actually monophyletic. A current study is addressing their phylogenetic relationship.

All four new species key out to couplet 11/12 of Scholtz's (1980) key to the subsaharan Trox and would be mistakenly identified as one of the subspecies of T. natalensis. T. ngomensis and T. sternbergi are easily distinguished from the T. natalensis group by virtue of their setose pronotal ridges. While they can be separated from each other by details of the male genitalia as illustrated in Figs 2.2a,b, and 2.4a,b. T. natalensis, T. elizabethae and T. elmariae have no setae on any of their pronotal ridges. T. elizabethae can be identified by the distinct "v"-shaped ridge on its pronotum which is absent in both the T. natalensis group and T. elmariae. T. natalensis, T. elizabethae and T. elmariae can be separated from each other by details of the male genitalia as well as illustrated in Figs 2.6a,b, 2.8a,b, and 2.9a,b.

ACKNOWLEDGEMENTS

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CHAPTER 3

Molecular Phylogeny Of The Family Trogidae (Coleoptera: Scarabaeoidea).

Y. van der Merwe, C.H. Scholtz & A.D.S. Bastos

INTRODUCTION

The Trogidae are a relatively small cosmopolitan family within Coleoptera comprising roughly 300 species. Both adults and larvae are facultative necrophages and may be found associated with virtually any animal remains. They feed primarily on keratin, and thus are usually the last of the succession of insects to invade carcasses, but have been recorded on many other sources of animal matter (Scholtz 1980, 1986, 1986[b], 1993).

The family currently consists of three recognized genera; Trox Fabricius, Polynoncus Burmeister and Omorgus Erichson (Scholtz 1986). Trox includes two subgenera, Trox s. str., which consists of 59 species restricted to the Holarctic region and Phoberus Macleay, which consists of approximately 40 species restricted to the Afrotropical region. Polynoncus occurs only in South America and includes 33 species. Omorgus has three subgenera. The nominate subgenus consists of 82 species spread across the southern Nearctic, Neotropical and Australasian regions. Afromorgus Scholtz's 45 species occur in the Oriental and Afrotropical regions.

Haroldomorgus Scholtz is a monotypic subgenus that occurs only in South America.

The Trogidae are regarded as an ancient group, based on various morphological clues (Caveney & Scholtz 1993; D'Hotman & Scholtz 1990; Nel & Scholtz 1990). Smith et al. (2006) has suggested that the Trogidae are basal to the Scarabaeoidea, based on 28S and 18S ribosomal DNA evidence.

The presently-accepted classification is the result of Scholtz's 1986 study, which was one of the first to attempt to determine relationships within the Trogidae on the basis of shared derived characters (or synapomorphies) using a morphological dataset as per the cladistic method. Previous studies (Baker 1968; Balthasar 1935; Nakane & Tsukamoto 1955; Paulian 1981; Paulus 1972; Scholtz 1979, 1980, 1980[b], 1980[c], 1986[b], 1990, 1991, 1991[b], 1993; Vaurie 1955) used only superficial similarity to construct their keys and group the different taxa into genera and species groups.

The resulting phylogram (Scholtz 1986; Figure 3.1) has been supported by subsequent cladistic studies (Browne et al. 1993; Caveney & Scholtz 1993; Scholtz 1993; Scholtz & Lumaret 1991; Scholtz & Peck 1990). Scholtz & Peck's (1990) study is especially important since a suite of larval characters produced a very similar phylogram to that of the 1986 study of adult characters. It lent unequivocal support to the hypothesis that the family is monophyletic as it stands and that the genera can be clearly defined on the basis of both larval and adult morphological autapomorphies (Scholtz 1990).

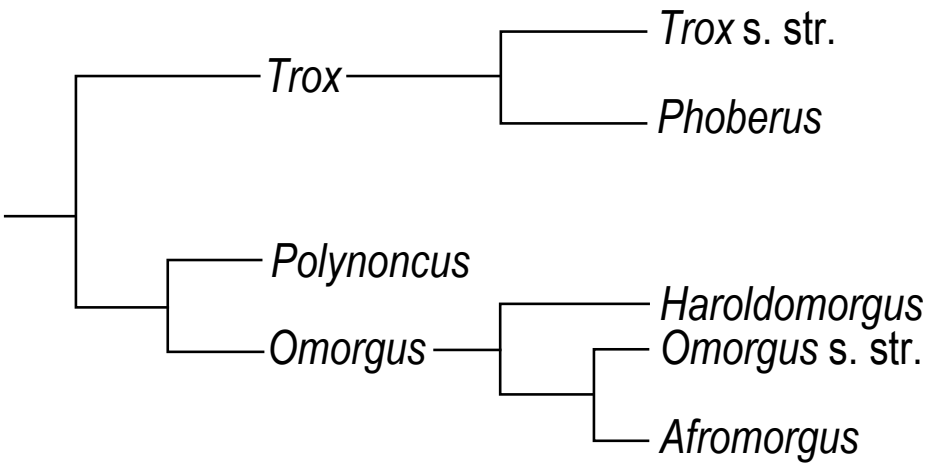

Fig. 3.1 Phylogram of the constituent elements of the Trogidae based on a morphological dataset from Scholtz (1986).

The reigning theory put forward by Scholtz's (1986) study is that, based on current distribution patterns, Trogids evolved in central Pangaea prior to the split that formed Laurasia and Gondwanaland. The separated populations evolved into several lineages.

The Trox lineage is thought to have speciated in temperate Laurasia. The morphologically most primitive nominate subgenus' occurrence in Europe and North America is evidence for this. Phoberus, the more derived subgenus under this hypothesis, occurs in Africa but not in Australia or South America. This suggests that the Trox lineage only invaded Africa after Gondwanaland had separated into the southern landmasses as we know them today. Eastern Zimbabwe, the Drakensberg system, the eastern highlands of the Democratic Republic of the Congo, and Ethiopia have, since the Tertiary, formed a temperate faunal exchange route along which the Trox lineage has

spread into Africa from southern Europe. Phoberus species are distributed along this route, with the greatest species radiation evident in southern Africa. The more primitive species keep to the temperate montane regions that characterise this extension line, while more derived species have radiated into arid regions secondarily.

Scholtz speculated that the radiation of Trox s. str. in southern and south-western North America coincided with a peak in the aridity of these areas about five million years ago. The radiation was not as dramatic as that of the species in southern Africa and the North American species are generally considered more primitive than the southern African ones.

The Omorgus lineage has its most likely origin in tropical and moist savanna such as that found in present day central-eastern South America and central West Africa. Once again, aridification of these continents may have lead to the radiation of this lineage as well as separation of populations during pluvials (Scholtz 1980). The most derived species still occur in deserts on all the continents. The diversification probably occurred only after the separation of the southern continents.

Radiation may have occurred during the Eocene, when the Omorgus lineage spread from South America via Antarctica to Australia. The Omorgus lineage spread northwards into North America from South America via the newly-formed land bridge approximately two million years ago. The Oriental Omorgus fauna has possible links with the African Omorgus fauna via Madagascar and India or across the Afro-Arabian plate.

The Polynoncus lineage may have been derived from an Omorgus-like ancestor and evolved in South America after the continental fragmentation as evidenced by its absence from the other continents. Competition with the mostly arid-adapted Omorgus may have prompted its radiation into the temperate regions of this continent.

Whilst the number of Scarabaeoidea DNA sequence entries are steadily increasing, there are presently (August 2008) just eight nucleotide sequence entries for Trogidae in the Genbank database. The aim of this study was to determine whether a phylogeny based on a mitochondrial DNA dataset would support the morphological phylogeny proposed by Scholtz (1986). To this end, the 16S ribosomal subunit gene on the mitochondrial genome was targeted for amplification due to its use for investigating sub-familial relationships (ie those between genera or subgenera) and its fairly conserved nature (Damgaard et al 2005; Forgie 2003; Page & Holmes 1998). The comparatively slower rate of evolution of this gene makes it possible to investigate the intermediate to deeper nodes in the evolutionary past of a family.

MATERIALS & METHODS

Taxon sampling.

We obtained whole specimens preserved in ethanol from all the major zoogeographic regions. An ingroup sample consisting of 51 individuals from 32 species was selected (Table 3.1) which represents roughly ten percent of the total trogid diversity known. Bolboceras was selected as an outgroup based on an unpublished phylogeny by David Hawks (2003) of higher coleopteran relationships constructed using 28S nuclear gene sequence data. Four specimens from two outgroup species were obtained. All samples were identified, catalogued and stored in a -20°C freezer upon arrival.

DNA extraction, PCR & nucleotide sequencing.

An entire metathoracic leg, as well as a mesothoracic leg for specimens smaller than 0.5 cm, was removed with sterilised forceps. Once excess ethanol had evaporated, the samples were frozen with liquid nitrogen and ground in separate 1.5 ml microfuge tubes using sterilised pestles. Extraction was accomplished using a Roche High Pure PCR Template Preparation Kit (Roche Diagnostics, Penzberg, Germany). A minimum incubation period of 24 hours was allowed instead of the recommended one hour, to ensure maximal template yield. Extracted samples were eluted in 100 μl of elution buffer and stored at 4°C until needed.

A fragment of approximately 420 bp corresponding to the 5' end of the mitochondrial 16S gene was amplified using 1U of Taq DNA polymerase, 10 mM dNTP (Abgene, Surrey, UK), 0.4µM of the 16Sb2 primer [5' TTT AAT CCA ACA TCG AGG 3'] (GibcoBRL) and 0.4µM of the reverse primer LRN-13398, also known as 16SaR [5' CGC CTG TTT AAC AAA AAC AT 3'] (GibcoBRL) (primer sequences from Simon *et al.* 1994), which target the 5' end of the 16S gene. PCR cycle conditions were 20 sec at 96°C for the initial denaturation, followed by two cycles of 96°C for 15 sec, 48°C for 25 sec, 72°C for 65 sec, five cycles of 96°C for 12 sec, 47°C for 20 sec, 72°C for 60 sec, 33 cycles of 96°C for 12 sec, 46°C for 20 sec, 72°C for 60 sec, and a final extension step at 72°C for 1 min, on a Perkin Elmer GeneAmp™ PCR system 2400 (Applied Biosystems, Foster City, USA). Successful PCR amplification was assessed by agarose gel electrophoresis of 5 µl of the product on a 1.5 % (W/V) gel (1 x TAE) (Bio-Rad laboratories, California, USA). The remaining PCR product from successfully amplified samples was purified using the Roche High Pure Product Purification Kit (Roche Diagnostics, Penzberg, Germany). DNA concentration of the purified product was estimated by comparing 2 µl of each sample against 25 ng of double-stranded lambda DNA (from bacteriophage lambda cI857 Sam 7) (Roche Diagnostics, Penzberg, Germany).

Between 50 and 100 ng of the purified product was cycle sequenced with 3.2 pmol of each PCR primer in separate reactions, 2 µl BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, USA)

in a final volume of 10 μl under the following thermal cycle conditions: 96°C for 10 sec, 47°C for 5 sec, 60°C for 4 min for 25 cycles.

Cycle sequencing products were precipitated by a standard sodium acetate/ethanol precipitation procedure. The dried pellet was resuspended and run on the capillary based ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA). Automated DNA sequences for each specimen were inspected and edited using Chromas Lite v.2.0 (Technelysium Pty Ltd, Tewantin, Qld, http://www.technelysium.com.au). All sequences generated in this study were submitted to GenBank under the accession numbers listed in Table 3.1.

Sequence Alignment.

Sequences were initially aligned in DAPSA v.4.91 (Harley 2001). The unaligned ends were trimmed, leaving a homologous region of 402 bp that was used for all subsequent analyses.

To further optimise the alignment and for weighting consideration, we obtained full-length mitochondrial 16S gene sequences for the firefly (Pyrocoelia rufa) (accession: NC_003970) and the red flour beetle (Tribolium castaneum) (accession: NC_003081) from GenBank. Using RNAFold (Hofacker et al 1994) via an Internet interface, we obtained the predicted secondary structures for both molecules. RNADraw v.1.1b2 (Matzura & Wennborg 1996) permitted stem and loop regions to be defined for each molecule. Both molecule sequences were aligned to an arbitrarily chosen ingroup sequence, the ends trimmed and the most likely conserved

Table 3.1 Trogid and outgroup specimens used in the analyses and their collection data.

Genus Species	Region.	Collection data.	Database code.	GenBank Accession #.
Bolboceras (outgroup)				
B. species 1		Botswana; Deschodt & Tshikae	B1	EF570408
B. species 1		Botswana; Deschodt & Tshikae	B2	EF570409
B. species 2		Botswana; Deschodt & Tshikae	B3	EF570410
B. species 2		Botswana; Deschodt & Tshikae	B4	EF570406
Polynoncus				
P. brevicollis	South America	Chile - San Antonio; Aguas Buenas; 3.ix.1999; Leg. V. Manuel Dieguez M.	3A	EF570366
P. brevicollis	South America	Chile - San Antonio; Aguas Buenas; 3.ix.1999; Leg. V. Manuel Dieguez M.	3B	EF570373
P. brevicollis	South America	Chile - San Antonio; Aguas Buenas; 3.ix.1999; Leg. V. Manuel Dieguez M.	3C	EF570385
P. brevicollis	South America	Chile - San Antonio; Aguas Buenas; 3.ix.1999; Leg. V. Manuel Dieguez M.	3D	EF570369
P. bullatus	South America	Chile - Curico; Rauco-fundo La Pancora; 31.vii.1999; Leg. Victor Manuel Dieguez	1	EF570378
Omorgus				
O. suberosus	South America	Argentina; La Roja; Aimogasta; Ruto 60; S28°35' - W66°44'; dead horse; 25.ii.2002; C. Medina & C.H. Scholtz	5A	EF570389
O. suberosus	South America	Argentina; La Roja; Aimogasta; Ruto 60; S28°35' - W66°44'; dead horse; 25.ii.2002; Claudia & Clarke	5B	EF570386
O. suberosus	South America	Argentina; La Roja; Aimogasta; Ruto 60; S28°35' - W66°44'; dead horse; 25.ii.2002; Claudia & Clarke	5C	EF570393
O. suberosus	South America	Argentina; La Roja; Aimogasta; Ruto 60; S28°35' - W66°44'; dead horse; 25.ii.2002; Claudia & Clarke	5D	EF570374
O. species 1	North America	USA: Arizona, Santa Cruz County; Pena Blanca Canyon; 17.viii.2002; W. Moore	8	EF570370
O. candidus	Australia	Yarramulla HS.; Undara Nat. Park, Q.; 9.ii.2003; G. Monteith; Dung trap	12B	EF570379
O. candidus	Australia	Qld: 26°23'Sx146°12'E; Charleville, 5km NW; 3-5Mar2003, 310m; G. Monteith, C. Burwell; Mulga 51123	19A	EF570390
O. costatus	Australia	Gilbert River nr. Georgetown, N.Qld.; 4.ii.2003; J. Hasenpusch	10	EF570391
O. demarzi	Australia	Garradunga, N.Qld.; 2.ii.2003; J. Hasenpusch	14	EF570367
O. demarzi	Australia	Qld: 26°23'Sx146°12'E; Charleville, 5km NW; 3-5Mar2003, 310m; G. Monteith, C. Burwell; Mulga 51123	17	EF570380
O. asperulatus	Africa	Nossob Camp; Kalahari Gemsbok Park; 25.i.2003	31	EF570412
O. radula	Africa	KNP: Skukuza Camp; 12.ii.2003	38A	EF570387
O. squalidus	Africa	Kgalagadi Transfrontier Park; S26°24'29.4" E20°42'32.7"; 913 m; K1S10; P. Tshikae	73A	EF570407
O. species 2	Africa	Kgalagadi Transfrontier Park; S26°24' 29.4" E20°42'32.7"; 913 m; K1S10; P. Tshikae	102A	EF570411
Trox (Holarctic)				
T. terrestris	North America	USA: Florida, Archbold, Biol. Stat., near L. Placid; 22-23.xi.2002; V. Grebennikov leg.	7A	EF570383
T. terrestris	North America	USA: Florida, Archbold, Biol. Stat., near L. Placid; 22-23.xi.2002; V. Grebennikov leg.	7B	EF570394
T. gemmulatus	North America	Mexico: Chihauhau; Creel, 11.vii.2002; W. Moore 100%EtOH	9A	EF570363
T. gemmulatus	North America	Mexico: Chihauhau; Creel, 11.vii.2002; W. Moore 100%EtOH	9B	EF570359
T. aequalis	North America	USA: Alabama; Madison County; Huntsville; Monte Sano State Park;	90	EF570375

		21.v.2005; Paul K. Lago		
T. hammatus	North America	USA: New Jersey; Somerset Co.; Hutcheson Memorial Forest; Disturbed field; 3-5.viii.2004; pitfall w/ dog faeces	83A	EF570388
T. hammatus	North America	USA: New Jersey; Somerset Co.; Hutcheson Memorial Forest; Disturbed field; 3-5.viii.2004; pitfall w/ dog faeces	83B	EF570381
T. tuberculatus	North America	USA: Mississippi; Coahoma County; Mississippi River; 10mi WNW Clarksdale; 10.v.2005; Jonas King	95	EF570364
T. spinulosis	North America	USA: Mississippi; Coahoma County; Mississippi River; 10mi WNW Clarksdale; 10.v.2005; Jonas King	96A	EF570362
T. spinulosis	North America	USA: Mississippi; Coahoma County; Mississippi River; 10mi WNW Clarksdale; 10.v.2005; Jonas King	96B	EF570376
T. niponensis	Japan	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.vi.2005; Satoru Nu leg.	100	EF570413
T. opacotuberculatus	Japan	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.vi.2005; Satoru Nu leg.	101A	EF570382
T. opacotuberculatus	Japan	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.vi.2005; Satoru Nu leg.	101B	EF570368
T. setifer setifer	Japan	Japan: Nara-ken; Kamikitayama-mura; Mt. Wasamatayama; 5.vi.2005; Satoru Nu leg.	99	EF570365
Trox (African)				
T. arcuatus	Africa	Kweekkraal farm; 9km west of Riversdal; lamb carcass; U. Kryger; 22.x-8.xi.2003	69	EF570395
T. capensis	Africa	RSA; Harkerville Forest; S34°02'04.8" E23°16'23.0"; 27-28.vi.2004; ODONTO 04; Deschodt & Momberg	106B	EF570392
T. capensis	Africa	RSA; Harkerville Forest; S34°01'59.9" E23°16'24.2"; 27-28.vi.2004; ODONTO 05; Deschodt & Momberg	107A	EF570360
T. caffer caffer	Africa	RSA, Northern Cape Prov.; 6km S of Kamieskroon, A. Frolov & C. Deschodt leg.; AF-0028(8); 1-13.IX.2003; S 30°15'58.15" E 17°55'30.19"	67B	EF570384
T. fascicularis rowei	Africa	KZN: Giant's Castle Reserve; 25.viii.2002	46A	EF570396
T. fascicularis rowei	Africa	KZN: Sani Pass; Middle Level; 29°35'57.3"S 29°18'25.3"E; 6.viii.2003	57A	EF570361
T. montanus	Africa	Kenya: Aberdare N.P.; 30.x.2002 - 03.xi.2002; H - 3100m; Between Kiandongoro & Mutobio gates, Fishing Lodge; V. Grebennikov leg.	22A	EF570377
T. montanus	Africa	Kenya: Aberdare N.P.; 30.x.2002 - 03.xi.2002; H - 3100m; Between Kiandongoro & Mutobio gates, Fishing Lodge; V. Grebennikov leg.	22B	EF570371
T. nanniscus	Africa	Baviaanskloof; Barolof; U. Kryger; 22.x-8.xi.2003	64	EF570401
T. planicollis	Africa	S. Namibia; Boom River, canyon ca. 10km of estuary; A. Frolov leg.; 30-31.iii.2003; S27°55'28.3" x E17°01'14.6"; h=590m, under stones	25A	EF570403
T. planicollis	Africa	ex. Parys; preserved 13.x.2003; (Clarke)	62	EF570397
T. rhyparoides	Africa	KZN: Ngome; A. Frolov	54A	EF570402
T. rhyparoides	Africa	KZN: Ngome; A. Frolov	55A	EF570404
T. rhyparoides	Africa	Baviaanskloof; Poortjies (Poort); U. Kryger; 22.x-8.xi.2003	70A	EF570372
T. rudebecki	Africa	KZN: Sani Pass; Middle Level; 29°35'57.3"S 29°18'25.3"E; 6.viii.2003	56	EF570405
T. squamiger	Africa	Willowmore; Timbi2; U. Kryger; 22.x-8.xi.2003	66	EF570398
T. sulcatus	Africa	Baviaanskloof; Poortjies (Poort); U. Kryger; 22.x-8.xi.2003	71	EF570399
T. talpa	Africa	Kweekkraal farm; 9km west of Riversdal; lamb carcass; U. Kryger; 22.x-8.xi.2003	68	EF570400

coleopteran stem regions approximated from areas where the stem regions of the complete molecule sequences overlapped (Appendix 3.1). The initial alignment was then optimised by eye on the basis of the predicted secondary structure of the molecule.

Model of sequence evolution.

Modeltest (Posada & Crandall 1998) uses explorative searches through PAUP4.0b10 and estimates the likelihood of up to 56 models in a hierarchical order of increasing complexity to select the model of sequence evolution that best fits the dataset. It also provides corrected parameters for the selected model to be used in subsequent phylogenetic analyses.

The General Time Reversible + Invariant sites + Gamma distribution (GTR+I+G) model – which takes into account that all six pairs of substitutions have unequal rates, that the bases occur at different frequencies, that there are invariant sites, and that mutation rates at variable sites are gamma distributed, was selected under the Akaike Information Criterion (AIC).

Testing for Rate Heterogeneity.

Since most tree drawing strategies assume that there is no rate heterogeneity among and along sequences being considered, and violation of this assumption can result in incorrect phylogenies, rate heterogeneity in the dataset was assessed.

First we performed an exploratory probe using Tajima's relative rate test (also known as the three taxon test) in MEGA v.3.1 (Kumar et al 2001). From an uncorrected p-distance tree, one ingroup taxon with the visually shortest branch-length, one ingroup taxon with the visually longest branch-length and an outgroup taxon was selected and used to evaluated whether the distance from the outgroup taxon to each of the ingroup taxa differed significantly. Other randomly selected ingroup combinations were also considered, in this simplistic and therefore preliminary assessment of rate heterogeneity.

In addition, RRTree v.1.1.11 which performs pairwise relative rate tests between user-defined lineages and is a much more robust test for rate heterogeneity than Tajima's relative rate test (Robinson-Rechavi & Huchon 2000) was used. Four ingroup lineages identified by an exploratory neighbour-joining tree were defined: Omorgus (1), Polynoncus (2), Holarctic Trox (3) and African Trox (4) for this test.

Lastly, we performed a rate likelihood test. The likelihood scores of two runs, one with a molecular clock imposed and one without, were compared as a final assessment of rate heterogeneity. These runs were performed in PAUP4.0b10 (Swofford 2003) under the model of sequence evolution selected in ModelTest under the AIC, detailed previously.

Phylogenetic analyses.

- Distance-based analyses (Neighbor-Joining & Minimum Evolution).

One of the two major approaches in analysing molecular data is to reduce all the variation seen to a single numerical value, often defining the degree of similarity or dissimilarity between a pair of sequences, and using this value to construct the phylogeny. This degree of similarity, or distance, is used in Neighbor-Joining (NJ) and Minimum-Evolution (ME) analyses.

Distance trees (NJ and ME) were constructed in MEGA v.3.1 with the Tamura-Nei model, complete deletion of gaps/missing data, and a gamma distribution of 0.3475. Node reliability was assessed by 10,000 bootstrap replications.

- Nucleotide-based analyses (Parsimony, Maximum Likelihood & Bayesian Inference).

These analyses consider each nucleotide site as a discrete entity and use each of these as discrete data points when constructing a phylogeny. Because of the nature of these analyses, they are often far more time-consuming and complex than distance-based methods but they retain valuable evolutionary information that would be lost otherwise.

PAUP4.0b10 was used for both the Parsimony and Maximum Likelihood analyses. For both analyses we defined the outgroup sequences as a monophyletic sistergroup to our ingroup sequences for rooting purposes.

Because of the relatively high number of taxa in the analyses (Page & Holmes 1998), we used only the full heuristic search option, employing bootstrapping to assess node support (1000 pseudo-replicates for the Parsimony runs and 100 pseudo-replicates for the Maximum Likelihood runs) due to of the computational complexity of analysing a large dataset. In both analyses, sequences were added randomly (100 random additions) and we used the tree-bisection-reconnection (TBR) branch swapping algorithm.

For the Parsimony analysis, gaps were treated as a fifth character state and the stem regions identified from the predicted secondary structure were upweighted relative to the loop regions, as stem regions are more conserved and less likely to reach a substitution saturation point that would make them misleading in our analysis (Page & Holmes 1998). In order to ascertain what effect a priori weighting would have on our results, three weighting schemes were investigated – no weighting (all bases have a weight of 1), differential stem:loop weighting (where bases identified as stem regions are assigned a weight of 2, and all other bases have a weight of 1) and differential stem:loop:gap weighting (where stem regions have a weight of 2, positions with a gap in any of the sequences have a weight of 0.5 and all remaining bases have a weight of 1). The values used for the reweighting were arbitrarily chosen. Successive weighting with the rescaled consistency (RC) index was also performed.

MrBayes v.3.0B4 (Huelsenbeck & Ronquist 2001) was used for the Bayesian analysis. We used likelihood settings that were in keeping with the

GTR+I+G model as selected by Modeltest (lset nst=6 rates=gamma). Each of a range of temperatures (from 0.0005 to 0.06) was assessed over 2,000,000 generations, with sampling and tree saving every 500 generations (mcmcp ngen=2000000 samplefreq=500 printfreq=500 temp=[0.0005, 0.001, 0.005, 0.008, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06] savebrlens=yes). A burn-in value of 50, effectively discarding the first 25,000 trees, was set for all runs.

RESULTS

Sequence statistics.

The 402 bp fragment of the 16S mitochondrial gene used for phylogenetic analyses was AT-rich (GC% = 22.9) as is the norm for insect mitochondrial sequences (Brower 1984, Damgaard et al 2005, Forgie 2003). The empirically determined transition:transversion ratio (R) was 0.5. There was a relatively high proportion of invariable sites (25.7%), and the shape of our gamma distribution was 0.3475 and skewed to the left indicating rate heterogeneity across the gene region sequenced.

Testing for rate heterogeneity.

We found no significant difference among taxa in the three-taxon test when using Trox fascicularis rowei and Trox sp. 2 as ingroup taxa and Bolboceras sp. 2 as the outgroup (p=0.869). Other randomly selected ingroup

taxa also displayed no significant difference using the Tajima relative rate test.

Since we defined four lineages for our RRTree analysis, the risk threshold for a type 1 error rises to 1.25% (ie in order for the p-value to be considered significant, it must be less than 0.0125). The resulting output table (Table 3.2) shows non-significant differences between all lineages compared.

Finally, given the chi-square value for 53 degrees of freedom, at p=0.05, is 70.99; the difference between the likelihood score for the run with no molecular clock imposed (-ln L 3094.20) and the likelihood score for the run with a molecular clock imposed (-ln L 3140.29), was not significant.

Table 3.2 RRTree output file with the p-values for each pairwise comparison of defined lineages.

Outgroup	# of seq.	Lineage A	# of seq.	Lineage B	# of seq.	p-value
Outgroup	4	Omorgus	14	Polynoncus	5	0.642649
Outgroup	4	Omorgus	14	Holarctic Trox	14	0.442906
Outgroup	4	Omorgus	14	African Trox	18	0.356013
Outgroup	4	Polynoncus	5	Holarctic Trox	14	0.244725
Outgroup	4	Polynoncus	5	African Trox	18	0.229065
Outgroup	4	Holarctic Trox	14	African Trox	18	0.786797

Phylogenetic analyses.

- Minimum Evolution analysis.

The Minimum Evolution analysis resulted in seventeen equally good trees of which the sum of branch lengths (SBL) was 1.84. Figure 3.2 shows the resulting tree with bootstrap support from 10 000 data replications. We can clearly define at least four moderately to well supported major groups – Polynoncus, Omorgus, Holarctic Trox, and African Trox – with bootstrap values of 98, 82, 60 and 67, respectively. Within groups, especially the two Trox groups, the branches are very short, often with low bootstrap support. There are moderately supported associations firstly between Polynoncus and Omorgus (bootstrap value of 71), but with each genus being well supported, and secondly between the Holarctic Trox group and the Polynoncus-Omorgus group (bootstrap value of 60). African Trox is basal to all other lineages.

- Parsimony analysis.

Of the 402 basepairs entered into the analysis, 245 were excluded as uninformative, leaving 157 sites that were parsimony informative.

The unweighted heuristic search resulted in sixteen equally parsimonious trees, 601 steps in length (Fig. 3.4). When we employed stem:loop differential weighting (132 characters with a weight of 1 and 25 characters with a weight of 2), we obtained fifteen equally parsimonious trees that were 689 steps long (Fig. 3.5). Only one tree of 626.5 steps was found

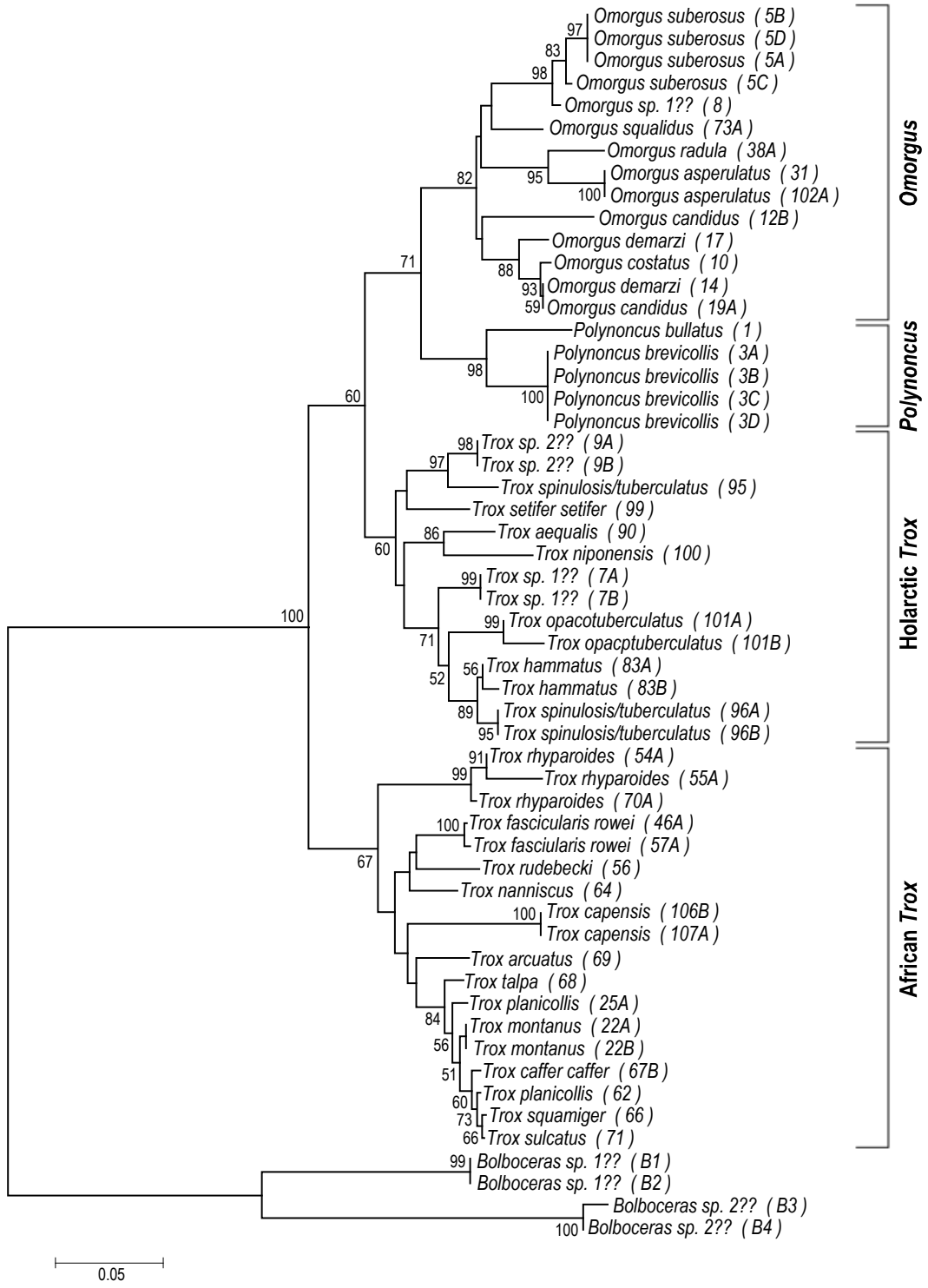

Figure 3.2 Bootstrap consensus tree for Minimum Evolution analysis (complete deletion of gaps, 10000 bootstrap pseudo-replicates, Tamura-Nei, gamma = 0.3475) showing four major groups.

when stem:loop:gap differential weighting (113 characters with a weight of 1, 25 characters with a weight of 2 and 19 characters with a weight of 0.5), was used (Fig. 3.6). The consistency index value (CI = 0.44), the retention index (RI = 0.82) and the rescaled consistency index (RC = 0.36) differed only very slightly between the unweighted and differential weighting schemes. The successive reweighted analysis also resulted in a single tree (Fig. 3.3), 239.2 steps long (CI = 0.61, RI = 0.88 and RC = 0.53).

Although the change from unweighted to differential weighting schemes did not have a marked effect on the homoplasy index (HI = 0.56 for all three schemes in question), it did reduce the number of equally parsimonious trees, as the stem:loop:gap weighting scheme produced one single best solution. However, the successive reweighted analysis resulted not only in a single best solution, but also reduced the homoplasy index to 0.39, and recovered four well-supported monophyletic lineages.

Figure 3.3 represents the 50% majority rule consensus tree (CI = 0.61, RI = 0.88 and RC = 0.53) for the successive reweighted analysis, which displays very few basal polytomies and is on the whole well-resolved. The 50% majority rule consensus trees (CI = 0.44, RI = 0.82, RC = 0.36) of both the unweighted and differentially weighted analyses are shown in Figures 3.4, 3.5 and 3.6 respectively. Differential weighting markedly improved the bootstrap support for the internal nodes, but weakened the support for certain terminal nodes to such an extent that resolution was lost and more polytomies formed.

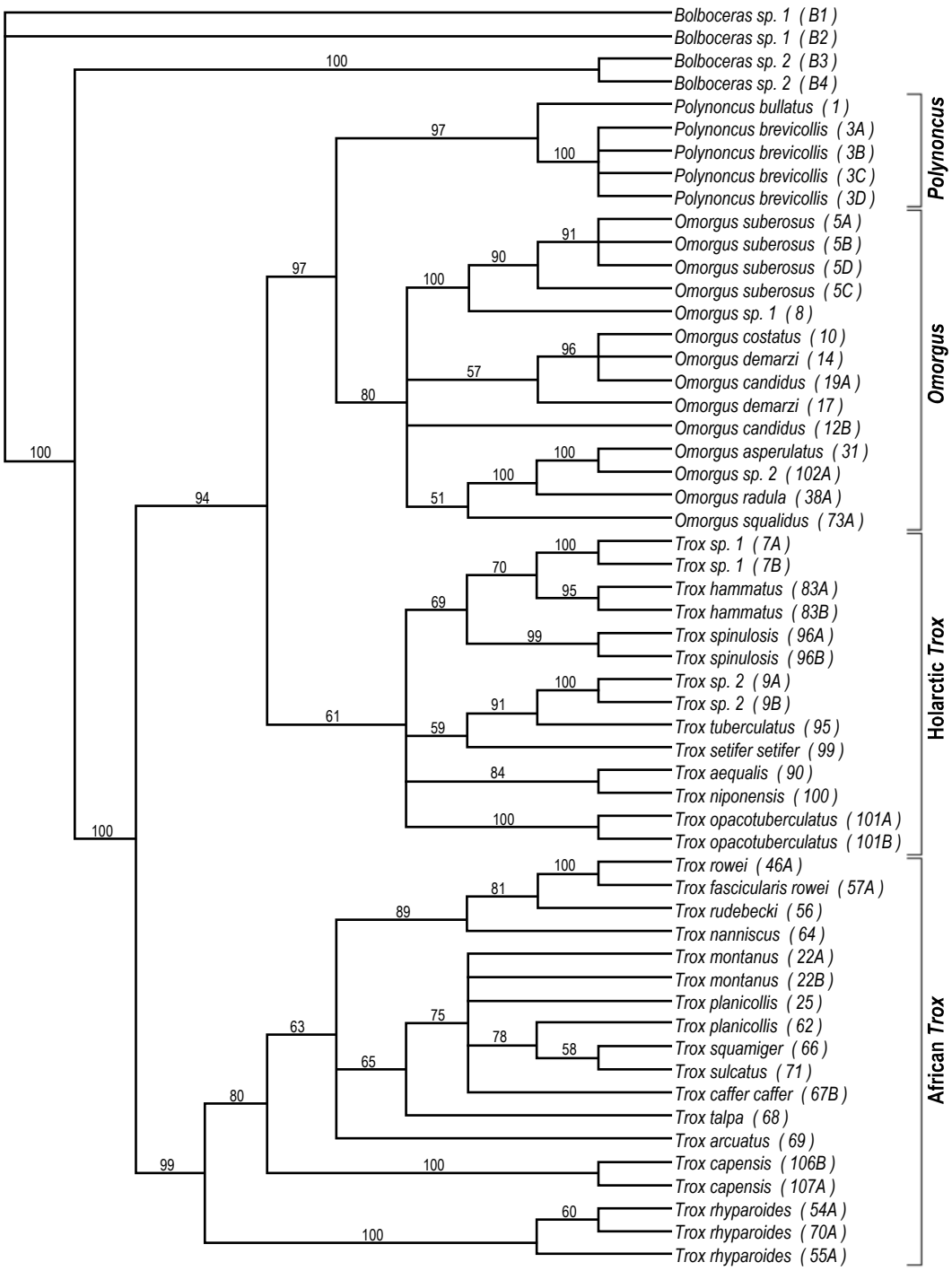

Figure 3.3 Bootstrap 50% majority rule tree for the successive weighted Parsimony analysis (full heuristic search, 1000 bootstrap pseudo-replicates, gaps = 5th character state, uninformative characters excluded). (CI = 0.61, RI = 0.88, RC = 0.53)

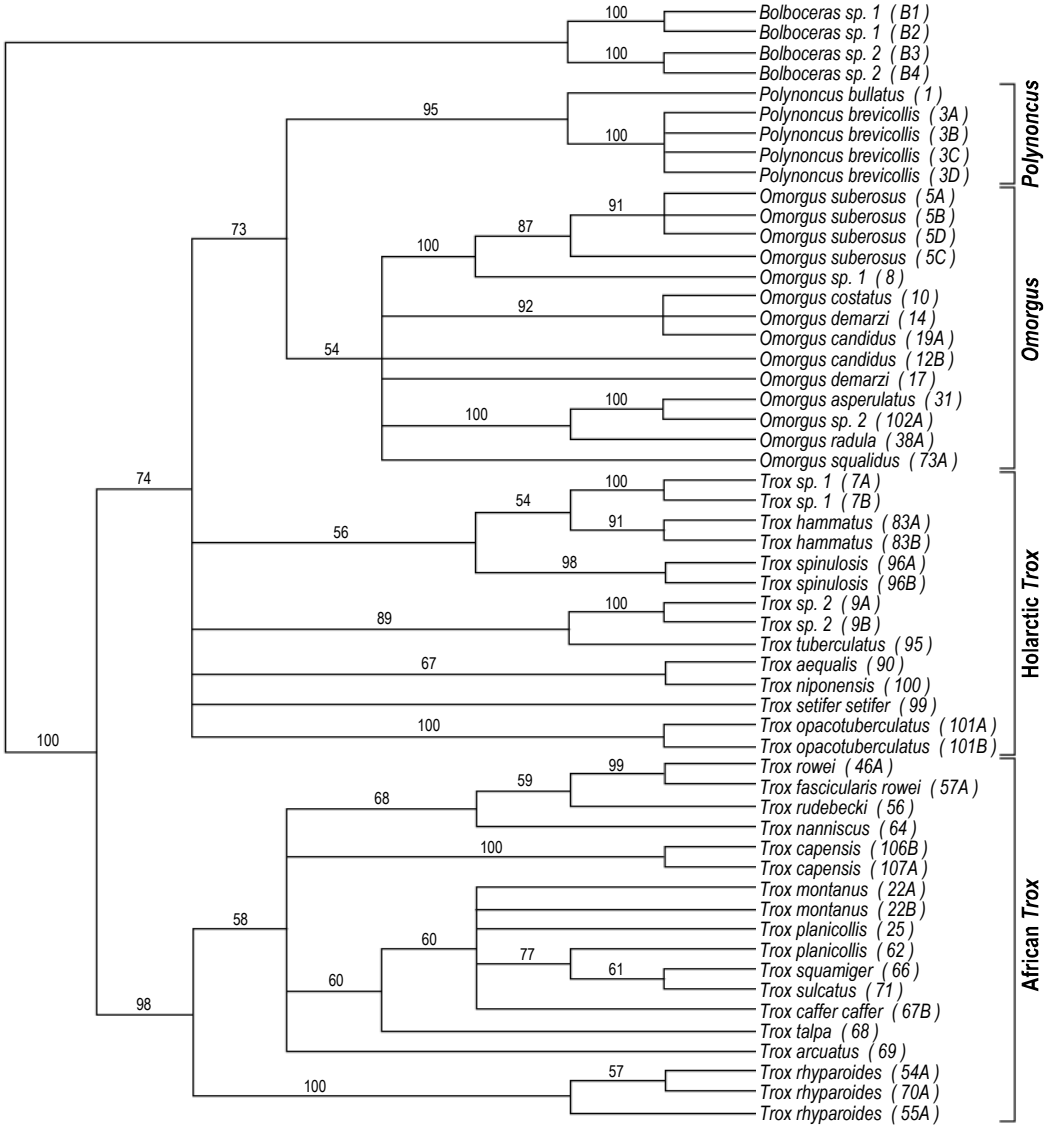

Figure 3.4 Bootstrap 50% majority rule tree for the unweighted Parsimony analysis (full heuristic search, 1000 bootstrap pseudo-replicates, gaps = 5th character state, uninformative characters excluded). (CI = 0.44, RI = 0.82, RC = 0.36)

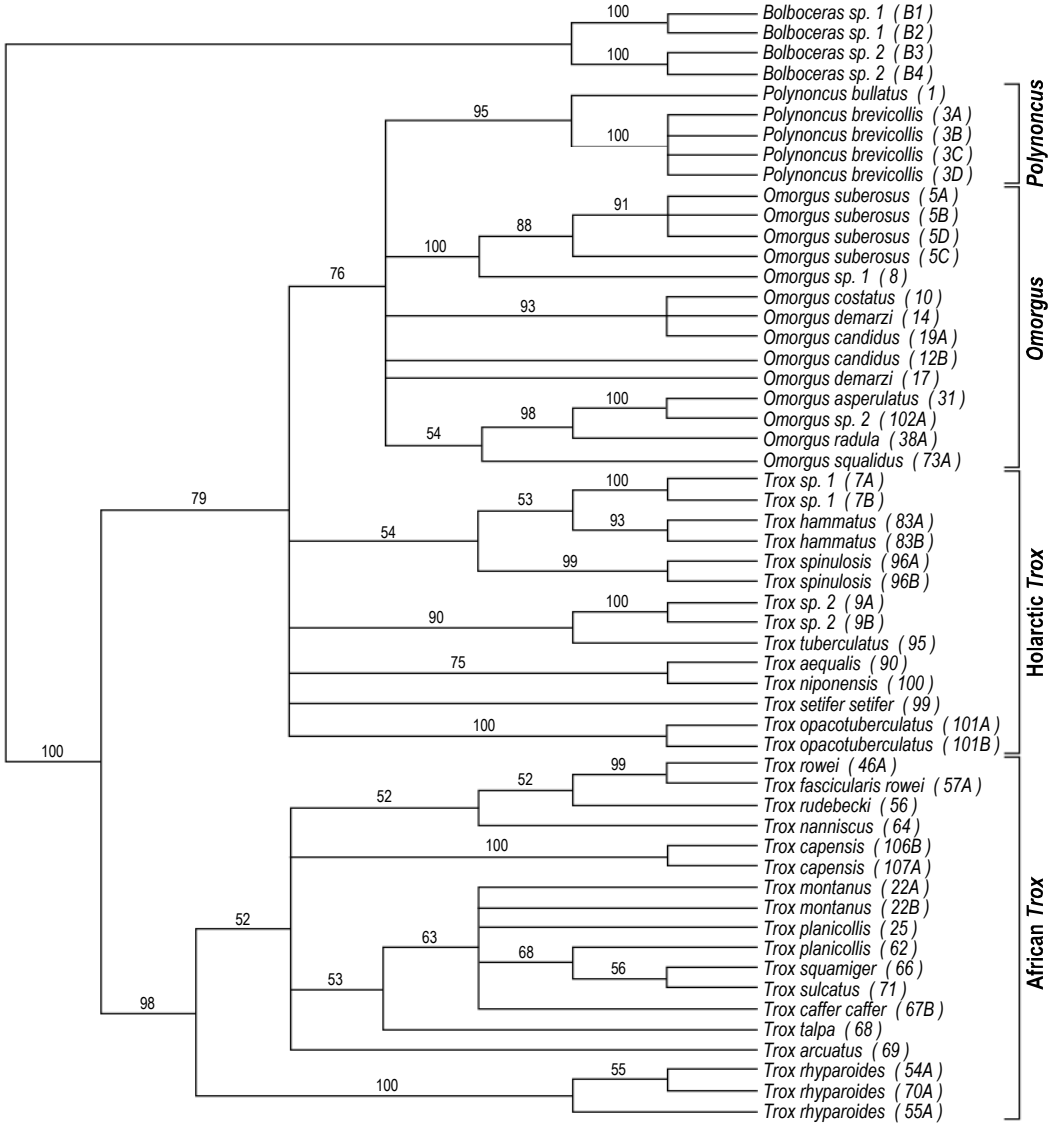

Figure 3.5 Bootstrap 50% majority rule tree for the 2:1 stem:loop reweighted Parsimony analysis (full heuristic search, 1000 bootstrap pseudo-replicates, gaps = 5th character state, uninformative characters excluded). (CI = 0.44, RI = 0.82, RC = 0.36)

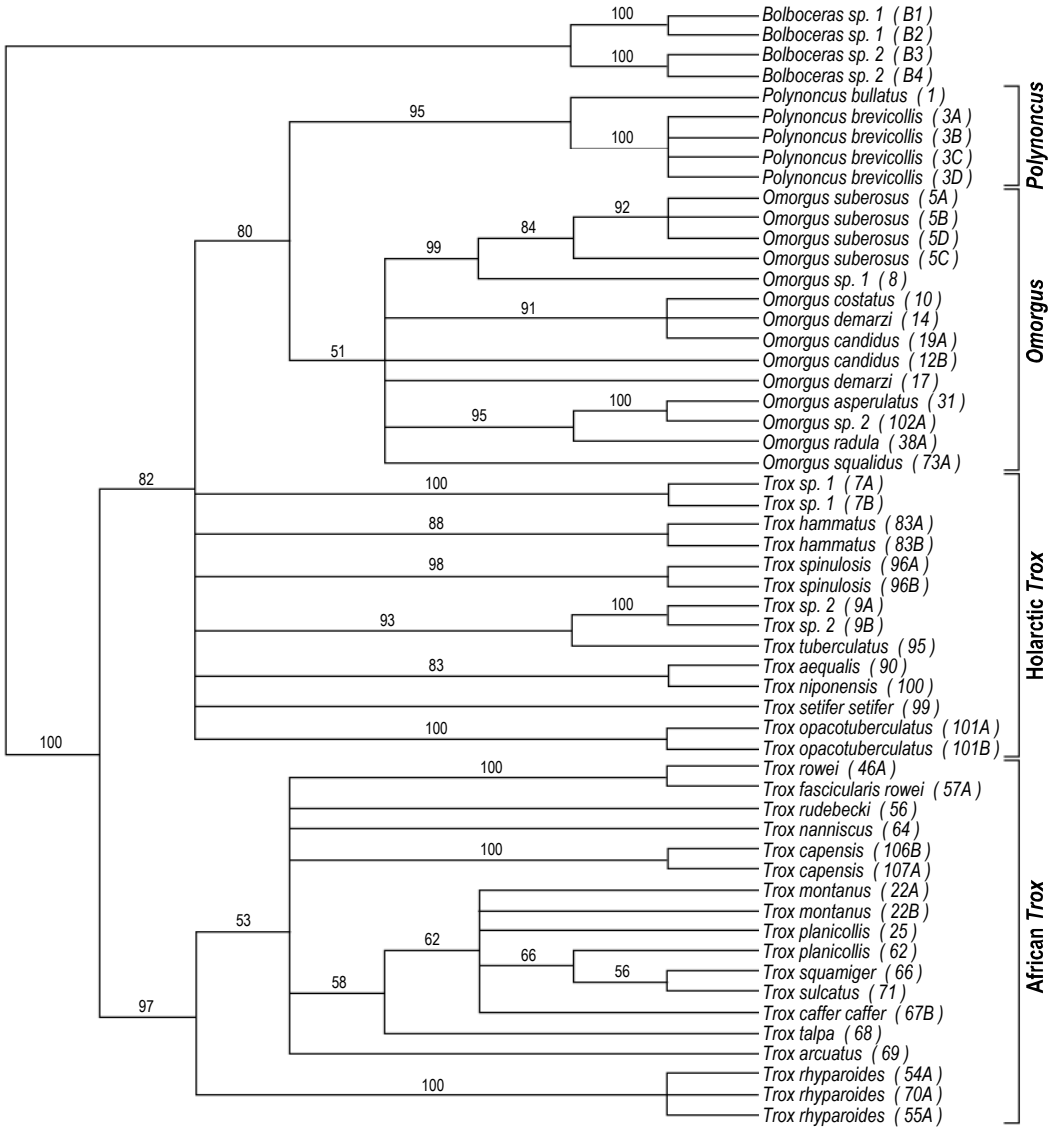

Figure 3.6 Bootstrap 50% majority rule tree for the 2:1:0.5 stem:loop:gap reweighted Parsimony analysis (full heuristic search, 1000 bootstrap pseudo-replicates, gaps = 5th character state, uninformative characters excluded). (CI = 0.44, RI = 0.82, RC = 0.36)

For all analyses, African Trox forms a strongly supported and separate basal group (bootstrap values of 97 – 99). Holarctic Trox presents as a poorly resolved polytomy and Omorgus forms a poorly supported group (bootstrap values of 54 and 51 for the unweighted and stem:loop:gap differentially weighted analyses respectively) that collapses into a polytomy under certain weighting conditions (the stem:loop differentially weighted analysis). For the successive reweighted analysis, however, there was 61 % bootstrap support for the monophyly of Holarctic Trox, whilst Omorgus, Polynoncus and African Trox had bootstrap values of 80, 97 and 80, respectively. In all cladistic analyses, Polynoncus and Omorgus grouped together with Holarctic Trox with bootstrap values ranging from 74 to 94 %, indicated a closer association of these taxa to each other than to African Trox.

- Maximum Likelihood.

Figure 3.7 shows the 50% majority rule consensus tree of the Maximum Likelihood analysis. An exploratory analysis of 10 replicates resulted in a single tree with a log likelihood score of 3094.20008. The final tree, the product of 100 replicates, shows much the same structure as those obtained with the phenetic and parsimony analyses. The sister-taxon grouping of Polynoncus and Omorgus is well supported (bootstrap value of 70), with the monophyly of each group being fairly well supported (bootstrap values of 97 and 63 respectively). African Trox was once again a strongly supported monophyletic group (bootstrap value of 81). Holarctic Trox, in

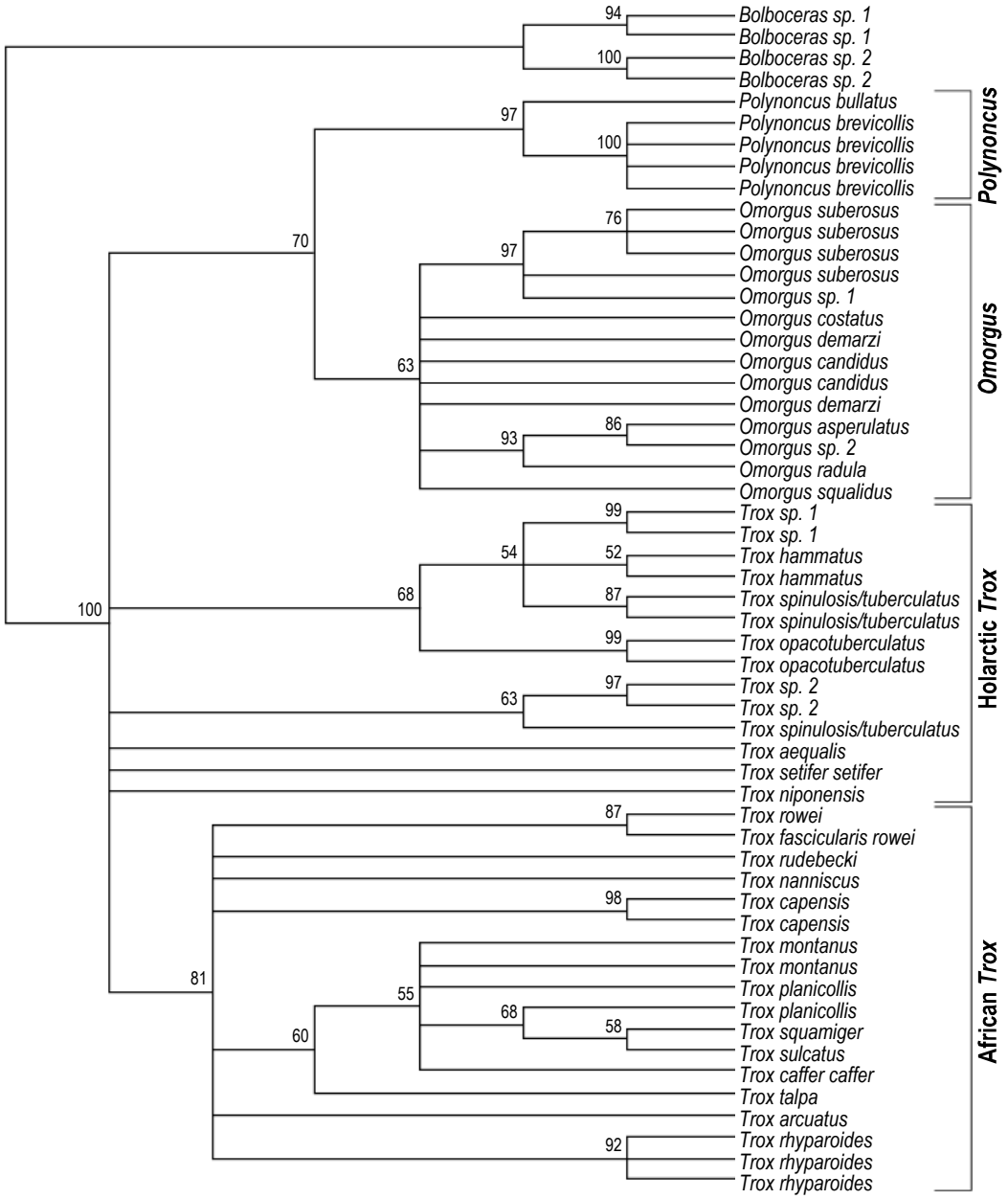

Figure 3.7 Bootstrap 50% majority rule consensus tree for Maximum Likelihood analysis (full heuristic search, 100 bootstrap pseudo-replicates).

contrast, is a mostly unresolved polytomy with no logical regional grouping evident for the representative species.

- Bayesian analysis.

A representative tree of all Bayesian runs is shown in Figure 3.8. Regardless of the temperature specified, the statistics and general topology of the trees remained the same, with posterior probabilites differing only very slightly, if at all.

Polynoncus and Omorgus each form a strongly supported group (posterior probabilities of 1.0 and 0.97 respectively) and have a strongly supported sister-taxon association with each other (posterior probability of 0.99). African Trox forms a very well supported monophyletic group (posterior probability of 0.97) but Holarctic Trox once more collapses into an ill-resolved polytomy.

DISCUSSION

Our molecular phylogeny only partially supports the morphological phylogeny proposed by Scholtz (1986). In all analyses, both distance- and nucleotide-based, we find four major groups (Polynoncus, Omorgus, Holarctic Trox (Trox s. str.) and African Trox (Phoberus)), not three as suggested by previous morphological studies. As with Scholtz's 1986 study, Polynoncus and Omorgus are sister taxa and the most derived groups, across all methods

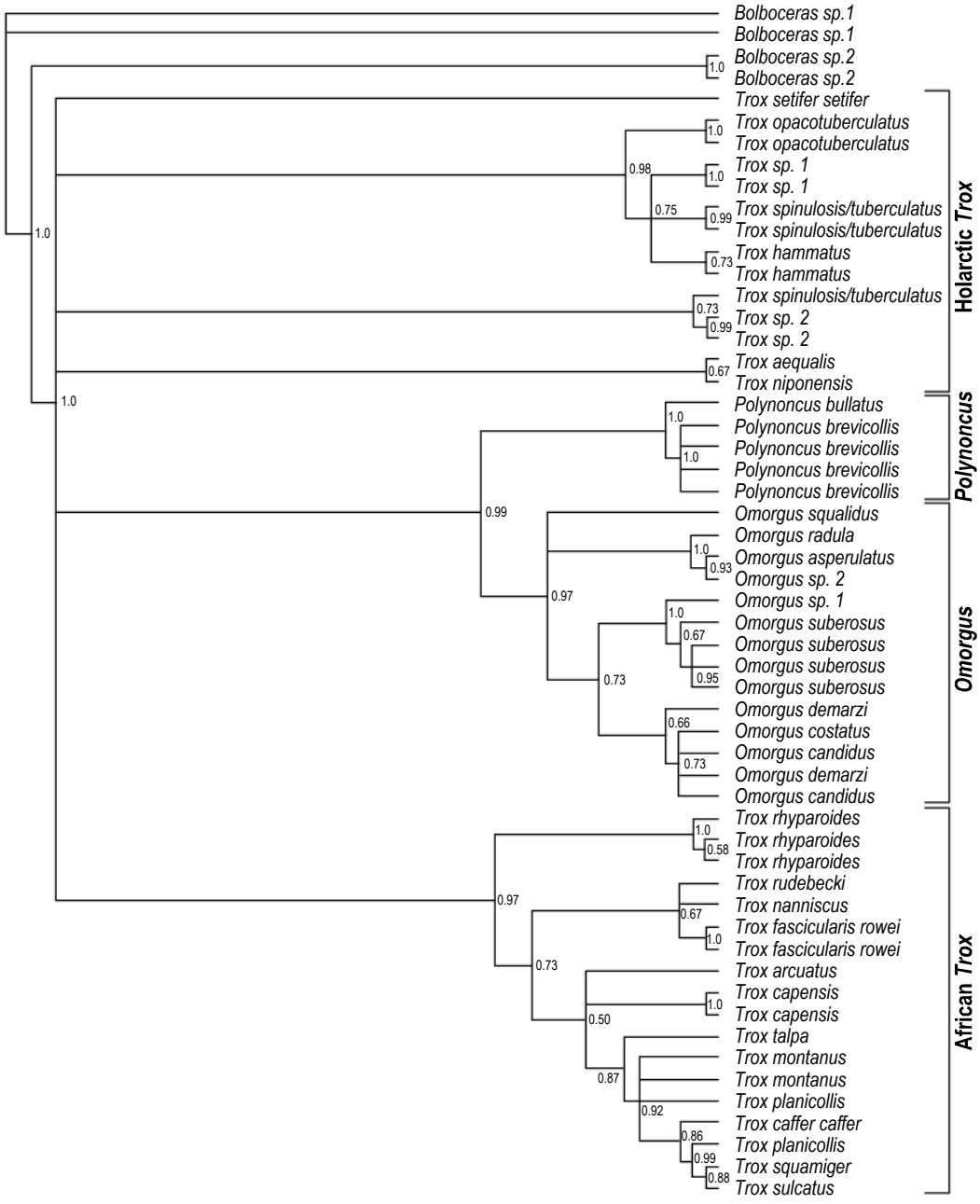

Figure 3.8 Representative tree of Bayesian analyses with posterior probabilities given and showing the four groups.

of analysis. The two Trox subgenera form distinct lineages in all analyses, with Holarctic Trox (Trox s. str.) often being more closely associated with the Polynoncus-Omorgus group than with the other Trox subgenus. This is contrary to what was expected from the morphological phylogeny.

Scholtz (1986) lists several characters that he investigated but found to be of little to no value to the study. Two of these characters, namely the size and sclerotization of the pars basalis of the aedeagus, become meaningful when our results are taken into consideration. Scholtz found that Trox sensu lato and Afromorgus, the African omorgid lineage, both share the derived conditions for the characters. Our results support the derivation of these character states from a common ancestor, rather than the independent acquisition suggested by Scholtz (1986).

We also lay to rest the polyphyly of Phoberus – in all our analyses, Phoberus (indicated African Trox in all the phylogenies) formed a well supported monophyletic group, with the problem species mentioned in the morphological study (most notably Trox capensis and Trox fascicularis rowei) falling well within the group and not sorting with Trox s. str. as predicted by the morphological data. The results of the molecular phylogeny indicate that promoting Phoberus to full generic status may be justified, and are not entirely novel. Scholtz (1980) proposed the subgeneric name for a small subset of African Trox confined to the Cape region of South Africa. In subsequent observations of sclerotised features of the endophallus, Scholtz

proposed the extension of the subgeneric name, which had been accepted by then, to include all African Trox.

Although the zoogeography proposed for Polynoncus and Omorgus remains plausible under our results, that proposed for the origin and spread of Trox sensu latu does not. Our results, with the exception of the Maximum Likelihood and Bayesian analyses, suggest that Phoberus (African Trox) is the ancestral, or more primitive, of the two Trox subgenera, and is indeed basal to all the other genera as a whole; the opposite to that proposed by the morphological dataset.

We still propose a Pangaean origin for this lineage, but suggest that Trox sensu lato might have had its origin in what would be latter-day North Africa. When Gondwana, Laurentia and other small landmasses that would eventually form Europe, Siberia, China and Kazakhstan combined 300 million years ago to form Pangaea, globe-spanning metamorphic belts formed at the sutures (Fig. 3.9). These metamorphic belts would have presented as global mountain chains, called the Pan-African Belts because of their extensive development on the African continent (McCarthy & Rubidge 2005). The Pan-African belts would have offered the ancestral temperate biome as suggested by Scholtz (1986). We propose that the Trox sensu lato lineage originated in the Pan-African Belt that covered most of North Africa about 180 million years ago. When Pangaea split into Gondwana and Laurasia roughly 150 million years ago, the lineage that would become Trox s. str. was separated from the

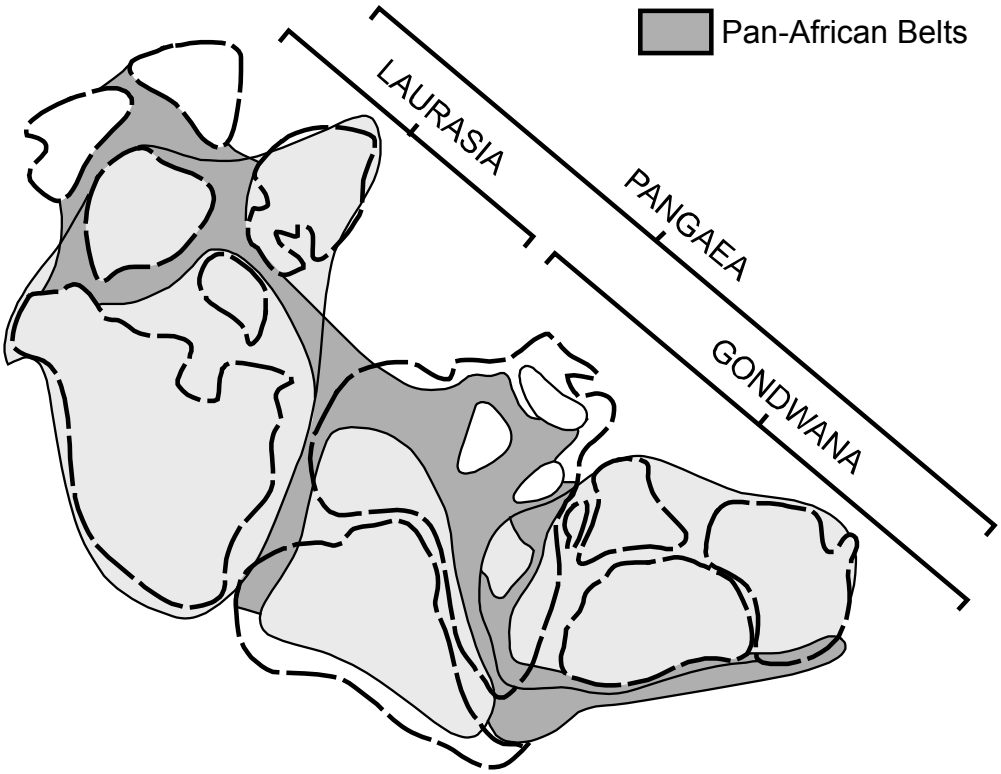

Figure 3.9 A representation of the Pangaean supercontinent as it was approximately 300 million years ago to show the extent of the Pan-African Belts.

Phoberus lineage by the encroaching Tethys Sea (Stanley 1986). The Phoberus lineage would later spread south towards southern Africa via the temperate faunal exchange route that formed during the Tertiary, rapidly colonizing its favoured biome.

The Omorgus lineage probably originated in the area enclosed by the Pan-African Belt (Fig. 3.9), spreading west into latter-day South America, down into Antarctica and across into Australia before Gondwana separated, as suggested by Scholtz (1986). The molecular data does not contest the

theory put forth by Scholtz that Polynoncus is probably closely related to Omorgus.

The short, poorly supported branches in our distance-based analyses, and the polytomies in our nucleotide-based analyses, suggest a period of rapid radiation which our gene was incapable of recording accurately, either because it evolved too slowly or because it evolved too quickly, with nucleotide sites rapidly reaching saturation and obscuring the phylogenetic signal. Because of our fairly high homoplasy values (RC ~0.36), we suspect that the latter might be more likely as addressing the high levels of homoplasy in the dataset through successive weighting with the RC, produced a monophyletic Holartic Trox lineage, supported by 61 %, and resulted in a single most parsimonious tree.

The theory of rapid radiation is further supported by the distribution of our Trox s. str. species in all our phylogenies. The American and Asian specimens are interspersed instead of forming distinct lineages, suggesting that these two groups diverged recently and are still fairly closely related despite their obvious geographical separation.

The partial congruence of the morphological and molecular phylogenies could be due to key differences regarding the two studies. The first and most conspicuous difference is the number of taxa involved. Due to a general lack of suitably preserved specimens, the current study had a small, but representative, sample of 30 species. The morphological study, on the other hand, had access to larger collections of pinned specimens from which

to choose its 230 species. Since molecular analysis techniques are still computationally very expensive and the complexity increases very rapidly the more taxa one includes (Page & Holmes 1998), a molecular study with the same scope as the morphological study would be time-consuming to perform at present. New analysis programs like MrBayes, which had comparatively short run times for this study's data sets, would make it easier to analyse samples that are more representative.

Our study could also have been improved by the inclusion of European samples, as, if our proposed phylogeny is correct, European species would represent a transitional group between the African and Holarctic lineages that our analyses suggest.

Although our sample size was much smaller than that of the morphological study, this is offset by the number of characters we analysed. In a DNA sequence, each nucleotide site is recognized as a discrete character, with the nucleotide present at that site representing the character state (Page & Holmes 1998). The molecular data set included 157 parsimony informative characters from a total homologous dataset of 402 sites – an order of magnitude greater than the 18 characters scored in the morphological study.

Access to suitably preserved specimens limited our choice of outgroup. We successfully sequenced representatives of four possible outgroup genera (Aphodius, Bolboceras, Frickius and Glaresis), of which only Bolboceras gave us satisfactory resolution in addition to forming a natural outgroup to our ingroup sequences. Scholtz's (1986) morphological study used

representatives from Ceratocanthidae to polarise its characters. In a recent phylogeny of higher scarabaeoid relationships based on the 28S and 18S ribosomal genes, Smith et al. (2006) suggest that the trogids represent a more primitive group than the ceratocanthids, and that the bolboceratids are in a clade closely related to the one that houses the trogids. This would suggest that our choice of outgroup was appropriate, and it is unlikely to have negatively affected our results.

Our tests for rate heterogeneity concluded that we were dealing with sequences that have similar rates of mutation across the different taxa. Although it is possible to impose a molecular clock on such sequences in order to calculate when taxa diverged, we were unable to find a satisfactory molecular clock estimate in order to test whether the times of divergence coincide with the formation of land bridges or periods of increased aridity as suggested by Scholtz's morphological study (1986). Brower (1994) suggests a general sequence divergence rate of 2.3 % per million years for insect mitochondrial genes. This value is however insufficiently precise to use for estimation, as the sequence divergence rate for the cytochrome oxidase I (COI) gene in a weevil genus is specified as 1.7 % per million years in the same paper. The only specific reference to the 16S mitochondrial gene is made for orthopteran sequences (2 % per million years). Since the COI gene is accepted as having a significantly faster rate of evolution than the 16S gene (Brower 1984, Page & Holmes 1998) and since coleopteran estimates would be more accurate for our data than orthopteran estimates, we believe

that using the general 2.3 % per million years or even the lower 2 % per million years rates of sequence divergence for our data set would result in a gross under-estimation of divergence times.

The best way forward would be to calculate a specific molecular clock rate for trogids. This has been done for both ratite birds (Cooper et al 2001, Van Tuinen & Hedge 2001) and rodents (Suzuki et al 2003) recently. Both groups used nuclear genes and fossil evidence, and even mitochondrial DNA extracted from sub-fossils in the case of the ratite birds (Cooper et al 2001), in order to extrapolate a molecular clock rate. The fossil record is almost non-existent for most insect groups (Howden 1966), with Trogidae being no exception. Since only a single Miocene trogid fossil (Trox antiquus) is known to this study (Wickham 1909), we suggest that more genes be sequenced (both nuclear and mitochondrial) and analysed in conjunction with our existing 16S data in order to improve phylogenetic resolution and possibly calculate a molecular clock estimate for this family.

We found that the phenetic analyses performed in this study seemed to provide consistently higher resolution than the likelihood analyses. This may be largely due to the different ways in which these methods handle sequence data in constructing trees.

As discussed earlier, phenetic methods reduce all similarity/dissimilarity between two sequences to a mathematical entity, which is then used to construct phylogenies in a pair-wise manner (Page & Holmes

1998). This would conceivably lead to less polytomies and present as a more resolved tree.

Likelihood methods, on the other hand, consider each nucleotide as a discrete unit and attempt to reconstruct a plausible evolutionary path for each sequence within its context. Given that each nucleotide is important in order for these methods to work optimally, the fragment used in this study may have been too short. This was compounded by high homoplasy (25.7%) and a high percentage of uninformative characters (roughly 61% of the sequence). It is possible that the likelihood analyses did not have adequate data to work with, which led to poor resolution.

Combining the current study's sequence data and additional nuclear molecular data with a morphological dataset (including new characters) for concurrent analysis could possibly further improve the resolution of the phylogenies. Damgaard et al (2005) found that comparing molecular phylogenies of water striders based on 16S, 28S or a joint analysis with both genes, returned very few of the clades proposed by the original morphological phylogeny. When the joint molecular data were combined with a rescored morphology matrix, many more clades were retrieved and resolution was improved.

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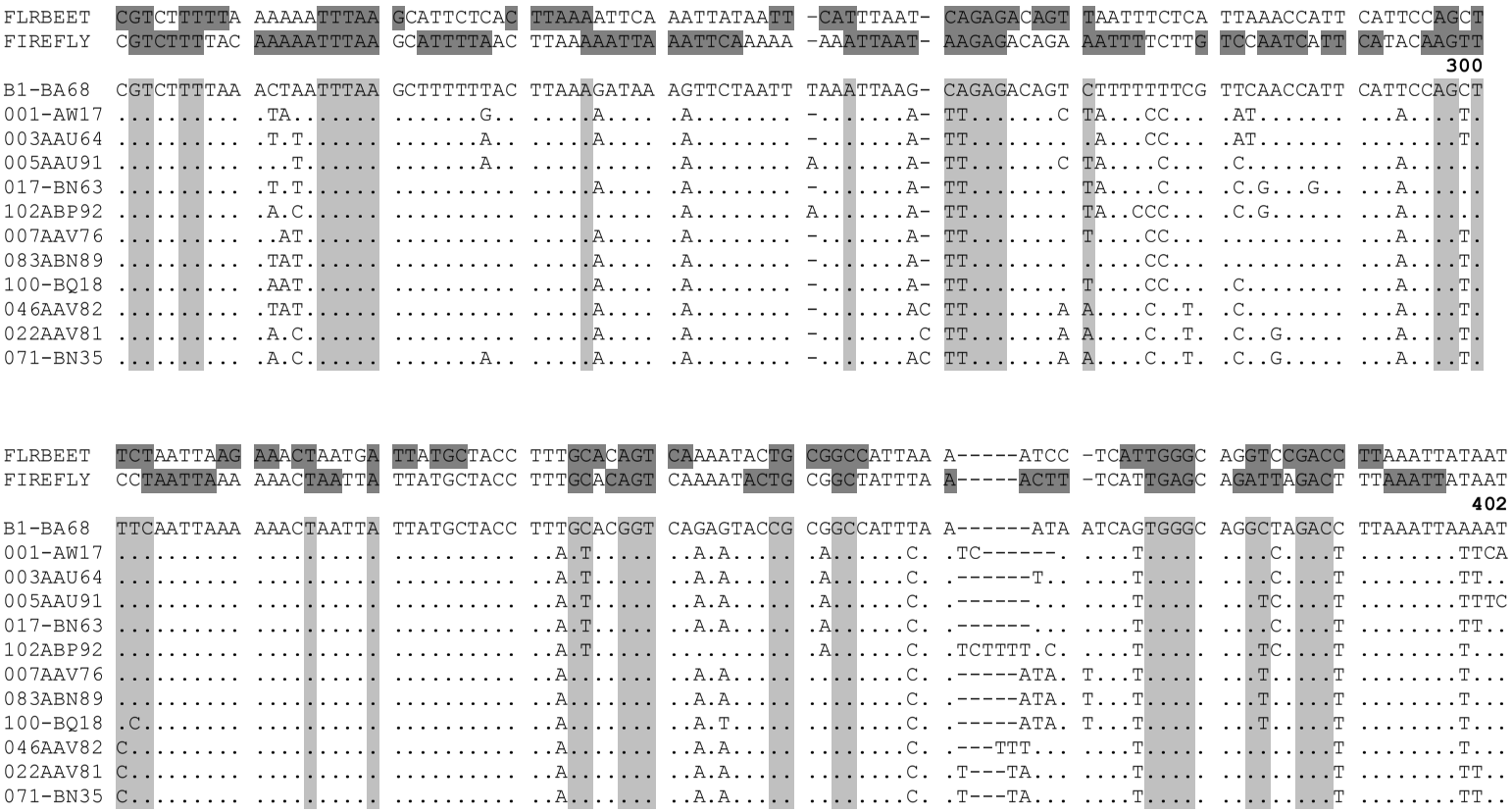
Appendix 3.1 Estimated stem-loop regions of the flourbeetle (FLRBEET) and firefly (FIREFLY) reference sequences. Nucleotides shaded in dark grey are the proposed stem regions identified from the secondary structure, whilst nucleotides shaded in light grey refer to the corresponding putative step structures in the sequences generated in this study. Representative of each of the genera characterised have been aligned to B1-BA68, a Bolbocerus sequence. The dataset comprises 402 nucleotide characteris with missing data being indicated by a ?, gaps inserted for alignment purposes being denoted by a - and nucleotide sites identical to the reference sequence B1-BA68 being indicated by .

```
FLRBEET  TTAC-GCTGT TATCCCTAAG GTAATTTAAT CTTTTAATCC AAAATTC-AG GATCA-ACAA TTCACTAATC AGTGTAAAAA -CAAAAAAAA AGTTAAATCA
FIREFLY  TTAC-GCTGT TATCCCCAAG GTAATTTAAT --TTAAACTC AAAAAAT-TA GAATC-ACAA TTCACAAATA AATGATCAAA -ATTTAAAGA -GTTTATTTA
                                                                                                                  100
B1-BA68  TAAC-GCTGT TATCCCTGGG GTAATTTAAT CTTTTAATCA TAAATGTATG GATCAACTAG CCATTAATTA ATGTTAAACC TTTAAAGAAA AGTTCATTCA
001-AW17 .T..-..... .......AA. .......... ...A...... ....AAA-.. .....-T.TA .TCA...A.C .ATGATT.AA A.AT..A... ....TG..A.
003AAU64 .T..-..... .......AA. .......... ...A...... ....AAA-.. .....-T.TA TTCA...A.T .ATG.TT.AA -..T..A... ........A.
005AAU91 .T..-..... .......AA. .......... .......... ...TAAA-.. .....-..TA TTCA...A.C .ATGATTTTA AAAT..A... ....TT..T.
017-BN63 .T..-..... .......AA. .......... .......... ....AAA-.. .....-..TA .TCA...A.C .ATGA.TTTA -.AT..A... ....AT..A.
102ABP92 .T..-..... .......AA. .......... .......... .....AA-.. .....-..TA .TCA...A.C .ATGATTCTA -.AT..A... .....T..A.
007AAV76 .T..-..... .......AA. .......... ...A...... .....CA-.. .....-..TA TTCA...A.T .ATGAT..AA ..-T..A... ....T.....
083ABN89 .T..G..... .......AA. .......... ...A...... .....TA-.. .....-..TA TTCA...A.C .ATGAT..AA ..-T..A... ....T.....
100-BQ18 .T..-..... .......AA. .......... ...A...... ....AAA-.. .....-T.TA .TCA.TCA.T .ATGAT..AA CA........ ....A...T.
046AAV82 .T..-..... .......AA. .......... .......... .....AA-.. .....-TCTA ATCA...A.C .ATGATTTTA -.AT..A... ....A...A.
022AAV81 .T..-..... .......AA. .......... ...A...... ....AAA-.. .....-..TA ATCA...A.C .ATGAT..AA -.AT..A... ....T...T.
071-BN35 .T..-..... .......AA. .......... ...A...... ....AAA-.. .....-..TA ATCA...A.T .ATGAT..AA -.AT..A... ........T.

FLRBEET  ATTTTCCTAT CACCCCAACA AAATTTTC-C AAGCAAATAA GAAAAAGAAA ACTT-CAAAA CCAATAAAAA CTTAGAAAAT AAAACTCTAT AGGGTCTTCT
FIREFLY  ATCTTTAAGT CACCCCAACT AATTTTTC-A ATTTTAATAA ATTATACTAA ---------- ------AAAT ATTTGAAAAT AAAACTCTAT GGGGTCTTCT
                                                                                                                  200
B1-BA68  ATTTTCTGAT CACCCCAACC AAATAGAAAT TTTCATACCC TAATAAACAA TACTTTAAAA AAGGATATAA AAACGGCTAT AAAACTCCAC AGGGTCTTCT
001-AW17 .....TCT.. ........TA .....TTT-. AA.AT..ATT A.....TTT. .T...A.T.T TTTA..TA.. .TTAAAA... .......T.T ..........
003AAU64 .....TCT.. ........TA .....TTT-. AA.ATA.ATT ........T.. .T.....T.T .TAA...A.T ..TAAAA... .......T.T ..........
005AAU91 .....T.TG. .........A .....TTT.- ...AT..ATA A..A...A.. .T.......T TTAC.ATA.T ...TAAA... .......T.T ..........
017-BN63 .....T.TG. .........A .....TTT-C A..ATC.TTT A..C.TTT.. .T...A...C TTAT.A.A.T ..TAAAA... .......T.T ..........
102ABP92 .....T.TG. .G.......A .....TTT-. CA.ATA.TT. A....TTT.. .T...A...T TTAT.AT..T .T.AAAA... T......T.T ..........
007AAV76 .....T.T.. ........TA .....CTTTA A..A.A.TTA A..-...A.. ..-.AAT..T TTA-..T.TT ..TAAAA... C......T.T ..........
083ABN89 .....T.T.. ........TA .....CTTTA A..A.A.-TT A.G-...A.. AT-..A.T.. TTTA..T.TT ..TAAAA... T......T.T ..........
100-BQ18 ......CT.. .........A .....CTTT. C-.A.A.ATT A..A...A.. .T..-A.T.. TTTA.AT..T ...AAAA... .......T.T ..........
046AAV82 .....T.TG. .........A .....TT.T. A--AT..TAT A..ATTTAT. ATTC..T... TTATT..... TT.ATAA... .......T.T ..........
022AAV81 .....T.TG. .........A .....CT.T. .AAT...TAA A.TAT.--T. ATTCC.T... TTATT..... ...ATAA... .......T.T ..........
071-BN35 .....T.TG. .........A .....CT.T. .AAT...TAA A.TAT.--T. ATTCC.T... TTATT..... T..ATAA... .......T.T ..........
```



Bolboceras: B1-BA68
Polynoncus: 001-AW17 & 003AAU64
Omorgus: 005AAU91, 017-BN63, 102ABP92
Holarctic Trox: 007AAV76 , 083ABN89, 100-BQ18
African Trox: 046AAV82, 022AAV81, 071-BN35

CHAPTER 4

Conclusions & Future Prospects.

Y. van der Merwe & A.D.S. Bastos

Although Trogidae is by no means the smallest beetle family (that honour belongs to the Meruidae, with a single constituent species)(Spangler & Steiner 2005), its importance should not be underestimated. All four of the novel species described in Chapter 2 would qualify as “vulnerable species”. Their overall population sizes are likely to be small; their habitat (the relict montane forests of KwaZulu-Natal) is fragmented, causing individual populations to be isolated; they have poor dispersal power, because of their small size and flightlessness; they are highly-specialised keratin-feeders; and their habitat is under threat from the encroachment of human land uses (Meffe et al. 1997).

In addition, it could be argued that trogids as a whole are keystone species: organisms in whose absence major ecological functions of the ecosystem would be significantly changed. True keratin feeding, involving enzymatic digestion rather than relying on symbiotic microorganisms (Hughes & Vogler 2006), is largely restricted, in terrestrial environments, to two moth sub-orders, the Tineidae and the Oecophoridae, and one beetle family, the Trogidae (Holloway et al. 1997, Kristensen 1999, Scoble 1992). In marine habitats, the niche is filled by certain species of a copepod genus,

Balaenophilus, which are ectoparasites of such diverse marine organisms as whales and sea turtles (Badillo et al. 2007).

Keratin is a remarkably resilient material. This resistance to degradation is largely due to its complex molecular structure, which is stabilized by durable disulphide bonds (Scott & Untereiner 2004). Hair, for example, is not an uncommon find at archaeological digs and is often used in forensic investigations, capable of being stored at room temperature for many years without ill effect.

Apart from the abovementioned keratin-feeding organisms, certain microorganisms are also responsible for the degradation of keratin. Under ideal conditions, it can take anything between seven days and six weeks for keratinolytic fungi, that is fungi that exhibit the enzymatic ability to attack and utilize keratin, to initiate the degradation of a keratin source (Scott & Untereiner 2004, Wilson et al.2007).

If wet and humid conditions constitute the “ideal conditions” for microorganisms to efficiently degrade keratin, it is reasonable to assume that this ability is much reduced in arid conditions. It is hypothesized that increased aridity was one of the factors that encouraged the diversification of trogids in particular, and the majority of the most derived species are found in present-day arid climates (Scholtz 1980, 1986). The significance of the role that trogids play in overall keratin degradation in arid environments merits further investigation.

The end of the previous century brought a challenge to established Linnaean nomenclature. In August of 1998, a new method of nomenclature was discussed at a workshop held at Harvard University. Focusing mainly on the monophyletic clades resulting from phylogenetic analyses, the International Code of Phylogenetic Nomenclature, abbreviated as the PhyloCode, proposed new guidelines to govern the naming of these clades (Cantino & de Queiroz 2004).

Although the naming of species will still be under the control of the various international codes for nomenclature, the PhyloCode will provide a formal set of rules to determine which combination of published names and definitions will be valid, if any of these names constitute homonyms or synonyms and, in the latter case, which of the published homonyms or synonyms will be considered valid.

Only monophyletic groups or clades will be considered under the PhyloCode – the naming of paraphyletic and polyphyletic groups will not be allowed. The use of the traditional ranks will still be allowed, but these will not have any impact on the spelling and application of names, and will most likely only be applied once nomenclature has been completed. For example, a name which ends in "-idae" will not necessarily refer to a traditional family.

Understandably, there has been a varied reaction to this controversial proposal. A recent updated classification of the Crustacea mentioned the PhyloCode but still opted to retain a “more classical approach for now” (Martin & Davis 2001). Parham et al. (2006), although not fully embracing the

PhyloCode for their study, did take it into consideration for what they termed "the transition from Linnaean taxonomy to PhyloCode". It is clear that the latter study considers it only a matter of time before the PhyloCode becomes commonplace in the taxonomic community. Dayrat (2005) makes full use of the PhyloCode for a problematic species. Unable to assign the species to a satisfactory genus-level monophyletic clade, he chose to attach it to the family, Discodorididae.

This is of particular interest to our study, specifically because of the ambiguity of the clades (the subgenera in particular) and how they relate to each other as suggested by Scholtz's (1986) morphological study as opposed to our current molecular study. Under the PhyloCode, the subgenus Phoberus would be completely justified in its full generic status due to its strong monophyletic grouping throughout all analyses. This would have repercussions for our four newly described species as well, as their binomials would most likely change to reflect this monophyletic grouping.

If the PhyloCode does achieve dominance in this field, studies of this kind will become increasingly important, as the identification of monophyletic groups, rather than simple classification, will become the primary goal of taxonomy.

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