Taxonomy, Systematics and Biogeography of the Scaritinae (Insecta, Coleoptera, Carabidae)

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Thesis submitted in partial fulfilment of the requirements of the award of Doctor of Philosophy

Oxford Brookes University

December 2012

Abstract

Scaritinae are a subfamily of ground beetles (Carabidae), containing about 1900 species and 125 genera. They share a distinctive body shape linked to a burrowing lifestyle. The diversity of Scaritinae is concentrated in the tropics and warmer regions of the southern hemisphere, particularly Southern Africa, Madagascar and Australia. The evolutionary history (phylogeny) of scaritines is unknown, leading to conflicting classification schemes and uncertainty over the definition of genera, especially in one sub-group of scaritines, the tribe Scaritini. In particular, it is unclear whether Scaritinae are descended from a common ancestor (monophyletic) or whether they are an artificial group defined by convergent adaptations to burrowing. Phylogenetic relationships of the Scaritinae were investigated in detail for the first time using morphological and molecular data.
Analysis of morphological characters resulted in multiple equally parsimonious trees. Bayesian analysis supported a monophyletic Scaritinae and within Scaritini, a basal position of subtribes Carenina and Pasimachina. Relationships of subtribe Scaritina were impossible to reconstruct due to a complex pattern of convergent evolution and character reversals. 18S rRNA gene sequences were aligned using ClustalX and by incorporating secondary structure information using MAFFT. Consistent results were obtained by Bayesian analysis of the MAFFT alignments, supporting the clades Scaritinae and Scaritini, Carenina and Scaritina. The Australian scaritines (Carenina) were found to be sister to all remaining Scaritini. An historical biogeographic reconstruction of the Scaritini was undertaken by incorporating evidence from extant distributions, fossils and the phylogenetic data. It is likely that evolution of the basal lineages of Scaritini occurred before the fragmentation of Gondwana and that the present-day distributions of the later radiation of Scaritina are due to dispersal. As with most groups of Carabidae, the lack of fossil evidence and molecular clock dating precludes any firm biogeographical conclusions.

Acknowledgements

It is no exaggeration to say this work would have been impossible without the help and tolerance of my family Rae, Maeve and Eddie.

Steve Simpson, George McGavin and my supervisor Tim Shreeve have encouraged me from the very beginning. David Maddison and George Ball offered sound advice during the early stages and Petr Bulirsch shared his great knowledge of scaritine taxonomy.

Special thanks must go to Darren Mann for help and advice on probably every aspect of this project and for gathering rare beetles from around the world. I also must thank my colleagues Zoë Simmons, Amoret Spooner and Katherine Child who were very supportive during the final stages of this work.

For help with translation I am indebted to my family, John Hogan (French), Pat Frith (Italian) and Sophie Hayes (German).

Alfried Vogler and John Day were very generous in providing facilities for DNA sequencing. Specimens for DNA work were graciously provided by Jose Galián, Mike Caterino, David Pryce, Daegan Inward and Dan Duran. Max Barclay, Christine Taylor and Beulah Garner kindly arranged loans of specimens from the Natural History Museum, London.

This thesis is dedicated to Elaine Hogan, who above all others wished to see its completion.

Contents

Chapter 1

Introduction

1.1 Introduction.

The family Carabidae, commonly known as ground beetles, is one of the larger families of Coleoptera, comprising about 35,000 species (Lorenz, 2005).

The focus of this research is on the Scaritinae, a subfamily of the Carabidae comprising about 1850 species and 125 genera (Lorenz, 2005).

Scaritinae are specialised for burrowing and are easily distinguished from other ground beetles both by their distinctive body shape and by their front legs modified for digging (figure 1.1).

Scaritines occur in a wide variety of habitats, from lowland tropical forest to semi-desert and from ocean beaches to the tops of mountains.

They are present on all continents except Antarctica and are most numerous in the tropics and warmer regions of the Southern Hemisphere. Some areas, such as Madagascar and Australia, have a large and diverse endemic fauna.

Scaritines are mostly generalist nocturnal predators and scavengers, consuming any available arthropod prey, sometimes in excess of their own size. In some ecosystems, such as the sandy marine beaches of the Mediterranean, they are the dominant insect predator.

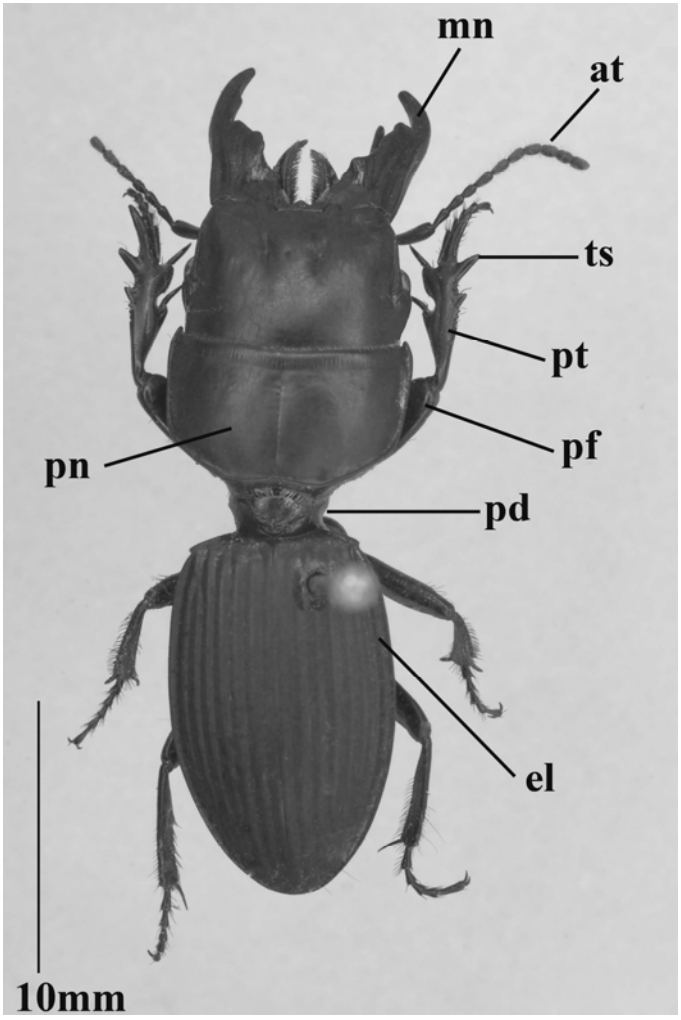

Figure 1.1. Dorsal view of the scaritine beetle *Taeniolobus rugicollis* Dejean. (Scaritini: Scaritina). Brazil. (mn = mandible; at = antenna; ts = tibial spine; pt = protibia; pf = profemora; pn = pronotum; pd = peduncle; el = elytron).

Scaritines very likely have a positive impact on agriculture by consuming large amounts of Lepidoptera larvae and other pest species, although this contribution has not been studied in detail. Conversely some scaritines are known to have a negative impact on human activities by consuming young crop seedlings (Larochelle and Larivière, 2003) or even by damaging golf courses (McQuillan, 1983).

Because of their characteristic appearance the Scaritinae have always been classified as a separate group within the Carabidae. P.A.Latreille in 1802 proposed the first comprehensive classification scheme of Carabidae, dividing the family into two parts, the 'Fossoyers' (scaritines) and the 'Celerigrades' (all other Carabidae) (Ball, 1979). In later classifications the scaritines were still maintained as a separate group but generally assigned to a lower rank. Modern classifications based on the work of Jeannel (1941) all place scaritines in the middle of an evolutionary series between the basal and derived groups of Carabidae. The evolutionary relationships of the Scaritinae are largely unresolved and are the subject of this work.

Biological classification, sometimes seen as a rather esoteric pursuit, actually has a clear purpose and function. Most importantly, classification provides a method of ordering the vast amount of information on biodiversity. Starting with the work of Aristotle and continuing to the nineteenth century, organisms were grouped together according to overall similarity. Since the work of Darwin modern classifications also enable species to be placed in a hierarchy of ranks according to their proposed evolutionary relationships. Hierarchical classification schemes based on evolution provide both a cataloguing system and information on how organisms are related to each other. Attributes used in defining a taxonomic group apply to all the members of that group, so that the classification hierarchy conveys a large amount of information.

Classification of organisms is however open to interpretation, dependent on the available evidence and the opinion of the author. Recent advances based

on molecular tools have led to revisions of some classifications and these revisions provide the basic framework for understanding evolutionary events. For example, the explosive bombardier defensive mechanism of certain ground beetles is a complex organisation of reactive chemical secretions and morphological structures, allowing a mixing of defensive compounds to create a controlled explosion. Such complexity led to classifications where the two groups possessing the bombardier mechanism, the brachinines and the paussines, being placed next to each other, as for example by Lorenz (2005). Molecular and other morphological evidence contradicts this placement (Maddison et al., 1999; Ober and Maddison, 2008) showing that brachinines and paussines are not closely related. This demonstrates that even complex morphological systems such as the bombardier mechanism can arise independently.

1.1.1 Classification of the Scaritinae.

The classification of carabid (ground) beetles, like many groups of organisms, has always been contentious and it has taken over two hundred years to arrive at a generally accepted scheme of how the family should be divided. The main divisions of ground beetles are assigned the taxonomic rank of subfamily by most modern authors.

All scaritines are classified together in the subfamily Scaritinae because they share a number of distinctive morphological adaptations to burrowing. These adaptations include enlarged pro-femora, flattened pro-tibiae armed

with spines and the peduncle, a constriction of the pro and meso-thorax (Figure 1.1).

A morphological diagnosis of the Scaritinae *sensu lato* is as follows. Body size ranging from very small (approximately 1.5 mm) to very large (approximately 70 mm). Mandibles with scrobe (lateral face) asetose. Antennal insertion hidden from above. Procoxal cavities closed by contact with projections of the proepimeron and prosternum (Bell, 1967). Mesocoxal cavities disjunct (Bell, 1967). Metacoxal cavities usually disjunct (Bell, 1967), rarely disjunct-lobate (for example *Passalidius fortipes* (Boheman)). Profemora enlarged. Protibiae flattened and armed with at least one but usually more spines. Body form pedunculate, with a constriction between the pro and mesothorax. Male genitalia with parameres of approximately similar size, endophallus with basal sclerite X (Ball, 1956) of variable form.

The subfamily Scaritinae is usually divided into four groups of tribal rank; Scaritini, Clivinini, Dyschiriini and Salcediini (Figure 1.2).

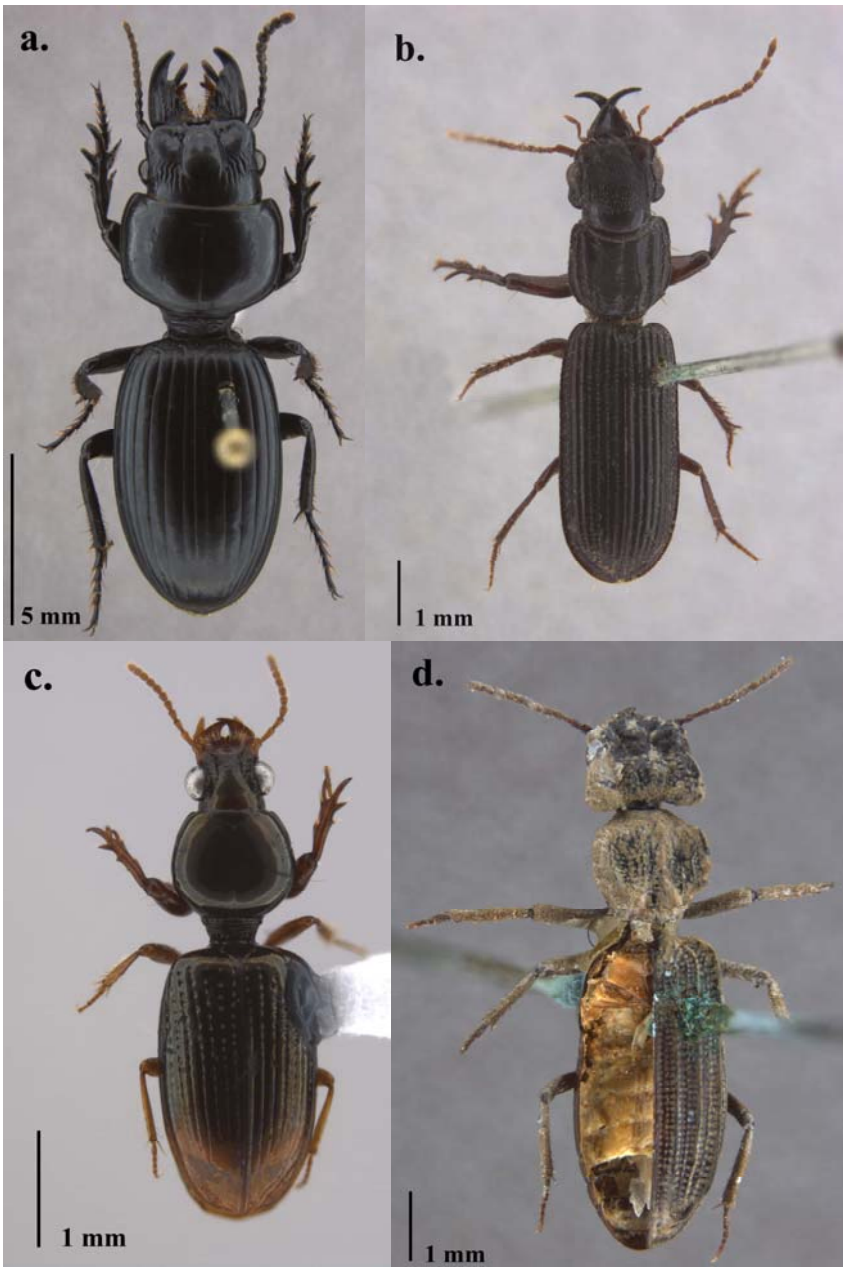

Figure 1.2. Examples of the four divisions of subfamily Scaritinae. (a) Scaritini: *Mamboicus afrellus* (Bates) (Zambia). (b) Clivinini: *Camptodontus* sp. (Brazil). (c) Dyschiriini: *Dyschirius analis* LeConte (U.S.A.). (d) Salcediini: *Solenogenys foeda* Westwood (Lectotype, Brazil).

Scaritini comprise a sizeable group of about 55 genera (Lorenz, 2005) with a comparatively large body size (8-70 mm long) and are often flightless. They occur in the warmer areas of all Zoogeographic regions but are concentrated in the tropics. The Clivinini is also a large group of 57 genera (Lorenz, 2005). Body size is smaller than Scaritini (2-30 mm long) and they are almost always capable of flight. They are distributed in all Zoogeographic regions but concentrated in the tropics. The Dyschiriini is a group of 8 genera (Fedorenko, 1996) with a small body size (1.5-8 mm long) and usually capable of flight. Although distributed in all Zoogeographic regions they are most numerous in the Northern Hemisphere, with a significant temperate fauna. The Salcediini is a small and poorly-known pan-tropical group of 4 genera (Reichardt, 1975) with small body size (4-15 mm long) and all capable of flight.

To complicate matters, the classification scheme outlined above is just one of many and almost every author has a slightly different system. This is due in part to a lack of knowledge about how scaritines are related to each other and to the rest of the Carabidae.

Classification schemes differ in the taxonomic ranks used, for example the Scaritinae *sensu lato* (in the sense of Lorenz, 2005) have also recently been classified as the family Scaritidae (Deuve, 2003), or as the supertribe Scarititae (Moore and Lawrence, 1994) or as the tribe Scaritini (Vieira and Bello, 2004). This confusion of names and taxonomic ranks means that communication about these organisms is sometimes ambiguous.

Alternative scaritine classifications can also differ more radically by inclusion of other groups of Carabidae. For example, Bell (1998) places the Rhysodinae (figure 1.3), an unusual carabid group of long disputed affinity, as part of the scaritines with a close relationship to Salcediini.

Figure 1.3. The rhysodid *Leoglymmnius lignarius* (Olliff) (Carabidae: Rhysodinae). Australia.

Also in dispute is the placement of the Promecognathinae, a small group of Carabidae with a scaritine-like appearance (figure 1.6). The promecognathines are either included within Scaritinae (for example Lindroth, 1961; Bouchard et al., 2011) or placed outside the Scaritinae as a separate subfamily (for example Ball and Bousquet, 2001; Lorenz, 2005). A summary of three different classification schemes is given in table 1.1 to illustrate how such schemes can vary.

Table 1.1. Three different classification schemes of the Scaritinae.

Basilewsky 1973b (Afrotropical Fauna)	Lorenz 2005 (World Fauna)	Bouchard et al. 2011 (World Fauna)
Subfamily Scaritinae	Subfamily Scaritinae	Subfamily Scaritinae
Tribe Scaritini	Tribe Scaritini	Tribe Carenini
Subtribe Scaritina s.str.	Subtribe Pasimachina	Tribe Clivinini
Subtribe Acanthoscelitina	Subtribe Carenina	Subtribe Ardistomina
Subtribe Dyscherina	Subtribe Acanthoscelitina	Subtribe Clivinina
Subtribe Storthodontina	Subtribe Scaritina s.str.	Subtribe Forcipatorina
Tribe Ochryopini	Subtribe Oxylobina	Tribe Dalyatini
Tribe Scapterini	Subtribe Scapterina	Tribe Dyschiriini
Tribe Corintascarini	Subtribe Clivinina	Tribe Pasimachini
Tribe Forcipatorini	Subtribe Dyschiriina	Tribe Promecognathini
Tribe Clivinini	Tribe Salcediini	Tribe Salcediini
Tribe Dyschiriini		Tribe Scaritini
Tribe Salcediini		Subtribe Acanthoscelitina
		Subtribe Corintascarina
		Subtribe Dyscherina
		Subtribe Ochryopina
		Subtribe Oxylobina
		Subtribe Scapterina
		Subtribe Scaritina

These alternative classifications are in part due to uncertainty over the status of morphological characters, use of alternative character sets, and a lack of a comprehensive analysis, rather than to simple differences of opinion.
As a step to resolving these issues this work focuses on one sub-group of scaritines, the tribe Scaritini *sensu stricto*.

The Scaritini *sensu stricto* are diagnosed as follows. Body size large (8-70 mm). Mandibles large and prominent. Antennae setose from article 4, 5 or 6, article 3 never densely setose. Median glabrous band of antennomeres present. Antennal scape elongate, longer than combined length of antennomeres 2 + 3. Setiform process between tarsal claws (arolium) absent. Median lobe tubular, not flattened laterally. Parameres long with numerous brush-like setae.

Examples of the major subtribes of Scaritini *sensu strico* are given in figure 1.4.

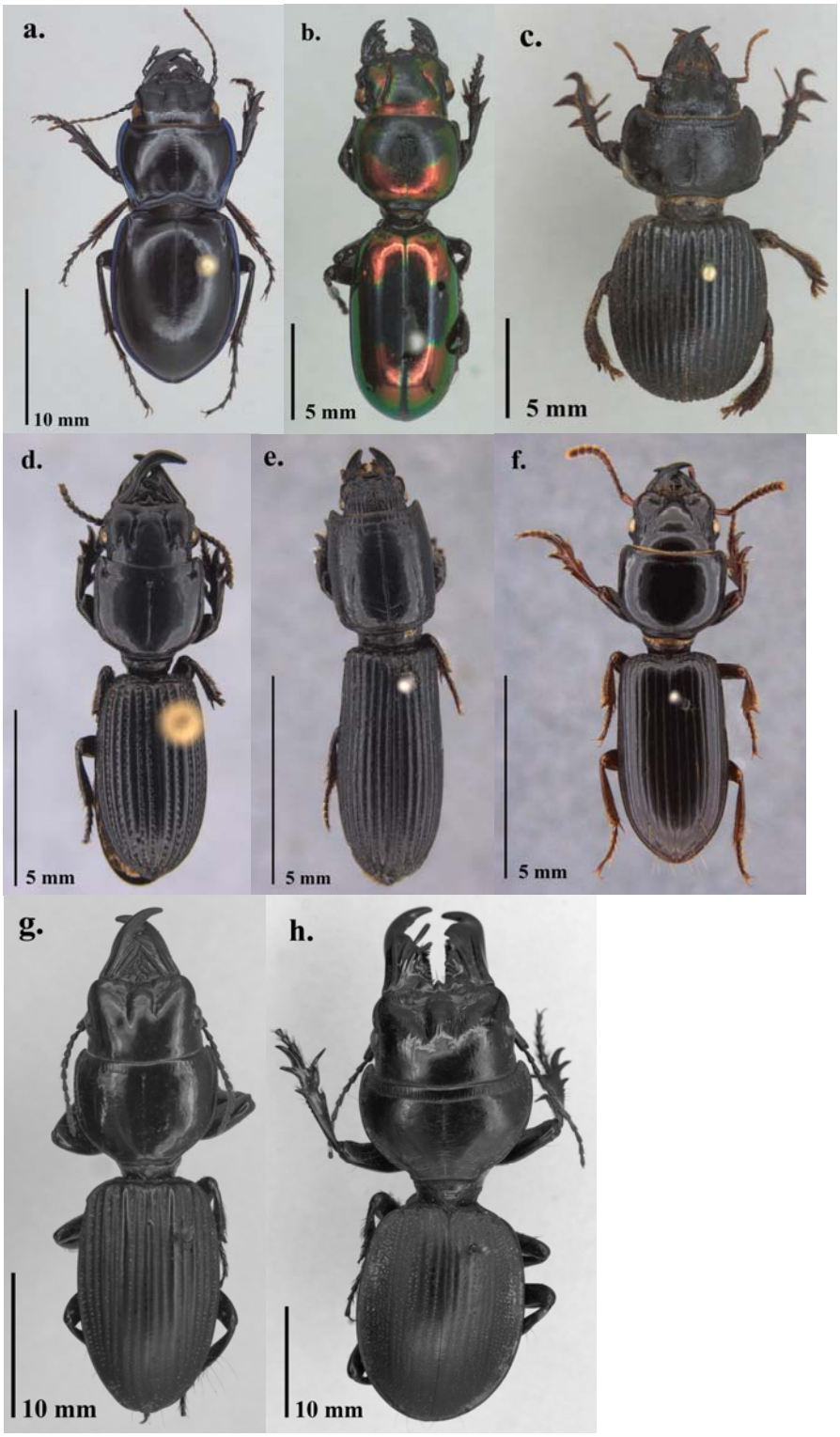

Figure 1.4. Examples of the subtribes of Scaritini. (a). Pasimachina: *Pasimachus* sp. (U.S.A.). (b). Carenina: *Carenum sumptuosum* Westwood (Holotype, Australia). (c). Acanthoscelitina: *Acanthoscelis ruficornis* (F.) (South Africa). (d). Oxylobina: *Oxylobus* sp. (undescribed species, India). (e). Scapterina: *Thlibops longicollis* (Putzeys) (Senegal). (f). Scaritina: *Distichus* sp. (Nepal). (g). Dyscherina: *Dyscherus costatus* (Klug) (Madagascar). (h). Storthodontina: *Crepidopterus goudotii* (Guérin) (Madagascar).

1.1.2 Evolution of the Carabidae.

The question of the evolutionary origins and affinities of the scaritines cannot be considered as a discrete problem, but must be considered in the context of the evolution of the Carabidae as a whole. Despite being well studied over many years, the finer details of carabid phylogeny are not completely resolved. Conflicting evidence from convergence and reversal of morphological characters makes the relationships between groups difficult to interpret (Maddison, 2006). Given the size and diversity of a group such as the Carabidae (approximately 35,000 species), it is unsurprising that a comprehensive, modern analysis of their phylogeny has not been attempted. Such a task would require a synthesis of traditional external morphological characters of adults and larvae, internal characters of the male and female genitalia and molecular data.

In spite of these problems there is at least general agreement as to the major lineages that make up the family. These lineages are in many cases well-defined groups based on morphological characters and are usually assigned the taxonomic rank of subfamily. Those groups displaying features thought to be closest to the common ancestor of all ground beetles are remnants of an early radiation and are regarded as 'basal-grade' carabids (Maddison et al., 1999). These tribes include some common and familiar extant genera such as *Carabus* and *Nebria*.

The majority of the species level diversity of Carabidae is a result of the most recent and extensive radiation of the subfamily Harpalinae, the so-

called ‘high-grade’ carabids. In between these grades is a smaller, disparate group of tribes, the ‘mid-grade’ carabids, in which the Scaritinae are placed in all modern classifications (Ball, 1979). It is important to note that the rank of ‘grade’ is a loose one and has no precise taxonomic definition, but nonetheless is a useful term to describe the main phases of carabid evolution.

The relationships between the major lineages of Carabidae are summarised in figure 1.5.

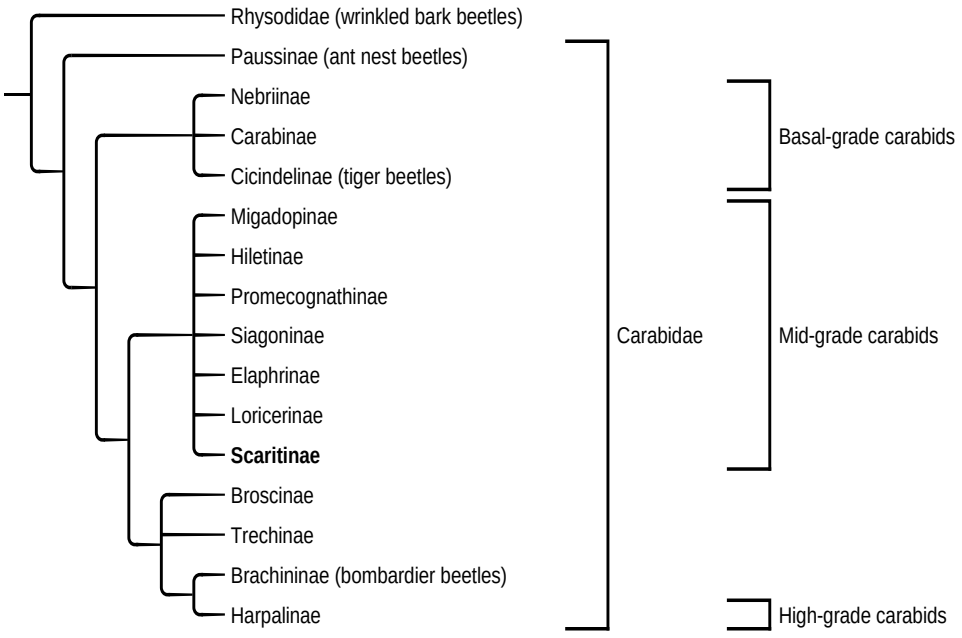

Figure 1.5. Diagram illustrating relationships of the major lineages or ‘grades’ of Carabidae (after Erwin (1985) and Maddison et al. (1999)).

1.1.3 Evolution of the Scaritinae.

When compared to other Carabidae (and in common with many other mid-grade tribes) Scaritinae are something of an enigma, possessing morphological specialisations without transitional forms linking them to other groups.

The scaritine sister group has not yet been identified with confidence, although several tribes of mid-grade carabids have been proposed at one time or another as a sister group to the Scaritinae (or have been placed next to them in classifications), including Siagonini (Andrewes, 1929; Erwin, 1978), Migadopini (Csiki, 1927), Promecognathini (Lindroth, 1961) and Hiletini (Erwin and Stork, 1985). These relationships have been suggested mostly on the basis of a small number of shared morphological structures with scaritines. In all these cases the morphological evidence supporting a relationship with scaritines is also contradicted by other characters.

Promecognathini, a small mid-grade tribe with a disjunct North American and South African distribution, construct burrows in soil (Larochelle and Larivière, 2003) and resemble scaritines in general form (figure 1.6).

Figure 1.6. *Promecognathus laevissimus* (Dejean) (Carabidae: Promecognathinae). U.S.A.

In common with scaritines, promecognathines possess prominent mandibles and a peduncle, but lack any major modifications to the pro-tibiae. In other respects there are significant differences to scaritines, for example promecognathines possess a seta in the mandibular scrobe, a character never present in scaritines. The presence or absence of this seta would appear to be somewhat trivial, but in other groups of Carabidae it can be a defining and invariable character. For example, the scrobal seta is absent in all members of the large subfamily Harpalinae. In addition, the structure of the male genitalia differs significantly; *Promecognathus* have two large plates covering the ostium and the parameres are glabrous and asymmetrical (Lindroth, 1961) while in Scaritini the ostium is simple and the parameres are setose and symmetrical. The classification of promecognathines within

Scaritinae appears to have occurred because of their similar appearance and there being is no strong evidence linking them to another group.

The mid-grade subfamily Siagoninae are another group with a general resemblance to scaritines (figure 1.7). Siagonines are strongly pedunculate but again lack any protibial modifications and differ from scaritines in other ways, notably by the absence of a submental suture and a strongly dorsoventrally flattened body. In contrast, Erwin (1978) suggested an interesting but untested association between *Enceladus* (Siagoninae) and *Pasimachus* (Scaritinae) based on a shared brush of setae on the mesotibia.

Figure 1.7.The siagonine *Enceladus gygas* Bonelli (Carabidae: Siagoninae). Venezuela.

Hiletinae are another mid-grade subfamily with a disjunct and possibly relict distribution (figure 1.8).

Figure 1.8. *Hiletus fossulatus* Jeannel (Carabidae: Hiletinae). Mozambique.

Based on tarsal characters, a close relationship between hiletines and clivinine scaritines has been inferred (Erwin and Stork, 1985). The long antennal scape of hiletines, a rather unusual character in Carabidae, is also similar to that seen in Scaritini, leading Jeannel (1941) to place both groups together in his division 'Scrobifera'. In contrast to this the procoxal cavities of Hiletini are of the open form while those of scaritines are closed (Bell, 1967).

The scaritines as a whole are classified together as a single group defined by a set of characters associated with burrowing. One of these characters, the presence of protibial spines, is so rare within the Carabidae that they appear to have evolved independently in only one other lineage, the broscine genus *Gnathoxys* (Roig-Juñent, 2000) (figure 1.9).

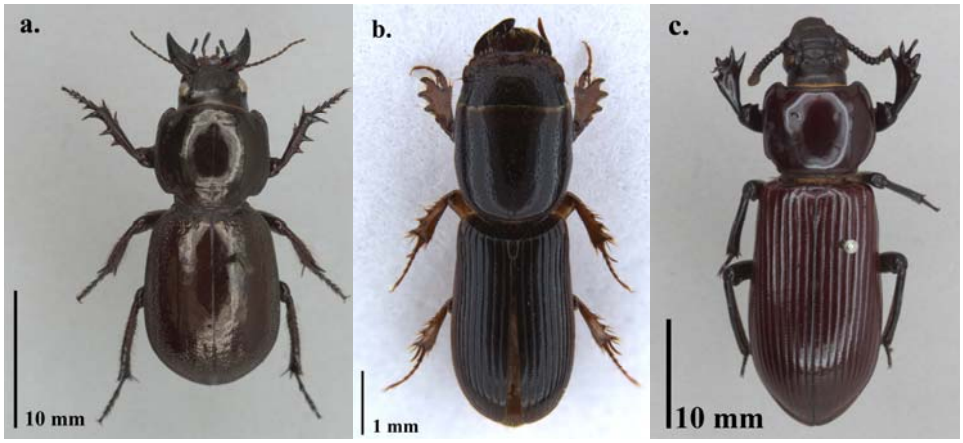

Figure 1.9. Burrowing Coleoptera showing convergent adaptations. (a.) *Gnathoxys* sp. (Carabidae: Broscinae). Australia. (b.) *Chiron* sp. (Scarabaeoidea: Chironidae). Gambia. (c.) *Chiroscelis* sp. (Tenebrionidae). Liberia.

When other morphological characters are considered, such as the form of the mesocoxal cavities, it is clear that despite their similar appearance *Gnathoxys* are not closely related to any of the scaritines. Importantly, the case of *Gnathoxys* demonstrates that specialised morphological structures associated with burrowing can evolve independently in different groups. Although rare in Carabidae, similar characters have also evolved independently in other burrowing Coleoptera. For example, modified front tibiae and a structure similar to the peduncle are present in the genera

Chiron (Scarabaeidae) and *Chiroscelis* (Tenebrionidae). In general form these burrowing Coleoptera superficially appear similar to scaritines (figure 1.9).

The scaritines have always been assumed to share a common ancestor (i.e. are monophyletic) and to have acquired their burrowing adaptations by inheritance from this ancestor. However, the previous examples given of other burrowing beetles demonstrate that morphological adaptations to similar environments or lifestyles can occur independently. Indeed, evolutionary convergence is a well-documented phenomenon observed in many animal and plant groups. Therefore the possibility exists that the scaritines are a mixed group of two or more separate and unrelated lineages (i.e. polyphyletic) that have independently evolved similar adaptations to burrowing. No study so far has addressed this possibility in detail, but limited data from wider studies on carabid evolution suggest that scaritines may not be a natural group.

Data from chemical defence compounds (Moore, 1979) and characters of the female reproductive tract (Liebherr and Will, 1998) both show that the scaritines form two distinct groups. One group is composed of the larger species (tribe Scaritini *sensu stricto*) and the other group the smaller species (tribes Clivinini, Dyschiriini and Salcediini). While these two groups almost certainly belong to the mid-grade carabids, it cannot be assumed they represent a sister group. Liebherr and Will (1998) postulated a close relationship between Clivinini and another mid-grade tribe, the Amblytelini,

based on a shared spermathecal gland. The Scaritini *sensu stricto* were placed as sister to the tribe Hiletini based on shared accessory glands. Since these studies outlined above have focused on single character systems and did not attempt to address scaritine relationships in detail, it is not possible to draw any firm conclusions.

1.1.4 Reconstructing the evolutionary history of scaritines.

The evolutionary history or phylogeny of the scaritines is not yet known. The accepted relationships between the different tribes, subtribes and genera of scaritines are based on a small number of characters and have not been tested.

The phylogeny of a group of organisms can be reconstructed using the method of cladistics. Cladistics is a way of classifying organisms according to their shared inherited characters (Forey et al., 1992). The three principle assumptions of cladistics are firstly, that all organisms are ultimately related to each other via common ancestors as part of a single tree of life. Secondly, over time the characteristics of organisms change and thirdly, that new species arise from an ancestor in a bifurcating pattern (Brinkman, 2001). Cladistic relationships are depicted graphically in a number of ways, most simply as a branching diagram or 'tree' called a cladogram. It is cladograms and other types of evolutionary trees which are the basis for interpreting and communicating the evolutionary relationships of organisms (Lipscomb,

1998). A cladogram can be considered a type of evolutionary tree in which only the relationships of the organisms are depicted, not other information such as the amount of evolutionary change which has occurred. The lines joining the taxa are known as branches and the points at which the branches meet are nodes (Brooks and McLennan, 1991). A hypothetical example of a cladogram is given in figure 1.10 depicting the relationships of four species A-D.

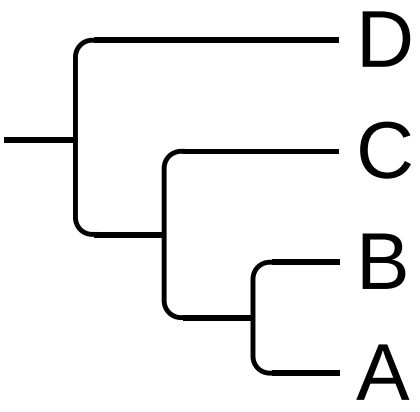

Figure 1.10. A simple cladogram depicting relationships between four hypothetical species A-D.

The branching pattern of the tree depicts a hypothesis of the relationships between the taxa, so that for example A-C share a common ancestor, and form a monophyletic group or clade. A and B are more closely related to each other than to any other taxon and are known as sister taxa. Cladograms also depict hierarchical relationships so that the sister group of the clade A+B is C.

An important point is that cladograms are not equivalent to the genealogical trees used to depict relationships between living people and their ancestors. Ancestors in cladograms are hypothetical, so that for example taxa A and B in figure 1.10 are descended from a hypothetical ancestor at their common node, and that A did not evolve directly from B and vice versa.

Clades are defined by shared, derived characters (a character in this sense is any heritable attribute of an organism). A derived or 'changed' character (a synapomorphy) is a character which has evolved in the recent ancestor of a particular group and that does not occur in more distant ancestors of that group. Taxa will always share many characters but only derived characters provide information on grouping (Quicke, 1993). For example, a synapomorphy defining the Coleoptera would be hardened fore-wings (elytra). Characters in their original states (or plesiomorphies) do not provide information on grouping because they are common to all members of the group. A plesiomorphic character of insects is the presence of six legs. As this character occurs in all insects it provides no information on grouping Coleoptera for example.

A real cladistic analysis can produce very large numbers of alternative cladograms (for example Naylor, 2002; Liebherr and Will, 1998). The branching order of different cladograms infers different amounts of evolutionary change to have occurred. On one particular tree a character can define a single clade and therefore the character changes only once from one state to another. On an alternative tree the branching order of taxa is

different and this character may change state a number of times or define several separate clades, requiring that the character changes several times from one state to another (Wiley et al., 1991).

Some criterion must be applied to choose between these alternative trees and traditionally the principle of parsimony has been used. Parsimony is a philosophical rule by which the preferred solution to a problem is the simplest one. Parsimony is applied in cladistics with the assumption that evolutionary change is a rare event, so that the tree requiring the least number of character state changes is the preferred tree (Kitching et al., 1998).

In addition to parsimony, statistical methods can also be used to infer phylogenies. One such method that has gained in popularity in recent years is Bayesian inference (Felsenstein, 2004). This method is based on Bayes rule, a probability theorem used to calculate the probability of an event by considering the data and prior evidence. When Bayes rule is applied to phylogeny reconstruction, the posterior probability distribution of a set of trees is given as:

$$Pr[Tree\,|Data] = \frac{Pr[Tree]\ Pr[Data\,|Tree]}{Pr[Data]}$$

(after Huelsenbeck et al., 2001)

Pr[*Tree*|*Data*] is the posterior probability of a tree given the data in the form of a matrix of DNA sequences or morphological character states.

Pr[*Tree*] is the prior probability of a tree, or simply the 'prior'. The prior is usually given an arbitrary value because there is usually no prior information about the phylogeny, or it may be undesirable to influence the results. For example, all possible trees could be given equal prior probability (Ronquist et al., 2009).

Pr[*Data*|*Tree*] is the likelihood of a tree (the likelihood of the data given a tree and a suitable DNA substitution model or model of morphological character change). Formulas exist to enable the likelihood to be calculated (for details see Felsenstein, 2004; Schmidt and von Haeseler, 2009).

Pr[*Data*] is the probability of the data. This is a complex multidimensional integral and in practice is impossible to calculate. To overcome this problem a mathematical technique known as Markov chain Monte Carlo (MCMC) is used to obtain a sample of the posterior probability distribution (Huelsenbeck et al., 2001).

Apart from providing information on evolutionary history itself, information derived from cladograms can be applied to many problems in biology, such as biogeography and epidemiology (Page and Holmes, 1998).
Of relevance to this study, cladograms can be used to test conflicting ideas about classification. New classification schemes supported by phylogenetic data are more likely to be adopted in the future because they are less subjective.

Initially phylogenies were reconstructed with morphological data but since the development of the polymerase chain reaction, DNA sequence data is now more widely used. The advent of these genetic data allows traditional classifications and phylogenies to be tested, sometimes with results that overturn established thinking. A good example of this is a recent comprehensive molecular phylogeny of the Dictyoptera (cockroaches, mantids and termites) (Inward et al., 2007). The results of this study conclusively showed that the termites form a clade nested within the cockroaches, leading to the conclusion that termites are in fact eusocial cockroaches. This result also led to major changes in the classification of Dictyoptera, the separate termite order Isoptera being discarded and the termites instead placed as the family Termitidae within the Blattodea (cockroaches as a whole). Morphological and molecular data have their own strengths and weaknesses and in this study their use is considered as complementary.

One of the main differences between these two types of data is the assignment of homology of characters, which can have a significant effect on the results of an analysis. With morphological data, homology is assigned at the stage when characters are defined, a process which can be subjective, perhaps leading different authors to arrive at different conclusions about homology. Homology of molecular characters is assigned at the stage of sequence alignment and can be objective and unambiguous for length invariable sequences but more problematic with length variable sequences (Sanderson and Shaffer, 2002).

Along with these theoretical differences there are practical considerations between the two methods which are no less important. The best quality DNA can only be obtained from specimens collected specifically for this purpose, by ensuring rapid dehydration, although it is possible to extract DNA from other material but with less success (Gilbert et al., 2007). This requirement limits the number of specimens available for any study, especially for a group such as the scaritines which have many species scattered across the globe. Morphological studies have the advantage that a wide range of specimens are often available from museum collections.

1.1.5 Morphology and scaritine phylogeny.

The only published work providing a morphological cladistic analysis of the genera of Scaritini is that of Nichols (1986b). Using larval characters the author attempted to determine the systematic position of the genus *Antilliscaris* from the mountains of Puerto Rico and Haiti (figure 1.11).

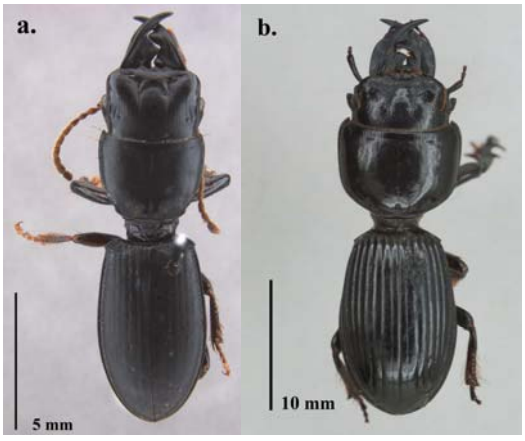

Figure 1.11. (a) *Antilliscaris mutchleri* (Bänninger). Puerto Rico. (Scaritini: Scaritina) (b.) *Prodyscherus pluto* (Künckel) (Scaritini: Scaritina). Madagascar.

The characters used and the results of the analysis by Nichols (1986b) are reproduced in figure 1.12.

The results show the closest relative of *Antilliscaris* to be the Madagascan genus *Prodyscherus* (figure 1.11), a rather surprising result given that *Antilliscaris* is endemic to the Caribbean. *Antilliscaris* also differs significantly to *Prodyscherus* in adult morphology, for example in the shape of the apex of the maxilla and in the form of elytral intervals seven and eight.

Because data on larval morphology are lacking for most genera of Scaritini, the sampling of taxa from this work is consequently very poor and precludes any meaningful hypotheses of relationships.

- Character 1: Number of lateral macrosetae on front anterior to antennal base: plesiomorphic (0): two; apomorphic (1): one.
- Character 2: Paralateral epicranial grooves of head capsule: plesiomorphic (0): present on dorsum of epicranium, running parallel to the longitudinal axis of the body; apomorphic (1): absent through loss.
- Character 3: Ocular grooves of head capsule: plesiomorphic (0): present – a semicircular groove on each side of epicranium, encircling area of head with stemmata; apomorphic (1): absent through loss.
- Character 4: Femoral spines: plesiomorphic (0): ventral rows in addition to apical whorl; apomorphic (1): apical whorl only.
- Character 5: Tarsus: plesiomorphic (0): normal – subequal in length to tibia; apomorphic (1): reduced – tarsus much shorter than tibia.
- Character 6: Retinaculum of mandible: plesiomorphic (0): relatively small and lacking denticulations along inner margin; apomorphic (1): enlarged and possessing denticulations along inner margin.
- Character 7: Cervical grooves: plesiomorphic (0): present – transverse groove located posteriorly on each side of the head; apomorphic (1): absent through loss.

Taxon	Character						
	1	2	3	4	5	6	7
Morion	0	0	0	0	0	0	0
Antilliscaris	1	1	1	1	1	0	0
Crepidopterus	1	1	1	1	0	1	1
Dinoscaris	1	1	1	1	0	1	1
Distichus	1	0	1	1	0	0	0
Dyscaris	1	0	1	1	0	0	0
Dyscherinus	1	1	1	1	0	1	0
Dyscherus	1	1	1	1	0	1	0
Madascaris	1	1	0	1	0	0	0
Paradyscherus	1	1	1	1	0	1	0
Parallelomorphus	0	0	0	0	0	0	0
Pilades	1	1	1	1	0	1	1
Prodyscherus	1	1	1	1	1	0	0
Scarites	0	0	0	0	0	0	0
Storthodontus	1	1	1	1	0	1	1
Tapinoscaris	1	1	1	1	0	1	1
Typhloscaris	1	1	1	0	0	0	0

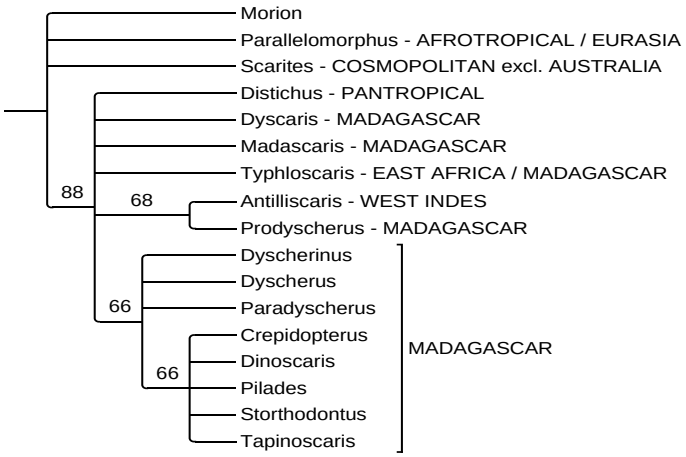

Figure 1.12. Summary of the characters, taxon/character matrix and cladogram of the analysis of Nichols (1986b) based on larval morphology. The cladogram is a strict consensus of 9 equally parsimonious trees. Numbers above nodes are bootstrap percentage values.

In the absence of any other phylogenetic analysis, various authors have published their own ideas about the relationships of selected scaritine genera. For example, based on larval and adult characters Moore and Lawrence (1994) proposed the elevation of the majority of the Australian scaritines to a new tribe, the Carenini. This new classification has not been adopted in subsequent works, for example Lorenz (2005), probably due to the omission of a comprehensive cladistic analysis.

In reality, different relationships can be proposed by picking and choosing from among any set of confirmatory or conflicting characters. A more rigorous approach is necessary to draw any firm conclusions. Cladistics provides a method whereby all of the available data (morphological and molecular) can be considered in an objective way.

1.1.6 Molecular systematics and scaritine phylogeny.

Several large scale studies have attempted to reconstruct the phylogeny of the Carabidae using DNA sequence data, either as a whole (Maddison et al., 1999) or for some major sections of the family (Ober, 2002; Ribera et al., 2005). Unfortunately the use of molecular data has presented new problems and a well-supported molecular phylogeny of the Carabidae has remained elusive.

In some cases the results of molecular phylogenetic analyses seriously contradict well established relationships derived from morphology.

Difficulties can arise because the chosen gene may evolve at an inappropriate rate, insertions or deletions can cause problems with alignment and unequal rates of sequence evolution among different taxa can cause artefacts such as long branch attraction.

A substitution occurs when a nucleotide changes from one to another. The most common type of substitution is a transition, where one purine changes to a different purine or one pyrimidine changes to a different pyrimidine. Another type of substitution is a transversion and is less common, involving a change from a purine to a pyrimidine or vice versa. If the substitution rate of the chosen gene is too slow there will not be enough genetic changes (characters) to enable the phylogeny to be fully resolved. If the substitution rate is too fast saturation may occur. Substitution saturation arises when multiple substitutions occur at the same site, masking the true phylogenetic signal and producing spurious relationships.

Errors in DNA replication can cause the insertion or deletion of regions of DNA ranging from a single base to whole sections of a chromosome. These insertions or deletions, known as indels, cause variation in the length of sequences and unambiguously aligning them may be difficult. As the critical step of homology assignment occurs at the alignment stage, different alignments may produce different results.

The phenomenon of long branch attraction (LBA) is commonly given as the cause of incorrect phylogenetic hypotheses (Sanderson and Shaffer, 2002).

LBA occurs as the result of substitution rate heterogeneity (meaning that different substitution rates occur in different taxa) or when long periods of time have elapsed in the evolution of a group. As there are only four possible bases at any single position of a DNA sequence, those sequences that evolve rapidly (high substitution rate) or over a longer time period are more likely to accumulate the same base at a given position by chance alone. When the criterion of parsimony is applied these changes are interpreted as being homologous and the long branches are joined together, producing an incorrect grouping.

Several potential solutions to the LBA problem have been proposed when parsimony fails to recover the 'correct' tree. Instead of parsimony a statistical method of phylogeny reconstruction such as Bayesian inference can be used. In theory Bayesian inference should be less susceptible to LBA by incorporating an appropriate value of rate heterogeneity as one of the model parameters.

Adding more taxa to an analysis can also be used to overcome LBA problems (Bergsten, 2005). Specifically, additional taxa closely related to those artificially grouped by LBA are added. Because they are closely related these taxa should share more characters with the close relative formerly grouped by LBA, allowing the correct relationship to emerge.

Despite these pitfalls the use of DNA sequence data has undoubtedly revolutionised the field of systematics and provides information that cannot be obtained from morphology alone.

The most comprehensive attempt at resolving carabid relationships with molecular data is that of Maddison et al. (1999) using 18S rRNA gene sequences. While making a significant contribution to the understanding of carabid phylogeny, this study was unable to address the relationships of scaritines to other Carabidae due to LBA. In the analysis of Maddison et al. (1999) four subfamilies of ground beetles, the Cicindelinae (tiger beetles), Rhysodinae (wrinkled bark beetles), Paussinae (ant beetles) and the Scaritinae formed a single group, the 'CRPS quartet', yet there is compelling morphological evidence to reject this hypothesis.

To date, only a single molecular phylogenetic study specifically of the Scaritinae has been published, based on seven West Mediterranean species of the subtribe Scaritina (Galián et al., 1999). The results were in general accordance with established relationships derived from morphology, but the limited taxon sampling provides data on only three genera.

1.1.7 Biogeography of the Scaritini.

As well as taxonomy, information from phylogenies can be applied to problems in biogeography. The distribution of an organism is determined both by ecological factors such as climate and competition and by historical factors (Ridley, 1993). Climatic factors are undoubtedly important in shaping the distribution of the Scaritini as they are only present in warmer parts of the world, between latitudes 50º north and 50º south. However, climatic factors alone do not provide a complete explanation of

biogeographic patterns and historical factors must also play a part, especially in accounting for the highly restricted ranges of some genera. Dispersal and vicariance are two historical processes important in shaping the extant distributions of organisms. Dispersal involves the active or passive movement of an organism from one area to another. Vicariance occurs when the range of an organism is split by a barrier caused by geological processes such as the emergence of a mountain range or continental break-up (Myers and Giller, 1990).

If the geographical distributions of Scaritines are examined it is immediately clear that they are not evenly distributed around the world. In particular, the larger and often flightless Scaritini are highly concentrated in some areas yet almost lacking in others, even though there may be apparently abundant suitable habitat. This tribe shows the greatest generic level diversity and endemicity in Madagascar, Australia, India and South Africa (Csiki, 1927). Complicating this situation is the fact that a small number of genera of the Scaritini, notably *Scarites* and *Distichus*, show the opposite pattern of distribution. Members of these genera are usually fully winged and individual species can have extremely wide ranges. For example, *Scarites terricola* Bonelli occurs in most of the warmer areas of the Palearctic region, from the Mediterranean and Central Europe to Japan (Balkenohl, 2003). Species of *Scarites* have even colonised the oceanic islands of Madeira and the Galapagos.

This study will investigate how historical factors have affected the distribution of the Scaritini.

There is anecdotal evidence to suggest that the present distributions of the Scaritini are due to vicariance arising from plate tectonics. Much of the generic level diversity of this tribe is concentrated on fragments of the former southern supercontinent Gondwana, especially Madagascar, India and Australia. When examining the fauna of these Gondwanan areas some striking similarities also become apparent. For example, members of the genus *Gnaphon* from India possess a distinctive modification of the elytra where intervals three, five and seven are raised into sharp ridges (Andrewes, 1929). The only other genus to exhibit this character is *Anomophaenus* from New Caledonia. Comparable distributions of extant and extinct taxa on remnants of Gondwana have also been identified for many groups including ratites (birds) (Cooper et al., 1992) and *Nothofagus* trees (Heads, 2006). However, both these groups, like scaritines, are difficult to reconstruct biogeographically, and have uncertain phylogenies (e.g. Knapp et al., 2005). The Scaritini are an ideal study group to test theories of vicariance by plate tectonics. They are hypothesised to belong to a lineage of sufficient age to have existed before the fragmentation of Gondwana during the Triassic (Erwin, 1985), they are well represented on Gondwanan fragments today and in the main have poor dispersal ability (Driscoll and Weir, 2005).

The three other tribes of the Scaritinae (Clivinini, Dyschiriini and Salcediini) are, with the exception of a few subterranean and cave inhabiting species, fully winged. Dispersal is probably a more important factor than vicariance in shaping their distributions and for this reason the biogeography of these tribes will not be addressed in this study.

Systematics can be applied to problems in biogeography, enabling testing of alternate biogeographical hypotheses using phylogenies. In a now classic paper, Brundin (1966) showed that the evolution of chironomid midges was driven by continental drift. He discovered that the sequence of disconnection of the southern supercontinent was exactly mirrored in the phylogeny of the midges. Recently, methods have been proposed to test the degree of historical association between organisms and areas (Page, 1994), allowing more rigorous testing of biogeographical scenarios.

The application of systematics has also overturned established theories of the biogeographical history of organisms. As with the work of Brundin on chironomids, pelomedusoid turtles were once cited as a good example of how plate tectonics can drive the evolution of a group. When a phylogeny of this superfamily was reconstructed with molecular data, no correlation between evolutionary history and current geography could be found. This led to the conclusion that events affecting the phylogeny of the Pelomedusoidea occurred before the break up of Gondwana and that the group was widely distributed before this time (Noonan, 2000).

1.2 Aims of this study.

Because of the uncertainty over the classification and evolutionary history of the Scaritini *sensu stricto*, the primary aim of this work is to:

- Reconstruct the phylogeny of the Scaritini at the generic level using morphological and molecular data.

The phylogenetic data is then used to:

- Test whether the Scaritinae *sensu lato* are a natural group derived from a single common ancestor.
- Attempt to identify the sister group of the Scaritinae among the mid-grade tribes of Carabidae.
- Review the higher-level classification of the Scaritini (relationships at and above the generic level).
- Produce a general biogeographical hypothesis explaining the origin and diversification of the Scaritini and investigate whether plate tectonics and movement of land masses could have influenced scaritine evolution.

Chapter 2

Phylogeny of the Scaritinae inferred from morphological data

2.1 Characters and character states.

The theoretical concepts employed in creating the morphological dataset are outlined in this section.

A cladistic character can be defined as an attribute of an organism existing in one or more alternative character states. This is a traditional definition of characters and states, and while not explicitly stated, is used by most modern authors in reconstructing morphological phylogenies.
Certain assumptions are required when defining cladistic characters and character states. These assumptions are given below.

Characters must have evolved independently from one another because dependent characters will be correlated by their function. For example, Carabidae with atrophied wings always have atrophied flight muscles. If dependent characters are used they may bias the analysis by giving undue weight to a single evolutionary event (Felsenstein, 1982).
Characters must also be homologous, and the concept of homology is fundamental to the study of evolution. Characters shared by organisms are homologous if they have been inherited from a common ancestor. These

characters need not be identical, but may exist as alternative forms or states, implying modification of the character through descent.

All characters used in a cladistic analysis should be homologous. If not, spurious results will be obtained because non-homologous (analogous) characters will support relationships based not on evolution, but on other processes such as ecological convergence (when analogy is discussed in the context of cladograms or other evolutionary trees it is often termed homoplasy).

The recognition of homology of morphological characters can most rigorously be viewed as a two-step process (de Pinna, 1991). A primary homology statement, or an initial hypothesis of homology based on similarity, is created by defining characters and character states.

A secondary homology statement then follows as a result of a phylogenetic analysis, where each primary homology statement (in the form of character state changes) is evaluated by congruence with other characters.

Primary homology assessment is a critical step in a cladistic analysis and this stage will have the greatest influence on the results. Primary homology assessment is also the most subjective stage of the analysis because different authors may interpret characters in different ways (Hawkins et al., 1997) and may also be influenced by pre-conceived ideas about the evolution of a group, whether subconsciously or not.

2.2 Methods.

2.2.1 Taxon sampling.

The Scaritini consist of 54 currently recognised genera (Lorenz, 2005). From each of these genera at least one species was sampled, the exceptions being: the Asian genus *Tonkinoscaris*; the Australian genera *Neoscaphus*, *Trichocarenum* and *Steganomma* and the Madagascan genera *Dyscherinus*, *Prodycherodes* and *Paradyscherus*. These genera are scarce in collections and were either not available for study or known only from type specimens. Details of the 86 taxa examined are provided in table 2.1.

Genera (or in fact any taxonomic rank above that of species) are artificial groups of species which may or may not reflect evolutionary relationships. Since it cannot be assumed *a priori* that each genus is monophyletic, each individual species was used as a terminal in the analysis, and where possible the type species of each genus was used. Every genus is defined by a type species, so that even if the limits of a genus are changed, and species added or removed by such action, the genus will always contain its 'genus type'.

As the sister group to the Scaritinae is unknown (section 1.1.3), representative species from the mid-grade carabid subfamilies Elaphrinae, Broscinae, Psydrinae, Siagoninae, Migadopinae and Hiletinae were included as outgroups in the analysis. The genus *Elaphrus* (Elaphrinae) was arbitrarily chosen to root the trees resulting from the morphological analysis in this chapter and the molecular analysis in chapter 4.

Table 2.1. Summary of specimens examined for the morphological analysis. Classification follows Lorenz (2005). OUMNH = Oxford University Museum of Natural History, BMNH = British Museum (Natural History).

Subfamily	Tribe	Subtribe	Genus	Subgenus	Species	Locality	Depository
Siagoninae	Enceladini		*Enceladus*		*gygas* Bonelli	Venezuela, Hato Pinero	OUMNH
Siagoninae	Siagonini		*Luperca*		*laevigata* (F.)	India, Madras	OUMNH
Siagoninae	Siagonini		*Siagona*		*dejeani* (Rambur)	Spain, Algeciras	OUMNH
Hiletinae	Hiletini		*Eucamaragnathus*		*batesi* Chaudoir	Peru, Rio Tambonata Reserve	BMNH
Hiletinae	Hiletini		*Hiletus*		*versutus* Schiödte	Ivory Coast, Lamto	BMNH
Migadopinae	Migadopini		*Lissopterus*		*quadrinotatus* Water.	Falkland Islands	OUMNH
Promecognathinae	Promecognathini		*Promecognathus*		*laevissimus* (Dejean)	USA, California	OUMNH
Psydrinae	Amblytelini		*Amblytelus*		sp.	Australia, Sydney	OUMNH
Psydrinae	Melisoderini		*Melisodera*		*picipennis* Westwood	Australia, Victoria	OUMNH
Psydrinae	Psydrini		*Psydrus*		*piceus* LeConte	USA, California	OUMNH
Psydrinae	Psydrini		*Meonis*		*niger* Laporte	Australia, Sydney	OUMNH
Elaphrinae	Elaphrini		*Blethisa*		*multipunctata* (L.)	UK	OUMNH
Elaphrinae	Elaphrini		*Elaphrus*		*riparius* (L.)	UK	OUMNH
Broscinae	Broscini		*Gnathoxys*		sp.	Australia, Swan River	OUMNH
Broscinae	Broscini		*Broscus*		*cephalotes* (L.)	UK, Isle of Wight	OUMNH
Pterostichinae	Cnemalobini		*Cnemalobus*		sp.	Argentina, Puerto Madryn	OUMNH
Scaritinae	Dyschiriini		*Dyschirius*	*Dyschirius* s.str.	*thoracicus* (Rossi)	France, Etaple	OUMNH
Scaritinae	Clivinini	Clivinina	*Clivina*		*fossor* (L.)	UK, Dartmoor	OUMNH
Scaritinae	Clivinini	Clivinina	*Schizogenius*		*lineolatus* (Say)	USA, Indiana	OUMNH
Scaritinae	Clivinini	Clivinina	*Bohemania*		*gigantea* (Boheman)	Namibia, Windhoek	OUMNH
Scaritinae	Clivinini	Clivinina	*Forcipator*		*cylindricus* (Dejean)	Brazil	OUMNH
Scaritinae	Clivinini	Ardistomina	*Aspidoglossa*		*subangulata* (Chaudoir)	USA, Florida	OUMNH
Scaritinae	Scaritini	Pasimachus	*Mouhotia*		*gloriosa* Castelnau	Thailand	BMNH
Scaritinae	Scaritini	Pasimachina	*Pasimachus*	*Emydopterus*	*purpuratus* (Putzeys)	Belize, Chiquibul Forest	OUMNH
Scaritinae	Scaritini	Carenina	*Carenum*		*tinctillatum* (Newman)	Australia, Queensland	BMNH
Scaritinae	Scaritini	Carenina	*Neocarenum*		*elongatum* (MacLeay)	Australia, Perth	BMNH
Scaritinae	Scaritini	Carenina	*Laccopterum*		*spencei* Westwood	Australia, Moreton Bay	BMNH

Table 2.1 (continued). Summary of specimens examined for the morphological analysis.

Subfamily	Tribe	Subtribe	Genus	Subgenus	Species	Locality	Depository
Scaritinae	Scaritini	Carenina	*Monocentrum*		*convexum* (Sloane)	Australia, Cairns	BMNH
Scaritinae	Scaritini	Carenina	*Carenidium*		*bicornutum* (MacLeay)	Australia, Queensland	BMNH
Scaritinae	Scaritini	Carenina	*Scaraphites*		*rotundipennis* (Dejean)	Australia, Sydney	BMNH
Scaritinae	Scaritini	Carenina	*Philoscaphus*		*tuberculatus* (MacLeay)	Australia, New South Wales	BMNH
Scaritinae	Scaritini	Carenina	*Epilectus*		*mastersi* (MacLeay)	Australia	BMNH
Scaritinae	Scaritini	Scapterina	*Thlibops*		*longicollis* (Putzeys)	Senegal, Bambey	BMNH
Scaritinae	Scaritini	Scapterina	*Scapterus*		*guerini* Dejean	N.W.India	BMNH
Scaritinae	Scaritini	Scapterina	*Passalidius*		*fortipes* (Boheman)	South Africa	OUMNH
Scaritinae	Scaritini	Scaritina	*Acanthoscelis*		*ruficornis* (F.)	South Africa	OUMNH
Scaritinae	Scaritini	Scaritina	*Corintascaris*		*ferreirae* Basilewsky	Malawi	BMNH
Scaritinae	Scaritini	Scaritina	*Ochryopus*		*gigas* (Schiödte)	Uganda, Makerere	OUMNH
Scaritinae	Scaritini	Oxylobina	*Oxylobus*		*porcatus* (F.)	India, Nilgiri Hills	BMNH
Scaritinae	Scaritini	Scaritina	*Neochryopus*		*savagei* (Hope)	Nigeria, Oban	BMNH
Scaritinae	Scaritini	Scaritina	*Baenningeria*		*galapagoensis* (Linell)	Galapagos, Chatham Island	BMNH
Scaritinae	Scaritini	Scaritina	*Haplotrachelus*	*Haplotrachelus* s.str.	*holcopleurus* Chaudoir	South Africa, Bedford	BMNH
Scaritinae	Scaritini	Scaritina	*Haplotrachelus*	*Haplotrachelinus*	*atropis* (Bates)	South Africa, Durban	BMNH
Scaritinae	Scaritini	Scaritina	*Mamboicus*		*lastii* Bates	Tanzania, Morogoro	BMNH
Scaritinae	Scaritini	Scaritina	*Typhloscaris*		*gracilis* Bänninger	Tanzania, Tanganyika Range	BMNH
Scaritinae	Scaritini	Scaritina	*Coptolobus*		*glabriculus* Chaudoir	Sri Lanka, Nawara	BMNH
Scaritinae	Scaritini	Scaritina	*Geoscaptus*		*laevissimus* Chaudoir	Australia	BMNH
Scaritinae	Scaritini	Scaritina	*Anomophaenus*		*costatogranulatus* (Ch.)	New Caledonia	BMNH
Scaritinae	Scaritini	Scaritina	*Gnaphon*		*loyolae* (Fairmaire)	India, Shambagunur	BMNH
Scaritinae	Scaritini	Scaritina	*Macromorphus*		*elongatus* (Chaudoir)	No Data	BMNH
Scaritinae	Scaritini	Scaritina	*Cryptoscaphus*		*lissonotus* Chaudoir	Malawi, Tanga Province	BMNH
Scaritinae	Scaritini	Scaritina	*Glyptogrus*		*molopinus* (Perty)	Paraguay, Asuncion	BMNH
Scaritinae	Scaritini	Scaritina	*Glyptogrus*		*glypticus* (Perty)	Brazil	BMNH
Scaritinae	Scaritini	Scaritina	*Antilliscaris*		*mutchleri* (Bänninger)	Puerto Rico, El Yunque	BMNH
Scaritinae	Scaritini	Scaritina	*Scarites*	*Taeniolobus*	*guerini* (Chaudoir)	Colombia	BMNH

Table 2.1 (continued). Summary of specimens examined for the morphological analysis.

Subfamily	Tribe	Subtribe	Genus	Subgenus	Species	Locality	Depository
Scaritinae	Scaritini	Scaritina	*Scarites*	*Taeniolobus*	*silvestris* Laporte	Brazil, Para	BMNH
Scaritinae	Scaritini	Scaritina	*Scarites*	*Parallelomorphus*	*terricola* Bonelli	France, Narbonne	BMNH
Scaritinae	Scaritini	Scaritina	*Scarites*	*Scarites* s.str.	*subterraneus* F.	USA, Florida	BMNH
Scaritinae	Scaritini	Scaritina	*Scarites*	*Scarites* s.str.	*striatus* Dejean	Saudi Arabia, Jebel Shammar	BMNH
Scaritinae	Scaritini	Scaritina	*Scarites*	*Scarites* s.str.	*buparius* (Forster)	Algeria	OUMNH
Scaritinae	Scaritini	Scaritina	*Menigius*		*schaumi* Chaudoir	Cameroon, Sardi	BMNH
Scaritinae	Scaritini	Scaritina	*Pachyodontus*		*languidius* (Wiedemann)	South Africa, Table Mountain	BMNH
Scaritinae	Scaritini	Scaritina	*Haplogaster*		*ovata* Chaudoir	India, Bengal	BMNH
Scaritinae	Scaritini	Scaritina	*Distichus*	*Distichus* s.str.	*planus* (Bonelli)	Spain, Algaida	BMNH
Scaritinae	Scaritini	Scaritina	*Tapinoscaris*		*raffrayi* (Fairmaire)	Madagascar	BMNH
Scaritinae	Scaritini	Scaritina	*Storthodontus*		*nimrod* Chaudoir	Madagascar	BMNH
Scaritinae	Scaritini	Scaritina	*Crepidopterus*		*goudotii* (Guérin)	Madagascar, Tananariva	BMNH
Scaritinae	Scaritini	Scaritina	*Pilades*		*coquereli* (Fairmaire)	Madagascar	BMNH
Scaritinae	Scaritini	Scaritina	*Dyscaris*		*mordax* (Fairmaire)	Madagascar	BMNH
Scaritinae	Scaritini	Scaritina	*Prodyscherus*		*pluto* (Künckel)	Madagascar, Ambovombe	BMNH
Scaritinae	Scaritini	Scaritina	*Mecynoscaris*		*longula* (Fairmaire)	Madagascar, Mt. De Ambre	BMNH
Scaritinae	Scaritini	Scaritina	*Dyscherus*		*costatus* (Klug)	Madagascar	BMNH
Scaritinae	Scaritini	Scaritina	*Dinoscaris*		*venator* (Chaudoir)	Madagascar	BMNH
Scaritinae	Scaritini	Scaritina	*Madascaris*		*enoplus* (Alluaud)	Madagascar	BMNH
Scaritinae	Scaritini	Carenina	*Euryscaphus*		*waterhousei* (MacLeay)	Australia	BMNH
Scaritinae	Scaritini	Scaritina	*Typhloscaris*		*hutchinsi* (Alluaud)	Kenya, Fort Hall	OUMNH
Scaritinae	Scaritini	Scaritina	*Mamboicus*		*afrellus* (Bates)	Zambia, Lusaka	OUMNH
Scaritinae	Scaritini	Scaritina	*Scarites*	*Parallelomorphus*	*aterrimus* (Morawitz)	Japan	OUMNH
Scaritinae	Scaritini	Scaritina	*Scarites*	*Scarites* s.str.	*molossus* (Bänninger)	Zambia, Lusaka	OUMNH
Scaritinae	Scaritini	Pasimachina	*Pasimachus*	*Pasimachus* s.str.	*depressus* (F.)	USA	OUMNH
Scaritinae	Scaritini	Oxylobina	*Oxylobus*		*lateralis* (Dejean)	India	OUMNH
Scaritinae	Scaritini	Carenina	*Carenum*		*politum* Westwood	Australia	OUMNH
Scaritinae	Scaritini	Carenina	*Laccopterum*		*doddi* (Sloane)	Australia	OUMNH
Scaritinae	Scaritini	Carenina	*Monocentrum*		*frenchii* (Sloane)	Australia	OUMNH
Scaritinae	Scaritini	Storthodontina	*Crepidopterus*		*pipitzi* Fairmaire	Madagascar	OUMNH
Scaritinae	Scaritini	Carenina	*Scaraphites*		*silenus* (Westwood)	Australia	OUMNH
Scaritinae	Scaritini	Pasimachina	*Pasimachus*	*Emydopterus*	*rotundipennis* Chevrolat	Mexico, Jalapa	OUMNH

2.2.2 Character definition and coding.

Primary homology statements were formed as follows, using a two-step process of character definition and character state coding as outlined by Brower and Schawaroch (1996).

2.2.2.1 Character definition.

Characters were defined from published sources such as identification keys, taxonomic revisions and phylogenetic works (table 2.2) and by critical examination of specimens.

Table 2.2. List of published sources used in the initial selection of characters.

Andrewes, H.E. (1929). The Fauna of British India including Ceylon and Burma, Coleoptera. Carabidae Volume 1 Carabinae. London, Taylor and Francis.
Baehr, M. (2006). The Australian scaritine genus *Steganomma* Macleay (Coleoptera, Carabidae, Scaritinae). Mittelungen München Entomologische Gesellschaft 95, 59-66.
Balkenohl, M. (1994). New species and records of Scaritinae from the Himalayas (Coleoptera, Carabidae). Revue Suisse de Zoologie 101(1), 19-41.
Bänninger, M. (1937). Monographie der Subtribus Scaritina I. Deutsch entomologische Zeitshrift. (3-4), 81-160.
Bänninger, M. (1950). The subtribe Pasimachina (Coleoptera, Carabidae, Scaritini). Revista de Entomologia 21(3), 481-511.
Basilewsky, P. (1973a). Contributions a l'etude des Scaritinae d'Afrique II. Tableau des genres afro-malgaches de la sous-tribu des Scaritina. Revue de Zoologie et de Botanique Africaine 87, 174-180.
Basilewsky, P. (1973b). Insectes Coleopteres Carabidae Scaritinae. Faune de Madagascar 37. Orstrom CNRS, Paris.
Bell, R. T. (1967). Coxal cavities and the classification of the Adephaga (Coleoptera). Annals of the Entomological Society of America 60(1), 101–107.
Dostal, A. (1996). Bermerkungen zur systematischen Stellung der Gattung *Distichus* Motschulsky, 1857 und deren nächstverwandten Gattungen (Coleoptera, Carabidae, Scaritini). Bulletin et Annales de la Societe Royale Belge d'Entomologie 132, 7-17.
Erwin, T.L. & Stork, N.E. (1985). The Hiletini, an ancient and enigmatic tribe of Carabidae with a pantropical distribution (Coleoptera). Systematic Entomology 10, 405-451.
Erwin, T.L. & Sims, L.L. (1984). Carabid beetles of the West Indies (Insecta: Coleoptera): a synopsis of the genera and checklists of tribes of Caraboidea, and of the West Indies species. Quaestiones Entomologicae 20, 351-466.
Fedorenko, D.N. (1996). Reclassification of world Dyschiriini, with a revision of the Palearctic fauna (Coleoptera, Carabidae). Pensoft Series Faunistica No.4. Pensoft, Moscow.
Hlavac, T.F. (1969). A review of the species of *Scarites* (*Antilliscaris*), (Coleoptera: Carabidae) with notes on their morphology and evolution. Psyche 76(1), 1-17.
Matthews, E.G. (1980). A guide to the genera of beetles of South Australia. Part 1. South Australian Museum, Adelaide.
Moore, B.P. and Lawrence, J.F. (1994). The extraordinary larval characters of *Carenum* Bonelli and their bearing on the phylogeny of the Scarititae (Coleoptera, Carabidae). The Canadian Entomologist 126, 503-514.
Nichols, S.W. (1986a). Two new flightless species of *Scarites* s.str inhabiting Florida and the West Indes. Proceedings of the Entomological Society of Washington 88(2), 257-264.
Peringuey, L. (1896). Descriptive Catalogue of the Coleoptera of South Africa Part II. Cicindelidae supplement, Carabidae. West, Newman & Co., London.
Perrault, G.G. (1994). Studies on Neotropical scaritini II. Forcipatorina and Clivinina limits, with description of new genera. The Canadian Entomologist 126, 683-693.
Purrington, F.F. and Drake, C.J. (2005). A key to adult Nearctic *Pasimachus* Bonelli (Coleoptera: Carabidae: Scaritini), with comments on their functional mouthpart morphology. Entomological News 116(4), 253-262.
Reichardt, H. (1977). A synopsis of the genera of neotropical Carabidae (Insecta: Coleoptera). Quaestiones Entomologicae 13, 346-493.
Roig-Juñent, S (1998). Cladistic relationships of the tribe Broscini (Coleoptera: Carabidae). In: Ball, G.E., Casale, A., Taglianti, V.T. (Eds.). Phylogeny and classification of Caraboidea (Coleoptera: Adephaga). Museo Regionale di Scienze Naturali, Torino.
Sloane, T.G. (1904). Revisional notes on Australian Carabidae. Part 1. Proceedings of the Linnean Society of New South Wales 4, 699-733.
Sloane, T.G. (1923). The classification of the family Carabidae. Transactions of the Entomological Society of London I, II, 234-250.
Westwood, J.O. (1842). On the Scaritideous beetles of New Holland. In: J. O. Westwood (Ed.) Arcana Entomologica or illustrations of new rare and interesting insects. William Smith, London.

Morphological features showing any degree of variation between taxa were initially considered for inclusion in the analysis. For example, this could be the presence or absence of an attribute at a particular position (such as setae, carinae (ridges) and sulci (depressions)), or variation in the shape or size of body parts such as the eyes, palpi or tibiae.

Homology of characters across different species was assigned using the criterion of 'topographical correspondence' (Brower and Schawaroch, 1996). Essentially, characters were considered homologous if they occupied a similar physical position in the organism. In many cases the initial assignment of homology was intuitive, but otherwise characters were only used when their homology was reasonably unambiguous.

The characters chosen were those with potential to group taxa above the species level. These are characters that show little variation between members of the same genus, but conversely exhibit variation between members of different genera. Some of these characters are those previously used to define genera or other higher taxa. These were extracted from the literature (table 2.2) and each was re-evaluated by reference to specimens. Other characters were initially included on the basis examination of specimens in this study.

Several types of characters were excluded from the analysis after initial consideration; highly variable characters at the species level, unique derived characters confined to only one taxon (autapomorphies) and continuous

characters. The rejected characters are summarised in table 2.3. Included characters are listed in table 2.4 and are elaborated in detail in section 2.2.3. Highly variable characters at the species level would be expected to have no phylogenetic signal above the generic level and to add only noise to the dataset. Many of these variable intraspecific characters are in fact continuous so were not used for this reason also.

An autapomorphy is a uniquely derived character possessed by only one taxon. Because of this, autapomorphies provide no information on grouping in a cladistic analysis and therefore were not used.

After scoring, several characters were linked to the loss of flight ability and were also excluded (section 2.2.2.7).

Table 2.3. Morphological characters discarded after initial consideration.

Character	Variation
Antero-lateral punctures of frons.	Present or absent in different species of *Mamboicus*.
Paralateral dorsal setae of labrum.	Present in all taxa except *Corintascaris ferreirae*. Parsimony uninformative.
Enlargement and modification of labial palpomere 2.	Autapomorphy for *Ochryopus gigas*. Parsimony uninformative.
Surface sculpture of mandibles.	Inconsistent. Smooth and striate mandibles occur in different species of *Pasimachus*.
Eye size	Continuous character.
Ocular callus (Basilewsky, 1973)	Not homologous between different subtribes and genera and both present or absent in other genera for example *Haplotrachelus*.
Length of antennae	Continuous character.
Insertion of antennomere 2 (pedicel) with antennomere 1 (scape).	Antennomere 2 is eccentrically attached to antennomere 1 in some genera of Clivinini only. Not relevant to ingroup.
Shape of apex of lateral lobe of mentum.	Inconsistent within several genera and difficult to assign to discrete states.
Lateral border of mentum	Impossible to assign to discrete states.
Shape of mentum tooth	Impossible to assign to discrete states.
Number of pronotal lateral setae	Variable at species level. Presence and number of lateral setae varies between species of *Gnaphon* (Andrewes, 1929) *Scarites* and *Steganomma* (Baehr, 2006).
Seta of hind angle of pronotum.	Present or absent in various species of *Scaraphites*.
Transverse ridges of prosternum.	Autapomorphy for *Ochryopus gigas*. Parsimony uninformative.
Prescutellar pore	Present in Dyschiriini only. Not relevant to ingroup.
Number of dorsal punctures of elytra.	Number of punctures can vary within a single species for example *Haplogaster ovata* (Balkenohl, 1994) and *Scarites striatus* (Tschitschérine, 1904).
Elytral pubescence	Autapomorphy for *Ochryopus gigas*. Parsimony uninformative.
Sculpture of lateral channel of elytron	Impossible to assign to discrete states.
Marginal umbillicate pores of elytra	Interrupted in Dyschiriini only. Not relevant to ingroup.
Accessory protibial spines.	In some *Scaritina* additional spines are present, proximal and in the same horizontal plane as the main protibial spines. These are present or absent in various species of *Scarites*. Additional protibial spines also occur in *Philoscaphus* and *Euryscaphus* but are not in the same horizontal plane and are non-homologous with the accessory spines of *Scarites*.
Position of terminal protibial spur relative to 2nd spur.	Inconsistent within *Scaraphites*, contrary to Moore and Lawrence, 1994.
Metatibial setae	Long metatibial setae present in some species of *Scarites* only.
Metallic coloration.	Species of *Carenum* and *Laccopterum* may be metallic blue or green or entirely black.

Table 2.4. Morphological characters used in the analysis.

Character number	Character
1	Posterior supraorbital callus.
2	Anterior supraorbital setae.
3	Posterior supraorbital setae.
4	Presence and form of frontal furrows.
5	Frons tubercle.
6	Antennal insertion.
7	Extent of antennal pubescence.
8	Presence of median band of antennomeres 5-11.
9	Length of the antennal scape (antennomere 1).
10	Presence of a seta on the antennal scape.
11	Number of clypeal setae.
12	Clypeal suture.
13	Shape of the labrum.
14	Medial dorsal setae of the labrum.
15	Shape of the terminal maxillary palpomere.
16	Shape of the terminal labial palpomere.
17	Number of setae on the inner margin of labial palpomere 2.
18	Shape of the maxillary lacinia.
19	Mandibular scrobal seta.
20	Length of the maxillary fissure.
21	Genal process.
22	Form of the mentum tooth.
23	Anterior mentum setae.
24	Posterior mentum setae.
25	Fusion of the mentum and submentum.
26	Setae of the submentum.
27	Form of the gula.
28	Antennal groove.
29	External carina.
30	Internal carina.
31	Lateral border of the pronotum.
32	Prosternal keel.
33	Raised border of the prosternal process.
34	Prosternal setae.
35	Closure of the pro-coxal cavities.
36	Mesocoxal cavities.
37	Mesosternal setae.
38	Metasternal setae.
39	Metacoxal cavities.
40	Separation of the metacoxae.
41	Anterior metacoxal seta.
42	Posterior metacoxal seta.
43	Inner-marginal metacoxal seta.
44	Number of protibial spines.
45	Mesotibial outer angle.
46	Dorsal mesotibial spines.
47	Metatibial outer angle.
48	Unguitractor plate.
49	Form of humeral region (humeral field) of elytra.
50	Width of the elytral epipleuron.
51	Ocellate punctures of the elytral base.
52	Foveate elytral pits.
53	Parascutellary stria.
54	3rd elytral interval.
55	5th elytral interval.
56	6th elytral interval.
57	7th elytral interval.
58	Punctures of the 7th elytral stria.
59	8th elytral interval.
60	Transverse sulci of the last 3 abdominal sternites.
61	Lateral setae of the third sternite.
62	Presence of ambulatory setae of abdominal sternites three to five.

2.2.2.2 Character coding.

Character coding is a process whereby the variation observed in a character is assigned to discrete states.

Characters were defined either as simple binary characters with two states or as multistate characters with three or more states.

2.2.2.3 Ordering of character states.

Multistate characters can be treated in a number of ways. Characters can be unordered, with transitions between any of the character states requiring the same number of steps. Characters may also be ordered as the result of a hypothesis of character state transformation, for example if an ordered character has states 0, 1, 2 and 3, a change from state 0 to state 3 requires three steps. These ordered characters are also variously termed additive or Wagner characters. Because character ordering requires an *a priori* hypothesis of character evolution it may be subjective, but potentially can improve tree resolution and reduce the number of equally parsimonious trees obtained (Hauser and Presch, 1991).

While not ordering characters may be a more objective approach it should be noted that an unordered character still provides an explicit definition of character state transitions (i.e. transitions between any state cost the same number of steps).

Watrous and Wheeler (1981) give a detailed account of the outgroup comparison method for ordering character states, which involves the comparison of character states between the ingroup and outgroup. Character states present in the outgroup and only some of the ingroup are considered plesiomorphic, while character states present in only some of the ingroup are considered apomorphic. As this method requires detailed comparison between ingroup and outgroup, if the outgroup is not known with confidence it may be difficult to unambiguously order characters.

Because the sister group to the Scaritinae is unknown, where possible multistate characters were ordered using the 'generalised outgroup comparison method' of Ball (1985). This method is in effect a less stringent modification of the method of Watrous and Wheeler (1981) where character states that are widely distributed in the outgroup taxa (and generally in other carabid groups) are considered plesiomorphic.

In practice, most multistate characters in this study were unordered because the direction of transition from one character state to another was unclear.

2.2.2.4 Missing and inapplicable data.

Missing data entries (coded as '?') were used when the physical condition of specimens prevented scoring of certain characters. For example, the only specimen available of *Macromorphus elongatus* (Chaudoir), a very rare species in collections, was missing the hind legs.

The ‘?’ symbol was also used for inapplicable characters. Inapplicable characters occur when a morphological feature is missing in certain taxa (Strong and Lipscomb, 1999). For example, the presence or absence of the external carina of the antennal groove (character 29) could not be scored for those species which lack the antennal groove.

2.2.2.5 Convergent and parallel evolution.

Convergent evolution is a process whereby two distantly related taxa independently acquire similar traits in response to similar environmental conditions. For example, the adaptation of mammals to a subterranean environment has resulted in convergence of morphological structures, such as well-developed incisors and fore-limbs and a cylindrical body shape (Nevo, 1979).

Parallel evolution can be considered a similar process to convergent evolution but occurs between closely related organisms. The terms ‘distantly related’ and ‘closely related’ are somewhat vague but the important point is that the independently acquired traits are not inherited from a common ancestor and therefore not homologous. For this reason, convergent or parallel evolution can seriously confound attempts to reconstruct evolutionary history.

Convergence may be detected at both the primary and secondary stages of homology assessment (Desutter-Grandcolas et al., 2005). At the primary stage, close and careful examination of characters may result in their failure

to meet the criterion of similarity. At the secondary stage of homology assessment, occurring after the analysis, convergence may be detected by incongruence with other characters.

The two most frequently used measures of the fit of a particular character to a tree are the per-character consistency index (*ci*) and the per-character retention index (*ri*).

The *ci* provides a measure of the amount of homoplasy exhibited by a character. It is defined as:

$$ci = \frac{m}{s}$$

(Farris, 1989).

Where *m* is the minimum possible number of character changes (steps) of the character on the tree (for example, for a binary character coded as 0 and 1 the minimum number of possible character state changes is a single change from 0 to 1 and therefore *m*=1).

and *s* is the actual number of steps on the tree.

A character with no homoplasy and perfectly fitting the tree has a *ci* of 1, while a character showing a large amount of homoplasy will have a value tending towards (but never actually reaching) zero.

The *ri* is defined as:

$$rt = \frac{(g - s)}{(g - m)}$$

(Farris, 1989).

Where *m* and *s* are the same as for the consistency index.

and *g* is the maximal number of steps for the character on the tree. For a binary character (coded as 0 or 1), the value of g is the number of taxa with state 0 or 1, whichever is lower.

The *ri* gives a measure of the amount of synapomorphy expected from the data that is retained as synapomorphy on the tree (Lipscomb, 1998). An *ri* of 1 indicates the character provides the maximum possible amount of synapomorphy while decreasing values indicate the character providing lower amounts of synapomorphy.

Both the *ci* and *ri* can be summed over all the characters to give the ensemble consistency index (*CI*) and the ensemble retention index (*RI*). These ensemble indices give a measure of how all the characters perform on the tree.

2.2.2.6 Convergent and Parallel evolution in the Scaritini.

The morphological adaptations to burrowing in scaritines generally involve modifications to the head, legs and thorax.

Locomotory adaptations for burrowing or moving through loose substrate include:

- Flattened protibiae armed with marginal spines for moving substrate and excavating burrows.
- Enlarged profemora to house the necessary musculature for digging.
- The larger species, of which most belong to the Scaritini *sensu stricto*, use their mandibles for excavating burrows in addition to capturing and consuming prey.
- A constriction of the mesothorax known as the peduncle. The pedunculate body form allows greater flexibility of movement in confined spaces such as between sand and gravel particles for smaller species or in burrows excavated by the larger species.

Adaptations protecting delicate sensory structures from abrasion include:

- An antennal plate, a plate-like structure derived from the anterior part of the frons which covers the antennal insertion.
- An antennal groove for housing the retracted antennae.
- An enlarged gena protecting the eye.

These adaptations could be the result of convergence, especially between the three main groups of scaritines (Scaritini, Clivinini and Dyschiriini). At first sight it would also appear that these characters are functionally linked to burrowing and therefore dependent, violating one of the key requirements of cladistic characters.

However, by comparison with other groups of Carabidae it is clear that these characters occur in different combinations in other groups, making any

conclusions about their function subjective. For example, species of *Siagona* (Siagoninae) and *Promecognathus* (Promecognathinae) possess an antennal plate, elongate scape and peduncle but lack antennal grooves and protibial spines. Conversely, *Gnathoxys* (Broscinae) lack antennal plates and an elongate scape but do possess a peduncle and protibial spines.

The question arises as to whether these putatively adaptive characters should be used in a phylogenetic analysis. On the one hand, including them increases the risk of using homoplasious and therefore misleading characters. On the other hand, excluding them involves making *a priori* subjective decisions about the adaptive function of characters (Liebherr, 2003). The approach taken in this study is to attempt to maximise objectivity, by including all potentially informative characters (with the exception of the two thoracic characters outlined in the following section) regardless of presumed adaptive function and perceived susceptibility to convergence. The adaptive function of each character is nonetheless discussed (section 2.2.3), because function may be linked to the amount of homoplasy displayed by a character.

2.2.2.7 Characters associated with flight.

It is hypothesised that two characters of the mesothorax, the length of the metepisternum and the length of the metasternum, are a result of the loss of flight ability in scaritines and are functionally linked. For these reasons they are excluded from the analysis.

The loss of flight ability is well documented in Carabidae and Coleoptera as a whole and is especially prevalent in species inhabiting mountains and islands (Darlington, 1943). The morphological changes associated with flight loss are varied and are presumably due to the length of time elapsed since flight ability has been lost. Flightlessness can occur initially by a reduction in the metathoracic flight muscles, with the wings and thoracic sclerites otherwise unchanged (Smith, 1964). Flight ability can also be lost as a result of brachyptery (reduction in the size of the wings).
One of the most dramatic morphological modifications caused by flightlessness is a reduction in the length of the metathoracic sclerites, the metasternum and metepisternum. This occurs as a consequence of the reduction in size of the tergosternal and pleural flight muscles and has been documented widely in Carabidae, Chrysomelidae, Tenebrionidae, Ptinidae and other families (Smith, 1964).

By comparing closely related species it is possible to clearly discern these changes in body shape associated with the loss of flight. For example, almost all members of the burrowing scarab beetle genus *Dichotomius* are winged and fly in order to locate their food source, animal dung. One species out of the 145 so far described, *D.comorapensis* Génier, is flightless and exhibits unusual characters not seen in the flying forms (D.J.Mann, pers. comm.). Here the humeral region and length of the elytra are reduced and the mesothorax and metathorax are shortened (Génier, 2000). The

reduction of the metathorax is clearly evident by comparing the lengths of the metepisterna (figure 2.1).

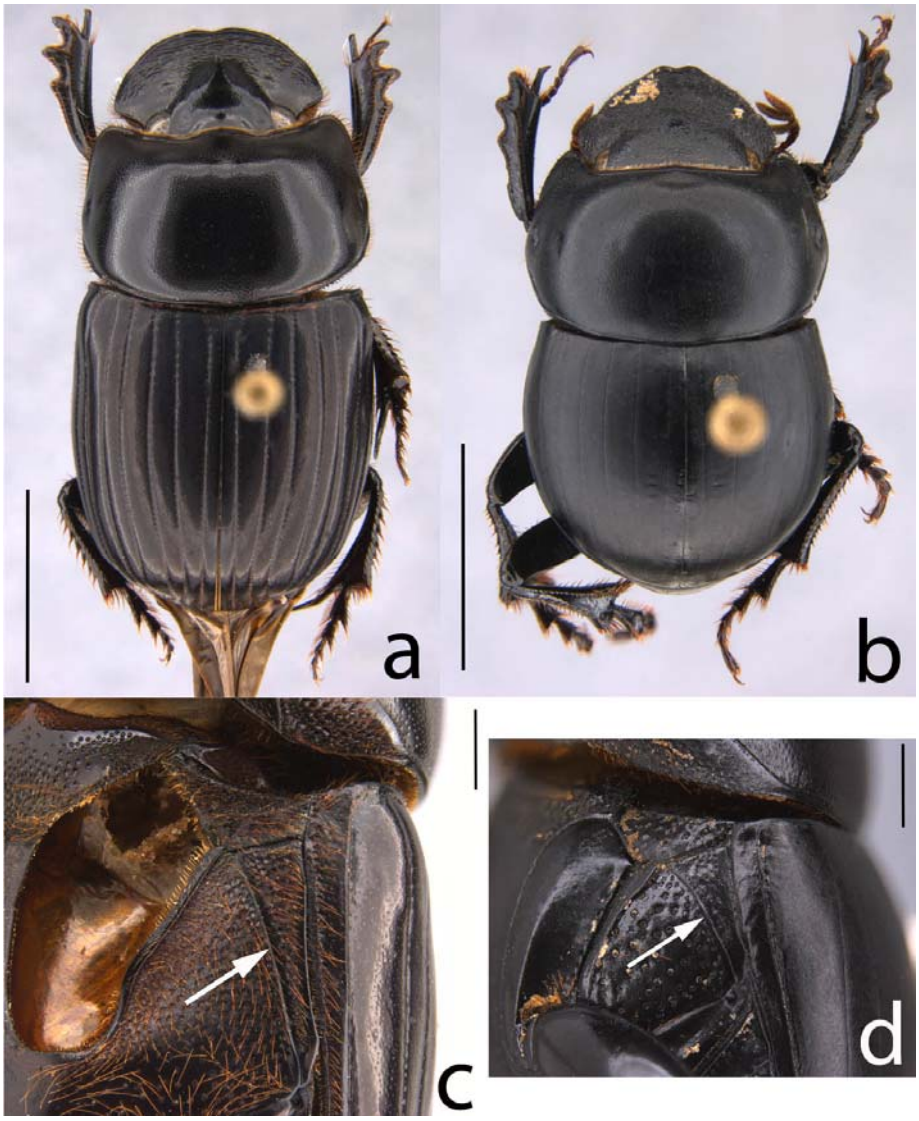

Figure 2.1. Flying and flightless species of *Dichotomius* (Coleoptera: Scarabaeidae). (a) *Dichotomius* sp. (Bolivia), fully winged and flying species. Scale bar = 5 mm. (b) *D.comorapensis* (Bolivia), a flightless species. Scale bar = 5 mm. (c) *Dichotomius* sp. ventro-lateral view showing long metepisternum (ratio anterior margin of metepisternum / exterior margin 0.29) (indicated by arrow). Scale bar = 1 mm. (d) *D.comorapensis* ventro-lateral view showing shortened metepisternum (ratio anterior margin / exterior margin 0.44) (indicated by arrow). Scale bar = 1 mm.

The same morphological modifications occur in closely related flying and flightless Scaritini.

For example, the flightless species *Scarites* (*Parallelomorphus) aterrimus* Morawitz has a short metasternum and metepisternum when compared to other flying species of the same subgenus (figure 2.3).

Nichols (1986a) gives similar examples for species of the *subterraneus* complex of *Scarites sensu stricto* where the metepisternum is progressively shortened in brachypterous and micropterous species.

Extreme examples of the reduction in length of the metepisternum (and the metathorax in general) occur mostly in species from montane habitats such as *Antilliscaris megacephala* (Hlavac) (Hlavac, 1969) from Mount El Yunque, Puerto Rico and *Pachyodontus languidus* (Wiedemann) from Table Mountain, South Africa (figure 2.2). The reduced hind-body of these species leads to a very peculiar body shape quite unlike any other Carabidae.

Figure 2.2. *Pachyodontus languidus* (Wiedemann) (Scaritini: Scaritina), a large species endemic to Table Mountain, South Africa.

Other examples of flightless Scaritini are numerous and some of the genera structurally similar to *Scarites* such as *Taeniolobus*, *Menigius* and *Mecynoscaris* are defined in part by a short metasternum and metepisternum. (Bänninger 1937; Dostal 1996).

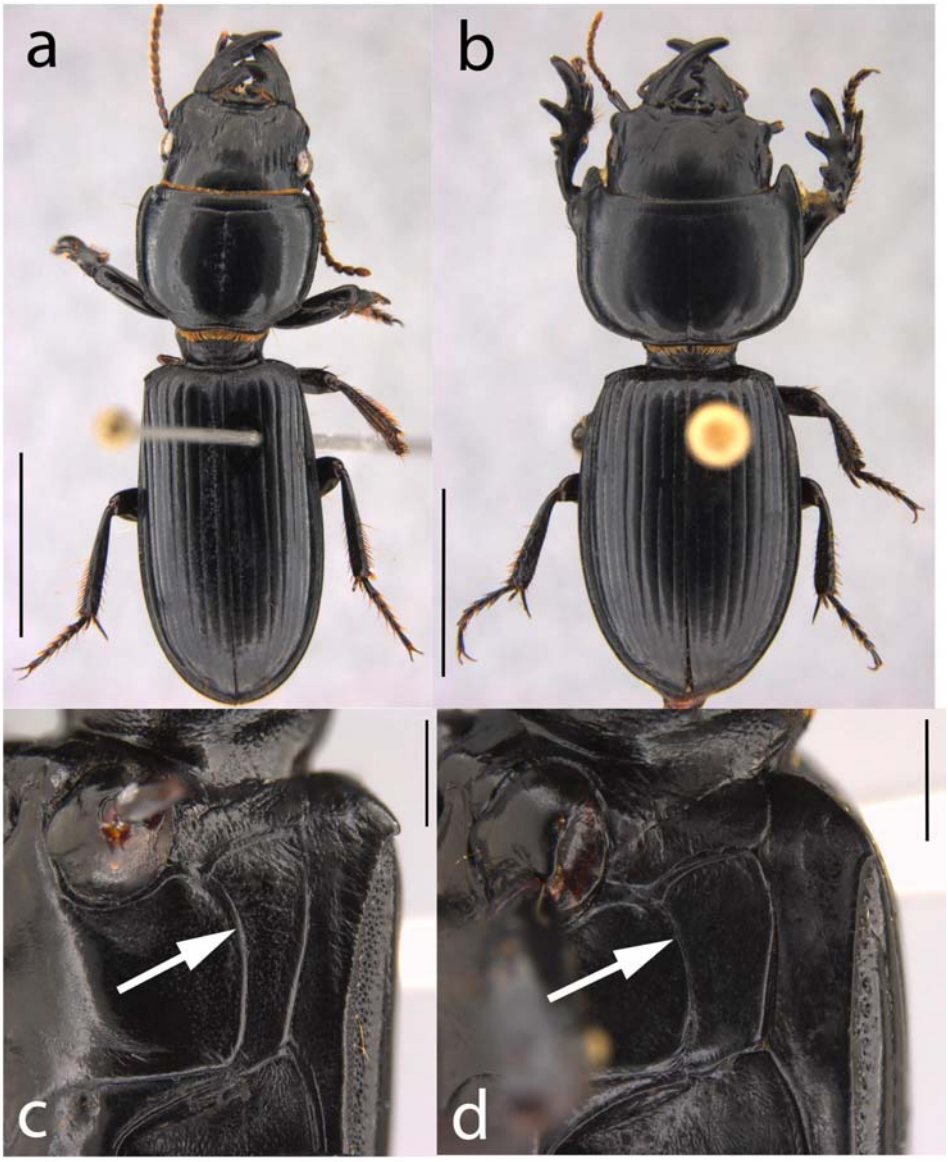

Figure 2.3. Flying and flightless species of *Scarites* subgenus *Parallelomorphus* (Scaritini: Scaritina). (a) *Scarites (Parallelomorphus) terricola ssp. pacificus* Bates (China), fully winged and flying species. Scale bar = 5 mm. (b) *Scarites (Parallelomorphus) aterrimus* Morawitz (Japan), a flightless species. Scale bar = 5 mm. (c) *S.terricola ssp. pacificus* ventro-lateral view showing long metepisternum (ratio anterior margin of metepisternum / exterior margin 0.44) (indicated by arrow). Scale bar = 1mm. (d) *S.aterrimus* ventro-lateral view showing shortened metepisternum (ratio anterior margin / exterior margin 0.56) (indicated by arrow). Scale bar = 1 mm.

Darlington (1943) estimated that of all the known species of Carabidae, one fifth to one quarter are flightless. Because the loss of flight ability occurs so often in Carabidae, characters associated with it are hypothesised to be particularly unreliable markers of evolutionary history.

2.2.3 The morphological characters.

Specimens were examined using a Leica Wild M3Z microscope at a magnification of between X13 and X80. Relative measurements were made using an eyepiece graticule. Photographs of small specimens (less than 20 mm) were taken with a Leica DFC 490 digital camera linked to a Leica M165C microscope. Stacks of images were acquired using the program Leica Application Suite version 3.7.0 (Leica Microsystems Limited) and combined into single montage images using Helicon Focus version 5.2.4 (Helicon Soft Limited). Photographs of larger specimens were taken with a Nikon D50 camera with a Sigma 105mm 1:2.8D DG lens.

A total of 62 characters were scored and included in the analysis. 45 of these were binary characters and 17 were multistate. Of these 17 multistate characters only 6 could be ordered either because it was not possible to determine the plesiomorphic state by outgroup comparison or the direction of character state transitions were uncertain. The ordered characters are characters 20 (length of the maxillary fissure), 44 (number of protibial spines), 46 (dorsal mesotibial spines), 49 (form of humeral region (humeral field) of elytra), 57 (7^{th} elytral interval) and 59 (8^{th} elytral interval).

A taxon versus character data matrix was constructed with WinClada version 1.00.08 (Nixon, 2002).

The following is an annotated list of the morphological characters scored in the analysis.

2.2.3.1 Dorsal surface of the head.

Examples of morphological structures of the dorsal surface of the head are given in figure 2.4.

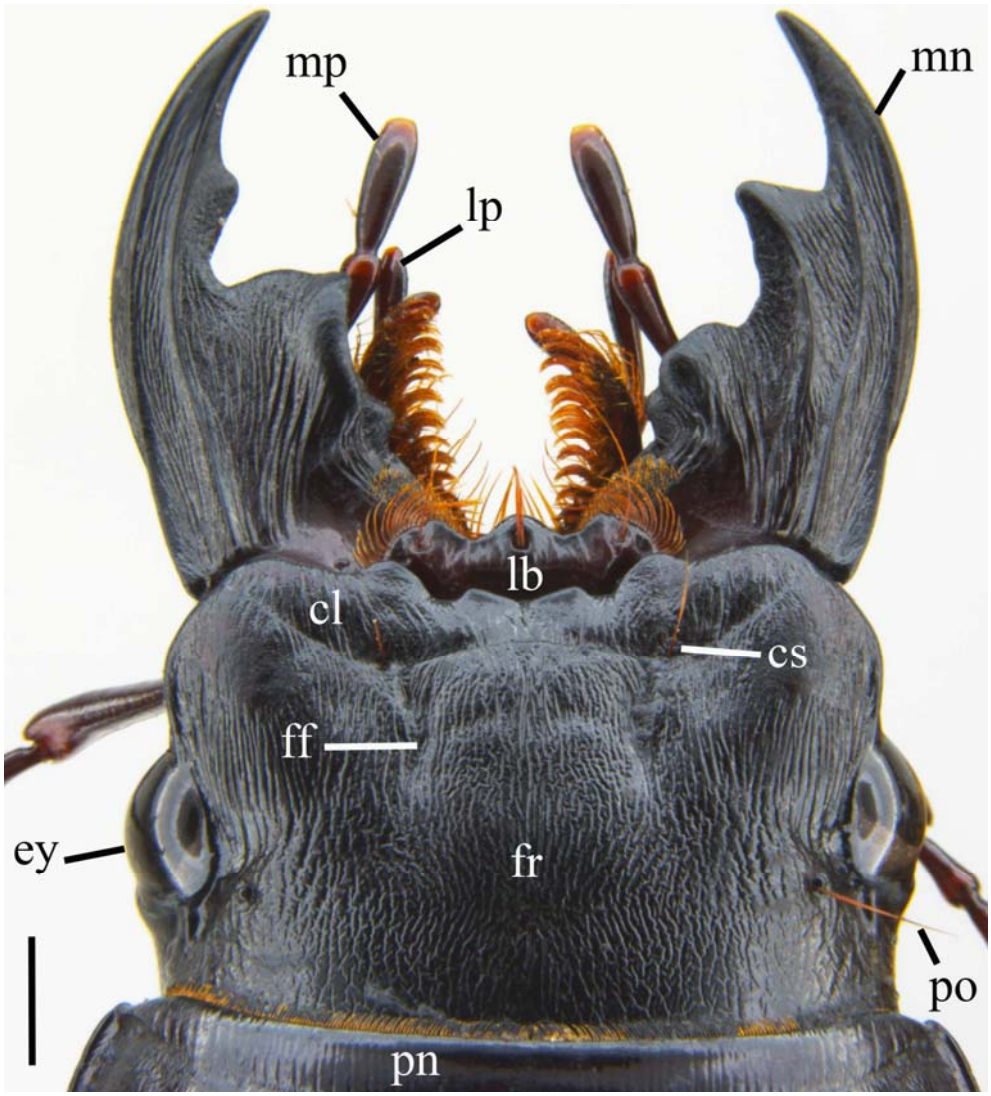

Figure 2.4. Dorsal view of the head of *Scarites* sp. (Scaritini: Scaritina). Myanmar. (mn = mandible; po = posterior supraorbital seta; pn = pronotum; fr = frons; ey = eye; ff = frontal furrow; cl = clypeus; cs = clypeal seta; lb = labrum; mp = maxillary palp; lp = labial palp). Scale bar = 1 mm.

Character 1. Posterior supraorbital callus.

Immediately underneath the posterior supraorbital seta of most scaritines is a small projecting tubercle. It is presumed to protect the attachment point of the seta from damage by abrasion.

0-Callus absent; 1-callus present.

Number and position of supraorbital setae.

Supraorbital sensory setae are present in nearly all Carabidae, located on the vertex of the head adjacent to the eye. The number and position of supraorbital setae is sometimes a very consistent and therefore defining character of many carabid tribes. Usually the supraorbital setae are present as anterior and posterior pairs, although the anterior pair is missing in some groups. In others the number and position of setae is variable, for example the mid-grade tribe Broscini shows all states from complete absence to 6 pairs of setae or more (Roig-Juñent, 2000).

Clivinini and Dyschiriini usually possess both anterior and posterior pairs of setae, although some Forcipatorina (Clivinini) have up to 7 pairs and other forcipatorine genera such as *Obadius*, *Camptodontus* and *Camptidius* have only 1 pair (Perrault, 1994). In Scaritina the anterior pair is always absent and rarely also the posterior pair. Complete absence of supraorbital setae occurs in the scaritine genera *Mouhotia*, *Passalidius, Corintascaris, Tibioscarites* (Basilewsky 1973a), some Australian Scaritini and some Salcediina. Conversely, Baehr (2006) describes 2 pairs of setae for the genus *Steganomma*, which could either be a true anterior and posterior pair

or a doubling of the posterior pair (this genus was unavailable for study). In some species of *Prodyscherus* the posterior supraorbital setae number 6 or more, but are scored here as 'posterior pair only' as they are hypothesized to arise by duplication. Sloane (1923) also notes that in the Australian Carenina (Scaritini) the number of supraorbital setae varies among species. Members of the scaritine genera *Forcipator* and *Ochryopus*, and the siagonine *Siagona* are impossible to score for these characters because the supraorbital setae are indistinguishable from the numerous other setae found on the surface of the head. Rather than assigning a third character state to these taxa, and therefore creating a statement of homology, they are scored as 'unknown' or 'missing data' using the '?' symbol.

According to Baehr (1997) the supraorbital setae and other fixed setae at various positions on the dorsal and ventral surface probably allow the insect to measure distances in confined spaces. The adaptive reasons for their loss in Scaritinae are unknown but suggests an unusual mode of life.

Character 2. Anterior supraorbital setae.

0- Anterior supraorbital setae absent; 1- anterior supraorbital setae present.

Character 3. Posterior supraorbital setae.

0- Posterior supraorbital setae present; 1- posterior supraorbital setae absent.

Character 4. Presence and form of frontal furrows.

Many Scaritinae possess a paralateral pair of longitudinal furrows on the vertex of the head, originating at the clypeal suture and terminating at the level of the eye or just before. The furrows show variation in depth and width between members of different genera. The assignment of this variation into discrete states is in most cases straightforward as there is little overlap between character states.

Similar furrows occur in the tribes Broscini (Roig-Juñent, 2000) and Hiletini, the shape of which is possibly correlated with mandible morphology (Erwin and Stork, 1985). By comparison of the form and position of the furrows it is hypothesized they are homologous across different groups of Carabidae.

This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0- Frontal furrows absent; 1- frontal furrows wide; 2-frontal furrows narrow with sharply defined edges.

Character 5. Frons tubercle.

The scapterine genera *Scapterus* and *Thlibops* (Scaritini) display a well-developed tubercle or small horn on the centre of the frons (Andrewes, 1929). This is a very unusual character in Carabidae, but also occurs in some genera of the unrelated Ditomina (Carabidae, Harpalini).

0- Central tubercle absent; 1- central tubercle present.

Character 6. Antennal insertion.

The antennal insertion is concealed from above by a lateral expansion of the anterior area of the frons in scaritines and some other groups of Carabidae, presumably to protect the antennae.

0-At least part of rounded base of scape visible from above; 1- concealed from above.

Character 7. Extent of antennal pubescence.

Small hairs covering at least part of the antennomeres are a usual feature of carabids and in many tribes there is a consistent pattern of pubescence, always starting from either the 3rd, 4th, 5th or 6th antennomere (this pubescence is not to be confused with the larger apical ring setae of the antennomeres).

In the scaritine genus *Mouhotia* the antennae are pubescent from antennomere 6, while in almost all other Scaritini the pubescence begins at antennomere 5. Complete absence of this pubescence is very rare in Carabidae but occurs in the scapterine *Passalidius*. Even though *Passalidius* has glabrous antennae there still remain 2 parallel carinae on each side of antennomeres 5-11, marking the position of an area of microsculpture where the pubescence was once attached. Because of this interpretation the antennae of *Passalidius* are scored as 'antennomeres pubescent from number 5'.

The function of the antennal pubescence may be sensory or water repellent (Erwin and Stork, 1985) and the loss of this pubescence in *Passalidius* may

be related to the arid habitats in Namibia and South Africa where the genus occurs.

This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0-Pubescent from antennomere three; 1-pubescent from antennomere four; 2-pubescent from antennomere five; 3-pubescent from antennomere six.

Character 8. Presence of median band of antennomeres 5-11.

In most Scaritini and larger Clivinini such as *Sparostes* the fine pubescence and granular areas of antennomeres 5-11 are interrupted on the dorsal and ventral surfaces by a glabrous median band; this band is lacking in smaller Clivinini such as *Clivina*. This character is coded simply as presence or absence of the median band, but in *Scarites* subgenus *Antilliscaris* (Hlavac, 1969) and possibly other taxa inhabiting high altitudes, the median band is reduced in width. This suggests an increased sensory function of the antennae in these species.

0-Median band absent, antennomeres 5-11 entirely pubescent; 1- median band present.

Character 9. Length of the antennal scape (antennomere 1).

Antennomere 1 is scapiform (longer than antennomeres 2 and 3 combined) in most Scaritini and also in the mid-grade tribes Hiletini and Siagonini. The other scaritines (Clivinini and Dyschiriini) have a scape of more or less normal length for Carabidae. The scapiform character state in Scaritinae

occurs with another character, the presence of the antennal groove (character 28 in this study). However, these two characters are not considered to be linked as in the outgroup *Siagona,* antennomere 1 is scapiform without a corresponding groove.

0-Scape 'normal' length (<= combined length of antennomeres 2 and 3); 1-scape long, scapiform (> combined length of antennomeres 2 and 3).

Character 10. Presence of a seta on the antennal scape.

A conspicuous long seta is present on the dorsal surface of the antennal scape of *Pasimachus* and *Corintascaris* (Basilewsky, 1952) but lacking in all other Scaritini except as an aberration in *Scaraphites lenaeus* Westwood (Bänninger, 1950). In the Clivinini the scape seta occurs in members of the subtribe Clivinina (Ball, 2001) but is absent in the subtribe Forcipatorina (Reichardt, 1977).

0- Long seta present on dorsal surface of antennal scape; 1-seta absent.

Character 11. Number of clypeal setae.

Most Scaritini and Clivinini have a paralateral pair of clypeal setae. Absence of the clypeal setae was used by Dostal (1999) as a character to define *Distichus* subgenus *Baenningostichus*. Absence of the clypeal setae is unusual in Carabidae, which is presumably why this character state has been used previously by various authors as a diagnostic character for other taxa of Scaritini, for example *Geoscaptus*, *Cryptoscaphus* and *Scarites* subgenus *Parallelomorphus*.

0-Two paralateral setae; 1- clypeal setae absent.

Character 12. Clypeal suture.

The clypeal suture is more or less impressed in all Scaritini, visibly separating the clypeus from the frons, but occasionally it is obsolete.

0-Clypeal suture impressed, sometimes feint or only partly impressed; 1-clypeal suture obsolete.

2.2.3.2 Mouthparts.

Mouthpart structures are illustrated in figure 2.5.

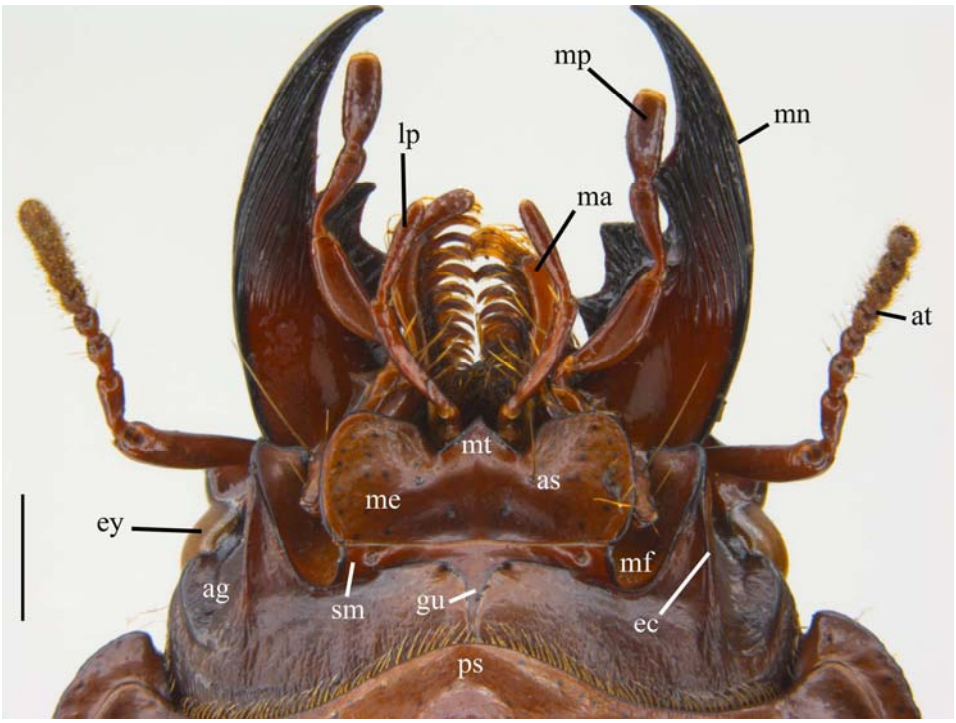

Figure 2.5. Ventral view of the head of *Acanthoscelis ruficornis* (F.) (Scaritini: Scaritina). South Africa. (mn = mandible; at = antenna; ec = external carina; mf = maxillary fissure; mp = maxillary palp; ma = maxillary lacinia; me = mentum; mt = mentum tooth; as = anterior mentum seta; sm = submentum; gu = gula; ps = prosternum; ag = antennal groove; ey = eye). Scale bar = 1 mm.

Character 13. Shape of the labrum.

Most Scaritini have a trilobed labrum, while in *Corintascaris* and in many Clivinini it is bilobed.

This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0-Labrum approximately rectangular; 1-bilobed (emarginate); 2-trilobed; 3-quadrilobed.

Character 14. Medial dorsal setae of the labrum.

Unordered as transition between character states unclear.

0-Medial setae absent; 1-two to five widely spaced setae, more or less aligned in a row; 2-three or more setae grouped closely to each other in the central area of the labrum.

Form of the maxillary and labial palpomeres.

Mouhotia and some Australian Scaritini possess very obvious securiform (hatchet-shaped) or sub-securiform terminal labial and maxillary palpomeres. Most other scaritines possess the normal cylindrical form of palpi for Carabidae.

The securiform shape increases the area of chemosensory pits (the papillate sensillae of Erwin and Stork (1985)) at the end of the palp and the securiform state has evidently evolved independently a number of times in ground beetles, for example in the tribes Cychrini and some Broscini (Roig-Juñent, 2000). In the basal-grade Cychrini it has been suggested that

securiform palpi are an aid to prey location by allowing the beetles to detect and follow the slime trails of molluscs (Forsythe, 1982) and similarly in other tribes to follow chemical trails of millipedes (Erwin, 1979).

Securiform palpi have evolved in some Scaritini and Broscini occurring in similar dry habitats in Australia, so an alternative (but purely speculative) possibility is that they are used as humidity sensors. In live beetles both sets of palps are directed downwards with the sensory areas pressed to the ground (Erwin, 1979). Whatever their exact use it is clear that expanded palpi allow some kind of enhanced sensory ability.

In examining a range of species it was evident that the labial and maxillary palpi of Carabidae vary continuously, from slightly dilated to very strongly dilated. Because it was not immediately clear whether the observed variation could be divided into discrete states, a histogram was plotted of frequency of occurrence versus relative width of the palp margin (figure 2.6).

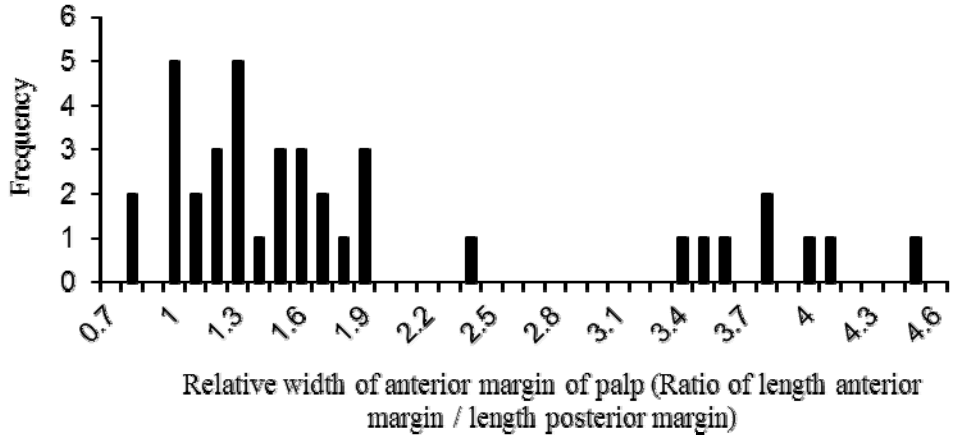

Figure 2.6. Histogram showing the distribution of labial and maxillary palp relative widths.

The histogram shows that variation in the relative width of the palps can be confidently divided into two discrete classes, 0.8-2.4 (the general unmodified palp shape of Carabidae) and 3.3-4.5 (securiform).

Character 15. Shape of the terminal maxillary palpomere.

0-Maxillary palp spindle-shaped (fusiform) to mildly dilated at apex. Ratio of length anterior margin / length posterior margin <2.6; 1- Maxillary palp hatched-shaped (securiform). Ratio of length anterior margin / length posterior margin >2.6.

Character 16. Shape of the terminal labial palpomere.

0-Labal palp spindle-shaped (fusiform) to mildly dilated at apex. Ratio of length anterior margin / length posterior margin <2.6; 1- Labial palp hatched-shaped (securiform). Ratio of length anterior margin / length posterior margin >2.6.

Character 17. Number of setae on the inner margin of labial palpomere 2.

The number of setae of labial palpomere 2 was noted by Andrewes (1929) as an apparent difference between the otherwise very similar genera *Gnaphon* (which have 'half a dozen' setae) and *Anomophaenus* (which have 2 setae). Palpomere 2 is usually bisetose in Clivinini and always so in the outgroup Hiletini (Erwin and Stork, 1985). A possible function of these setae is to prevent large fragments of food from falling away from the mouthparts (Forsythe, 1982).

Unordered as the transition between character states is unclear.

0-One seta on the inner margin of labial palpomere 2; 1-two setae; 2-three to four setae; 3-five setae; 4-six setae; 5-multisetose (>6).

Character 18. Shape of the maxillary lacinia.

The shape of the apex of the lacinia is a character given much weight in the differentiation of genera in the Scaritini (Chaudoir, 1879; Bänninger, 1937). This is especially so in the Scaritina, allowing this large subtribe to be conveniently divided into two groups. In *Scarites* and other genera the apex

of the lacinia is hooked while in many other Scaritina and Carenina it is rounded.

The hooked and densely setose lacinia of *Cychrus* (Carabidae, Carabini) apparently functions to break up soft bodied prey (Forsythe, 1982).

This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0- Hooked, pointed or truncate; 1-rounded; 2-asymmetric club bearing spicules.

Character 19. Mandibular scrobal seta.

The presence of a seta in the mandibular scrobe (the outer face of the mandible) is important in carabid classification as it is usually present in mid-grade carabids, but absent in most of the high-grade Harpalinae. A scrobal seta is absent in all scaritines.

0-Scrobal seta present; 1-absent.

Character 20. Length of the maxillary fissure.

Some scaritines possess a fissure separating the submentum from the genae, the function of which is unknown but is presumably associated with a particular mode of feeding. The absence of this fissure was proposed by Moore and Lawrence (1994) to be a synapomorphy for the subtribe Carenina, which was elevated to the rank of tribe (Carenini) on the basis of this and other characters. The absence of a fissure also occurs in other

Scaritini, so it is possible the fissure has been independently gained or lost a number of times in separate lineages.

In some genera, notably *Scaraphites* (Scaritini, Carenina), the fissure is present but extremely small and indistinct. In other Scaritini the fissure occurs in two discrete states, either long (extending to the base of the submentum) or short (not extending to the base of the submentum).

The fissure extends backwards beyond the base of the mentum in all Scaritina except *Distichus rectifrons* Bates (Andrewes, 1929) and a few related species (Balkenohl, 1994).

Ordered by outgroup comparison.

0-Wide (ratio width fissure/width mentum >0.1) and reaching at least 50% of the depth of the submentum; 1- narrow (ratio width fissure/width mentum <0.1) and reaching at least 50% of the depth of the submentum; 2-rudimentary (<50% of the depth of the submentum); 3-absent.

Character 21. Genal process.

In many Scaritina the area of the gena between the antennal groove and the mentum is developed into a dentiform process.

When coding this character it was initially suspected that it was linked to character 20, the length of the maxillary fissure. This is because without exception, all species with a wide maxillary fissure (character 20) lack a genal process. It appears that these two characters however are not linked because the reverse is not always true - species with a narrow and deep maxillary fissure do not always have a genal process, for example

Macromorphus elongatus Chaudoir, *Pachyodontus languidus*, *Dinoscaris venator* (Chaudoir) and *Crepidopterus goudoti* (Guérin-Méneville).

0-Process absent; 1-process present.

Character 22. Form of the mentum tooth.

In most scaritines the apex of the mentum tooth is unidentate, but is tridentate in *Macromorphus* (Peringuey, 1896).

This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0-Unidentate; 1-bidentate; 2-tridentate; 3-quadridentate.

Character 23. Anterior mentum setae.

The mentum of scaritines bears series of setae at various positions. As these setae are found in approximately similar positions they are considered to be homologous in different genera. In some genera, specific series of setae are absent.

The anterior mentum setae are located at the base of the mentum tooth or immediately adjacent to it. When present, they are represented by one or two pairs of setae.

This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0-Absent; 1-two anterior setae; 2-four anterior setae.

Character 24. Posterior mentum setae.

The posterior setae are located close to the hind margin of the mentum. This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0-Absent; 1-two posterior setae; 2-four posterior setae.

Character 25. Fusion of the mentum and submentum.

A fused mentum-submentum is a synapomorphy for the outgroup Siagonini. This character state is rare in scaritines but does occur in the dyschiriines *Clivinopsis* and *Antidyschirius* (Fedorenko, 1996).

0-Mentum and submentum not fused, submental suture visible; 1- mentum and submentum fused, submental suture not visible.

Character 26. Setae of the submentum.

This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0-Absent; 1-two submentum setae; 2-four submentum setae; 3-six submentum setae; 4-multisetose.

Character 27. Form of the gula.

The gula is the region of the head floor between the postoccipital (gular) ridges (Nichols, 1989). The external width of the gula reflects the internal separation of the gular ridges and the size of the stipal retractor muscles. A wide gula corresponds to large stipal retractor muscles and suggests an

important role for the maxillae in either breaking up prey or rapidly passing food to the mouth. A narrow gula suggests mandibular movements are more important in feeding, as is usually seen in phytophagous carabids (Forsythe, 1982).

In Carabidae the gula is usually characterised as 'wide' or 'narrow' in relation to the width of the mentum. Gula width is a character used in part to define some genera, for example the clivinine genus *Psammocoryza* (Hogan, 2006). In many Scaritini the gula is narrow, presumably reflecting the prominent role of the mandibles in feeding and burrowing.

0-Gula wide, at narrowest point >0.1 X maximum width of mentum; 1-gula narrow, at narrowest point <0.1 X maximum width of mentum.

2.2.3.3 Ventral surface of the head.

Character 28. Antennal groove.

In many Scaritini the antennal groove occurs as a longitudinal sulcus between the gena and the paragena, functioning to receive the elongated first antennal segment (the scape) when the antennae are retracted. The grooves are absent in those Scaritines with a shorter scape such as *Mouhotia* and *Pasimachus*. The presence of the antennal groove is not always linked to an elongate scape (character 9), for example the outgroup *Siagona* has a long scape but no groove, and hence these two characters are treated as independent.

0-Antennal groove absent; 1- antennal groove present.

Character 29. External carina.

The external carina is formed by the raised internal border of the antennal groove (illustrated in Basilewsky, 1973b) and occurs in some species of Scaritini. Species lacking the antennal groove are coded with a question mark and treated as missing data.

0-External carina absent; 1-external carina present.

Character 30. Internal carina.

The internal carina extends obliquely backwards and outwards, originating from an area close to the submental seta and extending backwards to the hind margin of the head (illustrated in Basilewsky, 1973b).

0- Internal carina absent; 1- internal carina present.

2.2.3.4 Thorax.

Ventral thoracic structures are illustrated in figure 2.7.

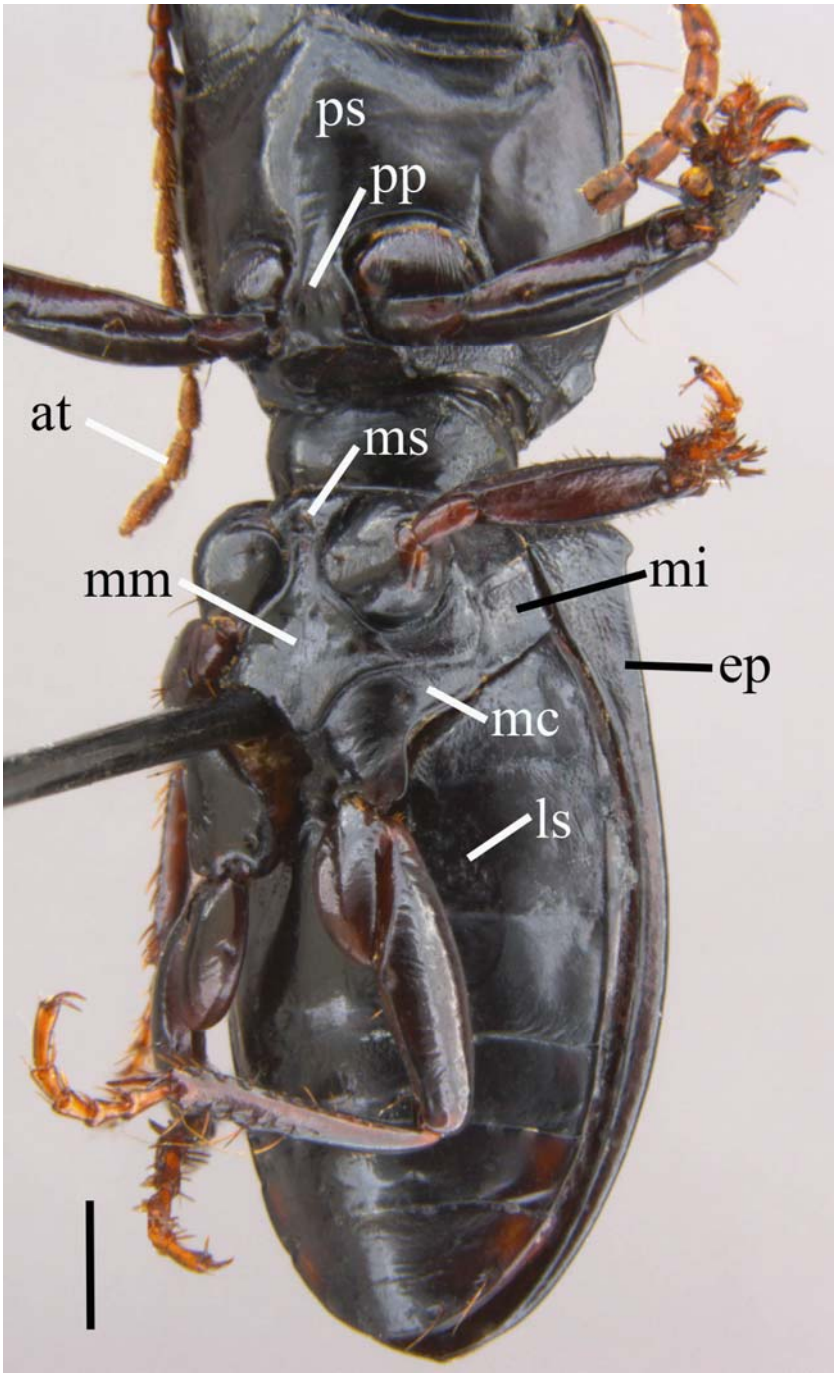

Figure 2.7. Latero-ventral view of *Antilliscaris mutchleri* (Bänninger). (Scaritini: Scaritina). Puerto Rico. (at = antenna; ps = prosternum; ms = mesosternum; mm = metasternum; ep = elytral epipleuron; mi = metepisternum; mc = metacoxa; ls = lateral setae of abdominal sternite 3; pp = prosternal process). Scale bar = 1 mm.

Character 31. Lateral border of the pronotum.

0-Lateral border of pronotum uninterrupted; 1-lateral border interrupted by a tubercle at the hind angle of the pronotum.

Character 32. Prosternal keel.

A prosternal keel, where the mid-line of the prosternum is produced ventrally, is present in some Scapterina.

0-Prosternal keel absent; 1-prosternal keel present.

Character 33. Raised border of the prosternal process.

0-Unbordered; 1-with raised external border.

Character 34. Prosternal setae.

The prosternal process is punctate and setose in some genera and subgenera of Scaritini, for example species of *Steganomma* have from 2 to 8 prosternal setae (Baehr, 2006) and *Antilliscaris* have 6-17 setae (Hlavac, 1969). In other genera this character varies, with the setae present or lacking in different species, for example the genera *Laccopterum*, *Monocentrum, Glyptogrus, Tapinoscaris* and *Storthodontus* (Basilewsky, 1973b). The prosternal setae take the form of long sensory setae, each arising from a pit on the ventral surface of the prosternal process.

0-Prosternal setae absent; 1-prosternal setae present.

Character 35. Closure of the pro-coxal cavities.

The procoxal cavities of Carabidae are either closed (completely surrounded by the prosternum posteriorly) or open (not completely surrounded by the prosternum posteriorly) (Bell, 1967).

0-Procoxal cavities open; 1-procoxal cavities closed.

Character 36. Mesocoxal cavities.

The mesocoxal cavities of ground beetles occur in two forms, disjunct and conjunct.

In the disjunct state the lateral wall of the mesocoxal cavity is formed in part by the mesepimeron. In the conjunct state the mesosternum and metasternum completely surround the mesocoxal cavity (Bell, 1967). The disjunct condition is almost certainly plesiomorphic and occurs in Scaritinae, Loricerini, Migadopini, Amarotypini, Promecognathini, Siagonini and Hiletini. In contrast the conjunct condition is considered apomorphic and occurs in Psydrini, Amblytelini and high-grade carabids. Despite this, Bell (1967) notes that the structure of the coxal cavities show evidence of parallel and convergent evolution.

0-Mesocoxal cavities disjunct; 1- mesocoxal cavities conjunct.

Character 37. Mesosternal setae.

0-Mesosternum glabrous; 1-mesosternum setose.

Character 38. Metasternal setae.

In some genera of Scaritini, for example *Distichus* and *Taeniolobus* (Reichardt, 1977), the metasternum in the area immediately behind the mesocoxa has one or two setae arising from a conspicuous puncture.

0-Metasternum glabrous; 1-metasternum with one or more setae.

Character 39. Metacoxal cavities.

Bell (1967) described the three configurations of the metacoxal cavities of Carabidae. Relevant to this study, the disjunct condition, where the metepimeron borders the lateral wall of the metacoxa, occurs in Scaritini, Elaphrini and Loricerini. The conjunct condition occurs in Promecognathini, where the metepimeron is absent, so the metepisternum is in contact with the first abdominal sternite. This character is unordered as it is not possible to determine the plesiomorphic state by outgroup comparison.

0-Conjunct (metepimeron missing, metasternum touches first abdominal sternum); 1-disjunct (metepimeron visible); 2-disjunct-lobate.

Character 40. Separation of the metacoxae.

Metacoxal separation possibly results from flightlessness (Holm and Scholtz, 1979) and in Scaritini this often appears to be the case, although flightless species with the metacoxae touching also occur, for example some species of *Oxylobus* and *Pasimachus*.

0-Metacoxae touching; 1-metacoxae separated by intercoxal projection

Character 41. Anterior metacoxal seta.

Metacoxal setae are found in 3 positions in Scaritini, anterior, posterior and inner-marginal (Sloane, 1904).

0- Anterior metacoxal seta present; 1- anterior metacoxal seta absent.

Character 42. Posterior metacoxal seta.

0- Posterior metacoxal seta absent; 1- posterior metacoxal seta present.

Character 43. Inner-marginal metacoxal seta.

0- Inner-marginal metacoxal seta absent; 1-inner-marginal metacoxal seta present.

2.2.3.5 Legs.

Figure 2.8. shows anatomical structures of the legs and elytra.

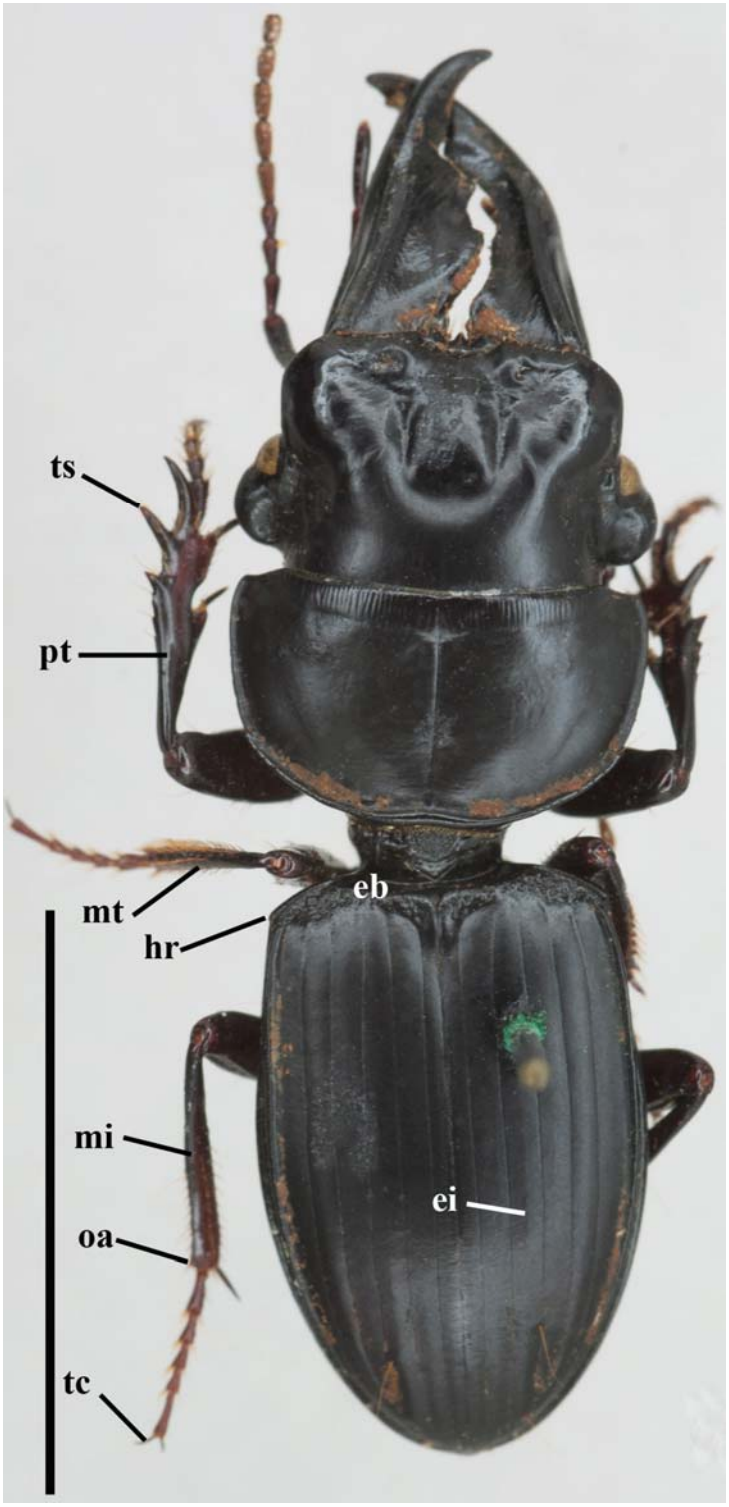

Figure 2.8. *Mamboicus ochryopoides* Bänninger (Scaritini: Scaritina). Tanzania. (pt = protibia; ts = tibial spine; mt = mesotibia; mi = metatibia; tc = tarsal claw; hr = humeral region of elytra; eb = elytral base; ei = elytral interval; oa = metatibial outer angle). Scale bar = 10 mm.

Character 44. Number of protibial spines.

The number of protibial spines varies across different groups of scaritines. All Scaritina and Pasimachina (Bänninger, 1950) possess 3 spines while in Carenina the number of spines varies, from *Monocentrum megacephalum* (Hope) with only one fully formed apical spine (Westwood, 1842) to species of *Carenum* with either 2 or 3 spines. Some Scapterina including *Thlibops*, *Scapterus* and *Passalidius* have 4 spines.

Ordered by outgroup comparison. It is hypothesised that the number of protibial spines represents a transformation series from zero to four spines. 0-protibiae lacking spines or projections, of normal carabid type; 1-One spine; 2-two spines: 3-three spines; 4-four spines.

Character 45. Mesotibial outer angle.

The Pasimachina and Carenina lack the dorsal mesotibial spines characteristic of some Scaritina (character 46). Instead the mesotibiae bear an apical spine formed by an extension of the outer angle of the mesotibia. 0-outer angle of mesotibia unmodified; 1-outer angle modified into a spine or projection.

Character 46. Dorsal mesotibial spines.

The dorsal spine(s) are located at the distal end of the mesotibia, but removed far enough from the apex of the mesotibia to be considered non-homologous to those spines formed by an extension of the mesotibial outer-angle (character 45). The dorsal spines also differ by the presence of an

excision approximately half way along the length of the spine from which a seta arises. Because of the presence of this seta it is likely that the dorsal spines are derived from one of the seta-bearing tubercles of the mesotibia.

The number of dorsal spines has been used in combination with other characters to define the large genus *Scarites*, and if the subgenus *Parallelomorphus* is excluded all *Scarites* have two spines. In other genera, for example *Haplogaster*, the number of mesotibial spines appears to vary between species; *H.ovata* Chaudoir has one spur, *H.granulipennis* Balkenohl has two (Balkenohl, 1994).

The structure of the legs of scaritines is complex and other forms of spurs and projections often occur. Members of the genus *Mouhotia* have a large rounded spathulate projection about half way along the length of the mesotibia. Because of differences in shape and position, this projection is considered not to be homologous to the spines found in other Scaritini and is a likely autapomorphy for the genus.

Rows of much smaller spines occur in some other genera such as *Neochryopus* and *Macromorphus*, but again these are considered non-homologous to the larger dorsal spines.

Ordered by outgroup comparison. It is hypothesised that the number of mesotibial spines represents a transformation series from zero to two spines.

0-dorsal spines absent; 1-one dorsal spine; 2-two dorsal spines.

Character 47. Metatibial outer angle.

Of similar form to the outer-angle spines of the mesotibia. Present in some storthodontines and scapterines.

0-Outer angle of metatibia unmodified; 1-outer angle of metatibia modified into a spine.

Character 48. Unguitractor plate.

The unguitractor plate is a ventral sclerite inside the final tarsomere, articulated with the claws distally and the unguitractor tendon proximally (Gorb, 1996). This plate is sometimes extended as a setiform process named variously as the arolium (Steinmann and Zombori, 1981) or empodium (Gorb, 1996), although the homology of these structures is uncertain between different groups of insects.

The arolium occurs as a leaf-like or setiform process between the tarsal claws of Clivinini (Erwin and Sims, 1984), with the exception of the genus *Kultianella* (Perrault, 1994). In Scaritini this process is always absent.

0-Unguitractor plate extended as a setiform process between the tarsal claws; 1-setiform extension of unguitractor plate absent.

2.2.3.6 Elytra.

Character 49. Form of humeral region (humeral field) of elytra.

The humeral region is the antero-lateral area of the elytron.

The two Madagascan endemic subtribes of Scaritini, Dyscherina and Storthodontina, have a modified form of the humeral region of the elytra and is illustrated and discussed in detail by Basilewsky (1973b).

In the usual form seen in Scaritina, the humeral region is formed by the raised edge of the epipleuron, which is folded over onto the dorsal surface of the elytron and is therefore visible from above. If a humeral tooth is present it is formed by a projection of the epipleuron. The external border of the elytron is delimited by the raised border of the epipleuron so that the marginal channel and umbillicate pores are usually visible from above.

In the Dyscherina, only the internal half of the humeral region is formed by a dorsal up-folding of the epipleuron. The outer half is composed of a ridge formed by the common origin of intervals 7 and 8. The external margin of the elytron is delimited by the carinate 8^{th} interval and the marginal channel and umbillicate pores are folded under to the ventral surface forming a pseudo epipleuron. If a humeral tooth is present it occurs at the point of bifurcation of the 7^{th} and 8^{th} intervals.

In the Storthodontina the humeral region is formed entirely by a carina originating from the 8^{th} interval. The epipleuron is therefore hidden from above. The marginal channel and umbillicate pores are folded under to the ventral surface forming a very wide false epipleuron, the true epipleuron being reduced to a narrow band.

These three types of humeral border appear to constitute a transformation series, with the border undergoing successive modification from the normal

Scaritina type to the Dyscherina type and finally to the Storthodontina type (Basilewsky, 1973b).

In most other Carabidae the humeral fold is absent and the edge of the elytron in the humeral (shoulder) region is formed by the raised lateral bead of the epipleuron.

Ordered by outgroup comparison.

0-Humeral fold absent. Edge of elytron in humeral (shoulder) region formed by the raised lateral bead of the epipleuron; 1-humeral region formed by the epipleuron folded over onto the dorsal surface of the elytron; 2- humeral region composed of the epipleural fold and a carina at the common origin of intervals 7 and 8; 3- humeral region composed entirely of a carina originating from the 8th interval.

Character 50. Width of the elytral epipleuron.

A wide epipleuron occurs often in flightless scaritines, for example species of *Coptolobus*, *Antilliscaris, Gnaphon* and *Mamboicus*. However some genera, for example *Dyscaris* and *Mecynoscaris*, are flightless with a narrow epipleuron.

For the purpose of standardisation the epipleuron was measured from the level of the anterior margin of the metepisternum to the humeral angle.

The subtribes Storthodontina and Dyscherina were scored as missing/inapplicable data as the epipleuron is modified at the humeral region (character 49) and not comparable with other Scaritini.

0-elytral epipleuron narrow (narrower than or at most as wide as the anterior margin of the metepisternum); 1- elytral epipleuron wide (wider than width of anterior margin of metepisternum).

Character 51. Ocellate punctures of the elytral base.

Series of large, unusually shaped pores are present at the elytral base (and sometimes also in the lateral channel) of some scaritines. These pores consist of a circular sunken area with a central seta and an outer rim sharply delimited by a circular raised carina. These ocellate pores are lacking in the Australian genus *Scaraphites,* leading Moore and Lawrence (1994) to postulate this genus belongs to the Scaritina and not the Carenina. However, ocellate pores are present in many other scaritine genera and are not a synapomorphy for the Carenina.

The ocellate punctures of Scaritini are located on the outer half of the base of the elytra adjacent to the elytral shoulder. Many groups of Carabidae have a similar single ocellate puncture at the base of the 1st or 2nd stria, the parascutellar pore. This is not treated as homologous to the basal punctures of Scaritini as it occupies a different position on the elytra.

0-ocellate punctures of elytral base absent; 1-ocellate punctures of elytral base present.

Character 52. Foveate elytral pits.

0-Foveate elytral pits absent; 1-deep foveate pits present.

Character 53. Parascutellary stria.

The parascutellary stria is lacking in almost all Scaritini, yet present in many other groups of Carabidae. This stria is clearly visible in *Mouhotia planipennis* Pouillade, contrary to Andrewes (1929).

Species with no visible elytral striae are coded with a question mark as missing or inapplicable data.

0-Scutellary stria present; 1-scutellary stria absent.

Character 54. 3rd elytral interval.

Modifications to the 3rd and 5th and 6th elytral intervals occur in some genera of Scaritini.

0-3rd interval the same in appearance as intervals 1, 2 and 4; 1-3rd interval raised and carinate as compared to intervals 1, 2 and 4.

Character 55. 5th elytral interval.

0-5th interval the same in appearance as intervals 1, 2 and 4

1-5th interval raised and carinate as compared to intervals 1, 2 and 4

Character 56. 6th elytral interval.

0-6th interval the same in appearance as intervals 1, 2 and 4

1-6th interval raised, carinate and acting as a false epipleuron, concealing the true lateral border of the elytron when viewed from above.

Character 57. 7th elytral interval.

Modifications to the 7th elytral interval, if present, always occur in flightless species, with the single exception of *Neochryopus savagei* (Hope). Species with no visible elytral striae are also coded, as species without visible striae may still have a careniform 7th interval.

Ordered by outgroup comparison. It is hypothesized that successive modifications to the 7th interval represent a transformation series.

0-7th interval not careniform; 1-7th interval careniform at least basally; 2-7th interval careniform at least basally and partially concealing the elytral border when viewed from above; 3-7th interval completely careniform and acting as a false epipleuron, concealing the true lateral border of the elytron when viewed from above.

Character 58. Punctures of the 7th elytral stria.

The 7th elytral stria of some Australian scaritines contains a conspicuous row of punctures.

0-7th stria punctures absent; 1-7th stria punctures present.

Character 59. 8th elytral interval.

Species with no visible elytral striae are also coded, as species without visible striae may still have a careniform 8th interval. (see also character 57).

Ordered by outgroup comparison. It is hypothesized that successive modifications to the 8th interval represent a transformation series.

0-8th interval unmodified; 1-8th interval careniform at least basally; 2-8th interval careniform at least basally and partially concealing the elytral border when viewed from above; 3-8th interval completely careniform and acting as a false epipleuron, concealing the true lateral border of the elytron when viewed from above.

2.2.3.7 Abdomen.

Character 60. Transverse sulci of the last 3 abdominal sternites.

Transverse sulci are present near the anterior margin of the last 3 abdominal sternites of *Neochryopus, Distichus, Lophogenius, Menigius, Taeniolobus* and other genera of Scaritini. Both character states are present in the genus *Coptolobus* (Andrewes, 1929).

0-Transverse sulci absent; 1-transverse sulci present at anterior margin of the last 3 abdominal sternites.

Character 61. Lateral setae of the third sternite.

Some taxa possess a patch of short setae on abdominal sternite 3. This character is usually hidden under the hind femur or trochanter and can be overlooked.

0- Lateral setae of sternite three absent; 1-lateral setae of sternite three present.

Character 62. Presence of ambulatory setae of abdominal sternites three to five.

0-Ambulatory setae present; 1-ambulatory setae absent.

2.2.4 Phylogenetic analysis.

Two methods of analysis were employed; a maximum parsimony (MP) analysis using PAUP* version 10beta (Swofford, 2003) for the unordered and ordered data and a Bayesian inference (BI) analysis using MrBayes version 3.2.1. (Ronquist et al., 2012) for the unordered data.

The data matrix was first exported from WinClada in nexus format to allow reading by PAUP* and MrBayes.

Commands were issued to both programs using batch files rather than at the command prompt. Batch files are useful because they enable long analyses to be performed without further input and they provide a record of the commands issued and files created.

Trees resulting from the analysis were annotated using the program TreeGraph version 2.0.47-206 beta (Stöver and Müller, 2010).

2.2.4.1 Maximum parsimony methods.

An example of a typical batch file is given below to show in detail the commands used in PAUP*.

```
#nexus
Begin paup;
set autoclose=yes warntree=no warnreset=no increase=auto
tcompress=yes torder=right;
log start file=filename.log;
execute filename.nex;
cstatus;
outgroup Elaphrus;
tstatus;
Set criterion=parsimony;
hsearch start=stepwise addseq=random nreps=4000 nchuck=2
chuckscore=2 swap=tbr;
hsearch start=current chuckscore=no;
set root=outgroup;
roottrees;
describetrees 1 /chglist=yes apolist=yes diag=yes;
savetrees file= filename.tre brlens=yes root=yes;
savetrees file= filename.tre brlens=no root=yes;
contree all / strict=yes treefile=filename.tre;
contree all / majrule=yes percent=50 treefile=filename.tre;
cleartrees;
bootstrap nreps=1000 search=heuristic/ addseq=random
nchuck=10 chuckscore=1 nreps=10;
savetrees file= filename.tre from=1 to=1 savebootp=nodelabels
maxdecimals=0;
log stop;
end;
```

‘hsearch’ begins a tree search using a heuristic algorithm. The tree search is a two-step process; an initial tree is generated which then undergoes branch swapping.

‘start=stepwise addseq=random’ are commands used to generate the starting trees. Initially the taxa are randomly ordered and a tree is constructed using the first three taxa, using the chosen optimality criterion (in this case maximum parsimony). Taxa are then added one at a time in a stepwise fashion, in the order randomly generated at the start. Each new taxon is added to the tree in the most parsimonious position (Kitching et al., 1998).

‘nreps=4000’ specifies 4000 replicates of the stepwise addition procedure.

‘nchuck=2 chuckscore=2 swap=tbr’ are commands controlling the branch swapping stage. ‘nchuck=2’ specifies that no more than two trees are retained with a score greater than the ‘chuckscore’ value. This effectively retains the two shortest trees from each replicate. ‘swap=tbr’ specifies the tree bisection and re-connection (TBR) method of branch swapping. The TBR algorithm as implemented in PAUP* removes a portion of the tree and re-connects it to each branch of the remaining tree. All possible bisections and re-connections are evaluated (Swofford and Olsen, 1990).

‘hsearch start=current chuckscore=no;’ performs further branch swapping on all the trees retained from the initial round of swapping.

The resulting shortest trees were rooted with the outgroup species *Elaphrus riparius* (L.) and summarised by calculation of a strict consensus tree (with

the '`contree all / strict=yes`' command) and a 50% majority rule consensus tree (using '`contree all /majrule=yes percent=50`').

If very large numbers of equally parsimonious trees are obtained from an analysis it is not practically possible to examine all of them. Instead consensus methods can be used to summarise the set of trees.

The strict consensus is the most conservative consensus method and is useful because only the nodes present in all of the trees are shown. However, strict consensus trees can also have low resolution, in which case a majority rule (with the majority rule typically set at 50%) consensus tree can also provide useful information. A 50% majority rule consensus tree shows only those nodes present in more than 50% of the individual trees.

To assess the amount of homoplasy in the data, PAUP* was used to generate values of the consistency index (*ci*) and retention index (*ri*) for each character using the '`describetrees`' command.

To obtain a measure of how well nodes on the resulting trees were supported by the data a bootstrap test was used with 1000 bootstrap replicates being performed using the '`bootstrap`' command in PAUP*. The bootstrap technique begins by randomly sampling characters in the original data matrix to generate a number of pseudoreplicate matrices. These are then analysed using a preferred optimality criterion. The bootstrap value for a clade is the frequency with which that clade occurs in all the replicates (Siddall, 2002). Essentially, clades supported by large numbers of characters are less affected by the randomisation procedure than clades supported by only one or a few characters.

Bootstrap values are most conveniently displayed on a 50% majority rule consensus tree. These values were then transcribed onto to the consensus trees with TreeGraph.

2.2.4.2 Bayesian inference methods.

Commands in MrBayes were issued from a MrBayes block added to the nexus data file generated by Winclada. Details of each command are available within the program using '`help <command>`'.

```
BEGIN MRBAYES;
set autoclose=yes;
log start filename=filename.log.txt;
outgroup Elaphrus;
lset nst=1 coding=variable rates=gamma;
showmodel;
mcmc ngen=10000000 samplefreq=1000 printfreq=1000 nruns=2
nchains=4 temp=0.1 stoprule=yes stopval=0.01 savebrlens=yes;
sumt relburnin=yes burninfrac=0.25 contype=halfcompat;
END;
```

The '`lset`' command controls the parameters of the model of character change chosen for the data. For morphological data there is in fact only one model available in MrBayes, the 'standard discrete' or Mk model (Lewis, 2001). This model specifies a single rate of change between character states and is implemented using '`nst=1`'. '`coding=variable`' indicates that only variable characters were sampled, as is usual for morphological data.

'`rates=gamma`' sets the parameter of how the rate of change of states varies across the characters. In this case a gamma distribution of rates was arbitrarily chosen.

The '`mcmc`' command instructs the program on how the analysis will be run. The Markov chain Monte Carlo (MCMC) method is a simulation technique used to estimate the posterior probability distribution of trees given the prior distribution, the model and the data. The Markov chain is initiated using a randomly generated tree, with associated branch lengths and model parameters. A new tree is proposed as the next state in the chain by changing the topology or model parameters. This new tree is accepted with a probability based on the ratio of the posterior probabilities of the existing and proposed trees (Ronquist and Huelsenbeck, 2003). Over a number of generations the chain converges and produces a good sample of the posterior probability distribution (the chains were sampled every 1000 generations using '`samplefreq=`1000').

The MCMC method has the crucial property that once convergence has been reached, the number of times the chain visits a particular tree is proportional to its posterior probability. In addition, when considering a pool of trees of similar likelihood, such as those produced by an MCMC run in MrBayes, the frequency with which a particular clade occurs in those trees is an approximation of the posterior probability of that clade (Kelly, 2005).

To accelerate convergence a technique known as metropolis coupling is used (Ronquist and Huelsenbeck, 2003). This involves running one or more additional chains that are 'heated' by having a raised posterior probability, allowing the heated chain to explore some areas of the probability distribution more easily. At intervals a swap can be made between the heated and the original or 'cold' chain. The end result is that the cold chain can more easily find trees with similar likelihoods but different topologies. Default values of the number of chains and the amount of heating were used by issuing the '`nchains=4`' and '`temp=0.1`' commands in MrBayes.
To confirm convergence MrBayes runs two or more independent analyses in parallel and compares them, by calculating the average standard deviation of the frequencies of particular clades (termed 'splits' in MrBayes) from the trees in each run. The default number of two runs was used with '`nruns=2`'. '`stopval=0.01`' stops the analysis when the average standard deviation of clade (split) frequencies reaches a value of less than 0.01.

The '`sumt`' command controls how the results of the analysis are reported. Firstly the program is instructed on how many of the initial tree samples to discard. Because the chain is initiated with a random tree, the initial samples have a low posterior probability. The posterior probability then increases until the chain has stabilised. The initial tree samples with low probability from the so called burn-in phase are discarded using the '`relburnin=yes`' and '`burninfrac=`*`value`*' commands. 25% of initial trees were discarded in the analysis.

A visual check of the burn-in phase and stability of likelihood values (stationarity) was also employed by plotting the likelihood scores against generation time in Tracer version 1.5 (Rambaut and Drummond, 2009). If stationarity has been reached there should be no trend of increasing or decreasing likelihood scores.

A 50% majority rule consensus tree was used to summarise the set of topologies obtained in the analysis with the command '`contype=halfcompat`'. The posterior probability of each clade is automatically calculated by MrBayes and displayed on this consensus in the output file.

To visualise the progress of the analysis a trace plot of log likelihood versus generation number was constructed by importing these values from the output file generated by MrBayes into Microsoft Excel.

2.3 Results.

2.3.1 Results of the character scoring.

The taxon versus character data matrix is given in table 2.5.

Table 2.5. Morphology data matrix.

Taxon \ Character number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Enceladus	0	0	0	1	0	0	2	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	1	0	1	2	0	1	0	0	0
Luperca	0	1	0	0	0	0	2	0	1	0	0	0	1	1	0	1	1	0	1	0	0	1	0	1	1	0	0	1	0	0	0
Siagona	0	0	0	0	0	0	2	0	1	0	0	0	2	1	0	1	1	0	1	0	0	1	?	?	1	?	0	1	0	0	0
Eucamaragnathus	0	1	0	2	0	1	2	0	1	1	0	0	0	0	0	0	1	2	1	0	0	1	0	0	0	?	0	1	0	0	0
Hiletus	0	0	0	2	0	1	2	0	1	1	0	0	0	0	1	1	1	2	1	0	0	3	0	0	0	?	0	1	0	0	0
Lissopterus	0	0	0	0	0	0	2	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	2	0	0	?	0	0
Promecognathus	0	1	0	2	0	1	2	1	1	0	0	0	3	1	0	0	1	1	1	2	1	0	1	0	0	1	1	0	?	0	0
Amblytelus	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	?	0	0
Melisiodera	0	1	0	0	0	1	2	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	2	0	1	0	0	0
Psydrus	0	1	0	0	0	0	2	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	1	0	0	1	0	0	?	0	0
Meonis	0	1	0	2	0	0	1	?	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	?	0	0
Blethisa	0	1	0	2	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	1	0	4	0	0	?	0	0
Elaphrus	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	4	0	0	?	0	0
Gnathoxys	0	0	0	1	0	0	2	0	0	0	0	0	1	1	0	0	1	0	0	0	0	?		0	0	1	0	0	?	0	0
Broscus	0	0	0	0	0	0	2	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	1	0	0	2	0	0	?	0	0
Cnemalobus	0	0	0	0	0	1	2	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	0	0
Dyschirius	0	1	0	2	0	1	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	1	1	0	1	0	0	?	0	0
Clivina	0	1	0	2	0	1	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	1	0	2	0	1	0	0	1
Schizogenius	0	1	0	?	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	2	0	1	0	0	1
Bohemania	0	1	0	1	0	1	1	1	1	0	0	0	1	1	0	0	1	1	1	0	0	0	1	1	0	2	0	1	0	0	1
Forcipator	0	1	0	1	0	1	2	0	1	1	0	0	2	1	0	0	4	0	1	0	0	0	0	0	0	0	1	1	0	0	0
Aspidoglossa	0	1	0	2	0	1	0	0	0	1	0	1	0	1	0	0	1	0	1	0	0	0	1	1	0	2	0	0	?	0	0
Mouhotia gloriosa	?	0	1	1	0	1	3	1	1	1	0	1	0	1	1	1	1	1	1	2	0	2	0	0	0	0	1	0	?	0	0
Pasimachus purpuratus	0	0	0	1	0	1	2	1	1	0	1	0	2	1	0	0	4	1	1	2	0	0	0	0	0	1	1	0	?	0	0
Carenum tinctilatum	1	1	0	2	0	1	2	1	1	1	0	0	2	1	0	1	5	1	1	3	0	0	1	0	0	3	1	1	0	0	0
Neocarenum elongatum	1	1	0	2	0	1	2	1	1	1	0	0	2	1	0	1	5	1	1	3	0	0	0	0	0	2	1	1	0	0	0
Laccopterum spencei	1	1	0	2	0	1	2	1	1	1	0	0	2	1	0	1	4	1	1	3	0	0	1	0	0	2	1	1	0	0	0
Monocentrum convexum	0	1	0	0	0	1	2	1	1	1	0	0	2	1	1	1	4	1	1	3	0	0	0	0	0	2	1	1	0	0	0
Carenidium bicornutum	1	1	0	2	0	1	2	1	1	1	0	0	2	1	1	1	5	1	1	3	0	0	0	0	0	2	1	1	0	0	0
Scaraphites rotundipennnis	1	0	0	1	0	1	2	1	1	1	0	0	2	1	0	0	5	1	1	1	0	0	0	0	0	3	1	1	0	0	0
Philoscaphus tuberculatus	1	1	0	2	0	1	2	1	1	1	0	0	2	1	0	0	5	1	1	3	0	0	0	0	0	2	1	1	0	0	0
Epilectus mastersi	1	0	0	2	0	1	?	?	1	1	0	0	2	1	1	1	2	1	1	3	0	0	0	0	0	3	1	1	0	0	0
Thlibops longicollis	1	0	0	0	1	1	2	1	1	1	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0
Scapteus guerini	1	0	0	0	1	1	2	1	1	1	0	0	2	1	0	0	4	0	1	1	0	0	0	0	0	0	1	1	0	0	0
Passalidius fortipes	1	0	0	1	0	1	2	1	1	1	0	0	2	1	0	0	4	0	1	3	0	1	0	0	0	1	1	1	1	0	0
Acanthoscelis ruficornis	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	4	0	1	0	0	0	1	0	0	1	1	1	1	0	0
Corintascaris ferreirae	?	0	1	0	0	1	2	1	1	0	1	0	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0
Ochryopus gigas	?	?	?	1	0	1	2	1	1	1	1	0	2	?	0	0	?	0	1	2	0	0	2	1	0	1	1	1	0	0	1
Oxylobus porcatus	0	0	0	2	0	1	2	1	1	1	1	0	2	2	0	0	2	1	1	3	0	0	0	0	0	1	1	1	0	0	0
Neochryopus savagei	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	0	1
Baenningeria galapagoensis	?	0	1	1	0	1	2	1	1	1	0	0	2	2	0	0	3	0	1	1	1	0	1	0	0	1	1	1	1	1	1
Haplotrachelus chalcopleurus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	0	0
Haplotrachelus atropis	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	0	0
Mamboicus lastii	1	0	0	2	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	1	0	1	0	0	1	1	1	0	0	1
Coptolobus glabriculus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	2	1	1	1	1	0	1	0	0	1	1	1	1	1	0
Geoscaptus laevissimus	1	0	0	2	0	1	2	1	1	1	1	0	2	2	0	0	2	1	1	1	1	0	0	0	0	2	1	1	1	0	0
Anomophaenus costatogranulatus	1	0	0	1	0	1	2	1	1	1	1	0	2	2	0	0	4	1	1	1	1	0	0	0	0	2	1	1	1	0	0
Gnaphon loyolae	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	0	1
Macromorphus elongatus	?	0	1	1	0	1	2	1	1	1	1	0	2	2	0	0	?	1	1	1	0	2	0	0	0	1	1	1	0	0	0
Cryptoscaphus lissonotus	1	0	0	1	0	1	2	1	1	1	1	0	2	2	0	0	3	1	1	1	1	0	0	0	0	1	1	1	1	0	1
Glyptogrus molopinus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	0	1
Glyptogrus glypticus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	0	1
Antilliscaris mutchleri	1	0	0	1	0	1	1	1	1	1	0	0	2	2	0	0	3	0	1	1	1	0	1	0	0	1	1	1	1	0	0
Taeniolobus guerini	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	2	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Taeniolobus silvestris	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	1	0	1	0	0	3	1	1	1	0	1
Parallelo. terricola	1	0	0	1	0	1	2	1	1	1	1	0	2	2	0	0	3	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Scarites subterraneus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	4	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Scalloph. striatus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	4	0	1	1	1	0	0	0	0	1	1	1	1	0	1
Scalloph. buparius	0	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Menigius schaumi	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	2	1	0	1	0	0	1	1	1	1	0	0
Pachyodontus languidius	0	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Haplogaster ovata	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	0	1
Distichus planus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	2	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Tapinoscaris raffrayi	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	1	0	1	0	0	1	1	1	1	1	0
Storthodontus nimrod	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	0	0	1	2	0	1	1	1	1	1	0
Crepidopterus goudotii	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	0	0	1	0	0	1	1	1	1	0	0
Pilades coquereli	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	0	0	0	0	0	1	1	1	1	1	0
Dyscaris mordax	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	0	1	0	1	0	0	1	1	1	1	1	0
Prodyscherus pluto	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	0	0	0	0	0	0	1	1	1	1	0	1
Mecynoscaris longula	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1	0	1
Dyscherus costatus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	1	1	1	0	0	1	0	0	1	1	1	1	0	0
Dinoscaris venator	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	0	0	1	0	0	1	1	1	1	0	0
Madascaris enoplus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	0	1	0	1	0	0	1	1	1	1	1	1
Euryscaphus waterhousei	1	1	0	2	0	1	2	1	1	1	0	0	3	1	0	0	5	1	1	3	0	0	1	0	0	2	1	1	0	0	0
Typhloscaris hutchinsi	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	2	0	1	1	1	0	0	0	0	1	1	1	1	0	0
Mamboicus afrellus	1	0	0	2	0	1	2	1	1	1	0	0	2	2	0	0	4	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Parallelo. aterrimus	1	0	0	1	0	1	2	1	1	1	1	0	2	2	0	0	1	0	1	0	1	0	1	0	0	1	1	1	1	0	1
Scarites molssus	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	1	0	1	0	0	1	1	1	1	0	1
Pasimachus depressus	0	0	0	1	0	1	2	1	1	0	1	0	3	1	0	0	2	1	1	2	0	1	0	0	0	1	1	0	?	0	0
Oxylobus lateralis	0	0	0	2	0	1	2	1	1	1	1	0	2	1	0	0	1	1	1	3	0	0	0	0	0	1	1	1	0	0	0
Carenum politum	1	0	0	2	0	1	?	1	1	1	0	0	2	1	1	1	5	1	1	3	0	?		1	0	0	2	1	1	0	0
Laccopterum doddi	1	1	0	2	0	1	2	1	1	1	0	0	2	1	0	1	2	1	1	3	0	0	1	0	0	1	1	1	0	0	0
Monocentrum frenchii	1	1	0	2	0	1	2	1	1	1	0	0	2	1	0	1	3	1	1	3	0	0	1	1	0	3	1	1	0	0	0
Crepidopterus pipitzi	1	0	0	1	0	1	2	1	1	1	0	0	2	2	0	0	5	0	1	1	0	0	1	0	0	1	1	1	1	0	0
Scaraphites silenus	1	0	0	1	0	1	2	1	1	1	0	0	2	1	0	0	5	1	1	2	0	0	0	0	0	3	1	1	0	0	0
Pasimachus rotundipennis	0	0	0	1	0	1	2	1	1	0	1	0	2	1	0	0	2	1	1	2	0	0	0	0	0	1	1	0	?	0	0

Table 2.5 (continued). Morphology data matrix.

Taxon \ Character number	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
Enceladus	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Luperca	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	?	0	0	0	0	0	0	0	1	0
Siagona	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	?	0	0	0	0	0	0	1	1	0
Eucamaragnathus	0	1	0	0	0	1	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hiletus	0	1	0	0	0	1	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lissopterus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Promecognathus	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	?	0	0	0	0	0	0	0	0	0
Amblytelus	0	0	0	1	1	0	0	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melisiodera	0	0	0	1	1	0	0	2	?	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Psydrus	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	?	0	0
Meonis	0	0	0	1	1	0	0	2	1	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Blethisa	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0
Elaphrus	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	?	0	0	0	0	0	0	1	0	0
Gnathoxys	0	0	0	1	1	0	0	2	1	0	1	1	3	1	0	0	1	0	0	0	0	?	0	0	0	0	0	0	0	0	0
Broscus	0	0	0	1	1	0	0	2	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cnemalobus	0	0	1	1	1	0	0	2	0	0	1	1	1	0	0	0	1	0	0	0	0	?	0	0	0	0	1	0	0	0	0
Dyschirius	0	0	0	1	0	0	0	1	?	?	?	?	3	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Clivina	0	0	0	1	0	0	0	1	0	0	1	0	3	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Schizogenius	0	0	0	1	0	0	0	1	0	0	1	0	4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Bohemania	0	0	0	1	0	0	0	1	0	0	1	0	4	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Forcipator	0	0	1	1	0	0	0	1	0	0	1	1	4	1	1	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0
Aspidoglossa	0	0	0	1	0	0	0	1	1	0	1	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Mouhotia gloriosa	0	0	0	1	0	0	0	1	0	1	0	0	3	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Pasimachus purpuratus	0	0	0	1	0	0	0	1	0	0	0	0	3	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Carenum tinctilatum	0	0	1	1	0	0	0	1	1	0	0	0	2	1	0	0	1	1	1	1	0	?	0	0	0	0	0	0	0	0	0
Neocarenum elongatum	0	0	0	1	0	0	0	1	1	0	0	0	2	1	0	0	1	1	1	1	0	?	0	0	0	0	1	0	0	0	0
Laccopterum spencei	0	0	0	1	0	0	0	1	1	1	0	0	3	1	0	1	1	1	1	1	1	?	0	0	0	0	0	0	0	0	0
Monocentrum convexum	0	0	0	1	0	0	0	1	1	1	0	0	1	1	0	0	1	1	1	1	0	?	0	0	0	0	0	0	0	0	0
Carenidium bicornutum	0	0	1	1	0	0	0	1	1	0	0	0	2	1	0	0	1	1	1	1	0	?	0	0	0	0	0	0	0	0	0
Scaraphites rotundipennnis	0	0	0	1	0	0	0	1	1	0	0	0	3	1	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0
Philoscaphus tuberculatus	0	0	0	1	0	0	0	1	1	0	0	0	3	1	0	0	1	1	1	1	0	1	0	0	0	0	0	2	0	0	0
Epilectus mastersi	0	0	0	1	0	0	0	1	1	1	0	0	2	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1
Thlibops longicollis	1	0	0	1	0	0	0	?	1	0	0	1	4	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0
Scapteus guerini	1	0	0	1	0	0	0	1	0	0	0	0	4	0	2	1	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Passalidius fortipes	0	1	0	1	0	0	0	2	1	1	0	0	4	1	0	1	1	1	0	0	0	1	0	0	0	1	0	2	0	0	1
Acanthoscelis ruficornis	1	0	0	1	0	0	0	1	1	0	1	0	3	1	1	1	1	1	0	0	0	1	0	0	0	1	0	0	0	1	0
Corintascaris ferreirae	1	0	0	1	0	0	0	1	?	1	0	0	4	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	1
Ochryopus gigas	0	0	0	1	0	0	0	1	0	1	0	0	3	0	1	0	1	1	0	1	0	?	0	0	0	0	0	0	0	1	0
Oxylobus porcatus	0	0	0	1	0	0	0	1	1	1	1	1	3	1	0	0	1	0	1	1	1	1	1	1	1	1	0	?	0	0	1
Neochryopus savagei	0	0	0	1	0	0	0	1	0	0	1	0	3	0	1	0	1	1	1	1	0	1	0	0	0	2	0	0	1	1	0
Baenningeria galapagoensis	0	0	0	1	0	0	0	1	0	0	1	0	3	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0
Haplotrachelus chalcopleurus	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	0	1	1	1	1	0	1	0	0	0	1	0	2	0	1	0
Haplotrachelus atropis	0	0	0	1	0	0	0	1	1	0	1	1	3	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	1	0
Mamboicus lastii	0	0	0	1	0	0	0	1	0	0	1	0	3	0	1	0	1	1	1	1	0	1	0	0	0	3	0	0	0	1	0
Coptolobus glabriculus	0	0	1	1	0	1	0	1	0	1	0	0	3	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
Geoscaptus laevissimus	0	0	0	1	0	0	0	1	0	0	1	0	3	0	1	0	1	1	1	1	0	?	0	0	0	0	0	0	0	0	0
Anomophaenus costatogranulatus	0	0	0	1	0	0	0	1	0	0	1	1	3	0	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	0	0
Gnaphon loyolae	0	0	1	1	0	0	0	1	0	0	1	1	3	0	1	0	1	1	1	1	0	1	1	1	0	2	0	0	0	0	0
Macromorphus elongatus	0	0	0	1	0	0	0	1	1	1	0	0	3	0	1	?	1	1	1	0	0	?	0	0	0	0	0	2	0	1	1
Cryptoscaphus lissonotus	0	0	0	1	0	0	0	1	0	0	0	0	3	1	1	1	1	1	0	1	0	?	0	0	0	0	0	0	0	1	0
Glyptogrus molopinus	0	0	0	1	0	0	0	1	0	0	1	0	3	0	2	0	1	1	1	1	0	1	0	0	0	1	0	0	1	1	0
Glyptogrus glypticus	0	0	0	1	0	0	0	1	1	0	1	0	3	0	2	0	1	1	1	1	1	1	0	0	0	1	0	3	1	1	0
Antilliscaris mutchleri	0	0	1	1	0	1	1	1	?	0	1	1	3	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	0
Taeniolobus guerini	0	0	1	1	0	0	1	1	1	0	1	1	3	0	2	?	1	1	0	1	0	1	0	0	0	2	0	1	1	1	0
Taeniolobus silvestris	0	0	0	1	0	0	1	1	0	0	1	1	3	0	2	0	1	1	0	1	0	1	0	0	0	1	0	1	1	1	0
Parallelo. terricola	0	0	0	1	0	0	0	1	0	0	0	0	3	0	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0
Scarites subterraneus	0	0	0	1	0	0	0	1	0	0	1	0	3	0	2	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	0
Scalloph. striatus	0	0	0	1	0	0	0	1	0	0	1	0	3	0	2	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	0
Scalloph. buparius	0	0	0	1	0	0	0	1	0	0	1	0	3	0	2	0	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0
Menigius schaumi	0	0	0	1	0	0	1	1	0	0	1	1	3	0	1	0	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0
Pachyodontus languidius	0	0	1	1	0	0	1	1	1	0	1	0	3	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	0
Haplogaster ovata	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	0
Distichus planus	0	0	1	1	0	1	1	1	0	0	1	1	3	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	1	0	0
Tapinoscaris raffrayi	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	1	1	3	?	1	0	1	1	1	1	0	1	0	3	0	1
Storthodontus nimrod	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	1	1	3	?	1	0	1	0	0	0	1	0	3	0	1	0
Crepidopterus goudotii	0	0	0	1	0	0	0	1	0	0	1	0	3	0	1	1	1	3	?	1	0	1	0	0	0	0	0	3	0	1	0
Pilades coquereli	0	1	0	1	0	0	0	1	1	0	1	0	3	0	1	1	1	3	?	1	0	1	0	0	0	0	0	3	0	1	0
Dyscaris mordax	0	0	1	1	0	0	0	1	0	0	1	1	3	0	1	1	1	1	0	1	0	1	0	0	0	2	0	0	0	1	0
Prodyscherus pluto	0	1	0	1	0	0	0	1	1	0	0	0	3	0	1	1	1	1	1	0	0	1	0	0	0	1	0	2	0	1	0
Mecynoscaris longula	0	0	0	1	0	0	0	1	1	1	1	1	3	0	2	1	1	1	0	1	0	1	0	0	0	0	0	0	0	1	0
Dyscherus costatus	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	1	1	2	?	1	0	1	1	1	0	1	0	3	0	1	0
Dinoscaris venator	0	0	0	1	0	0	0	1	1	0	1	0	3	0	2	1	1	3	?	1	0	1	0	0	0	1	0	3	0	1	0
Madascaris enoplus	0	0	1	1	0	0	0	1	1	0	1	1	3	0	1	0	1	1	1	1	0	1	1	1	0	1	0	3	0	1	0
Euryscaphus waterhousei	0	0	1	1	0	0	0	1	1	0	0	0	2	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
Typhloscaris hutchinsi	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
Mamboicus afrellus	0	0	0	1	0	0	0	1	0	0	1	0	3	0	1	0	1	1	1	1	0	1	0	0	0	2	0	0	0	1	0
Parallelo. aterrimus	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0
Scarites molssus	0	0	0	1	0	0	0	1	0	0	1	0	3	0	2	0	1	1	1	1	0	1	0	0	0	0	0	2	0	1	0
Pasimachus depressus	0	0	0	1	0	0	0	1	0	0	0	0	2	1	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0
Oxylobus lateralis	0	0	0	1	0	0	0	1	0	1	0	1	3	1	0	0	1	0	1	1	0	1	0	0	1	1	0	?	0	0	1
Carenum politum	0	0	1	1	0	1	0	1	1	0	0	0	2	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
Laccopterum doddi	0	0	1	1	0	0	0	1	1	0	1	0	3	1	0	0	1	1	1	1	1	?	0	0	0	0	0	0	0	0	0
Monocentrum frenchii	0	0	1	1	0	0	0	1	?	0	0	0	2	1	0	0	1	1	1	1	0	?	0	0	0	0	0	0	0	0	0
Crepidopterus pipitzi	0	0	0	1	0	0	0	1	1	0	1	0	3	0	1	1	1	3	?	1	0	?	1	0	0	0	0	3	0	1	0
Scaraphites silenus	0	0	1	1	0	0	0	1	1	0	0	0	3	1	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	?	0
Pasimachus rotundipennis	0	0	0	1	0	0	0	1	1	0	0	0	3	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0

2.3.2 Results of the parsimony analysis.

Analysis of both the unordered and ordered data resulted in an extremely large number of equally parsimonious trees.

The unordered data matrix produced 569,176 trees of length 417, *CI* of 0.22 and *RI* of 0.70. The strict consensus of these trees is given in figure 2.9 and the 50% majority rule consensus in figure 2.10.

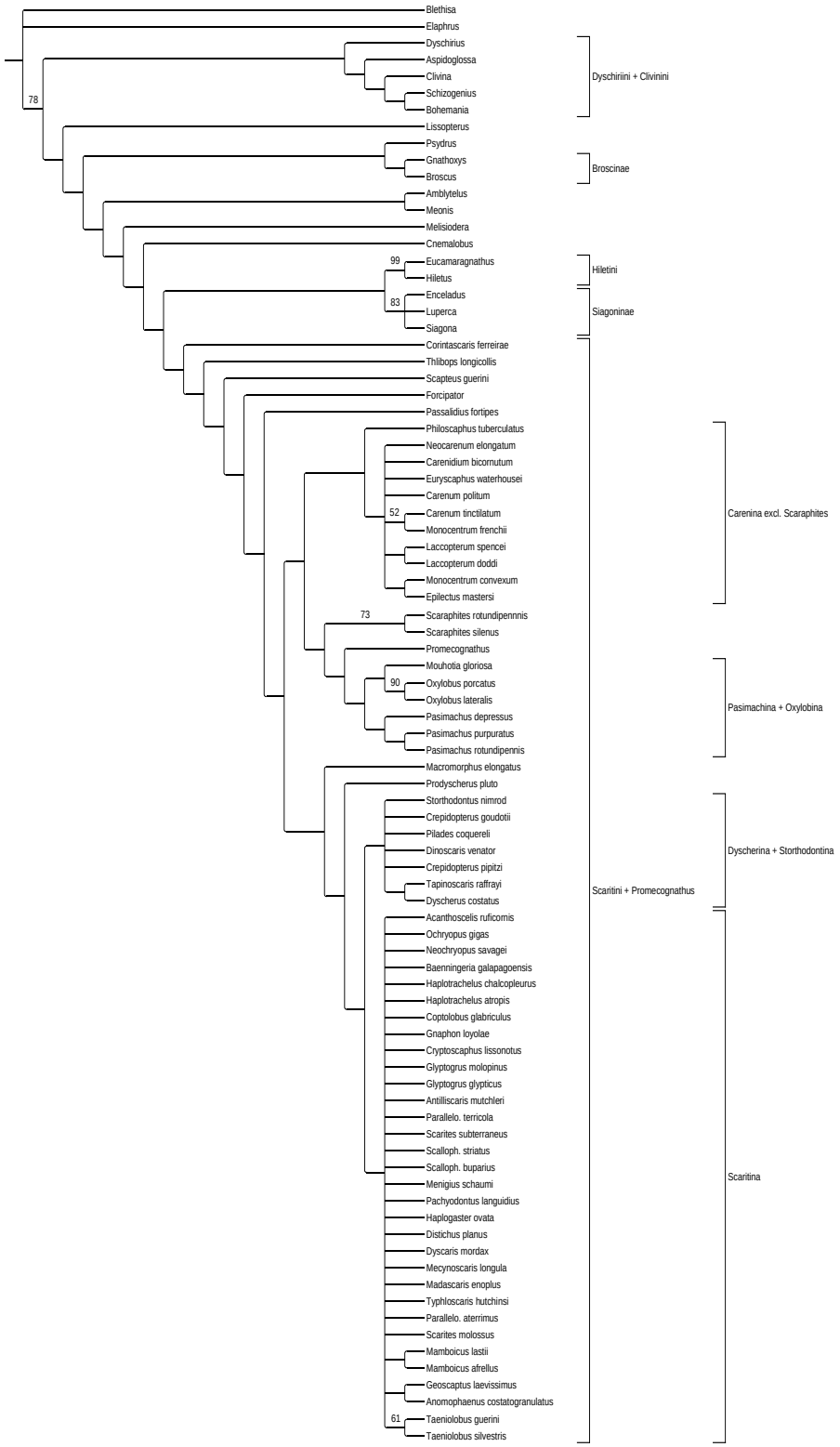

Figure 2.9. Strict consensus of 569,176 equally parsimonious trees of length 417 from the unordered data. Bootstrap percentage values above 50% are given next to the relevant nodes.

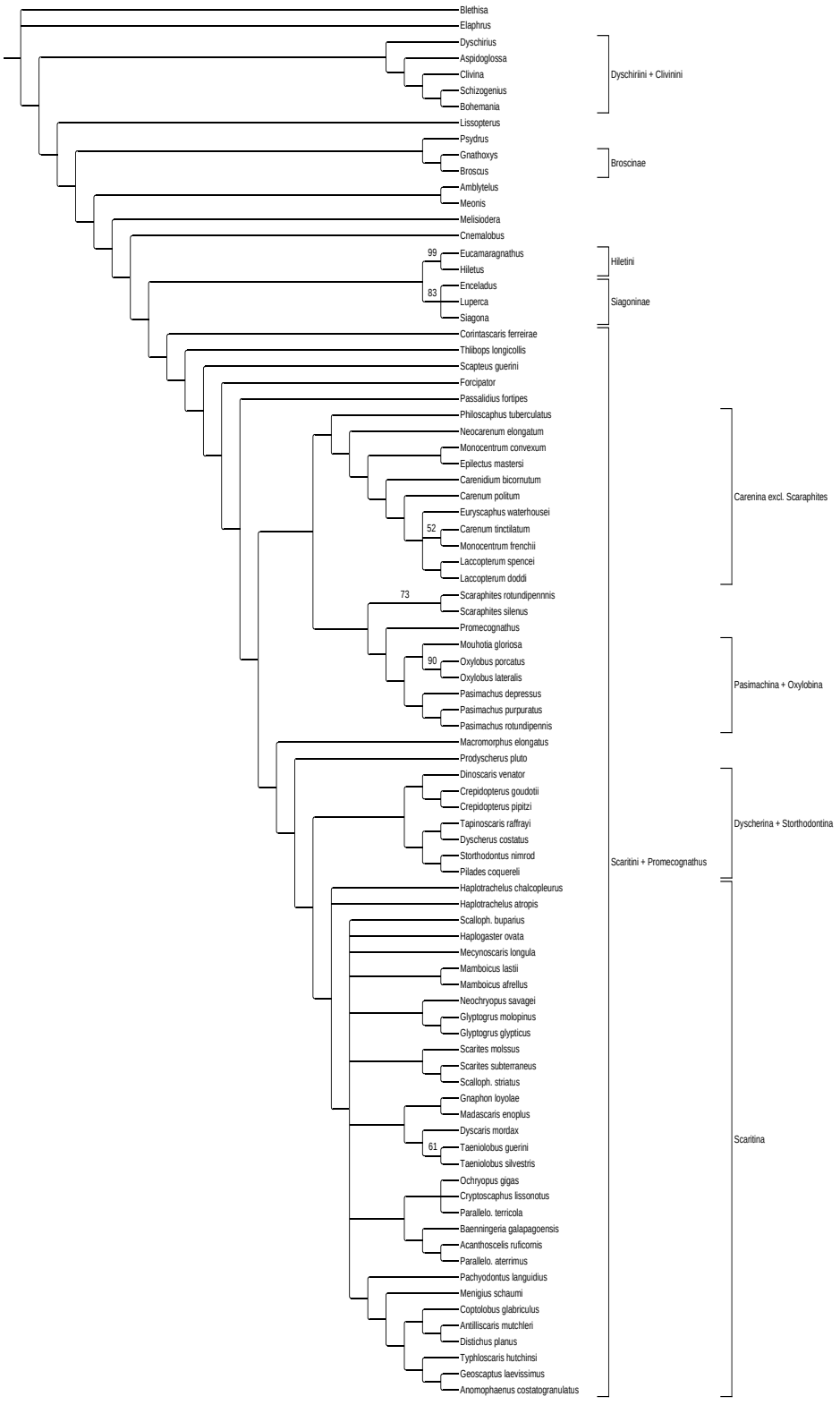

Figure 2.10. 50% majority rule consensus of 569,176 equally parsimonious trees of length 417 from the unordered data. Bootstrap percentage values above 50% are given next to the relevant nodes.

Analysis of the data matrix containing the 6 ordered and 56 unordered characters produced even more equally parsimonious trees than the unordered data, in excess of 800,000 trees. Beyond this number of trees PAUP* began to run extremely slowly and the analysis could not be completed. A second analysis with the number of trees limited to 500,000 (set using the '`maxtrees`' command) produced 500,000 trees of length 434, *CI* of 0.21 and *RI* of 0.71.

The strict consensus of these trees is given in figure 2.11 and the 50% majority rule consensus in figure 2.12.

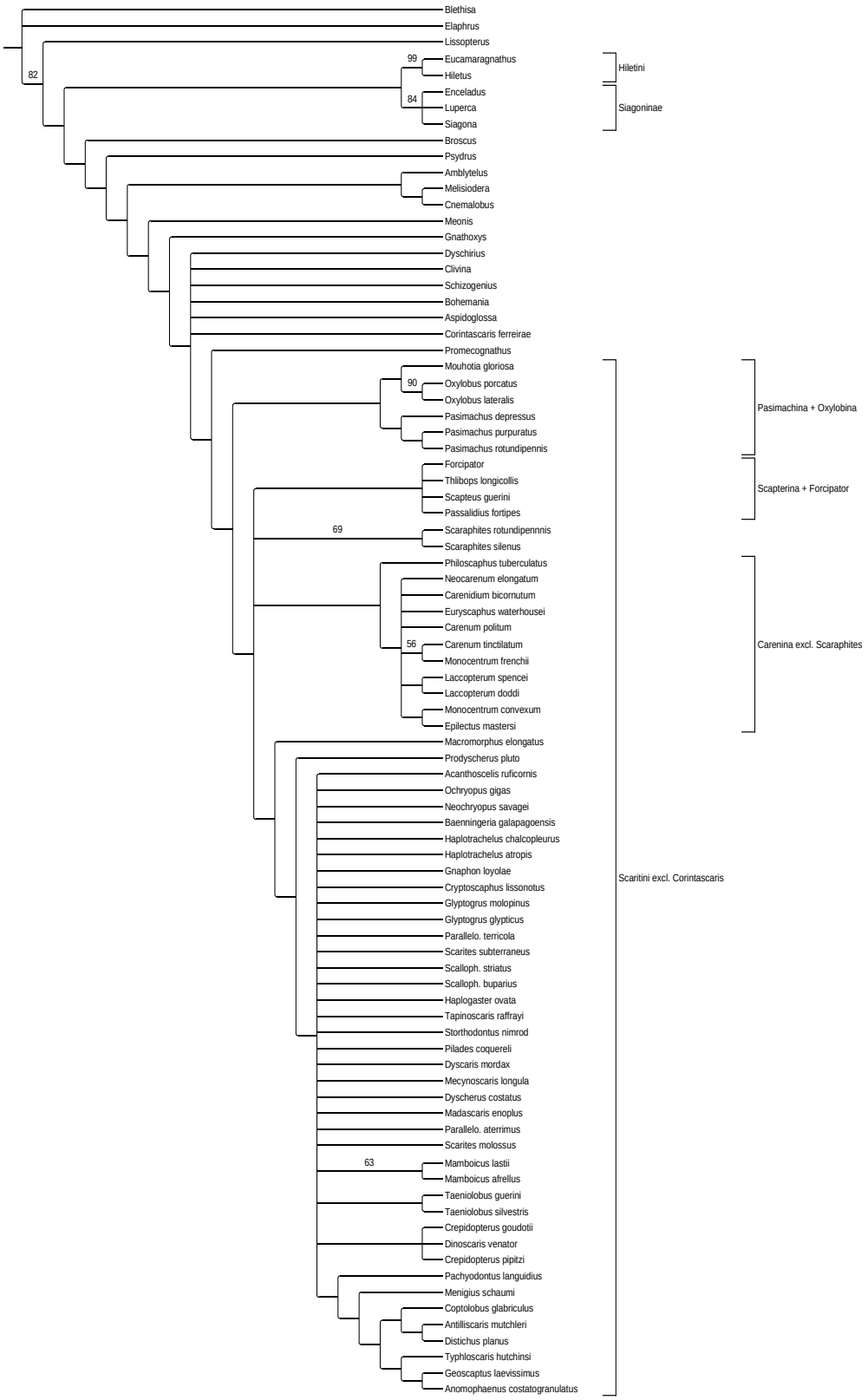

Figure 2.11. Strict consensus of 500,000 equally parsimonious trees of length 434 from the ordered data. Bootstrap percentage values above 50% are given next to the relevant nodes.

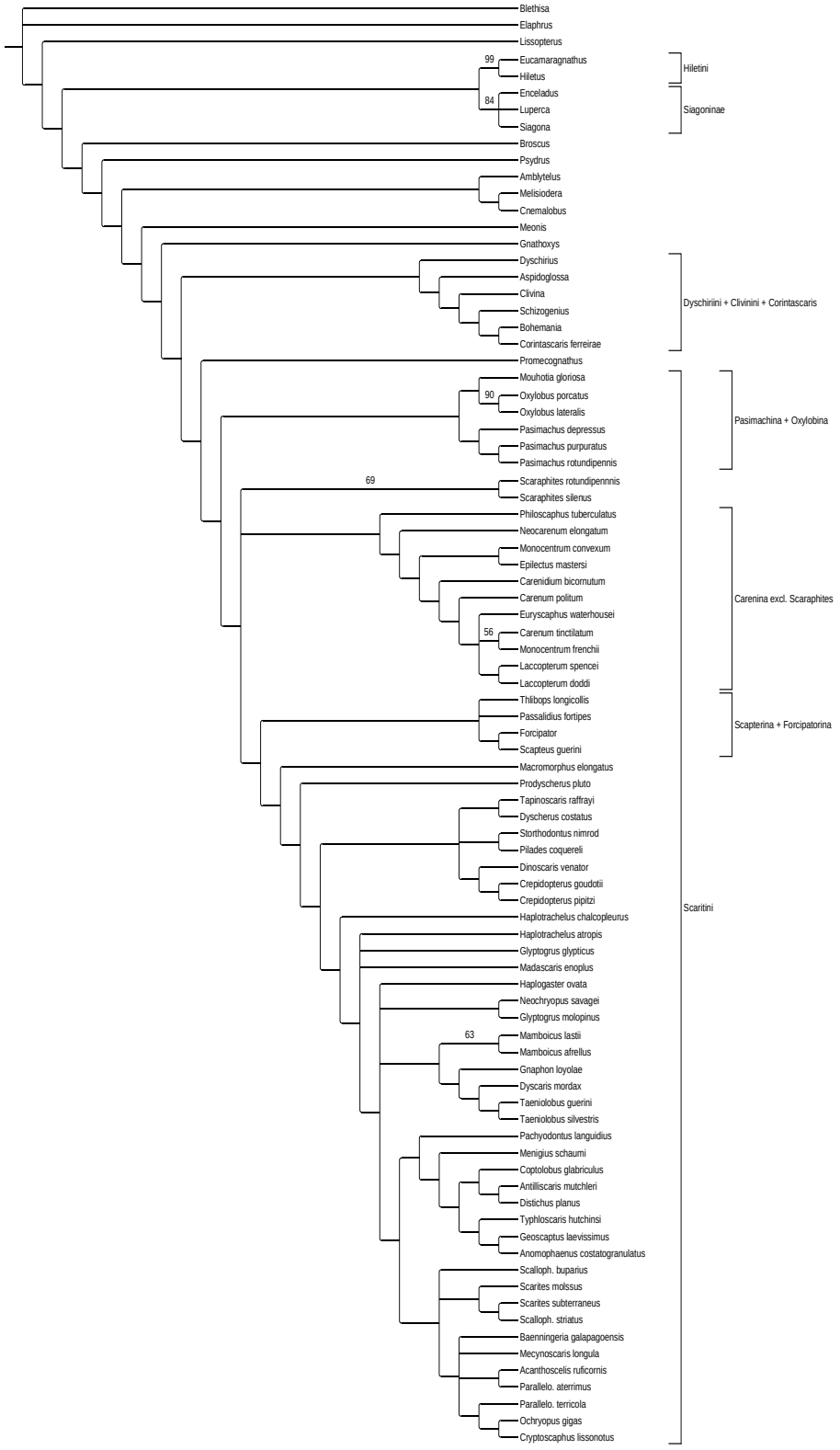

Figure 2.12. 50% majority rule consensus of 500,000 equally parsimonious trees of length 434 from the ordered data. Bootstrap percentage values above 50% are given next to the relevant nodes.

2.3.3 Results of the character ordering.

The ordering of only six of the sixty two characters had some significant effects on the results. Ordering resulted in many more equally parsimonious trees and the strict consensus of 500,000 of these had lower resolution than the consensus from the unordered data. In particular, the basal relationships between the Carenina and Scaritina are less resolved with the ordered data. In most respects the relationships inferred by the consensus trees from the ordered and unordered data are similar with one significant exception, Scaritinae *sensu lato* (Scaritini + Clivinini + Dyschiriini) and the broscine *Gnathoxys* are recovered as a monophyletic group with the ordered data (figure 2.11 and figure 2.12). With the unordered data however, the clade Clivinini + Dyschiriini occupies a basal position widely separated from Scaritini *sensu stricto* and *Gnathoxys* is placed as sister to the other broscine *Broscus* (figure 2.9 and figure 2.10). Even though these two relationships differ, neither is strongly supported by the data as none of these particular nodes have bootstrap support.

PAUP* can be used to obtain information on the nodes at which a particular character changes state (using the '`describetrees` *`treelist`* `/labelnode=yes chglist=yes`' commands) and all the character state changes supporting a particular node (using '`describetrees` *`treelist`* `/labelnode=yes apolist=yes`'). From this, it can be shown that these two different relationships are the result of ordering character 44 (number of protibial spines). When character 44 is ordered it supports a clade

containing all the taxa with protibial spines (*Gnathoxys* + Scaritinae *sensu lato*).

2.3.4 Results of the Bayesian analysis.

The MCMC procedure ran for 4,270,000 generations before terminating when the average standard deviation of split frequencies reached 0.01. Trace plots from run 1 are shown in figures 2.13 and 2.14.

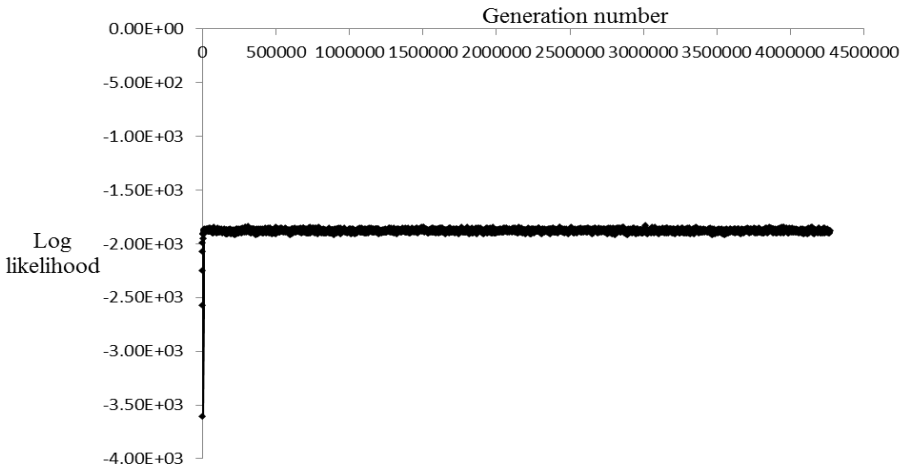

Figure 2.13. Trace plot from the unordered Bayesian analysis.

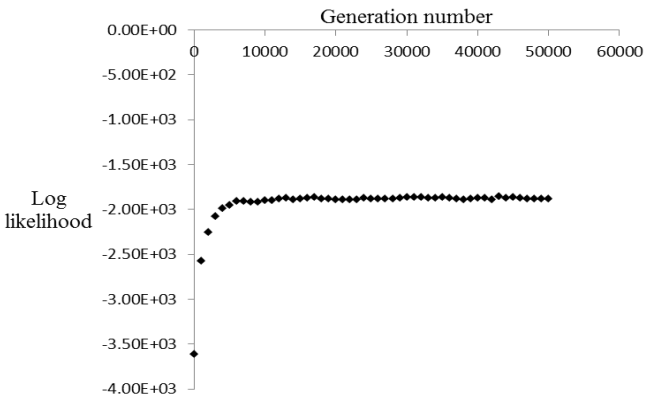

Figure 2.14. Trace plot from the unordered Bayesian analysis showing details of the the burn-in phase.

After 25% of the trees were discarded, 6408 trees from both runs were used to construct the consensus (figure 2.15).

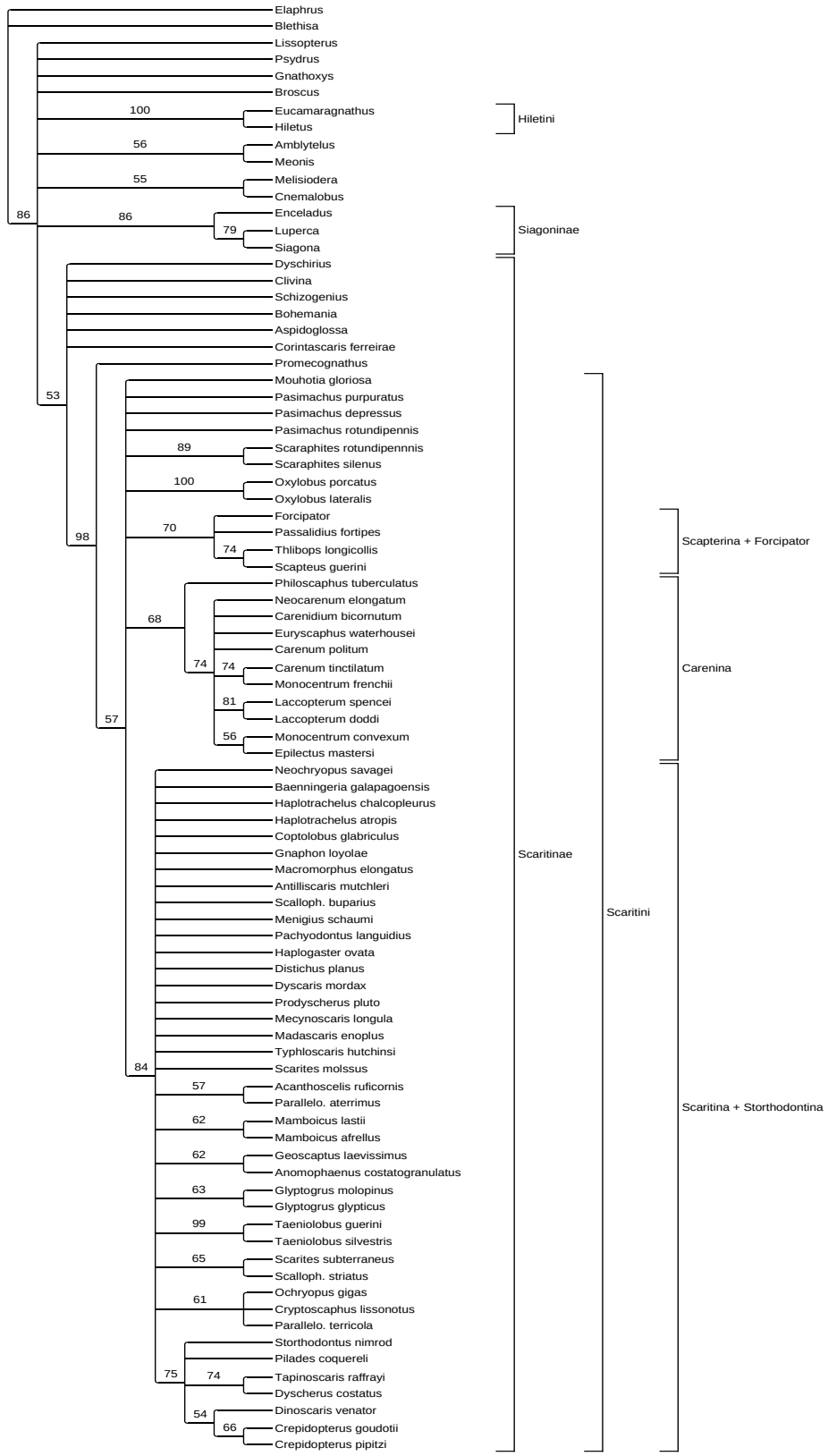

Figure 2.15. Bayesian 50% majority rule consensus tree constructed from 6408 trees. Numbers above nodes indicate clade credibility values greater than 50%.

2.3.5 Per-character statistics.

Table 2.6 shows the *ci* and *ri* scores of each character arbitrarily taken from the first tree of each parsimony analysis (tree number 1 reported by PAUP*). For the other equally parsimonious trees from each analysis some of these scores were different, therefore it is not possible to obtain *ci* and *ri* scores averaged across all the trees. Despite this, a good indication of the homoplasy displayed by characters over all the trees should still be possible by examining the character scores from a single tree.

Table 2.6. Per-character consistency index (*ci*) and retention index (*ri*) scores for the first tree from the unordered and ordered data.

Character number	Character	Consistency index (ci) (Unordered data)	Consistency index (ci) (Ordered data)	Retention index (ri) (Unordered data)	Retention index (ri) (Ordered data)
1	POSTERIOR SUPRAORBITAL CALUS	0.167	0.2	0.828	0.862
2	ANTERIOR SUPRAORBITAL SETAE	0.1	0.083	0.609	0.522
3	POSTERIOR SUPARORBITAL SETAE	0.25	0.25	0	0
4	FRONTAL FURROWS	0.143	0.143	0.647	0.647
5	FRONS TUBERCLE	0.5	1	0	1
6	ANTENNAL INSERTION	0.333	0.333	0.8	0.8
7	ANTENNAL PUBESCENCE	0.5	0.375	0.625	0.375
8	MEDIAN BAND OF ANTENNOMERE	0.25	0.25	0.8	0.8
9	LENGTH SCAPE	0.333	0.25	0.857	0.786
10	SCAPE SETA	0.2	0.25	0.81	0.857
11	CLYPEAL SETAE	0.167	0.167	0.615	0.615
12	CLYPEAL SUTURE	0.5	0.5	0	0
13	LABRUM SHAPE	0.3	0.3	0.667	0.667
14	MEDIAL DORSAL SETAE OF LABRUM	0.333	0.333	0.905	0.905
15	MAXILLARY PALP SHAPE	0.25	0.25	0.4	0.4
16	LABIAL PALP SHAPE	0.2	0.2	0.692	0.692
17	SETAE LABIAL PALPOMERE 2	0.238	0.238	0.667	0.667
18	LACINIA APEX	0.154	0.154	0.732	0.732
19	SCROBAL SETA	0.5	0.333	0.875	0.75
20	MAXILLARY FISSURE	0.25	0.188	0.809	0.787
21	GENAL PROCESS	0.2	0.2	0.871	0.871
22	MENTUM TOOTH DENTITION	0.375	0.429	0.5	0.6
23	ANTERIOR MENTUM SETAE	0.154	0.167	0.621	0.655
24	POSTERIOR MENTUM SETAE	0.286	0.333	0.444	0.556
25	MENTUM-SUBMENTUM FUSION	1	1	1	1
26	SUBMENTUM SETAE	0.235	0.25	0.5	0.538
27	GULA WIDTH	1	1	1	1
28	ANTENNAL GROOVE	0.25	0.2	0.786	0.714
29	EXTERNAL CARINA	0.25	0.25	0.903	0.903
30	INTERNAL CARINA	0.2	0.25	0.2	0.4
31	PRONOTUM LATERAL BORDER	0.167	0.143	0.808	0.769
32	PROSTERNAL KEEL	0.333	0.333	0.333	0.333
33	PROSTERNAL BORDER	0.25	0.25	0.25	0.25
34	PROSTERNAL SETAE	0.111	0.111	0.5	0.5
35	PROCOXAL CAVITIES	1	1	1	1
36	MESOCOXAL CAVITIES	0.5	0.5	0.833	0.833
37	MESOSTERNAL SETAE	0.333	0.333	0.75	0.75
38	METASTERNAL SETAE	0.25	0.25	0.5	0.5
39	METACOXAL CAVITIES	0.333	0.333	0.667	0.667
40	METACOXAL SEPARATION	0.059	0.059	0.568	0.568
41	ANTERIOR METACOXAL SETA	0.125	0.125	0.3	0.3
42	POSTERIOR METACOXAL SETA	0.111	0.1	0.742	0.71
43	INNER-MARGINAL METACOXAL SETA	0.091	0.091	0.375	0.375
44	NUMBER PROTIBIAL SPINES	0.364	0.308	0.741	0.842
45	MESOTIBIAL OUTER ANGLE	0.111	0.125	0.692	0.731
46	MESOTIBIAL DORSAL SPINE	0.2	0.182	0.81	0.824
47	METATIBIAL OUTER ANGLE	0.111	0.111	0.5	0.5
48	UNGUITRACTOR PLATE	0.25	0.2	0.571	0.429
49	HUMERAL FOLD	0.3	0.3	0.75	0.794
50	EPIPLEURON WIDTH	0.333	0.25	0.943	0.914
51	ELYTRAL BASE PUNCTURES	0.125	0.125	0.811	0.811
52	FOVEATE ELYTRAL PUNCTURES	0.25	0.25	0.4	0.4
53	SCUTELLARY STRIOLE	0.167	0.2	0.286	0.429
54	3RD ELYTRAL INTERVAL	0.2	0.167	0.333	0.167
55	5TH INTERVAL	0.25	0.2	0.4	0.2
56	6TH INTERVAL	1	1	1	1
57	7TH INTERVAL	0.2	0.2	0.478	0.6
58	7TH STRIA PUNCTURES	0.333	0.333	0.333	0.333
59	8TH INTERVAL	0.273	0.167	0.529	0.634
60	STERNITE TRANSVERSE SULCI	0.143	0.143	0.6	0.6
61	SETAE OF STERNITE 3	0.2	0.2	0.889	0.889
62	AMBULATORY SETAE	0.167	0.167	0.286	0.286

The *ci* scores indicate most characters exhibit a large amount of homoplasy. For the unordered *ci* values for example, over half of the characters have a *ci* of 0.3 or less.

Four characters exhibit no homoplasy (*ci*=1) and therefore represent good secondary homologies. These are character 25 (fusion of the mentum-submentum is a unique synapomorphy for Siagoninae), character 27 (a wide gula is a synapomorphy for the clade Scaritini (excluding *Corintascaris*) + *Forcipator* + *Promecognathus*)), character 35 (open procoxal cavities are a unique synapomorphy for Hiletinae) and character 56 (modification of the 6th elytral interval is a unique synapomorphy for *Oxylobus*).

The *ri* scores on the other hand show that despite much homoplasy, most characters contribute a significant amount of synapomorphy and for both unordered and ordered datasets over half the characters have an *ri* of 0.6 or greater. Character 1 for example (presence of the supraorbital callus) has a low *ci* of 0.17 but a high *ri* of 0.83. This is because the supraorbital callus is a synapomorphy for most Scaritini, resulting in a high *ri*, but is absent in various genera within the Scaritini clade leading to a low *ci*.

2.3.6 Phylogenetic relationships.

Very few nodes of any of the MP consensus trees had bootstrap support greater than 50%, and none of these were deep nodes. The Bayesian consensus, although showing less resolution than each MP consensus, did show support for some of the deeper nodes.

In the following section the relationships of specific taxa are discussed in more detail.

Hiletini and Siagoninae.

The monophyly of both Hiletini and Siagoninae is traditionally uncontentious and is supported by strong morphological evidence. This is confirmed by the high bootstrap support and clade credibility values for these clades.

A close relationship of Hiletini and Scaritini was proposed by Erwin and Stork (1985) but there is no strong evidence for this in any of the analyses. At best, the unordered MP data places the clade (Hiletini + Siagoninae) as sister to the Scaritini, but without bootstrap support (figure 2.9).

Scaritinae *sensu lato*.

The important question of the monophyly of the Scaritinae is not conclusively answered with the morphological data, but nonetheless there is weak support for a scaritine clade.

The unordered MP analysis provides no evidence for a monophyletic Scaritinae and the Dyschiriini + Clivinini and Scaritini clades are widely separated on all the trees (figure 2.9 and figure 2.10).

In contrast, the recovery of a monophyletic Scaritinae is evident from the ordered MP analysis and with the Bayesian analysis of the unordered data.

The evidence for this relationship is however not well supported statistically; the scaritine clade has bootstrap support of less than 50% (figure 2.11) and the Bayesian clade credibility value is also low at 53% (figure 2.15).

Promecognathus.

Lindroth (1961) incorporates *Promecognathus* as part of the scaritines. The MP analyses result in an unsupported placement of *Promecognathus* near the base of the Scaritini clade or as sister to it. The Bayesian analysis provides stronger evidence for this relationship, where *Promecognathus* is included within Scaritinae and placed as the sister to Scaritini with a high clade credibility value.

Scaritini *sensu stricto*.

Regardless of the analysis method or the way the characters are ordered, in all the trees obtained the Scaritini *sensu stricto* form a clade, although this is only supported statistically in the Bayesian analysis (clade credibility 57%). The relationships between the various subtribes of Scaritini are mostly unresolved or unsupported, but despite this, it still possible to draw the following conclusions.

Carenina.

The Carenina (an example is shown in figure 2.16) are recovered as a clade in all analyses and on this basis the subtribe almost certainly constitutes a natural group. With MP this clade is unsupported but with Bayesian inference it has reasonable support (clade credibility 68%). In the context of Scaritini, the Carenina are defined by a number of synapomorphies, including presence of anterior supraorbital setae (character 2), narrow and well defined frontal furrows (character 4), medial dorsal setae of the labrum aligned in a row (character 14), securiform terminal labial palpomeres (character 16) and the maxillary fissure absent (character 20).

The exact relationship of the Carenina clade to other Scaritini is ambiguous, but their placement always in a basal position. MP analysis of the unordered data places the Carenina as sister to the clade (*Scaraphites* + *Promecognathus* + Oxylobina + Pasimachina), but with bootstrap support of <50%. In the other analyses the Carenina occupies an unresolved basal position.

Figure 2.16. *Carenum coruscum* Macleay (Scaritini: Carenina). Scale bar = 10 mm.

Scaraphites.

Scaraphites (figure 2.17) has traditionally been classified with most of the other Australian scaritines as part of the Carenina, but is nonetheless something of an enigma, lacking many of the apomorphies defining this clade. The systematic position of *Scaraphites* in relation to the Carenina has been discussed previously by Sloane (1904) and Moore and Lawrence (1994). Both hypothesised that *Scaraphites* is an isolated genus and not

closely related to the other Carenina or indeed any other Scaritina. The results of this analysis support a placement of *Scaraphites* outside of Carenina, but do not suggest an alternative placement. The unordered data under MP gives an unsupported placement of *Scaraphites* at the base of a clade sister to the Carenina, containing *Scaraphites* + Pasimachina + Oxylobina (figure 2.9). The ordered data under MP and the BI analysis of the unordered data show an unresolved relationship of *Scaraphites* at the base of the Scaritini clade.

Figure 2.17. *Scaraphites* sp. (Scaritini: Carenina). Sydney, Australia. Scale bar = 10 mm.

Pasimachina and Oxylobina.

The overall similarity of *Pasimachus* (figure 1.4 (a)) and *Mouhotia* (figure 2.18) is readily apparent and has been noted previously (for example Nichols, 1988). However, in all MP trees *Mouhotia* has a sister relationship to *Oxylobus*, another Asian genus of uncertain affinity. The *Mouhotia* + *Oxylobus* clade is then in turn sister to *Pasimachus*. Again, none of these relationships have bootstrap support, precluding any firm conclusions about relationships. With BI each of these three genera occupies an unresolved basal relationship in Scaritini (figure 2.15).

Figure 2.18. *Mouhotia gloriosa* Castelnau (Scaritini: Pasimachina). Thailand. Scale bar = 10 mm.

Corintascaris

Corintascaris (figure 2.19) is a very unusual and taxonomically isolated genus, first established by Basilewsky (1952) to receive the single species *C.ferreirae* Basilewsky. The genus is defined mostly by autapomorphies and it shares few synapomorphies with other scaritines. Examples of these

autapomorphies are the absence of any supraorbital or clypeal setae and the massively enlarged and bulbous antennal scape, a character unique also in Carabidae as a whole. Regardless of analysis method, *Corintascaris* is always placed in a basal position in the scaritine clade.

Figure 2.19. *Corintascaris ferreirae* Basilewsky (Scaritini: Subtribe *incertae sedis*). Zambia. Scale bar =5 mm.

Scapterina.

This subtribe is an eclectic and rather ill-defined group morphologically. It is recovered as a clade, albeit without bootstrap support, in the ordered parsimony analyses and the Bayesian consensus gives this clade with a 70% clade credibility value. In both these analyses a sister relationship between

Thlibops and *Scapterus* is apparent due to these genera sharing an unusual synapomorphy; a tubercle on the front of the head (frons) (character 5). On the other hand MP analysis of the unordered data places the Scapterina in a paraphyletic series sister to all the other Scaritini.

Clivinini subtribe Forcipatorina.

Forcipator (figure 2.20) is a clivinine belonging to the subtribe Forcipatorina, a group with an uncertain relationship to the remainder of the Clivinini. An unexpected placement, apparent in all the trees obtained, is the inclusion of the genus *Forcipator* within Scaritini *sensu stricto*.

Figure 2.20. *Forcipator cylindricus* (Dejean) (Clivinini: Forcipatorina). Brazil. Scale bar = 10mm.

The Madagascan subtribes Dyscherina and Storthodontina.

Members of this this group (for example figure 2.21) are either contained within the largely unresolved Scaritina clade (unordered MP) or as a monophyletic group sister to the Scaritina (ordered MP and BI analyses). This clade is defined by two characters; progressive modifications to the humeral region of the elytra (see character 49) and the metatibial outer-angle modified into a spine (character 47). The Dyscherina + Storthodontina clade receives reasonable support in the Bayesian analysis with a clade credibility value of 75%. As the elytral characters are also not evident in any other Scaritini outside of Madagascar, the Dyscherina + Storthodontina clade probably represents a natural group.

Scaritina.

The Scaritina (or Scaritina + Storthodontina) are present as a clade in all the trees obtained, and in the Bayesian analysis there is reasonable statistical support for this group (clade credibility 84%).
While the subtribe as a whole would appear to be well founded, relationships within the Scaritina are almost completely unresolved in all the analyses. By examining each strict consensus from the parsimony analyses (figures 2.9 and 2.11) it is clear that multiple equally parsimonious arrangements within Scaritina are primarily responsible for the proliferation of equally parsimonious trees.

Figure 2.21. *Crepidopterus pipitzi* Fairmaire (Scaritini: Storthodontina). Madagascar. Scale bar = 10 mm.

2.4 Discussion.

2.4.1 Homoplasy and the problem of large numbers of equally parsimonious trees.

Large numbers of equally parsimonious trees obtained from an analysis could be due to missing entries or high levels of homoplasy in the data. Missing and inapplicable entries (coded as '?') are treated by PAUP* in the same way and are allowed by the program to have any of the possible states for the character. The particular character state assigned to the taxon is then the most parsimonious one when considering all the other characters. This potentially allows missing data entries to be optimised a number of different ways, producing more alternative trees. However in this study missing data entries made up only 1.2% of the total data points so they are not believed to have a great effect on the number of trees obtained (Wiens, 2003).

The low *ci* scores obtained for most characters suggest that homoplasy is the cause of the very large number of trees obtained.

Homoplasy can be the result of convergence or character reversal. Reversal is probably more likely in simple characters, such as the presence or absence of setae at certain positions, because they would be expected to be affected by relatively simple genetic changes. But, coding of these simple characters is more objective as they are easily assigned to discrete states and primary homology statements are less ambiguous. Conversely, complex characters

should be less prone to reversal and convergence but harder to assign to discrete states and correct homology.

After scoring the morphological characters it is obvious that many genera are defined only by a combination of characters and lack any unique synapomorphies. This is most evident in the large subtribe Scaritina. The unresolved relationships from the consensus trees and the low per-character *ci* scores suggest that characters traditionally used to define scaritine genera are highly labile, being lost and gained multiple times in separate lineages.

Following on from this, the next section outlines problems of how the genera of the Scaritina are currently defined, taking evidence from the degree of homoplasy shown by the data and by evaluating the characters used to define the individual genera. This analysis has concentrated on the Scaritina because it is by far the largest group of Scaritini, but the same principles and problems appear also to apply to the other main groups of Scaritini, the Australian Carenina and the Madagascan Storthodontina.

It is proposed that some of the genera of Scaritina, as currently defined, are artificial groups based on easily observable but ultimately homoplasious characters. These characters are obviously unsuitable for defining natural groups.

These artificial groups or genera have come to be defined for two reasons. Firstly, some genera or subgenera, for example *Scarites*, *Distichus*,

Menigius and *Mecynoscaris*, are diagnosed at least partly by labile (highly reversible) characters. The nature of these characters only becomes evident when they are examined in the context of the Scaritini as a whole. Secondly, separate groups of species with similar character states have probably arisen due to ecological convergence.

2.4.2 Genera defined by labile characters.

The characters used to define the subgenera of *Scarites* and other similar genera illustrate this point well, because in these groups large numbers of closely related species are to be found.

Table 2.7 illustrates how different character states are distributed among *Scarites sensu lato* and some other genera chosen because of their close similarity to *Scarites*. Each group is defined by a different combination of character states, but if taken individually no group has any unique character states (synapomorphies). The same principle holds for many of the other genera of Scaritina if additional characters are considered, for example modifications to elytral stria seven, loss of the clypeal setae and the form of the frontal furrows.

The character states shown in table 2.7 are those traditionally used to define *Scarites* and other similar genera. In addition to the variability of these character states *between* these genera, these character states can vary *within* other genera. Because these characters are variable within other genera they are likely to be unreliable when defining natural groups.

Table 2.7. Mosaic of character states defining the subgenera of *Scarites sensu lato* other similar genera. A white square indicates absence of the character state, black indicates presence and grey indicates presence of both character states. 1. In *Distichus sensu stricto* and *Distichus* subgenus *Lophogenius* the prosternum can be either glabrous or setose. 2. Species of *Menigius* have a glabrous prosternal process except *M.burgeoni* (Dostal, 1996). 3. *Mecynoscaris* have an elongate body, so that even though flightless they have a relatively long mesosternum. 4. *Scarites* can be either winged flyers or flightless. 5. *Parallelomorphus* can have one or two spines.

Genus	Subgenus	Prosternal process glabrous	Prosternal process setose	One mesotibial spine	Two mesotibial spines	Meso-sternum long (flyer)	Meso-sternum short (flightless)	Sulci of last 3 sternites present	Sulci of last 3 sternites absent	Maxilla hooked	Maxilla rounded	2nd sternite punctures absent	2nd sternite punctures present
Scarites	*Scarites* s.str					4.	4.						
Scarites	*Parallelomorphus*			5.	5.								
Scarites	*Taeniolobus*												
Scarites	*Orientolobus*												
Distichus	*Distichus* s.str.	1.	1.										
Distichus	*Lophogenius*	1.	1.										
Menigius			2.										
Mecynoscaris						3							

The following three examples of these types of characters are given to illustrate this further.

The presence of prosternal setae (character 34) is used as a diagnostic character for *Scarites* subgenus *Taeniolobus* (Bänninger, 1938) and the genus *Madascaris* (Basilewsky, 1973b), but these setae are both present and absent in various species of other genera such as *Typhloscaris*, *Distichus sensu lato*, *Menigius* and *Dyscaris*. In the unordered analysis this character has particularly low *ci* (0.11) and *ri* (0.5) scores and exhibits a great deal of homoplasy.

Most Scaritina have only a single dorsal mesotibial spine (character 46), while the presence of two spines is a diagnostic character for *Scarites sensu stricto* (Bänninger, 1938), *Glyptogrus* (Bänninger, 1938) and *Mecynoscaris* (Basilewsky, 1973b). However, in *Scarites* subgenus *Parallelomorphus* this character is inconsistent and either one or two spines are present.

The presence of transverse sulci near the anterior margin of the last three abdominal sternites (character 60) is used (in combination with other characters) to define *Scarites* subgenera *Taeniolobus* and *Orientolobus* and the genera *Distichus* and *Menigius* (Bänninger, 1938; Dostal, 1996). However the sulci are clearly present or absent in different species of *Coptolobus* (Andrewes, 1929) and more or less visible in other genera (P.Bulirsch, pers. comm.).

2.4.3 Ecological convergence.

When the character states of various genera and species are enumerated, especially in the speciose subtribe Scaritina (table 2.5), it becomes apparent that particular genera are very similar to others occurring in different parts of the world, with very few good external characters separating them. It is possible that this resemblance is due to convergent evolution, but this is not investigated further in this study other than to highlight the strong resemblance of various genera.

For example, *Antilliscaris* (from the mountains of Puerto Rico and Haiti) and *Typhloscaris* (from mountains in East Africa and Madagascar) are morphologically very similar (Nichols, 1986b). Except for fusion of the elytra and the presence of setae on antennomere 4, *Antilliscaris* are practically identical to *Typhloscaris* in terms of the external morphological characters scored in this study. Members of both genera in turn resemble flightless forms of other Scaritina such as various species of *Scarites* and *Parallelomorphus aterrimus* Morawitz (taxon 77, this study). As *Antilliscaris* and *Typhloscaris* both inhabit high-altitude environments in widely separated areas it is possible that their close resemblance is due to convergent evolution.

The close similarity between another pair of genera, *Haplotrachelus* from South Africa (figure 2.22) and *Glyptogrus* from the Neotropical region (figure 2.23), was also highlighted by Nichols (1986b) but without giving details. Both are lowland inhabitants and flightless. Again, based on the

external morphological characters scored in this analysis (table 2.5), the two species of each genus (*H.atropis*, *H.chalcopleurus*, *G.glyticus* and *G.molopinus*) were practically indistinguishable.

Figure 2.22. *Haplotrachelus atropis* (Bates) (Scaritini: Scaritina). South Africa. Scale bar = 10 mm.

Figure 2.23. *Glyptogrus molopinus* (Perty) (Scaritini: Scaritina). Paraguay. Scale bar = 10 mm.

Two flightless genera endemic to the mountains of Northern Madagascar, *Madascaris* (figure 2.24) and *Mecynoscaris*, are also similar and both could be considered flightless forms of *Scarites*. Aside from characters resulting from the loss of flight, *Madascaris* differs from *Scarites sensu lato* only by the discal setae touching the fourth elytral stria. Basilewsky (1973b) also gives lack of the clypeal setae (character 11) as an apomorphy for this genus, but in the single specimen examined in this study the setae were present. *Mecynoscaris* are even more similar to *Scarites*, the only significant difference being the rounded apex of the maxilla (character 18).

Figure 2.24. *Madascaris enoplus* (Alluaud) (Scaritini:Scaritina). Madagascar. Scale bar = 10 mm.

It is beyond the scope of this study to test the monophyly of all scaritine genera, as this would require large numbers of species to be scored.

However, despite apparent problems with some genera, there are also genera which appear to be monophyletic groups based on synapomorphies. An example is the New Caledonian *Anomophaenus*, with elytral intervals three, five and seven carinate and with labial palpomere two with six setae (although the elytral modifications are also present in the Indian genus *Gnaphon*). There are also monotypic genera which have unique synapomorphies and are deserving of generic status, for example *Ochryopus*, *Neochryopus* and *Acanthoscelis*.

In other cases, but in the absence of good character data, genera intuitively appear monophyletic and also have clearly delimited distributions. For example, species of *Geoscaptus* have the same general appearance and are all endemic to Australia (and marginally New Guinea). *Geoscaptus* species are also missing the clypeal setae, but this character state also occurs in other genera. Similarly, species of *Coptolobus* have a strong overall resemblance and are endemic to Sri Lanka. As proposing monophyly of these genera is speculative at this time they are not elaborated here in detail, but analysis of these putatively monophyletic groups would be a logical starting point for future studies.

2.5 Conclusions and future directions.

It is clear that the Scaritini as a whole are in need of a modern taxonomic treatment, with genera defined in an evolutionary framework.

The current classification and monophyly of genera should be tested using new morphological characters not yet applied to Scaritini, for example the structure of the mandibles and the male genitalia (chapter 3), along with DNA sequence data (chapter 4).

All Scaritini possess large mandibles for the purposes of feeding and burrowing (Hlavac, 1967), and sometimes in the male, for mating (Eberhard, 2004; Hlavac, 1967). The mandibles are complex structures, with different areas of the mandible shaped for different tasks, and have potential to yield useful characters for phylogenetic analysis. The apex is generally hooked to prevent the escape of prey and for loosening the substrate. The distal terebral region is smooth and acts as a cutting surface. The proximal molar region is usually armed with thick blunt teeth for breaking the exoskeleton of prey. The mandibles of some Scaritini, for example *Scapterus* and *Passalidius* also have additional teeth projecting in an upward or downward plane.

A whole suite of characters will probably be apparent after detailed comparative analysis of scaritine mandibles. Acorn and Ball (1991) provide a sound basis for the study of carabid mandibles and propose homology of the different mandibular structures. This work highlights the need for mandibular characters to be interpreted carefully, because similarities in

mandible structures could be the result of convergence due to similar diets or substrate preference. Recent work on tiger beetles (Carabidae, Cicindelinae) (Ball et al., 2011) provides an interpretation of mandibular characters in an evolutionary framework, highlighting their potential use in Scaritini.

In conclusion, based on morphological evidence, the current classification of Scaritina is probably a mix of monophyletic and polyphyletic groups. Even though the polyphyletic groups are based on highly reversible characters and the general intuition of past authors, these groups do at least allow ordering of the species into easily manageable units for the purposes of communication and species identification. However, to address questions about character evolution, phylogeny and biogeography a natural classification system is required.

Chapter 3

A study of the male genitalia of Scaritini

3.1 Introduction.

The male genital organ of Carabidae (the median lobe or aedeagus) is a chitinous tube containing the endophallus, a complex sac of folded membranes and sclerotized regions. During mating the endophallus is everted from the tip of the aedeagus to form an irregular balloon-like structure.

In some genera of Scaritini the external shape of the aedeagus exhibits sufficient variation to allow discrimination of closely related species (van Etten, 1984). Illustrations of the aedeagus of many Madagascan Scaritini shows there is sometimes great variation in shape within a single genus, for example *Storthodontus* (Basilewsky, 1973b). In other genera, for example *Scarites*, closely related species show no discernible differences in the male and female genitalia (Nichols, 1986a).

Due to this uncertain pattern of variation the external form of the aedeagus is not investigated further in this study.

3.1.1 Internal structure of the aedeagus.

The form of the endophallus has been used most comprehensively in the classification of the basal tribe Carabini. Once everted and unfolded (for

method see Meurgues and Ledoux, 1966) the endophallus is revealed as a very complex structure. The presence or absence and position of certain sclerites have been used to define the major divisions of the large genus *Carabus* (Deuve, 2004). Furthermore, characters of the endophallus of *Pamborus* are coded in detail by Sota et al. (2005) and those of *Platycarabus* by Casale et al. (1998). Problems of interpretation and homology assessment still remain however, and molecular analyses have shown that parallel evolution can occur in genitalic structures (Osawa et al., 2004).

Sclerites X and Y are sclerotized regions of the endophallus described for the mid-grade tribes Broscini (Ball, 1956), Melaenini (Ball and Shpeley, 2005) and Elaphrini (Goulet, 1983). Whitehead (1972) also described sclerotized structures ('basal stylets') of the male genitalia of the clivinine *Schizogenius*, which were interpreted by Roig-Juñent (1998) as homologous to sclerite X. Fedorenko (1996) also studied the internal structure of the genitalia of Dyschiriini and described two sclerotized regions of the endophallus. The distal part of the endophallus contains four sclerites: a, b, c and d and evolutionary transitions can be implied according to the shapes or secondary loss of one or more sclerites. However, sclerites a-d do not appear to have homologues in other groups of scaritines, but the basal region of the endophallus contains a Y-shaped sclerite that may be homologous to sclerite X of Clivinini.

The internal structure of the aedeagus of Scaritini has not been studied in detail. It has been illustrated without further elaboration for a few

Madagascan species (Basilewsky, 1973b) and Baehr (2006) described internal sclerites of the genitalia of the Australian genus *Steganomma*: both show a presumably homologous spinose apical sclerite. A similar sclerite is also visible in figures of the genitalia of *Haplogaster* given by Balkenohl (1994). Roig-Juñent (1998) interpreted the basal sclerite of the endophallus of *Scarites anthracinus* Dejean as being homologous to sclerite X in other mid-grade tribes. However, as sclerite X is present in several distantly related carabid tribes it may either be a plesiomorphic structure secondarily lost several times, or it is not homologous across different groups.

The aim of this chapter is to investigate the form of the endophallus in each of the subtribes of Scaritini.

3.2 Method.

External secondary sexual characters are lacking or very subtle in many scaritines, so that dissection is required to determine the sex of an individual.

Specimens were initially softened by immersion for one hour in hot distilled water. Once softened, the aedeagus was extracted from the abdomen using a fine pin. Any external membranes and fat were removed and the aedeagus was transferred to 10% potassium hydroxide solution and digested for two hours at 50 degrees.

As the aedeagus began to clear and reveal details of the internal structure it was washed in acid-alcohol (5% acetic acid in 75% ethanol) and stored in 70% ethanol.

At this stage details of the endophallus and in particular the form of sclerite X were visible.

Several attempts were made to inflate the endophallus to enable further study of this complex structure. A fine syringe needle was inserted into the basal orifice of the aedeagus and attempts made to force liquid into the aedeagus. This method was unsuccessful, but with ethanol-preserved specimens of *Pasimachus purpuratus* (Putzeys) the endophallus could be everted by very gentle extrusion with fine forceps. Using this technique with older dried material from museum collections failed and always ended with the membranes tearing. As the supply of specimens was limited, no more attempts at inflation were made and further study was confined to examination of the endophallus in the folded state. Because detail was obscured by the somewhat opaque wall of the aedeagus the endophallus was removed entire. The preparations were photographed in ethanol (for photographic method see section 2.2.3) and held in the required orientation using glass micro-beads.

3.3 Results.

Photographs showing details of the endophallus of thirteen species of Scaritini are given in figures 3.1 to 3.5.

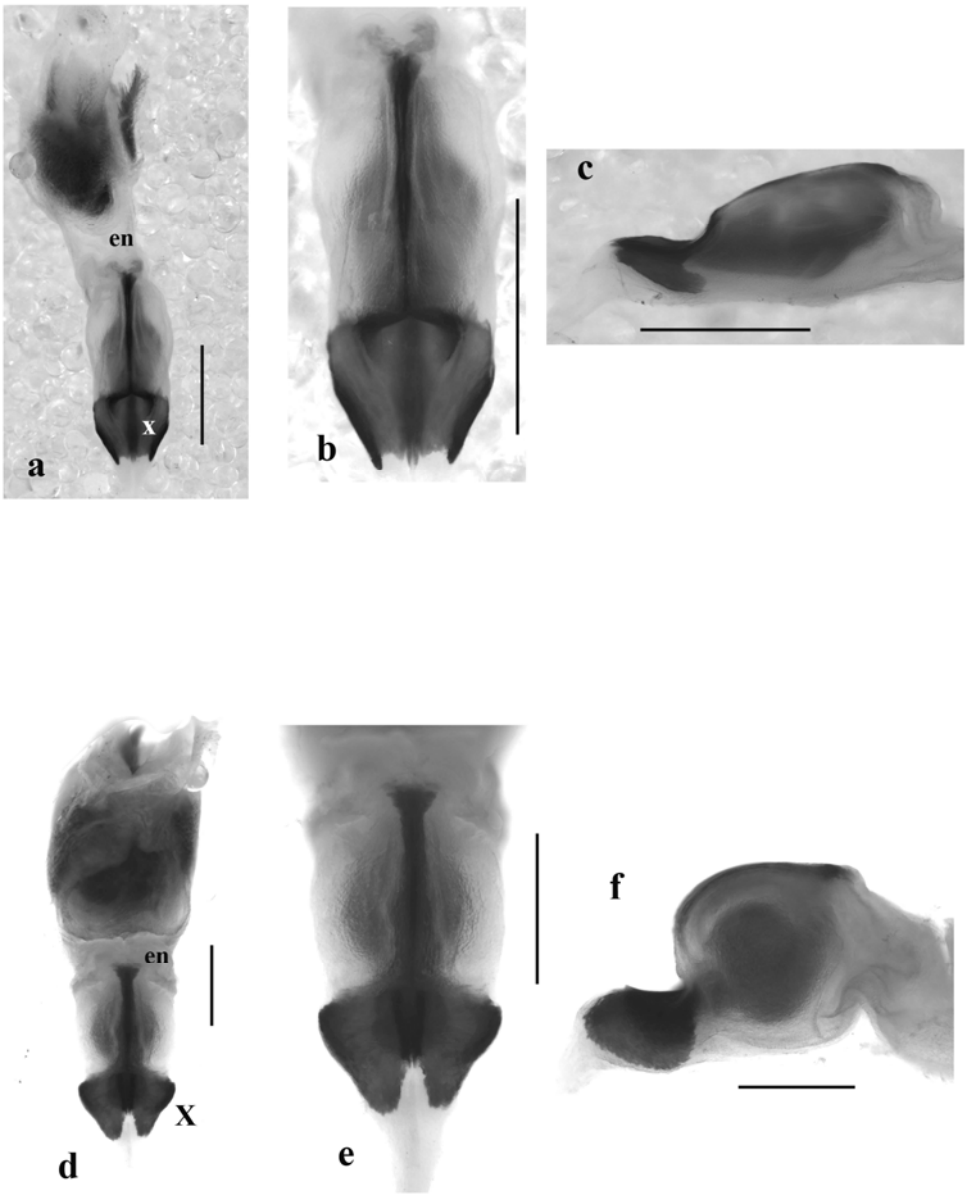

Figure 3.1. Photographs of the endophallus of two species of Carenina. a-c Endophallus of *Carenum* sp. (a). Dorsal view of endophallus. (b). Dorsal view of endophallus with detail of sclerite X. (c). Lateral view of sclerite X. d-f Endophallus of *Philoscaphus tuberculatus* (MacLeay). (d). Dorsal view of endophallus. (e). Dorsal view of endophallus with detail of sclerite X. (f). Lateral view of sclerite X. (Scale bar = 0.5 mm. en = endophallus; X = sclerite X).

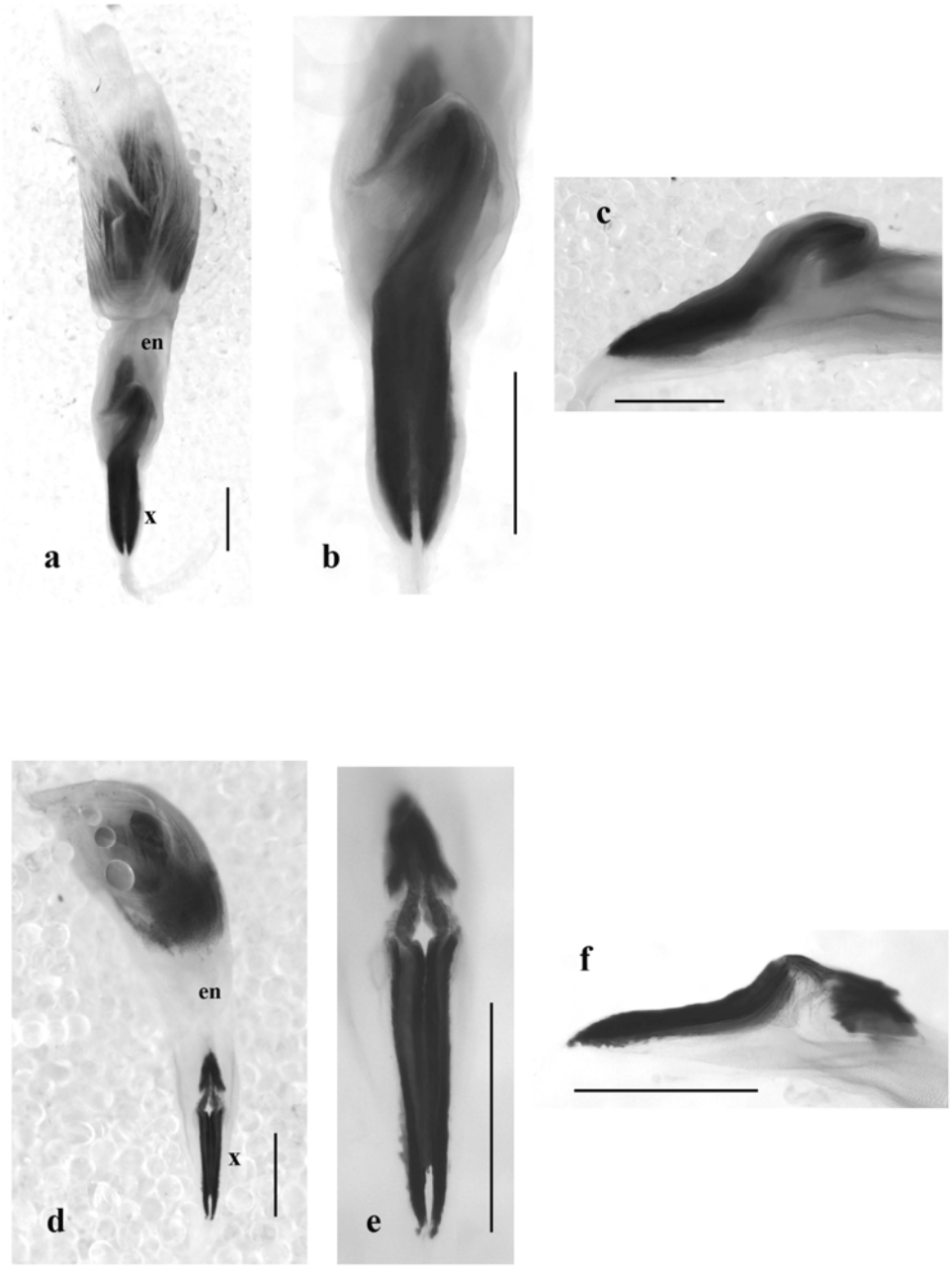

Figure 3.2. Photographs of the endophallus of two species of Pasimachina. a-c Endophallus of *Mouhotia gloriosa* Castelnau. (a). Dorsal view of endophallus. (b). Dorsal view of endophallus with detail of sclerite X. (c). Lateral view of sclerite X.
d-f Endophallus of *Pasimachus purpuratus rodruiguezi* (Putzeys). (d). Dorsal view of endophallus. (e). Dorsal view of endophallus with detail of sclerite X. (f). Lateral view of sclerite X. (Scale bar = 0.5 mm. en = endophallus; X = sclerite X).

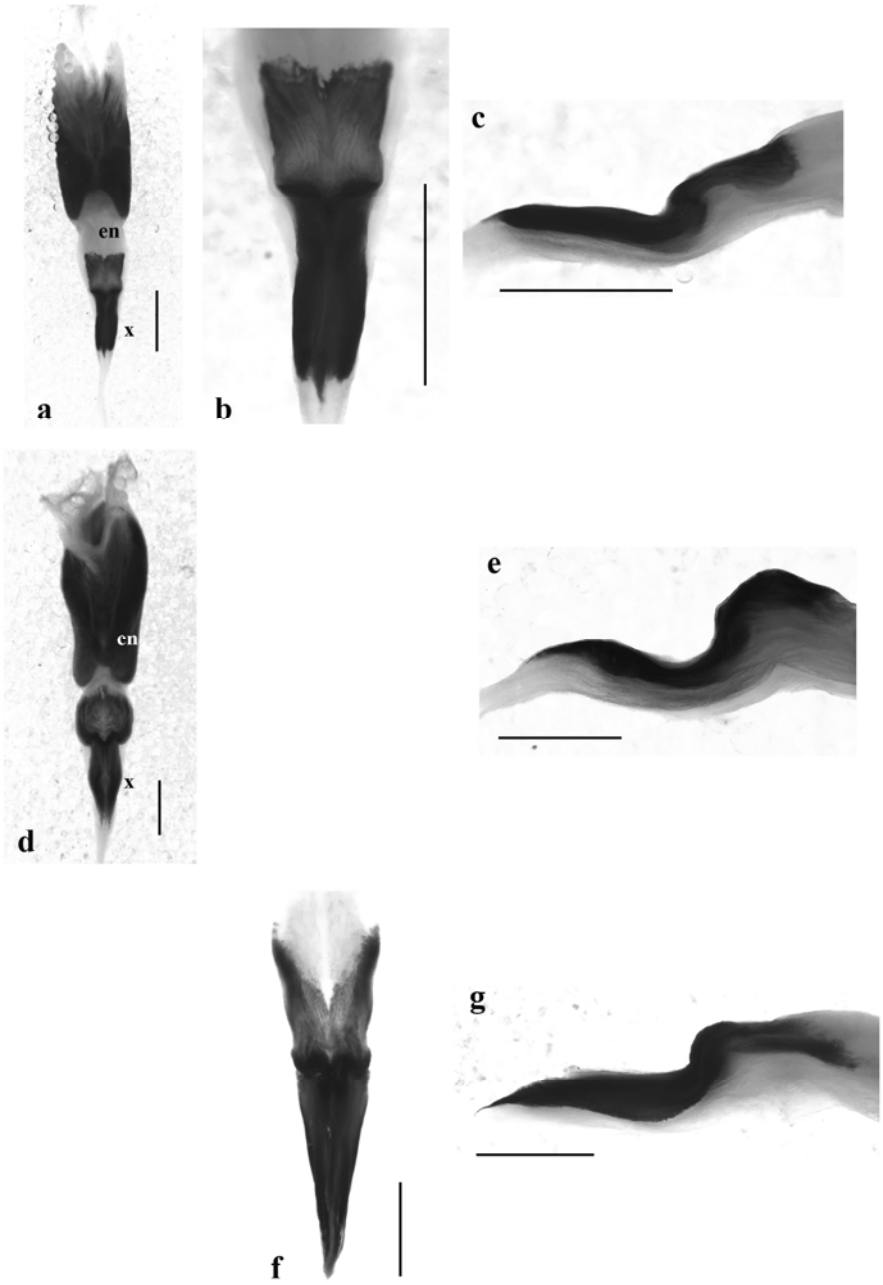

Figure 3.3. Photographs of the endophallus of Dyscherina and Storthodontina. a-c Endophallus of *Dyscherus costatus* (Klug). (a). Dorsal view of endophallus. (b). Dorsal view of endophallus with detail of sclerite X. (c). Lateral view of sclerite X. d-e Endophallus of *Crepidopterus pipitzi* Fairmaire. (d). Dorsal view of endophallus. (e). Lateral view of sclerite X. f-g Endophallus of *Pilades coquereli* Fairmaire . (f). Dorsal view of endophallus with detail of sclerite X. (g). Lateral view of sclerite X. (Scale bar = 0.5 mm. en = endophallus; X = sclerite X).

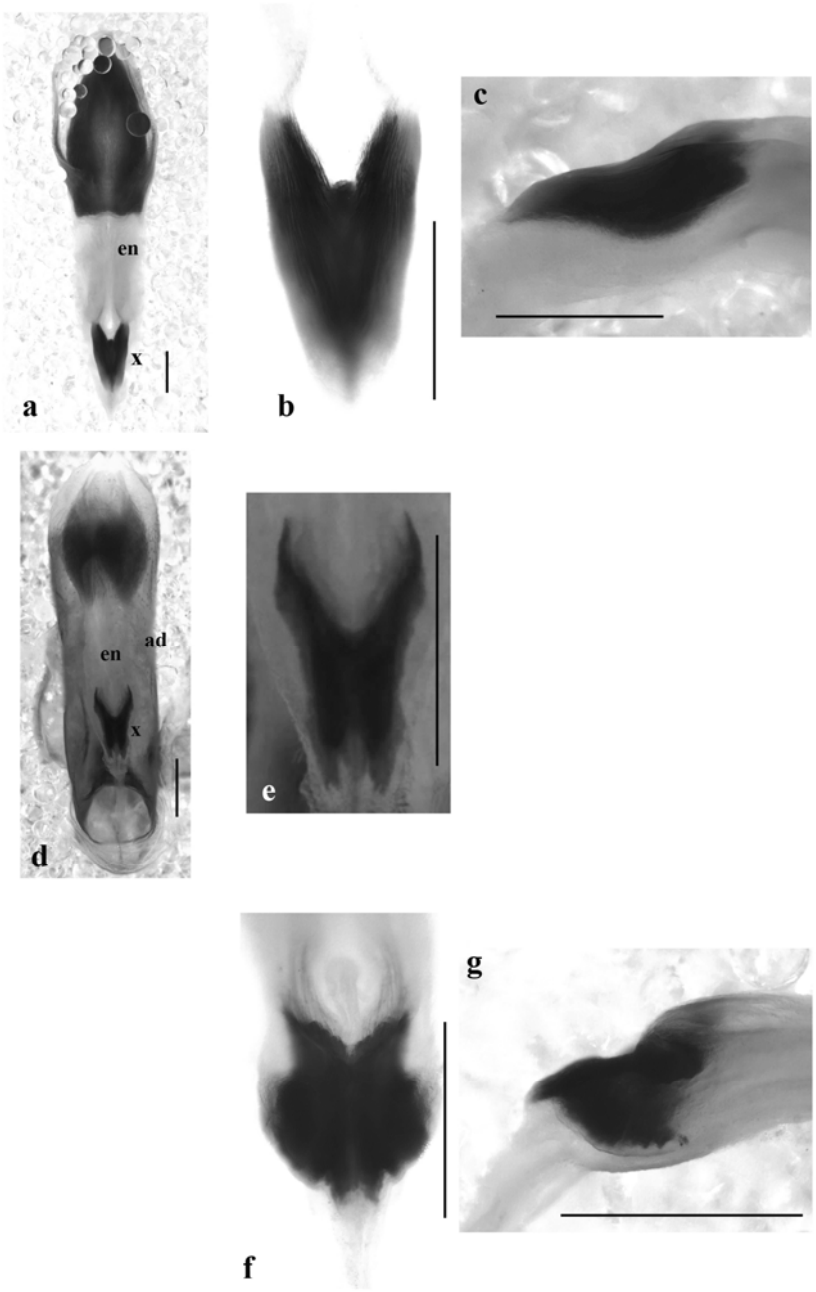

Figure 3.4. Photographs of the endophallus of Scapterina and Scaritina. a-c Endophallus of *Passalidius fortipes* (Boheman). a. Dorsal view of endophallus. b. Dorsal view of endophallus with detail of sclerite X. c. Lateral view of sclerite X. d-e Aedeagus and endophallus of *Acanthoscelis ruficornis* (F.). d. Cleared aedeagus preparation showing endophallus and sclerite X in situ. e. Detail of sclerite x in dorsal view. f-g Sclerite X of *Neochryopus savagei* (Hope). f. Detail of sclerite x in dorsal view. g. Lateral view of sclerite X. (Scale bar = 0.5 mm. en = endophallus; X = sclerite X; ad = aedeagus).

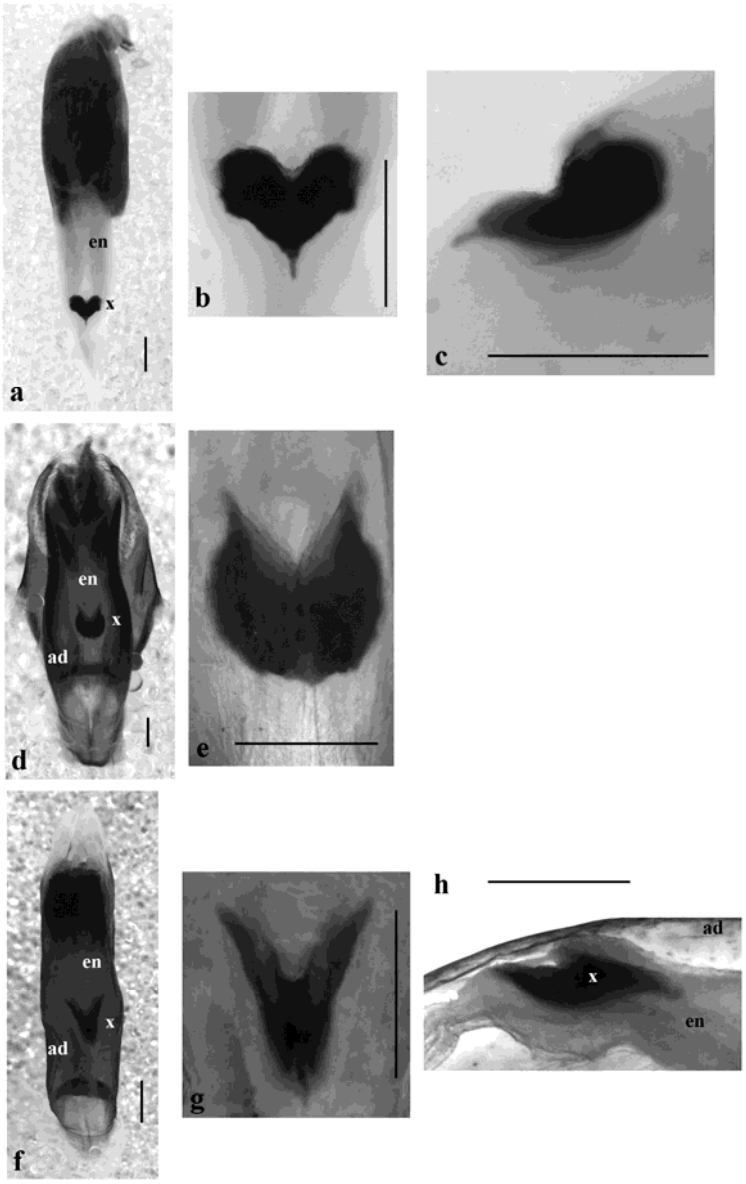

Figure 3.5. Photographs of the endophallus of three species of Scaritina. a-c Endophallus of *Haplogaster ovata* Chaudoir. (a). Dorsal view of endophallus. (b). Dorsal view of endophallus with detail of sclerite X. (c). Lateral view of sclerite X. d-e Aedeagus and endophallus of *Mamboicus ochryopoides* Bänninger. (d). Cleared aedeagus preparation showing endophallus and sclerite X in situ. (e). Detail of sclerite x in dorsal view. f-h Aedeagus and endophallus of *Scarites bucida* (Pallas). (f). Cleared aedeagus preparation showing endophallus and sclerite X in situ. (g). Detail of sclerite x in dorsal view. (h). Detail of sclerite x in lateral view. (Scale bar = 0.5 mm. en = endophallus; X = sclerite X; ad = aedeagus).

In the inverted and folded state two separate groups of structures are evident in the endophalli of all the Scaritini studied; a basal sclerotized region and a distal region of tightly folded membranes and microtrichia. It was not possible to examine this distal region without inflating the endophallus, so only the variation in the basal sclerotized region was studied.

This basal sclerotized region of Scaritini occupies a similar position to the sclerites X present in Broscinae, Elaphrinae and Clivinini. Sclerite X of Scaritini is considered homologous to the sclerites of these other groups, based on the criterion of topological correspondence.

Sclerites X of both genera of Pasimachina studied (figure 3.2) are long and thin in shape. The sclerite of *Mouhotia*, though somewhat twisted, is similar in shape to that of *Pasimachus*. The sclerite of *Pasimachus* is clearly composed of two separate regions, a parallel sided basal (proximal) area and an arrow-shaped apical (distal) region. It is possible this arrangement actually represents two separate sclerites, only one of which is homologous to sclerite X.

Sclerites X of the Madagascan *Dyscherus*, *Storthodontus* and *Pilades* are large and occupy up to a quarter of the total length of the endophallus. Again, it appears to be composed of two separate regions (figure 3.3).

Six genera of Scaritina were examined (figures 3.4 and 3.5) and in this subtribe there is a reduction in the size and complexity of sclerite X. The sclerite is smaller in relation to the total length of the endophallus,

composed of a single region and is approximately V shaped in all the species examined.

The sclerites of the two genera of Carenina (figure 3.1) are similar to each other and are quite unlike any of those of the other Scaritini studied. In this case sclerite X is a complex structure, with a wide basal area connected to an apical region folded along its length into a tube.

3.4 Conclusions.

Even though the overall number of species sampled was small, consistent differences in the form of sclerite X are apparent between the various subtribes of Scaritini. This provides evidence that the subtribes Carenina, Pasimachina, Storthodontina and Scaritina represent monophyletic groups. The structure of the endophallus of Carenina was found to be very different from all other Scaritini investigated, suggesting a more distant relationship between this group and the rest of the Scaritini.

The results of this initial investigation are encouraging and further study of the structure of the endophallus of Scaritini and other mid-grade carabid groups would be worthwhile. In particular, variation in the shape of sclerite X among the five genera of Scaritina investigated so far may help to interpret the complex relationships of this subtribe revealed by the morphological analysis (chapter 2).

The challenge remains to interpret the homology and variation of the internal sclerites to allow coding for cladistics analysis. If this can be

achieved, such characters are likely to be good indicators of evolutionary history. The distal region of the endophallus also has the potential to yield further characters if the technical difficulty of inflating the endophallus of dry-preserved specimens can be overcome.

Chapter 4

Phylogeny of the Scaritinae inferred from molecular data

4.1 Introduction.

This chapter deals with the sequencing, alignment and analysis of the small subunit 18S rRNA (18S) gene of a selection of Scaritinae *sensu lato* and other mid-grade Carabidae.

4.1.1 The small subunit 18S rRNA gene.

18S sequences are commonly used to resolve the higher-level relationships (relationships at or above the rank of tribe) of organisms. There are several reasons for the popularity of this marker. Most importantly, the analysis of 18S sequences produces meaningful results broadly in agreement with established morphological classifications (Caterino et al., 2000). The large number of studies using 18S also means that large numbers of sequences are publically available (Benson et al., 2012), and for this reason a good taxonomic coverage of mid-grade carabid outgroups was possible for this analysis. Finally, there are practical reasons for the popularity of 18S because the gene is easy to amplify and sequence across a wide range of taxa.

The main disadvantage of using 18S sequences for phylogenetic analysis is that variation in the length of the molecule causes problems with alignment. The lengths of most 18S rRNAs of Coleoptera are between 2-2.5kb but the total range for Eukaryotes is in the order of 1.5-4.5kb (Xie et al., 2011).

4.1.2 Structure of the 18S rRNA molecule.

The 18S molecule is part of the ribosomal functional core used in protein synthesis. The molecule is folded into a complex secondary structure of stems and loops and with further folding to create tertiary structures.
The primary structure of the 18S rRNA sequence comprises well demarcated slow and fast evolving regions (Hwang et al., 2000). The slow evolving regions form the stems of the secondary structure, and when folded may undergo complimentary base pairing with more distant regions of the molecule (figure 4.1).
The sequences of the fast evolving loop regions are less constrained than those of the stems (Hwang et al., 2000) and across different taxa these loop regions can vary greatly in length and nucleotide composition. Information from secondary structure can be used to guide the alignment process and has the potential to produce more accurate alignments.

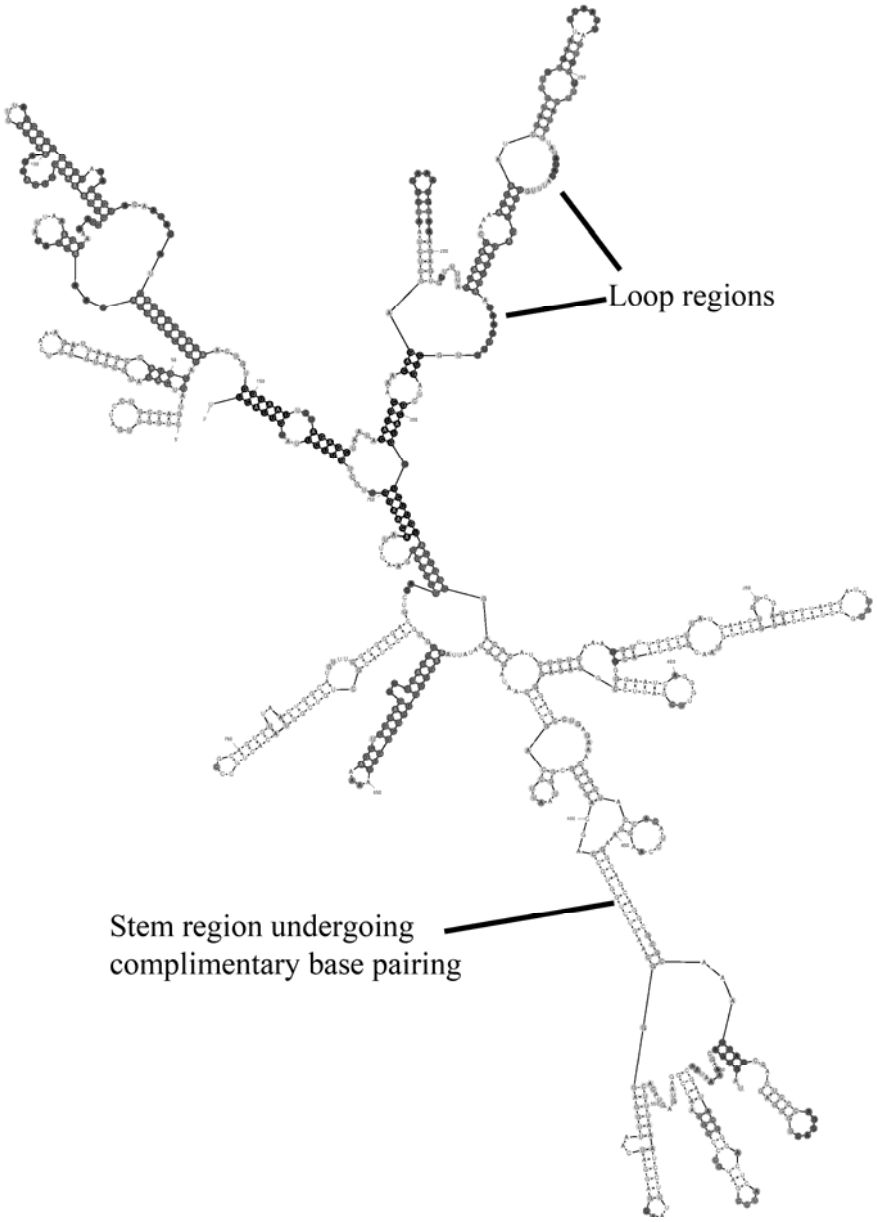

Figure 4.1. A section of the 18S rRNA sequence of the scaritine *Storthodontus reticulatus* Basilewsky showing the stem and loop regions of the secondary structure. The structure was produced using the mfold web server at http://mfold.rna.albany.edu/?q=mfold

4.1.3 Sequence Alignment.

Alignment is an important step in phylogenetic analysis using molecular data. As homology is assigned by alignment, this step is critical to the outcome of the analysis.

Alignment of the conserved regions of 18S is usually straightforward as these regions are similar in sequence and contain few insertions or deletions. In the case of the loop regions the opposite is true and it is usually difficult to unambiguously align these sections (Sanderson and Shaffer, 2002). This is because there may be a number of equally plausible placements of bases and gaps.

With this problem in mind there are a multitude of different approaches to the alignment of 18S sequences, although most methods have in common a separate alignment and analysis stage.

Clustal is the most widely used alignment program (Higgins and Lemey, 2009) as it can produce alignments quickly via a user-friendly interface. It provides a useful benchmark to compare the performance of other programs and for these reasons is used in this study.

There are three separate steps to the alignment process of Clustal (Chenna et al., 2003; Higgins and Sharp, 1988):

- All possible pairwise comparisons between sequences are made and a distance matrix is calculated on the basis of the proportion of nucleotide positions that differ between the sequences.
- The distance matrix is used to construct a guide tree using the neighbour joining method (Saitou and Nei, 1987).
- The progressive alignment method is then used to produce a multiple alignment of the input sequences.

The progressive alignment process proceeds as follows:

- The two 2 closest sequences are aligned first, using a *dynamic programming* algorithm (for details of the algorithm see Eddy, 2004) and the gap penalty (GP).
- These initial 2 closest sequences are then combined into a single consensus sequence or sub-alignment. An important consequence of this process is that this sub-alignment is now fixed and cannot be changed when considering the remaining sequences (Felsenstein, 2004).
- The next two closest sequences are then aligned. Depending on the branching order of the guide tree these could be two single

sequences, a single sequence added to a sub-alignment or two sub-alignments. This process continues until the alignment is complete.

The gap penalty is given as:

$$GP = GOP + GEP(l - 1)$$

Where l is the gap length, *GOP* is the gap opening penalty and *GEP* is the gap extension penalty (Higgins and Lemey, 2009).

The GOP defines the cost of opening a gap in the alignment and increasing the GOP results in a decrease in the number of gaps. The GEP defines the cost of extending an existing gap by one residue and increasing the value of this parameter results in shorter gaps.

The values of the GOP and GEP must be defined by the user and the choice of these values can have a substantial effect on the alignment and resulting phylogenetic trees. However, for any particular dataset there is no way to know *a priori* the optimum values of these parameters.

4.1.3.1 Incorporating secondary structure information into the alignment process.

Information from secondary structure, for example by identifying conserved structural motifs, can be used to guide and improve alignments (Letsch, et al., 2009). Secondary structure alignments can be performed manually by reference to a secondary structure model of a related organism (for example

Jordal et al., 2007) or better as a completely automated process (Stocsits et al., 2009).

Secondary structure information may be incorporated into the automated alignment process in a number of ways. For example, a single externally created secondary structure is used as a reference to guide the alignment of the sequences of interest. The program RNAsalsa (Stocsits et al., 2009) uses this approach. One drawback of this method is that there may not be a secondary structure reference available from a group taxonomically close to the one of interest.

An alternative approach is to generate information on secondary structure using internal algorithms without the use of an external guide structure. The Q-INS-i algorithm implemented in the program MAFFT (Katoh et al., 2002; Katoh and Toh, 2008a) is used in this study and follows this principle. MAFFT uses a progressive alignment method similar to that of Clustal, but with some important refinements that have been shown empirically to improve its accuracy (Katoh and Toh, 2008a).

Firstly, the progressive method is performed twice. After the initial alignment is created, new distance scores are used to calculate a second guide tree and a second cycle of progressive alignment is performed (Katoh and Toh, 2008b).

Secondary structure information is also incorporated at the progressive alignment stage with MAFFT (Q-INS-i), using the Four-way Consistency function of Katoh and Toh (2008a). This function identifies regions of

complementary base pairing to guide the alignment. The principle of Four-way Consistency is as follows.

- The function initially selects a small region of the first sequence.
- A second region further along the sequence is then identified that undergoes complementary base pairing with the first region. This is achieved by calculating base pairing probabilities with the McCaskill algorithm (for details see McCaskill, 1990).
- A match is then made between the two complementary pairing regions in the first sequence to the two corresponding homologous regions in the second sequence using similarity scores (Katoh and Toh, 2008a).

The complete process is illustrated in figure 4.2.

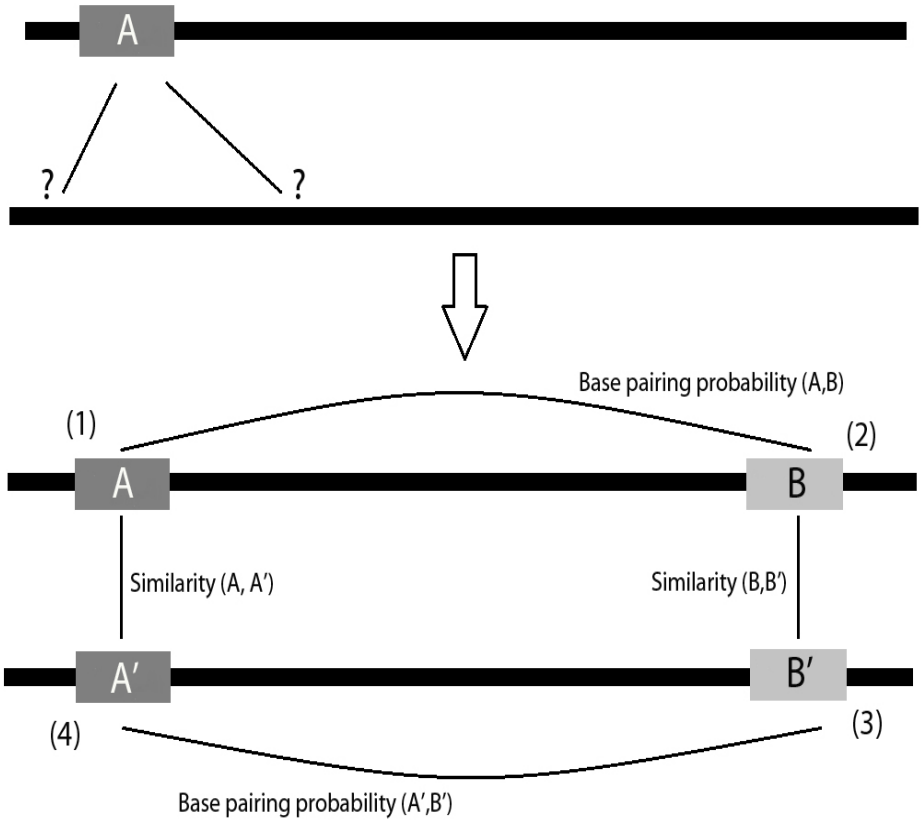

Figure 4.2. Diagram illustrating the Four-way Consistency method. Region A represents a length of sequence to be aligned with a homologous region A' in another sequence. (1) Region B undergoing complementary base pairing with A is identified by a base pairing probability score. (2) B', the homologous region to B in the second sequence, is identified by a similarity score. Homologous region A' is then identified using the base pairing probability between A' and B' (3) and the similarity score between A and A' (4). Re-drawn from Katoh and Toh (2008a).

An alternative to separate alignment and analysis steps is the method of direct optimisation, as implemented by the program POY (Wheeler et al., 1996-2003). POY combines alignment and tree searching in a single process to circumvent the problematic alignment stage. Despite this innovation POY

has not been shown to conclusively out-perform other methods (Ogden and Rosenberg, 2007) and is limited to maximum parsimony tree searches.

4.1.3.2 The problem of ambiguous alignment.

The simplest way to deal with ambiguously aligned characters is to exclude them from the analysis. This can be performed manually, but the process may also be automated in order to preserve repeatability, for example with the program ALISCORE (Misof and Misof, 2009). The great advantage of the exclusion method is that ambiguous homologies are removed. This essentially conservative approach may be at the cost of throwing away considerable amounts of data. For example, in a phylogenetic study of the higher-level relationships of adephagan beetles based in part on 18S sequences (Maddison et al., 2009), between 19% and 36% of the 18S sequence was discarded.

Exclusion of the regions of ambiguous alignment is based on the assumption that these sites contain misleading information due to the incorrect assignment of homology. However, studies have shown that these regions do contain useful phylogenetic information, particularly for resolving relationships among more closely related species (Smythe et al., 2006). For example, Lindgren and Daly (2007) used 18S sequences to reconstruct the phylogeny of oceanic squid (Decapodiformes). By analysing the length-variable regions separately it was shown that they contain a substantial number of informative characters with a consistent signal. A similar

conclusion was reached by Ruiz et al. (2009) and Jordal et al. (2007) by including regions of ambiguous alignment of the 28S rRNA gene in reconstructing the phylogenies of Sphodrini (Coleoptera, Carabidae) and Scolytinae (Coleoptera, Curculionidae) respectively.

4.2 Methods.

4.2.1 Taxon Sampling.

Attempts were made to directly obtain 18S sequences from 24 species of Scaritinae *sensu lato* and the outgroup *Eucamaragnathus brasiliensis* (Négre) (table 4.1). Most of the specimens used were collected in such a way as to maximise preservation of DNA; by dehydration either with silica gel or absolute ethanol. The exceptions to this were dried and pinned specimens of *Mouhotia planipennis* Pouillade and *Oxylobus punctatosulcatus* Chaudoir.

A further 30 scaritine and outgroup sequences were obtained from the GenBank database (Benson et al., 2012) (table 4.2).

Table 4.1. Specimens used for DNA extraction and sequencing.

Subfamily	Tribe	Subtribe	Species	Locality	Collector	Collection date	Preservation	Notes
Hiletinae	Hiletini		*Eucamaragnathus brasiliensis* (Négre)	Bolivia	D.Mann	01.xii.2003	Ethanol	
Scaritinae	Dyschiriini	Dyschiriina	*Dyschirius arenosus* Stephens	France, Pas de Calais	J.Hogan	12.vi.2002	Ethanol	
Scaritinae	Dyschiriini	Dyschiriina	*Akephorus marinus* LeConte	USA, California	M.Caterino	20.viii.2004	Ethanol	
Scaritinae	Clivinini	Clivinina	*Scolyptus angustatus* Dejean	Gambia, Yundum	D.Mann	02.viii.1997	Frozen, Dry	
Scaritinae	Clivinini	Clivinina	*Halocoryza arenaria* (Darl.)	Barbados, Bathsheba	J.Hogan	Unknown	Ethanol	
Scaritinae	Clivinini	Clivinina	*Clivina fossor* (L.)	UK, Warwickshire	D.Mann	Unknown	Ethanol	
Scaritinae	Clivinini	Forcipatorina	*Camptodontus* sp.	Bolivia	Mann & Hamel	01.xii.2003	Ethanol	
Scaritinae	Scaritini	Carenina	*Scaraphites* sp.	Australia, Perth	D.Pryce	28.i.2003	Ethanol	
Scaritinae	Scaritini	Pasimachina	*Pasimachus purpuratus* ssp. *rodruiguezi* (Putzeys.)	Belize, Las Cuevas	R.Pateman	ii.2004	Ethanol	
Scaritinae	Scaritini	Pasimachina	*Mouhotia planipennis* Pouillade	Thailand, Chiang Mai	Unknown	1998	Dry	Amplification failed
Scaritinae	Scaritini	Oxylobina	*Oxylobus punctatosulcatus* Chaudoir	India, Karnataka	M.Halada	14.v.2005	Frozen, Dry	Amplification failed
Scaritinae	Scaritini	Scapterina	*Passalidius fortipes* (Boh.)	Namibia, Caprivi Park	D.Mann	17.xii.1999	Ethanol	Partial amplification
Scaritinae	Scaritini	Storthodontina	*Storthodontus reticulatus* Basilewsky	Madagascar	D.Maddison via J.Galián	Unknown	Ethanol	DRM99001

Table 4.1 (continued). Specimens used for DNA extraction and sequencing.

Subfamily	Tribe	Subtribe	Species	Locality	Collector	Collection date	Preservation	Notes
Scaritinae	Scaritini	Scaritina	*Gnaphon loyolae* (Fairmaire)	India, Vellagan	D.Maddison via J.Galián	Unknown	Ethanol	DRM99025. Galian 6
Scaritinae	Scaritini	Scaritina	*Ochryopus gigas* Schiödte	Guinea	Leonard & Vingerhoedt	26.viii.2003	Frozen, Dry	
Scaritinae	Scaritini	Scaritina	*Distichus planus* (Bonelli)	Spain	J.Galián	Unknown	Frozen, Dry	'85'
Scaritinae	Scaritini	Scaritina	*Pachyodontus languidus* Wiedemann	South Africa, Table Mountain	D.Mann	ix.2003	Ethanol	
Scaritinae	Scaritini	Scaritina	*Scarites buparius* (Forster)	Spain	J.Galián	i.1996	Ethanol	SC13
Scaritinae	Scaritini	Scaritina	*Scarites buparius* (Forster)	Spain, Alicante	J.Hogan	iv.2002	Ethanol	SC25
Scaritinae	Scaritini	Scaritina	*Scarites eurytus* Waldheim	Spain	J.Galián	'10/1'	Ethanol	
Scaritinae	Scaritini	Scaritina	*Scarites hepericus* Dejean	Spain	J.Galián	Unknown	Ethanol	'145'
Scaritinae	Scaritini	Scaritina	*Scarites subterraneus* F.	USA, NJ, Freehold	D.Duran	2004	Ethanol	SC21
Scaritinae	Scaritini	Scaritina	*Scarites* sp.1	South Africa	D.Maddison via J.Galián	Unknown	Ethanol	DRM98054. (SA1)
Scaritinae	Scaritini	Scaritina	*Scarites* sp.2	South Africa	D.Maddison via J.Galián	Unknown	Ethanol	DRM98054. (SA2)
Scaritinae	Scaritini	Scaritina	*Scarites* sp.3	RSA, Kruger	D.Inward	Unknown	Ethanol	(SA3)

Table 4.2. 18S rRNA sequences retrieved from the GenBank nucleotide database.

Taxon	GenBank accession number	Reference
Amblytelus curtus	AF012484.1	Maddison et al. (1999)
Apotomus rufithorax	AF012497.1	Maddison et al. (1999)
Bembidion mexicanum	AF012490.1	Maddison et al. (1999)
Blethisa multipunctata aurata	AF002803	Maddison et al. (1998)
Broscosoma relictum	AF012502.1	Maddison et al. (1999)
Carenum interruptum	AF012491	Maddison et al. (1999)
Clivina ferrea	AF002796	Maddison et al. (1998)
Clivinini sp. (Florida)	AF201400	Shull et al. (2001)
Creobius eydouxi	AF012498.1	Maddison et al. (1999)
Dyschirius aeneus	AF201401	Shull et al. (2001)
Dyschirius sphaericollis	AF002798	Maddison et al. (1998)
Elaphrus californicus	AF012514.1	Maddison et al. (1999)
Elaphrus clairvillei	AF002802.1	Maddison et al. (1998)
Laccocenus ambiguus	AF012486.1	Maddison et al. (1999)
Loricera foveata	AF012503.1	Maddison et al. (1999)
Loricera pilicornis pilicornis	AF002799.1	Maddison et al. (1998)
Mecodema fulgidum	AF012501.1	Maddison et al. (1999)
Mecyclothorax vulcans	AF012482.1	Maddison et al. (1999)
Melisodera picipennis	AF012481.1	Maddison et al. (1999)
Meonis sp.	AF398722.1	Ober (2002)
Oregus aereus	AF012500.1	Maddison et al. (1999)
Pasimachus atronitens	AF002794	Maddison et al. (1998)
Pasimachus californicus	AF201399	Shull et al. (2001)
Promecoderus sp. nr *brunnicornis*	AF012499.1	Maddison et al. (1999)
Promecognathus crassus	AF012492.1	Maddison et al. (1999)
Psydrus piceus	AF002784.1	Maddison et al. (1998)
Scarites subterraneus	AF002795	Maddison et al. (1998)
Schizogenius falli	AF002797	Maddison et al. (1998)
Siagona europaea	AF012493.1	Maddison et al. (1999)
Tropopterus sp.	AF012483.1	Maddison et al. (1999)

4.2.2 DNA extraction, amplification and sequencing.

Total DNA extraction and 18S amplification of the species *Oxylobus punctatosulcatus*, *Mouhotia planipennis*, *Eucamaragnathus brasiliensis* and *Camptodontus* sp. was performed by J.Day in the laboratory of the Centre for Ecology and Hydrology, Oxford during October 2009. DNA extraction and amplification of the remaining twenty species (table 4.1) was performed by the author at the Molecular Systematics Laboratory of the Natural History Museum, London during November 2004. In both cases the final sequencing step was performed by staff at the DNA sequencing facility of the Natural History Museum.

4.2.2.1 DNA extraction.

Total DNA was extracted with a Qiagen DNeasy Blood & Tissue Kit (Cat. No. 69504) by following the manufacturer's protocol. To visualise the yield and quality of the DNA, 1 µl of each extraction was electrophoresed on an agarose gel and compared to a DNA size and quantity standard (Bioline Hyperladder^{TM} I).

4.2.2.2 DNA Amplification.

The 18S rRNA gene was sequenced in four overlapping regions using the primer pairs 18S5'/18Sb5.0, 18Sai/18Sb2.5, 18S1.0/18Sbi and 18Sa2.0/18S3'I (table 4.3).

Table 4.3. Primers used in the amplification and sequencing of the 18S r RNA gene.

Primer	Sequence	Direction	Reference
18S5'	5'GACAACCTGGTTGATCCTGCCAGT	Forward	Shull et al., 2001
18Sb5.0	5'TAACCGCAACAACTTTAAT	Reverse	Shull et al., 2001
18Sai	5'CCTGAGAAACGGCTACCACATC	Forward	Whiting et al., 1997
18Sb2.5	5'TCTTTGGCAAATGCTTTCGC	Reverse	Shull et al., 2001
18S1.0	5'GGTGAAATTCTTGGACCGTC	Forward	Whiting et al., 1997
18Sbi	5'GAGTCTCGTTCGTTATCGGA	Reverse	Whiting et al., 1997
18Sa2.0	5'ATGGTTGCAAAGCTGAAAC	Forward	Shull et al., 2001
18S3'I	5'CACCTACGGAAACCTTGTTACGAC	Reverse	Shull et al., 2001

Each PCR reaction contained 2.5 µl 10X BioTaq NH_4 buffer, 1.8 µl 50 mM $MgCl_2$, 0.25 µl each of 10 µM forward and reverse primer, 0.5 µl 10 mM d'NTP'S, 0.055 µl Bioline BIOTAQ™ DNA polymerase, 19.145 µl water and 0.5 µl of template DNA.

PCR cycling conditions comprised an initial denaturation of 94ºC for 2 minutes followed by 35 cycles of 94ºC denaturation for 30 seconds, 50ºC annealing for 30 seconds and 72ºC extension for 1 minute, with a final extension of 72ºC for 10 minutes.

PCR reactions were purified with Millipore MutiScreen-FB 96 well plates, following the manufacturer's protocol.

4.2.2.3 DNA Sequencing.

Cycle sequencing was performed using ABI PRISM® BigDye™ Terminators V.1.1. Reactions were purified by precipitating with a mixture of absolute ethanol and 3M sodium acetate and sequences were read using an ABI 3730 capillary DNA sequencer.

4.2.2.4 DNA sequence editing.

Raw sequence files were edited using ChromasPro version 1.3 beta (Technelysium Ltd.). Sequencing errors were corrected by comparing the forward and reverse sequences and contigs of the complete 18S gene sequence were exported as individual fasta files. The fasta files were then assembled into an un-aligned data matrix using Bioedit version 7.0.9 (Hall, 2007).

4.2.3 Analysis methods.

4.2.3.1 The 'multiple analysis' method.

This study uses automated sequence alignment. The alignments are analysed without any further modification or correction and no data is discarded. The aim is to produce repeatable alignments, while acknowledging there may be some error in assigning homology. In an effort to mitigate this, the

results across a range of arbitrarily chosen alignment parameters are compared and summarised.

The results of the Bayesian analysis are summarised with a table of the key nodes and relationships recovered. With maximum parsimony this is extended further using consensus trees across all the alignments. This general approach was first proposed by Lee (2001) as the 'multiple analysis' method. With this method a number of alignments are generated with different values of the user-defined parameters, for example the GOP and GEP of Clustal. The alignments are then separately analysed and a consensus constructed from all the resulting trees from all alignments. Only those relationships common to all (or subjectively a majority) of the trees are accepted.

This method has the advantage that it will reveal relationships that are insensitive (or subjectively less sensitive) to user-defined variation of the alignment parameters. However, by reliance on consensus methods there is the danger that the resulting topologies may lack resolution, leading to overly conservative phylogenetic hypotheses.

4.2.3.2 Multiple sequence alignment.

Sequences were aligned using the programs ClustalX version 2.0.12 (Larkin et al., 2007) and the Q-INS-i variant of MAFFT version 6.901b (Katoh and Toh, 2008a) using the MAFFT online server at http://mafft.cbrc.jp/alignment/server/.

Before producing an alignment both programs require the user to define several parameters.

Two parameters were defined for ClustalX, the GOP and the GEP. Both have a permissible range from 0-100. Ten different alignments were created using the following GOP:GEP values; 1:0.25, 1:0.5, 2:1, 5:1, 4:2, 7:2, 8:3, 10:2, 10:5 and 15:6.66 (default).

The choice of values was arbitrary, though guided by results of previous studies. For example, Hickson et al. (2000) showed that GOP values between 4 and 7 and a GEP of 2 gave alignments most comparable to an accurate secondary structure alignment of 12s rRNA. Other values, chosen on the basis of performing well in other phylogenetic studies of Coleoptera using 18S, were 1:0.25 (Ruiz et al., 2009), 10:2 (Maddison et al., 1999) and 15:6.66 (Bocakova et al., 2007).

For all the ClustalX alignments the DNA transition weight was arbitrarily held constant at the default value 0.5, giving transitions half the weight of transversions.

Similarly, two parameters were defined for MAFFT, the GOP and the offset value (OV), equivalent to the GEP of ClustalX. In MAFFT the permissible range of the GOP is 1 – 3 and for the OV 0 – 1.

Ten different alignments were created using the following arbitrary GOP:OV values; 1:0, 1.53:0 (default), 1.53:0.5, 1.53:1, 2:0, 2:0.5, 2:1, 3:0, 3:0.5 and 3:1.

For all the MAFFT alignments the nucleotide scoring matrix was held at the default value of 300 PAM.

Aligned sequences in fasta format were imported into Winclada (Nixon, 2002) and the start and end of each alignment was trimmed to avoid missing data from some taxa. The removed sequence corresponds to nucleotide positions 1-54 and 1916-1995 of the *Drosophila melanogaster* 18S rDNA sequence of Tautz et al. (1988). For the purposes of this analysis, the trimmed sequences are named 'full' 18S rRNA sequences, even though they are in fact about 130 bases short of the actual full sequence.
Data matrices were exported from Winclada in nexus format to enable reading by PAUP* and MrBayes.

4.2.3.3 Data partitioning.

The data were partitioned in order to investigate the phylogenetic signal present in the length-conserved (LC) and length-variable (V) regions of the 18S sequence.
The limits of the LC and V regions were set arbitrarily for all the alignments by visual inspection of the 1:0.25 ClustalX matrix. In theory the boundaries between the LC and V regions will be most ambiguous in this alignment because it has the highest number of gaps. However, in actuality the

boundary between the different regions could be easily identified and was unambiguous.

For the purposes of repeatability and to characterise the LC and V regions, an additional alignment was created by including the 18S sequence of *D.melanogaster* (Tautz et al., 1988; Genbank accession number M21017.1). Alignment with this reference sequence (results not shown) indicates the V regions are equivalent to the central sections of expansion segments V2, V4, V6 and V7 of *D.melanogaster* (Hancock et al., 1988).

The limits of each region relative to the *D.melanogaster* sequence are given in table 4.4.

Table 4.4. Limits of the LC and V regions standardised against the *Drosophila melanogaster* 18S rRNA sequence.

Region	Nucleotide position in the *Drosophila* 18S sequence
LC1	55-224
V2	225-267
LC2	268-723
V4	724-780
LC3	781-1440
V6	1441-1568
LC4	1569-1880
V7	1881-1916

For each pair of alignment parameter values the partitioning resulted in three datasets; the full length 18S sequence, the combined conserved regions (LC1 + LC2 + LC3 + LC4) and the combined variable regions (V2 + V4 + V6 + V7) (table 4.5).

4.2.3.4 Phylogenetic analysis.

Two methods of phylogenetic inference were employed; maximum parsimony (MP) using PAUP* version beta 10 (Swofford, 2003) and Bayesian inference (BI) using MrBayes version 3.2.1. (Ronquist et al., 2012).

MP was used to analyse all the alignments and partitions, while BI was used to analyse the full length 18S alignments only (table 4.5).

It was considered that MP was sufficient to explore the phylogenetic signal of the LC and V partitions and had the additional practical advantage of speed.

4.2.3.5 Phylogenetic analysis with PAUP*.

Commands were issued to PAUP* with batch files, using the same search strategy as used previously for the morphological data (section 2.2.4.1). Trees were rooted using the outgroup taxon *Elaphrus clairvillei* Kirby.

Values of ensemble consistency index (*CI*), retention index (*RI*) and rescaled consistency index (*RC*) were obtained from PAUP* with the 'describe trees' command.

4.2.3.6 Phylogenetic analysis with MrBayes.

An example MrBayes data block is as given below.

```
BEGIN MRBAYES;
set autoclose=yes;
log start filename=filename.log;
outgroup Elaphrusclairvillei18S;
lset nst=mixed rates=invgamma;
showmodel;
mcmc ngen=50000000 printfreq=1000 samplefreq=1000 nchains=4
temp=0.1 stoprule=yes stopval=0.01;
sump relburnin=yes burninfrac=0.25;
sumt relburnin=yes burninfrac=0.25 contype=halfcompat;
log stop;
END;
```

Rather than using a DNA substitution model *a priori*, the Markov chain Monte Carlo (MCMC) procedure was used directly to explore the posterior probability distribution of alternative substitution models (Huelsenbeck et al., 2004). This procedure, known as 'reversible-jump MCMC', is implemented in the latest version of MrBayes (Ronquist et al., 2012) with the '`lset nst=mixed`' command.

The rate variation across sites was arbitrarily set to a gamma distribution with a proportion of invariable sites, since these parameters cannot yet be estimated using reversible-jump MCMC with MrBayes. The other MrBayes commands and methods are discussed in detail in section 2.2.4.2.

4.2.3.7 Gap coding.

MrBayes only allows gaps to be interpreted as missing data, but PAUP* can treat gaps in one of two ways, either as missing data or as an additional 5th character state.

One of the assumptions of parsimony and model-based analysis is that each character is independent. Gaps coded as a 5th state are potentially misleading, because each separate position in the gap is treated as an independent character. If treated as a 5th state, gaps longer than 1 base artificially introduce bias by implying that taxa sharing multiple gap positions share multiple characters.

This effect of coding gaps as a 5th state has also been shown empirically to produce spurious relationships, for example Bocakova et al., 2007 and Ogden and Rosenberg, 2007. Therefore in this analysis all gaps were treated as missing data.

4.2.3.8 Construction of the combined consensus trees.

'Combined consensus trees' were obtained by creating a consensus of a number of individual strict consensus trees. The method employed was as follows.

- Ten alignments were created with ClustalX and MAFFT using different gap cost ratios, as outlined in section 4.2.3.2.
- Each alignment was analysed using maximum parsimony to obtain a strict consensus tree.
- The combined consensus was created by making a consensus of all 10 individual consensus trees.

The strict combined consensus therefore reveals only the relationships common to all trees produced from all alignments.

Because the resolution of strict combined consensus trees is often poor a 50% majority-rule combined consensus was also used. This tree shows only those relationships occurring in 50% or more of the individual strict consensus trees.

It is not possible to construct combined consensus trees automatically with PAUP* as the trees held in memory are re-set every time a new tree file is loaded. Instead, new tree files were created manually by combining the individual PAUP* tree files.

An overall summary of the analysis is given in table 4.5.

Table 4.5. Summary of the analysis showing the alignments, method of phylogenetic inference and selected trees displayed in the results section.

Alignment method gap cost	Length-conserved regions (LC)		Length-variable regions (V)		Full 18S sequence		
	Maximum parsimony		Maximum parsimony		Maximum parsimony		Bayesian inference
ClustalX 1:0.25		Strict and majority-rule combined consensus figures 4.5 and 4.6		Strict and majority-rule combined consensus figures 4.9 and 4.10		Strict and majority-rule combined consensus figures 4.13 and 4.14	
1:0.5							
2:1							
4:2							
5:1							
7:2							
8:3							
10:2							
10:5							
(Default) 15:6.66	Fig. 4.8		Fig. 4.12		Fig. 4.16		Fig. 4.17 and 4.18
MAFFT 1:0		Strict and majority-rule combined consensus figures 4.5 and 4.7		Strict and majority-rule combined consensus figures 4.9 and 4.11		Strict and majority-rule combined consensus figures 4.13 and 4.15	
(Default) 1.53:0					Fig. 4.19 and 4.20		Fig. 4.21 and 4.22
1.53:0.5							
1.53:1							
2:0							
2:0.5							
2:1							
3:0							
3:0.5							
3:1							

4.3 Results.

4.3.1 The sequence data.

DNA amplification and sequencing was successful in nearly all cases, but attempts to extract DNA from two pinned specimens of *Mouhotia planipennis* and one of *Oxylobus punctatosulcatus* failed. Most likely these specimens were not treated in the correct way to ensure preservation of DNA.

Only partial sequencing of the 18S gene was possible for *Passalidius fortipes* (Boheman) as DNA amplification with primer pair a2.0/3'1 failed. Because the remainder (1500 bp) of the *P.fortipes* sequence was obtained it was included in the analysis.

After trimming, the sequences varied in length from 1896bp (*Elaphrus californicus* (Mannerheim) and *Blethisa multipunctata* (L.)) to 2090bp (*Tropopterus* sp.).

4.3.2 The alignments and parameters.

A summary of the alignments and parsimony analyses is given in tables 4.6 and 4.7.

The effect of the different gap opening and gap extension costs is evident by comparing the total number of characters in each alignment. As expected,

low gap costs produced long alignments with more gaps and as the gap costs were increased the alignments became shorter.

The ClustalX alignments varied in length by 440 characters, from 2632 characters for GOP:GEP 1:0.25 to 2192 characters with GOP:GEP 10:6.66. In contrast MAFFT produced shorter alignments, varying in length by only 214 characters across the range of chosen parameter values. Figure 4.3 illustrates this difference with an excerpt of the V2 region aligned by Clustal and MAFFT with the lowest gap penalty chosen for each program.

Table 4.6. Summary of the ClustalX alignments.

Alignment parameters GOP:GEP	Partitioned data														Full length 18S sequence						
	Length conserved (LC) partition							Length variable (V) partition													
	No. chars.	No. inform. chars. (%)	MP tree length	No. MP trees	CI	RI	RC	No. chars.	No. inform. chars. (%)	MP tree length	No. MP trees	CI	RI	RC	No. chars.	No. inform. chars. (%)	MP tree length	No. MP trees	CI	RI	RC
1:0.25	1635	212 (13.0)	781	140	0.491	0.786	0.387	997	403 (40.4)	1517	88	0.587	0.750	0.440	2632	615 (23.4)	2343	80	0.543	0.755	0.411
1:0.5	1633	212 (13.0)	783	140	0.491	0.788	0.387	989	404 (40.8)	1513	18	0.583	0.741	0.433	2622	616 (23.5)	2335	8	0.543	0.752	0.409
2:1	1621	217 (13.4)	802	1980	0.491	0.793	0.390	865	430 (49.7)	1672	42	0.544	0.729	0.396	2486	647 (26.0)	2522	100	0.517	0.746	0.385
4:2	1615	224 (13.9)	816	417	0.493	0.796	0.392	711	443 (62.3)	1950	21	0.513	0.725	0.373	2326	667 (28.7)	2807	16	0.500	0.744	0.372
5:1	1613	227 (14.0)	822	56	0.492	0.792	0.393	786	434 (55.2)	1997	498	0.504	0.717	0.361	2399	661 (27.6)	2861	27	0.493	0.740	0.365
7:2	1612	226 (14.0)	822	4969	0.490	0.797	0.391	693	434 (62.6)	2140	112	0.477	0.715	0.341	2305	660 (28.6)	3001	1	0.474	0.737	0.350
8:3	1611	227 (14.1)	832	10002	0.488	0.796	0.389	629	442 (70.3)	2192	9	0.470	0.716	0.337	2240	669 (29.9)	3067	48	0.469	0.736	0.345
10:2	1610	225 (14.0)	834	8165	0.492	0.795	0.391	637	436 (68.4)	2262	207	0.466	0.705	0.329	2247	661 (29.4)	3140	16	0.467	0.728	0.340
10:5	1607	228 (14.2)	842	5083	0.493	0.798	0.393	595	437 (73.4)	2350	18	0.459	0.713	0.328	2202	665 (30.2)	3226	15	0.463	0.736	0.341
15:6.66	1606	229 (14.3)	852	5597	0.494	0.795	0.393	586	447 (76.3)	2482	4	0.429	0.685	0.294	2192	676 (30.8)	3378	8	0.440	0.714	0.314

Table 4.7. Summary of the MAFFT alignments.

Alignment parameters GOP:OV	Partitioned data													Full length 18S sequence						
	Length conserved (LC) partition							Length variable (V) partition												
	No. chars.	No. inform. chars. (%)	MP tree length	No. MP trees	CI	RI	RC	No. chars.	No. inform. chars. (%)	MP tree length	No. MP trees	CI	RI	RC	No. chars.	No. inform. chars. (%)	MP tree length	No. MP trees	CI	RI	RC
1:0	1611	222 (13.7)	841	1840	0.490	0.791	0.387	826	407 (49.3)	2397	70	0.430	0.660	0.284	2437	629 (25.8)	3281	1	0.440	0.697	0.307
1.53:0	1611	228 (14.1)	848	4078	0.494	0.796	0.394	759	408 (53.8)	2495	4	0.431	0.675	0.291	2370	636 (26.8)	3382	5	0.442	0.708	0.313
1.53:0.5	1608	227 (14.1)	842	2320	0.494	0.795	0.398	682	396 (58.1)	2569	10	0.402	0.662	0.267	2290	623 (27.2)	3459	6	0.419	0.697	0.292
1.53:1	1606	229 (14.2)	850	8398	0.494	0.800	0.395	650	400 (61.5)	2626	4	0.401	0.660	0.265	2256	629 (27.9)	3514	3	0.419	0.699	0.293
2:0	1611	227 (14.1)	848	3599	0.493	0.796	0.392	747	404 (54.1)	2561	20	0.419	0.666	0.279	2358	631 (26.7)	3449	18	0.432	0.702	0.303
2:0.5	1608	228 (14.2)	851	18172	0.492	0.795	0.392	660	408 (61.8)	2631	54	0.408	0.658	0.269	2268	636 (28.0)	3519	9	0.424	0.696	0.295
2:1	1605	228 (14.2)	850	3359	0.493	0.800	0.394	624	396 (63.5)	2712	8	0.388	0.651	0.253	2229	624 (28.0)	3599	2	0.409	0.692	0.283
3.0:0	1608	226 (14.1)	849	2880	0.491	0.800	0.393	716	411 (57.4)	2709	3	0.408	0.648	0.265	2324	637 (27.4)	3604	1	0.423	0.690	0.291
3:0.5	1607	227 (14.1)	856	40477	0.490	0.799	0.391	670	414 (61.8)	2775	2	0.386	0.647	0.252	2277	641 (28.2)	3678	12	0.407	0.688	0.280
3:1	1605	227 (14.1)	849	3720	0.492	0.799	0.394	618	404 (65.4)	2781	66	0.381	0.643	0.245	2223	631 (28.4)	3678	1	0.402	0.684	0.275

(a)

```
PasimachuspurpuratusSC518S  ----TTA---TATA--T------------------------------T--------CCTG---T
PasimachuscalifornicusSC18S ----TTT---T-T---T------------------------------T--------CC-G---T
PasimachusSC2718S           ----TTT---T-T---T------------------------------T--------CC-G---T
ScaritesSAfrica1SC2418S     ----TTT---TAT---TATG---AT----A------------A-----T--------CC-A---T
ScaritesbupariusSC1318S     ----TAT---TAC---TA-G---A-----A------------A-----T--------CC-A---T
ScariteshespericusSC1518S   ----TGT---TAC---TT-T---A-----A------------A-----T--------CC-A---T
ScariteseurytusSC1418S      ----TTT---TAT---TTTG---A-----A------------A-----T--------CC-A---T
ScaritessubterraneusSC2118S ----TTT---T-T---TT-----A-----A-TT-T----T--A-----T--------CC-A---T
PachyodontusSC418S          ----TTT---T-T---TT-----A-----A-TT-TCTTTT--A-----T--------CC-A---T
OchryopusSC3418S            ----TTT---T-T---TT-----A-----A-TTGT---TT--A-----T--------CC-A---T
DistichusplanusSC1118S      ----TTT---T-T---TT-----A-----AATT-T---TT--A-----T--------CT-A---T
GnaphonSC2218S              ----TTTCT-TATGAATTT----AT----A--T-T--C----A-----T---------T-A---T
PassalidiusSC718S           ----TTTGTATAT--ATTT----AT----A--TATA-CTTGGA-----T--------CT-A---T
StorthodontusSC318S         ----T-TA---AT---T------------A--T---------------T--------CC-T---T
CarenuminterruptumSC18S     A---CGT---T-T---TTT----A-----A--C---------G-----T--------TT-A---T
ScaraphitesSC818S           A---TGT---T-T---TT-----A-----A--C---------G-----T--------TT-T---T
```

(b)

```
PasimachuspurpuratusSC518S  ATAT-----------------AT-----------TCCTGT
PasimachuscalifornicusSC18S TTT-------------------T-----------TCC-GT
PasimachusSC2718S           TTT-------------------T-----------TCC-GT
ScaritesSAfrica1SC2418S     TATTT---TTA-TTA-----TGA----TAA----TCC-AT
ScaritesbupariusSC1318S     AGGAT---TAT-TAC-----TAG----AAA----TCC-AT
ScariteshespericusSC1518S   AGGGT---TGT-TAC-----TTT----AAA----TCC-AT
ScariteseurytusSC1418S      ATGAA---TTT-TTA-----TTTTG--AAA----TCC-AT
ScaritessubterraneusSC2118S TTTTT---TTT-TAA----TTTT------A----TCC-AT
PachyodontusSC418S          TTTTT---TTT-TAATTTCTTTT------A----TCC-AT
OchryopusSC3418S            ATTTT---TTT-TAATTG--TTT------A----TCC-AT
DistichusplanusSC1118S      TTTTT---TTTTTAAATT--TTT------A----TCT-AT
GnaphonSC2218S              ATTTT---CTTATGAATT--TAT------A----TTC-AT
PassalidiusSC718S           TATATATTTATATAT------AC----TTGGA--TCT-AT
StorthodontusSC318S         ------------TAA------TT------A----TTC-CT
CarenuminterruptumSC18S     ACGTT---TTT-TAA-----CGT----TTAT---TAC-GT
ScaraphitesSC818S           ATGTT---TT--TAA-----CGT---TTTT----TAC-G
```

Figure 4.3. Excerpt of the V2 region of 18S aligned by ClustalX and MAFFT. (a) ClustalX alignment with parameter values GOP:GEP 1:0.25. (b) MAFFT alignment with parameter values GOP:OV 1:0.

The length of the LC regions is relatively insensitive to alignment parameter values and alignment method. ClustalX produced LV alignments varying by 29 characters and MAFFT by only 6 characters (tables 4.6 and 4.7).

The variation in the total length of the alignments is almost entirely caused by differences in length and nucleotide composition of the V regions. These regions varied substantially in length, for example ClustalX with GOP:GEP set to 1:0.25 produced an alignment of 997 characters, almost twice as long as the 586 characters produced by the highest GOP:GEP cost of 10:6.66. The MAFFT alignments showed similar variation, although the difference between the longest and shortest alignment was less.

Despite the marked effect of parameter values on the length of the LV regions, the number of parsimony informative characters showed only a slight increase as gap penalty was increased. For example, the lowest GOP:GEP penalty (1:0.25) produced 403 informative characters from the LV region while the highest penalty (10:6.66) yielded 447 informative characters.

This is because although long alignments have more character positions, many of these extra positions are constant or parsimony uninformative. Importantly, although the number of parsimony informative characters is approximately equal between long and short alignments, the assignment of homology is different (figure 4.4).

Similarly, values of the ensemble consistency index (*CI*), retention index (*RI*) and re-scaled consistency index (*RC*) of the resulting trees did not

differ markedly considering the substantial difference in total length of the alignments. With increasing gap costs these tree statistics tend to decrease somewhat, although this trend is more marked with ClustalX than MAFFT.

(a)

```
Elaphrusclairvillei18S      ----------G--C---------------C--------C--G---T-T---C----GC
Tropopterus18S              ------G-----GG-T---------G-----TGAA-----CT-G---TGT---TT---GT
Meonis18S                   ------G-----GACT--------------TGA------CT-G---TGT---T----GT
Amblytelus                  ------G-----GG-T---------G-----TGA------TT-G---TGT---TT---GT
Melisodera                  ------G----GG-T---------G-----TGT------CT-G---TGT---TT---GT
Elaphruscalifornicus18S     ----------G--C---------------T--------C--G---T-T---C----GC
Blethisa                    ----------G--C---------------T--------C--G---T-T---C----GC
Laccocenus                  TCATTCGTTAACG--C---------G-----TTTGGTTCCC--G---T-T---C----GT
Apotomus                    ------GT----G--T---------G-----TATATG---CT-GCCGT-T---C----AT
Bembidion                   ------G-----G--C---------G-----T-TA-----CT-G---T-T---C----GT
Mecodema                    G---CCAT-------TAC-------G-----TTCA-----CC-G---T----------AT
Oregusaereus18S             G---CCAT-------TAC-------G-----TTCA-----CC-G---T-----C----AT
AkephorusSC2818S            ------G-----G--C---------A-----T--------C--G---T-T---T----GT
EucamaragnathusSC2918S      ------G-----G--CTG-------G-----C--------CT-G---T-T---C----GT
HalocoryzaSC1618S           TAA---G-----GG-T---------A-T--AT--------C--G---T-T---C----GT
Schizogenius                TAA---G-----GG-T---------T-T--AT--------C--G---T-T---C----GT
CamptodontusSC3018S         CAA---G-----TG-T---------T-TTAAT--------C--G---T-T---C----GT
ClivininiFlorida18S         TAA---G-----GG-T---------T-TT-AT--------C--G---T-T---C----GT
ClivinafossorSC1718S        ------G-----GG-T---------AAT---T--------T--G---T-T---T----TT
ScolyptusSC918S             ------G-----GG-T---------AGT---C--------C--G---T-T---C----GT
Clivinaferrea18S            ------G-----GA-T---------AAT---T--------C--G---TAT--------GT
Promecoderus18S             T-----A-----G--T---------------T-TA-----CTAG---T-TCCAC----AT
Creobius                    C-----A-----G--T---------------T-TA-----CTGG---T-TTCAC----AT
Broscosoma                  ------A-----G--T---------------T-CG-----CT-G---T-T--GC----GT
Loricerafoveata18S          ------G-----G--CG--------G-----TCT------CC-G---T-T---T----AT
Loricerapilicornis18S       ------G-----G--CG--------G-----TCT------CC-G---T-T---T----AT
PasimachuscalifornicusSC18S ------------------------------T--------CC-G---T-T---T----AT
PasimachusSC2718S           ------------------------------T--------CC-G---T-T---T----AT
PasimachusatronitensSC18S   ------------------------------T--------CC-G---T-T---T----AT
PasimachuspurpuratusSC518S  ------------------------------T--------CCTG---T-T---C----AT
ScaritesbupariusSC2518S     A-G---A-----A------------A-----T--------CC-A---T-TA--C----AT
ScaritesbupariusSC1318S     A-G---A-----A------------A-----T--------CC-A---T-TA--C----AT
ScariteshespericusSC1518S   T-T---A-----A------------A-----T--------CC-A---T-TA--C----AT
ScariteseurytusSC1418S      TTG---A-----A------------A-----T--------CC-A---T-TA--C----AT
ScaritesSAfrica3SC2018S     TTGGA-A-----A------------A-----T--------TC-A---T-TA--C----AT
ScaritessubterraneusSC2118S T-----A-----A-TT-T----T--A-----T--------CC-A---T-TA--C----AT
ScaritessubterraneusSC18S   T-----A-----A-TT-T----T--A-----T--------CC-A---T-TA--C----AT
PachyodontusSC418S          T-----A-----A-TT-TCTTTT--A-----T--------CC-A---T-TA--C----AT
OchryopusSC3418S            T-----A-----A-TTGT---TT--A-----T--------CC-A---T-TA--T----AT
DistichusplanusSC1118S      T-----A-----AATT-T---TT--A-----T--------CT-A---T-TA--T----AT
GnaphonSC2218S              TT----AT----A--T-T--C----A-----T---------T-A---T----------AT
PassalidiusSC718S           TT----AT----A--TATA-CTTGGA-----T--------CT-A---T-TA--C----AT
StorthodontusSC318S         ------------A--T---------------T--------CC-T---T-T---C----AT
Carenuminterruptum18S       TT----A-----A--C---------G-----T--------TT-A---T-TA--CGTTTGT
ScaraphitesSC818S           T-----A-----A--C---------G-----T--------TT-T---T-TA--CGTTTGT
```

(b)

```
Elaphrusclairvillei18S      GC---------------------CCGTTCGC
Melisodera                  -CGGGTGTGT-----------CTGTGTTTGT
Meonis18S                   -CGGACTTGA-----------CTGTGTT-GT
Amblytelus                  ACGGGTGTGA-----------TTGTGTTTGT
Tropopterus18S              -CGGGTGTGAA----------CTGTGTTTGT
HalocoryzaSC1618S           -TAAGGGTAT-----------ATCGTTCGT
Schizogenius                -TAAGGGTTT-----------ATCGTTCGT
CamptodontusSC3018S         -CAAGTGTTTT----------AATCGTTCGT
ClivinafossorSC1718S        -CGGGTAATT-------------TGTTTTT
ScolyptusSC918S             -CGGGTAGTC-------------CGTTCGT
ClivininiFlorida18S         -TAAGGGTTTT----------ATCGTTCGT
Clivinaferrea18S            -CGGATAATT-------------CGTATGT
Elaphruscalifornicus18S     GC---------------------TCGTTCGC
Blethisa                    GC---------------------TCGTTCGC
Laccocenus                  TCATTCGTTAACGCGTTTGGTTCCCGTTCGT
Mecodema                    GGTTC------------GCCATTACGTTCAC
Oregusaereus18S             GGTTC------------GCCATTACGTTCAC
EucamaragnathusSC2918S      AGTTTCG----------GCTGGCCTGTTCGT
Apotomus                    ACATTCGTGTGTA---TATGCTGCCGTTCAT
Bembidion                   ACGTTTCGGCGT---------TACTGTTCGT
Promecognathus              ACGCA-------------GTGCTCGTTCGT
Promecoderus18S             ACTAGTT-----------CCACATTTGCAC
Creobius                    ACTGGTT-----------TCACATAATCAC
Broscosoma                  GCTGTT-----------------GCGTTCAC
Siagona                     TTCACAAGGTATG------TTCTCTGTTCGT
Loricerafoveata18S          ACGGCGGTCT-------------CCGTTTAT
Loricerapilicornis18S       ACGGCGGTCT-------------CCGTTTAT
PasimachuscalifornicusSC18S ----TTTT---T---------TTCCGTTTAT
PasimachusSC2718S           ----TTTT---T---------TTCCGTTTAT
PasimachusatronitensSC18S   ----TTTT---T---------TTCCGTTTAT
PasimachuspurpuratusSC518S  ----TTATATAT---------TCCTGTTCAT
ScariteshespericusSC1518S   GGGTTGTTACTT----TA-AATCCATTACAT
ScaritesbupariusSC1318S     GGATTATTACTA----GA-AATCCATTACAT
ScaritesbupariusSC2518S     GGATTATTACTA----GA-AATCCATTACAT
ScariteseurytusSC1418S      GAATTTTTATTTT---GA-AATCCATTACAT
ScaritesSAfrica3SC2018S     GAACTTAAACTTTG--GAAAATTCATTACAT
ScaritessubterraneusSC2118S TTTTTTTTAATTT------TATCCATTACAT
ScaritessubterraneusSC18S   TTTTTTTTAATTT------TATCCATTACAT
PachyodontusSC418S          TTTTTTTTAATTTC--TTTTATCCATTACAT
OchryopusSC3418S            TTTTTTTTAATTG----TTTATCCATTATAT
DistichusplanusSC1118S      TTTTTTTTAAATT----TTTATCTATTATAT
GnaphonSC2218S              ATTTTCTTATGAAT--TTATATTCATTATAT
PassalidiusSC718S           ATATTTATATATAC--TTGGATCTATTACAT
StorthodontusSC318S         -----TTTAATT--------ATTCCTTTCAT
Carenuminterruptum18S       -----TACGTTTTT--TAACGTTTATTACGT
ScaraphitesSC818S           -----TATGTTTTT---AACGTTTTTTACGT
```

Figure 4.4. The same section of the V2 region of 18S aligned by ClustalX with different gap costs. (a) 60 characters obtained from gap costs 1:0.25. 24 characters are constant, 13 variable characters are parsimony uninformative and 23 characters are parsimony informative. (b) 31 characters obtained from gap costs 15:6.66. 2 characters are constant, 1 variable character is parsimony uninformative and 28 characters are parsimony informative.

4.3.3 The data partitions.

4.3.3.1 Phylogenetic signal of the length-conserved regions.

Analysis of most of the LC partitions produced large numbers (>100) of equally parsimonious trees (tables 4.6 and 4.7) and produced a range of conflicting topologies. Consequently, many of the deeper nodes of the strict consensus trees from individual alignments were unresolved.
However, irrespective of gap penalty or alignment method, some consistent and well-supported relationships were nonetheless recovered. These relationships are summarised with the strict combined consensus trees in figure 4.5 and the majority rule combined consensus trees in figures 4.6 and 4.7.

The ClustalX and MAFFT strict combined consensus trees differ in only minor details and in both cases Scaritini was recovered as a monophyletic group. The ClustalX alignments all infer subtribe Carenina as sister to the remaining Scaritini, while the sister taxon to the subtribe Scaritina is unresolved. Analysis of the LC MAFFT alignments does not produce contradictory relationships to those obtained with ClustalX, but differ in details of resolution. With MAFFT the basal node in Scaritini is unresolved but the sister to Scaritina is unambiguously resolved as *Storthodontus reticulatus* Basilewsky.

(a) (b)

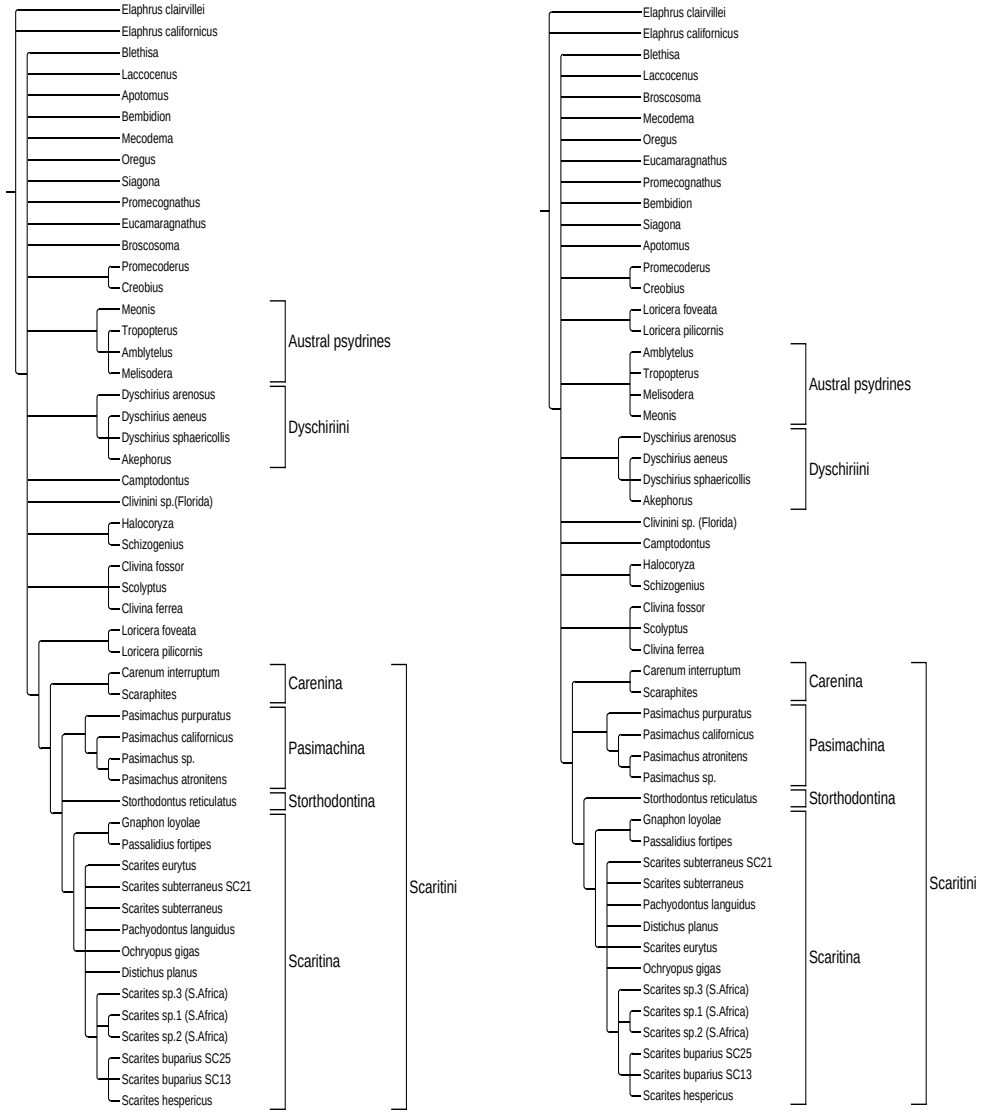

Figure 4.5. Strict combined consensus trees produced by parsimony analysis of the LC regions using (a) ClustalX and (b) MAFFT.

The majority-rule combined consensus trees of ClustalX and MAFFT differ more substantially, with the MAFFT alignments (figure 4.7) producing trees with more consistently resolved nodes than those of ClustalX (figure 4.6). The ClustalX majority rule combined tree is in fact little more resolved than the strict combined tree. This suggests that for the particular gap penalties used, MAFFT produces more consistent homology statements. In addition to the unambiguous relationships resolved by the combined strict consensus, the combined majority rule consensus (figure 4.7) shows that 80% of the MAFFT alignments also recover the outgroup Broscinae as a clade and 80% support a clade containing Dyschiriini + Clivinini.

Despite not supporting a monophyletic Broscinae, in common with MAFFT, ClustalX was at least able to resolve the Austral psydrine clade.

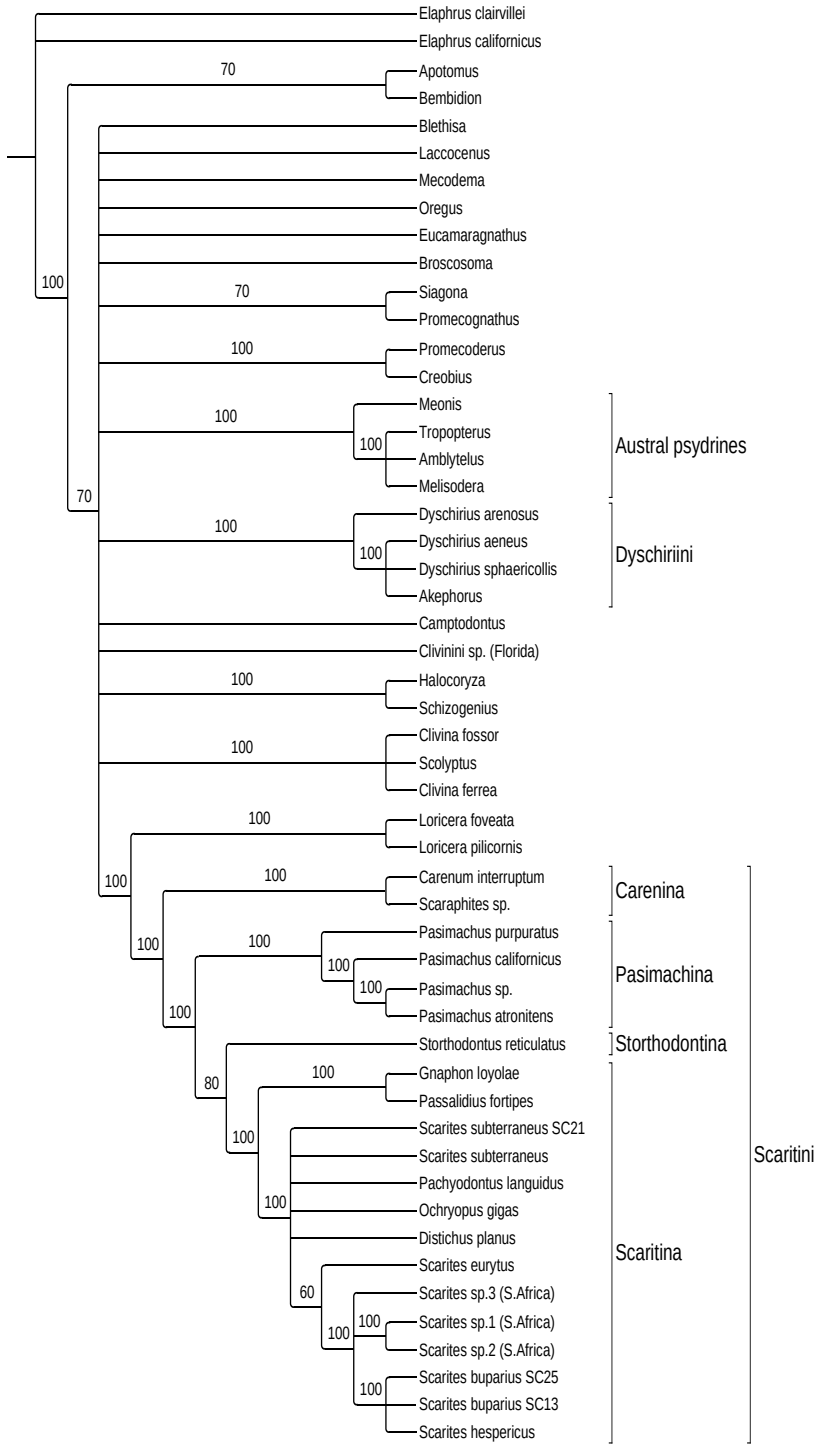

Figure 4.6. 50% majority-rule combined consensus tree produced by parsimony analysis of the LC regions using ClustalX. Numbers to the left of nodes indicate the percentage of strict consensus trees containing the node.

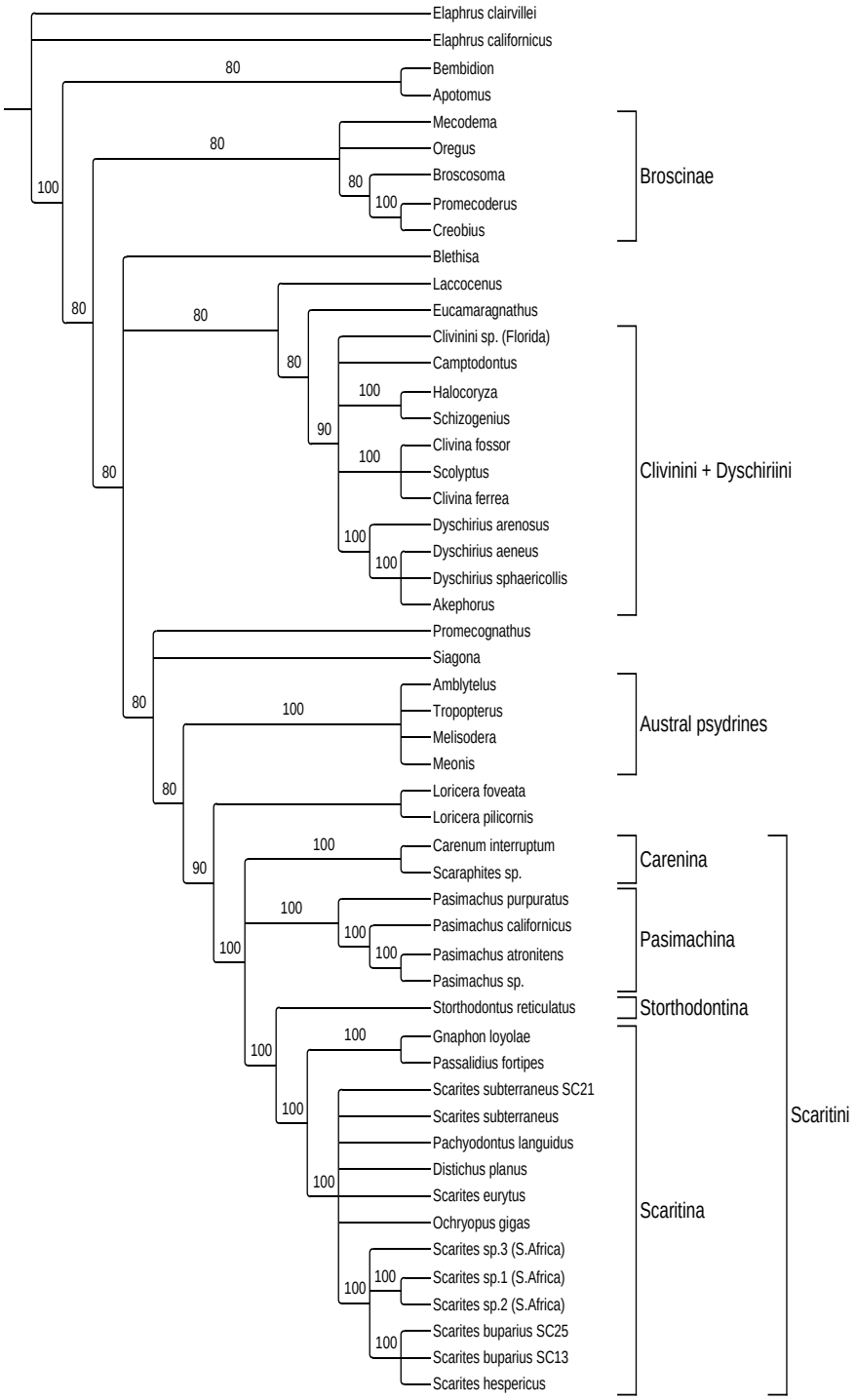

Figure 4.7. 50% majority-rule combined consensus tree produced by parsimony analysis of the LC regions using MAFFT. Numbers to the left of nodes indicate the percentage of strict consensus trees containing the node.

As a typical example and to give an indication of bootstrap support for particular clades, the LC consensus tree obtained with the default parameter values of ClustalX (GOP:GEP 15:6.66) is shown in figure 4.8.

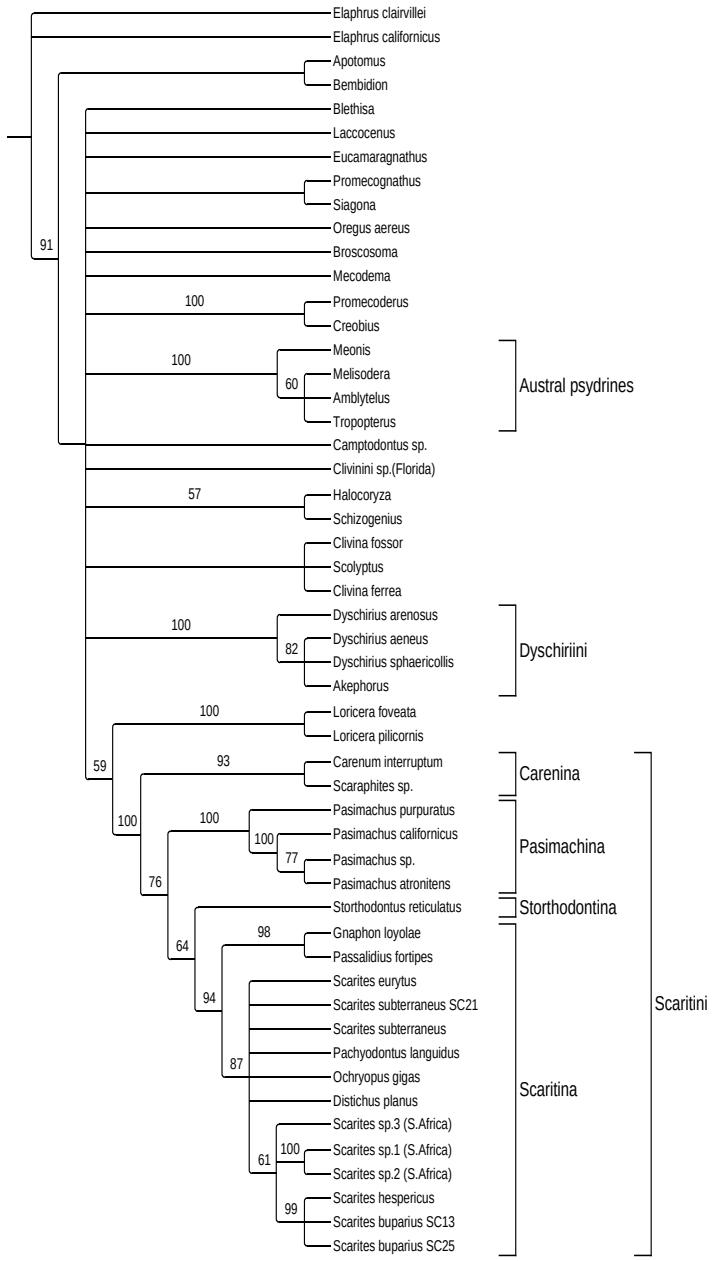

Figure 4.8. Strict consensus of 5597 equally parsimonious trees resulting from analysis of the LC regions aligned using the default ClustalX gap penalty 15:6.66. Numbers to the left of nodes are percentage bootstrap support values.

4.3.3.2 Phylogenetic signal of the length-variable regions.

In common with the results of the LC analysis, parsimony analysis of the V regions aligned under different gap penalties produced trees differing substantially in topology. This was true for both the ClustalX and MAFFT alignments.

To summarise the relationships obtained with ClustalX and MAFFT a strict (figure 4.9) and majority-rule combined consensus tree was produced for each (figures 4.10 and 4.11).

The clades unambiguously recovered from alignments by either program were the same; the Austral psydrines, Dyschiriini, Clivinini, Carenina, Pasimachina (as the genus *Pasimachus*) and Scaritini (figure 4.9). With the exception of Clivinini, these clades were also obtained from all trees resulting from parsimony analysis of the LC alignments (figure 4.5). This means that regardless of gap cost or data partition, both ClustalX and MAFFT always yield these relationships. It is therefore evident that the LC and V regions contain at least some congruent phylogenetic signal.

(a) (b)

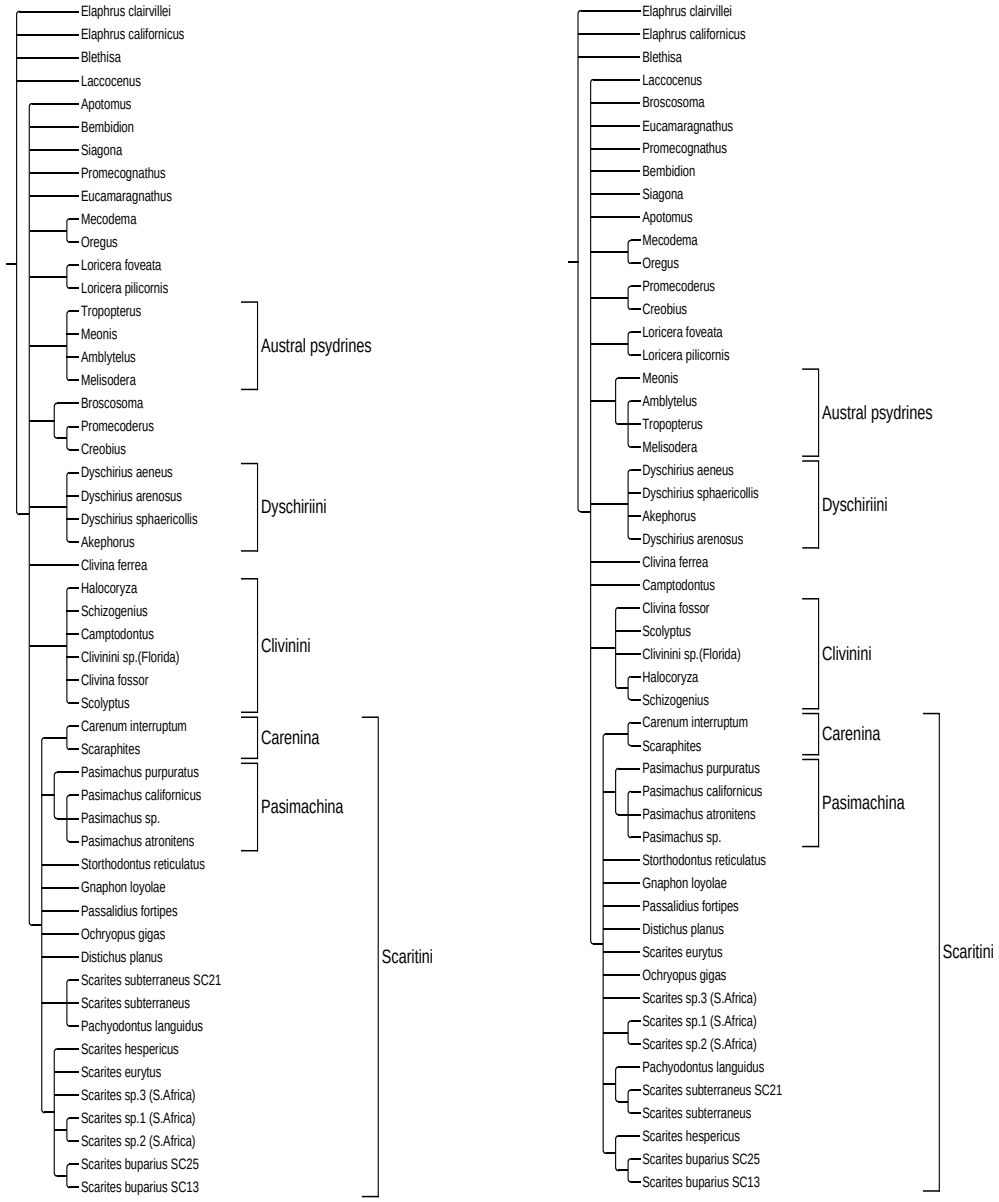

Figure 4.9. Strict combined consensus trees resulting from parsimony analysis of the V regions using (a) ClustalX and (b) MAFFT.

Again, the superior resolution of the majority-rule combined consensus of MAFFT (figure 4.11) over ClustalX (figure 4.10) shows that in this analysis this program is able to produce more consistent results. In addition to nodes recovered in a majority of the ClustalX alignments, 70% of the MAFFT alignments produce a monophyletic Broscinae and Scaritinae and place *Storthodontus reticulatus* as sister to Scaritina.

Both alignment methods did however produce a conflicting result to that obtained from the LC partition. 80% of the ClustalX and 90% of the MAFFT alignments placed *Pasimachus* and not Carenina as the sister to the remaining Scaritini (figures 4.10 and 4.11).

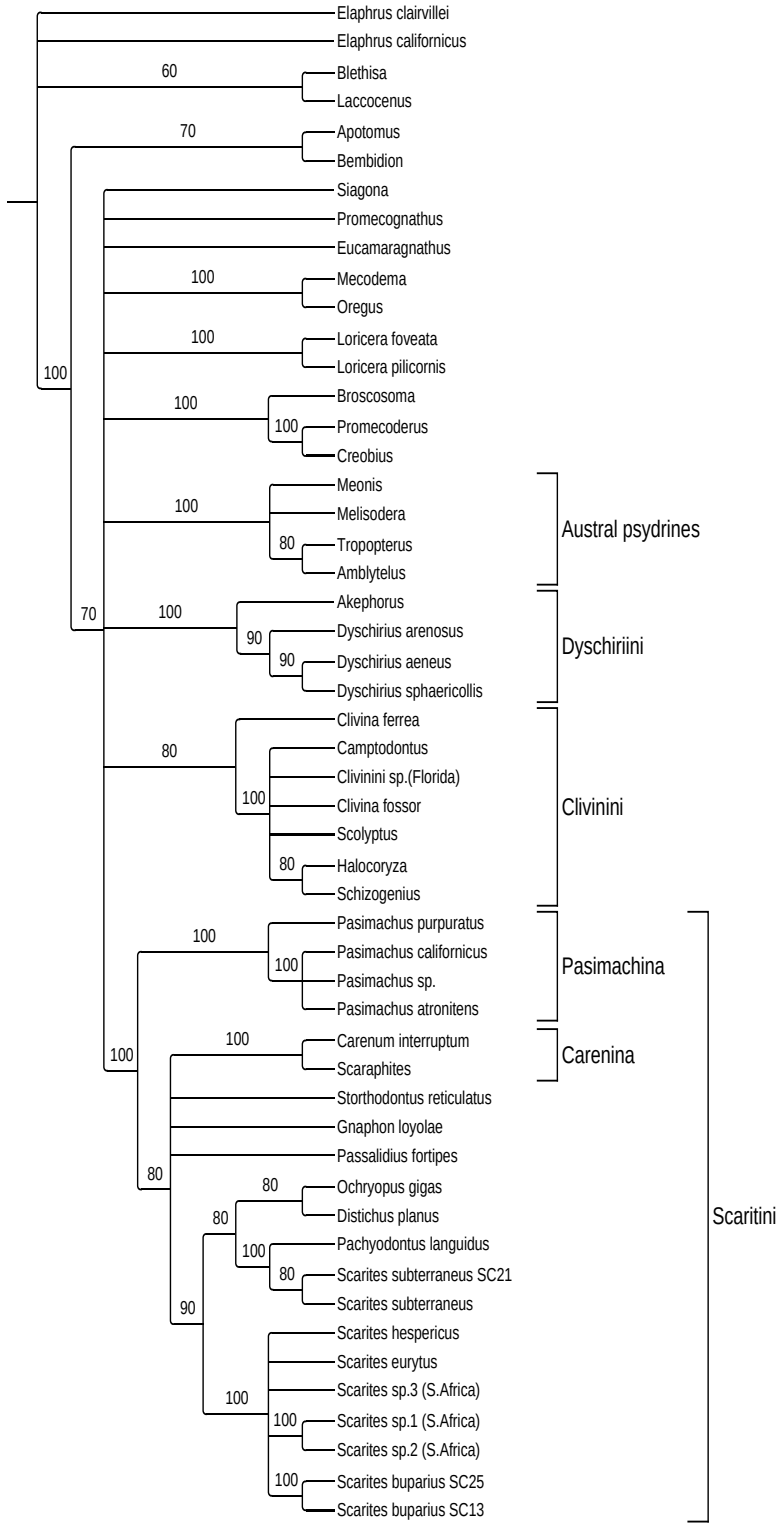

Figure 4.10. 50% majority-rule combined consensus tree produced by parsimony analysis of the V regions using ClustalX. Numbers to the left of nodes indicate the percentage of strict consensus trees containing the node.

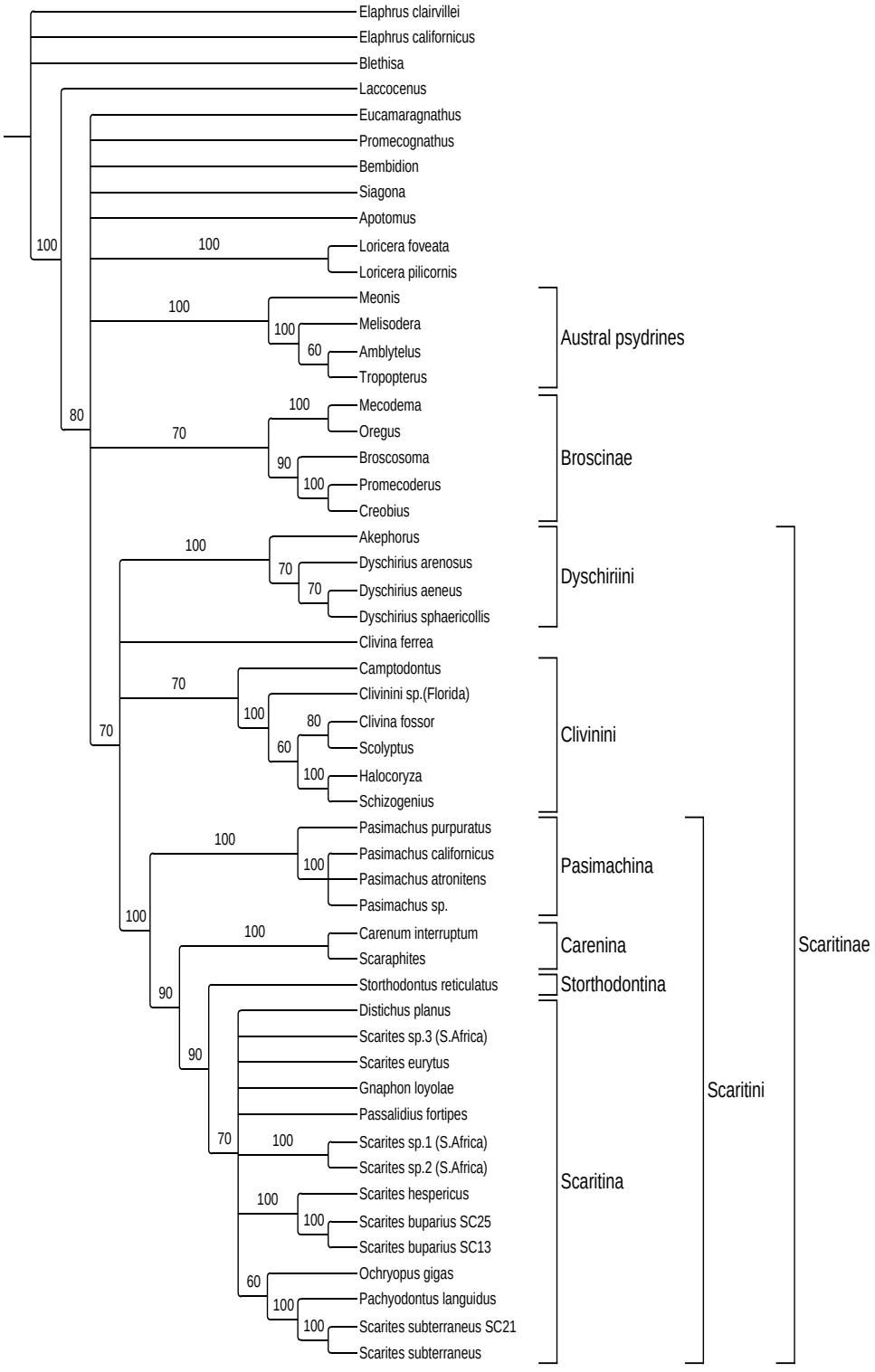

Figure 4.11. 50% majority-rule combined consensus tree produced by parsimony analysis of the V regions using MAFFT. Numbers to the left of nodes indicate the percentage of strict consensus trees containing the node.

An example of one of the alternative topologies produced from analysis of the V regions is given in figure 4.12. This is the result of alignment of the V regions using the default ClustalX GOP:GEP 15:6.66. It is shown to enable comparison with the LC tree obtained with the same gap penalty (figure 4.8) and to provide an indication of typical bootstrap support.

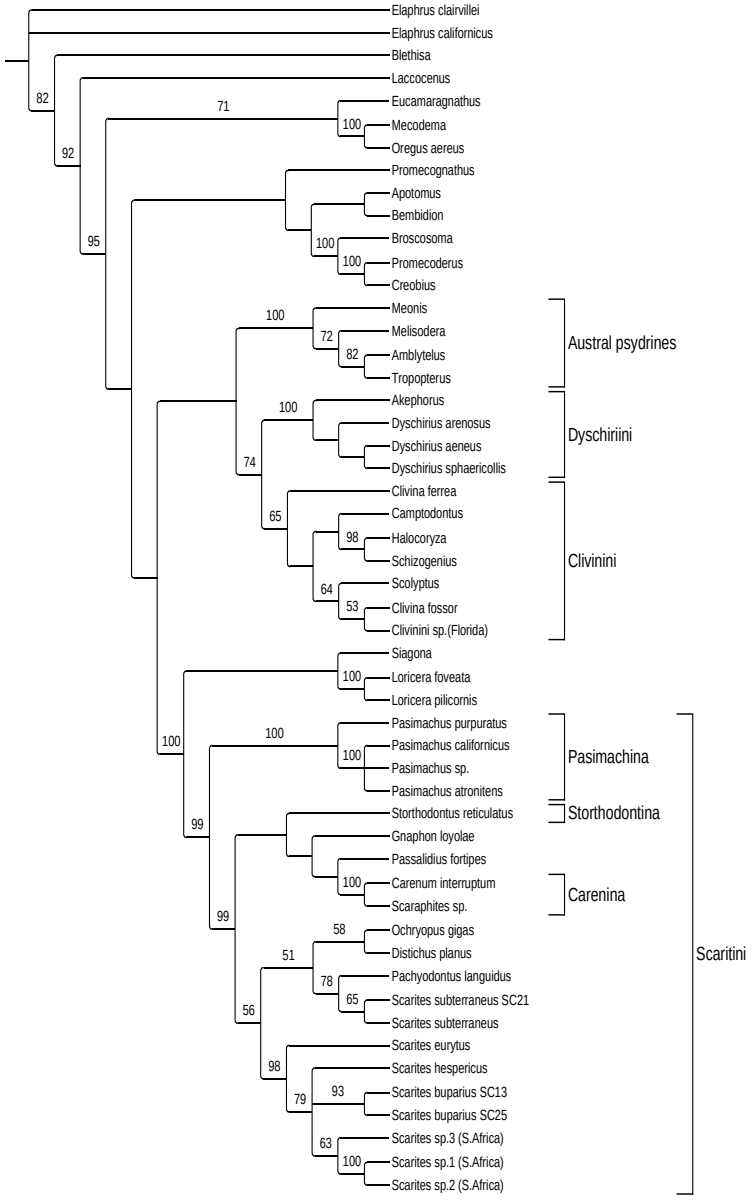

Figure 4.12. Strict consensus of 4 equally parsimonious trees resulting from parsimony analysis of the V regions aligned using the default ClustalX gap penalty 15:6.66. Numbers to the left of nodes are percentage bootstrap support values.

The topology of this consensus tree is somewhat at odds with the consensus trees resulting from the LC partition. Instead of Carenina it is *Pasimachus* which is placed as sister to the remaining Scaritini. Furthermore, *Storthodontus reticulatus*, placed as sister to Scaritina in the LC analysis, here occupies an unsupported position within Scaritina, sister to *Passalidius fortipes*.

4.3.4 Phylogeny inferred from the full length 18S sequence.

Analysis of the full length 18S sequence in general resulted in fewer equally parsimonious trees when compared to separate analysis of the LC and V regions (tables 4.6 and 4.7). In this regard there was also a marked difference in the performance of ClustalX and MAFFT. Across all ten gap penalties the ClustalX alignments produced a total of 319 equally parsimonious trees whereas the ten MAFFT alignments produced only 58.

The large number of trees produced by this analysis are summarised as follows:

Parsimony analysis of the full-length ClustalX and MAFFT alignments are summarised with the combined consensus trees in figures 4.13, 4.14 and 4.15.

Key nodes and relationships obtained from the parsimony and Bayesian analysis of each individual alignment follow in table 4.8.

Consensus trees inferred from the default ClustalX and MAFFT alignments by maximum parsimony and Bayesian inference are given in figures 4.16-4.22.

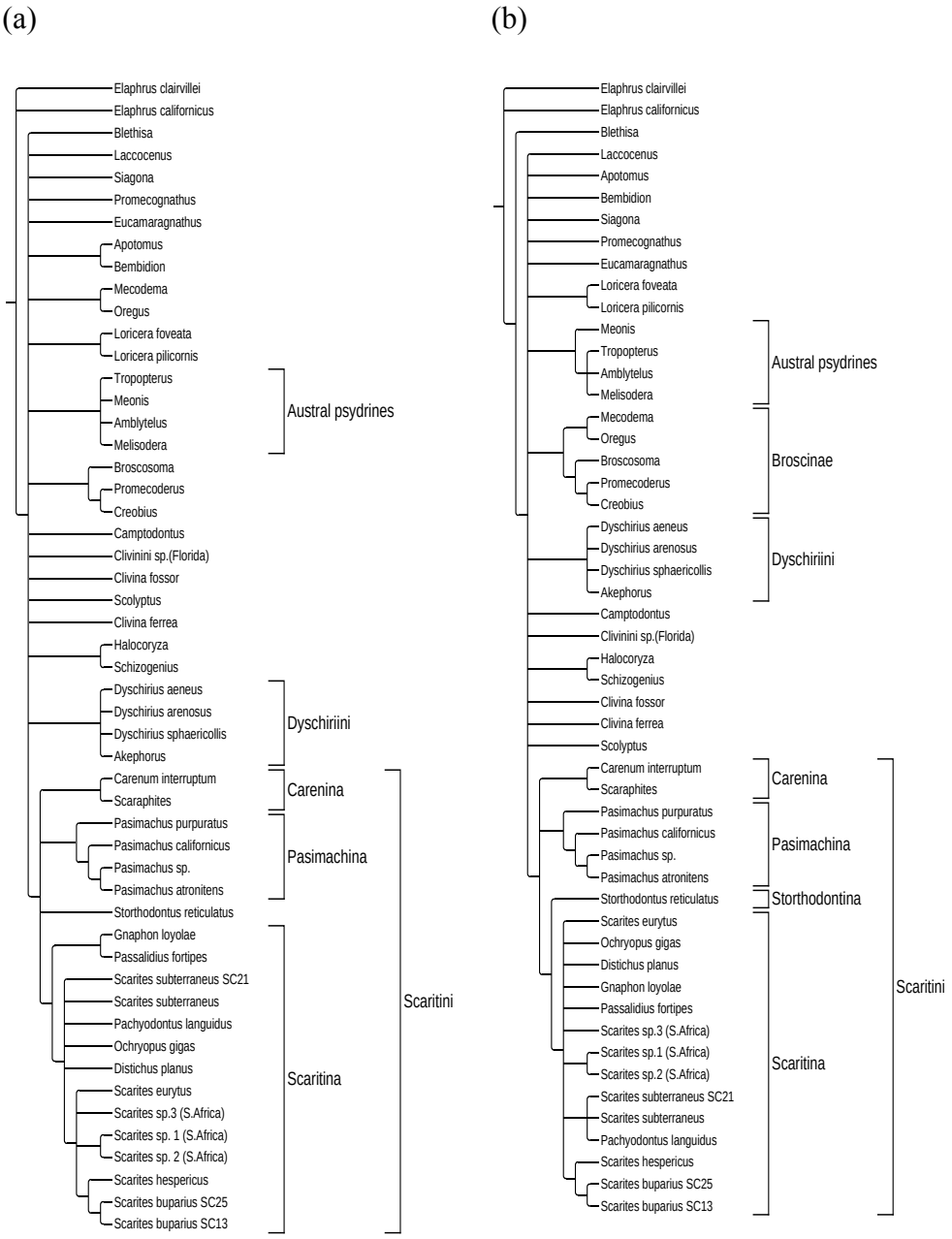

Figure 4.13. Strict combined consensus trees resulting from parsimony analysis of the full 18S sequence using (a) ClustalX and (b) MAFFT.

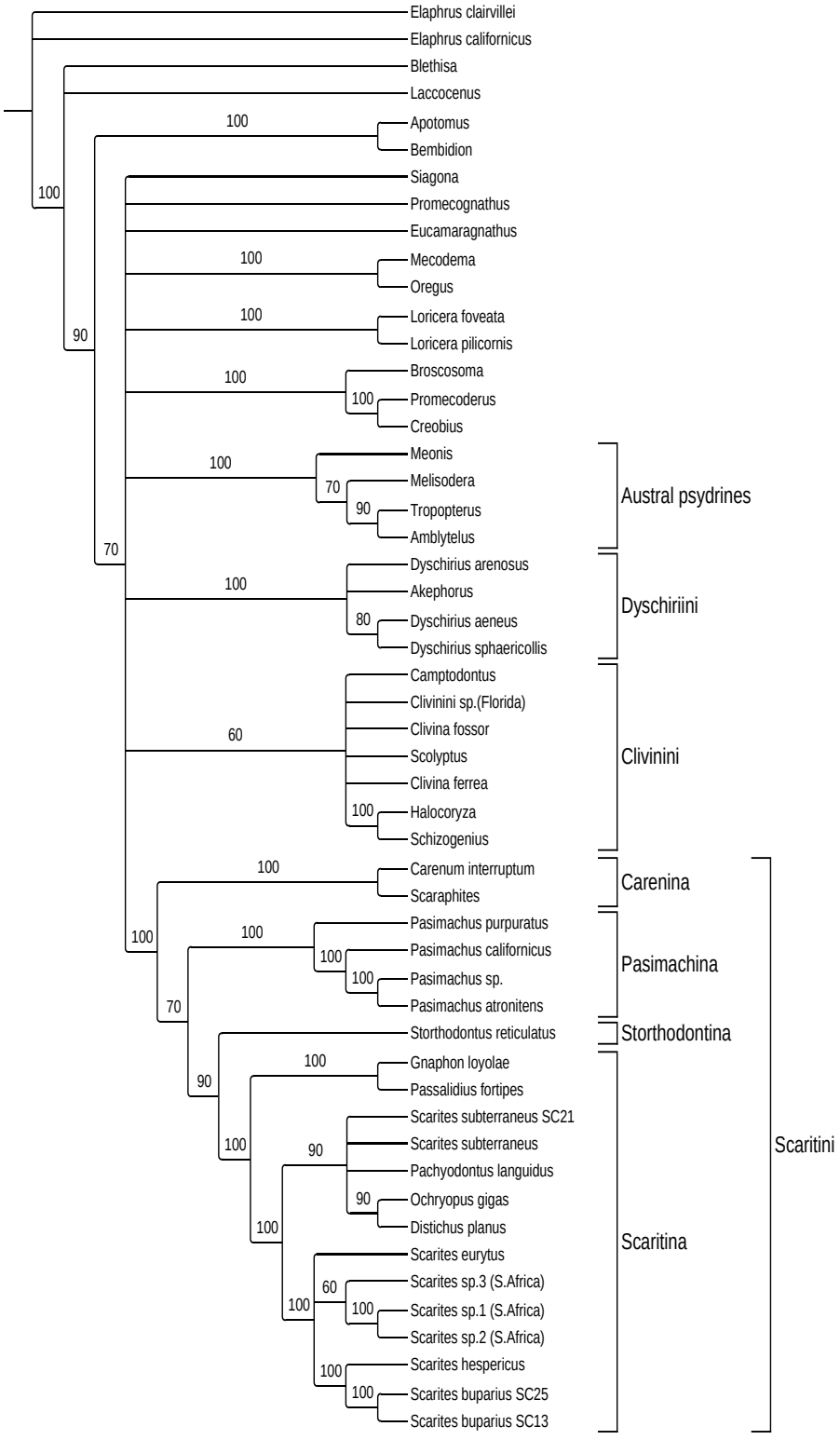

Figure 4.14. 50% majority-rule combined consensus tree produced by parsimony analysis of the full 18S sequence using ClustalX. Numbers to the left of nodes indicate the percentage of strict consensus trees containing the node.

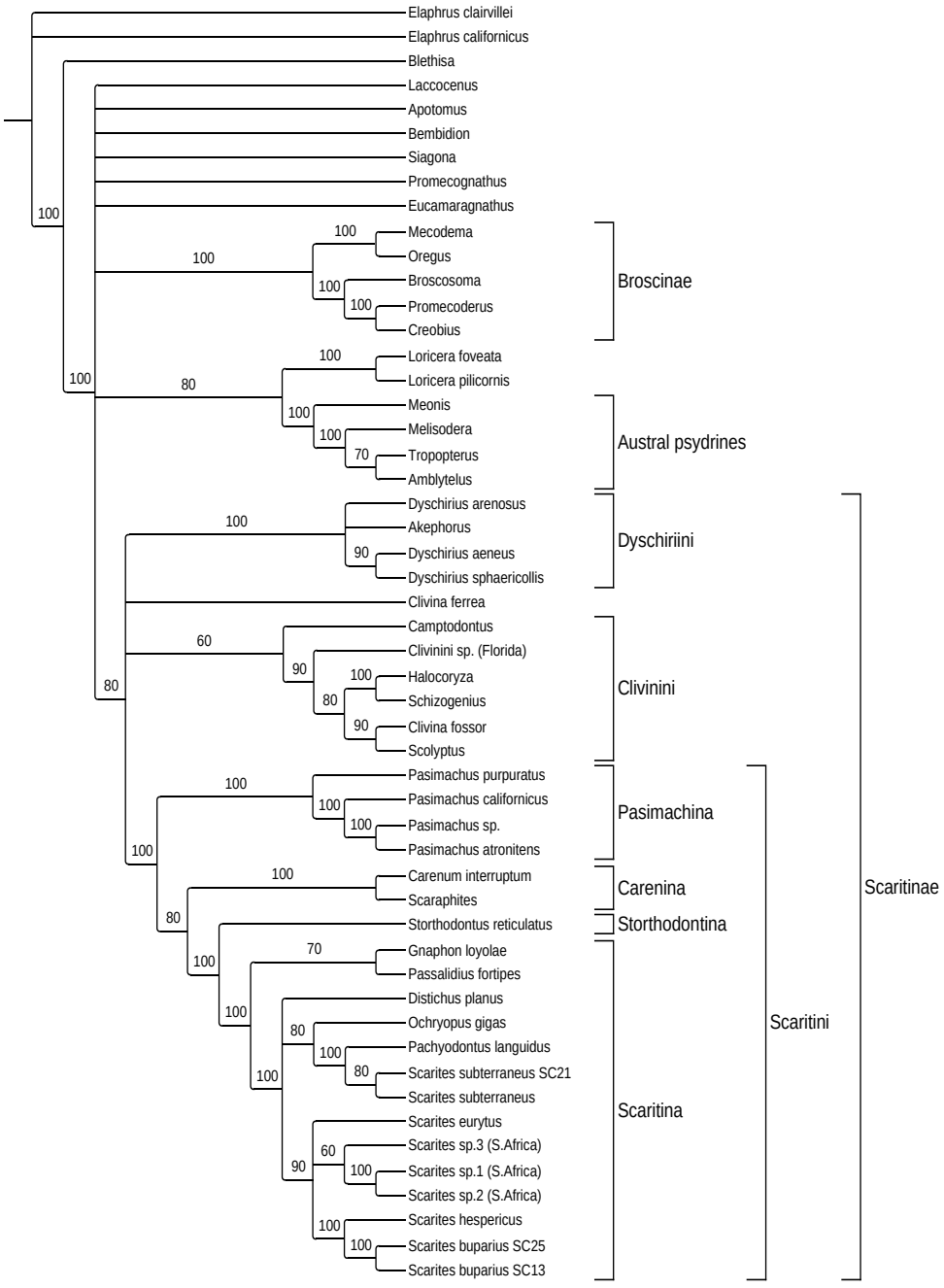

Figure 4.15. 50% majority-rule combined consensus tree produced by parsimony analysis of the full 18S sequence using MAFFT. Numbers to the left of nodes indicate the percentage of strict consensus trees containing the node.

Table 4.8. Summary of key nodes and relationships recovered from parsimony (P) and Bayesian analysis (B) of the full-length 18S sequence. ▒ indicates presence of the clade or relationship in the strict (P) or 50% majority rule (B) consensus tree. U indicates an unresolved but compatible relationship and an empty cell indicates absence of the clade or relationship.

Clade or relationship	ClustalX GOP:GEP																				MAFFT GOP:OV																	
	1:0.25		1:0.5		2:1		4:2		5:1		7:2		8:3		10:2		10:5		15:6.66		1:0		1.53:0		1.53:.5		1.53:1		2:0		2:0.5		2:1		3:0		3:0.5		3:1	
	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B
Austral psydrines	▒	▒	▒		▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Broscinae				▒	U														▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Promecognathus within Scaritinae																																							
Hiletinae sister to Scaritinae s.l.		▒		▒			▒	▒		▒	▒	▒			▒	▒						▒		U		U				U	▒				▒	▒	▒		▒
Dyschiriini	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Dyschiriini + Clivinini	U		▒		▒				▒				▒				▒		▒						▒		▒				▒								
Clivinini	U	▒	▒	▒	▒	U		▒	▒				U		▒	U	▒		▒			U					▒		▒		▒				▒		▒	
Schizogenius + *Halocoryza*	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Scaritinae s.l.	U	▒		▒	U	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒		▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒		▒	▒	▒	▒	▒	▒	▒
Pasimachus sister to Scaritini					U								▒						▒	▒	▒		▒	U			▒	▒			▒		▒	▒	▒	▒	▒	▒	
Carenina sister to Scaritini	▒	▒	▒	▒	U	▒	▒	▒	▒	▒	▒	▒		▒	▒	▒	▒	▒		▒		▒		U	▒	▒			▒	▒		▒						▒	▒
Storthodontus sister to Scaritina	▒		U		▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Scaritina	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒
Scarites sensu lato																																							

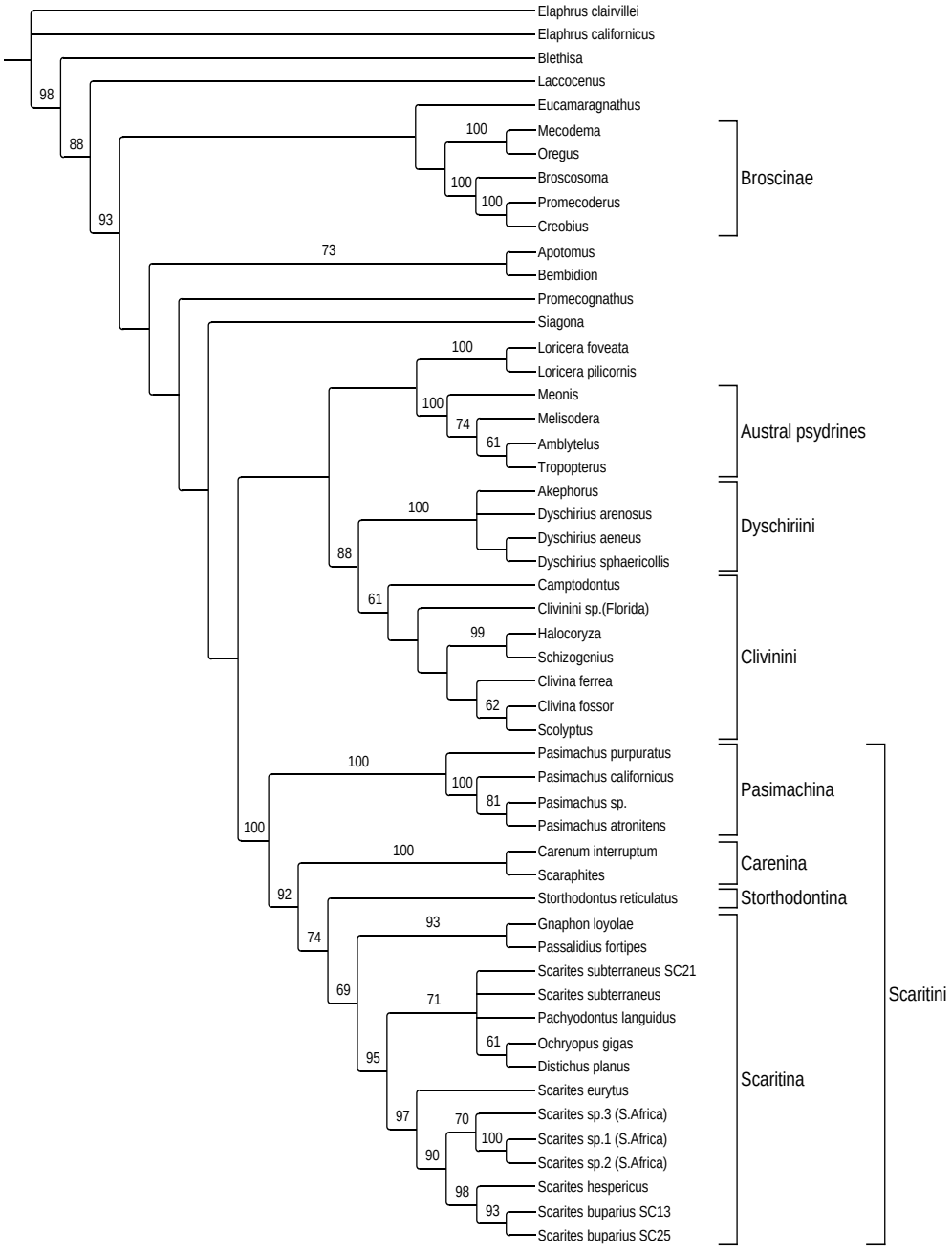

Figure 4.16. Strict consensus of 8 equally parsimonious trees resulting from analysis of the full 18S sequence aligned using the default ClustalX gap penalty 15:6.66. Numbers to the left of nodes are percentage bootstrap support values.

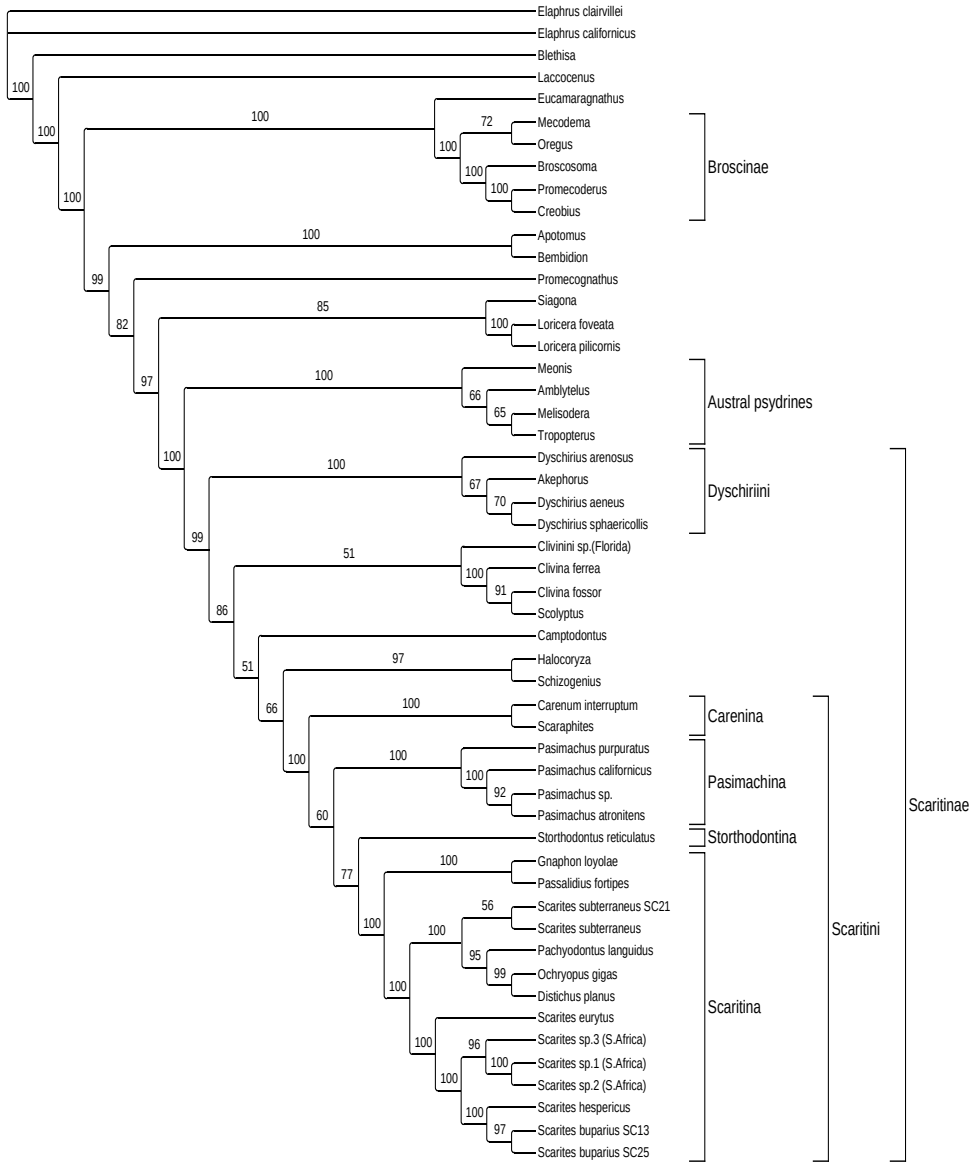

Figure 4.17 Bayesian 50% majority rule consensus of 752 trees resulting from analysis of the full 18S sequence aligned using the default ClustalX gap penalty 15:6.66. Numbers above nodes indicate clade credibility values greater than 50%.

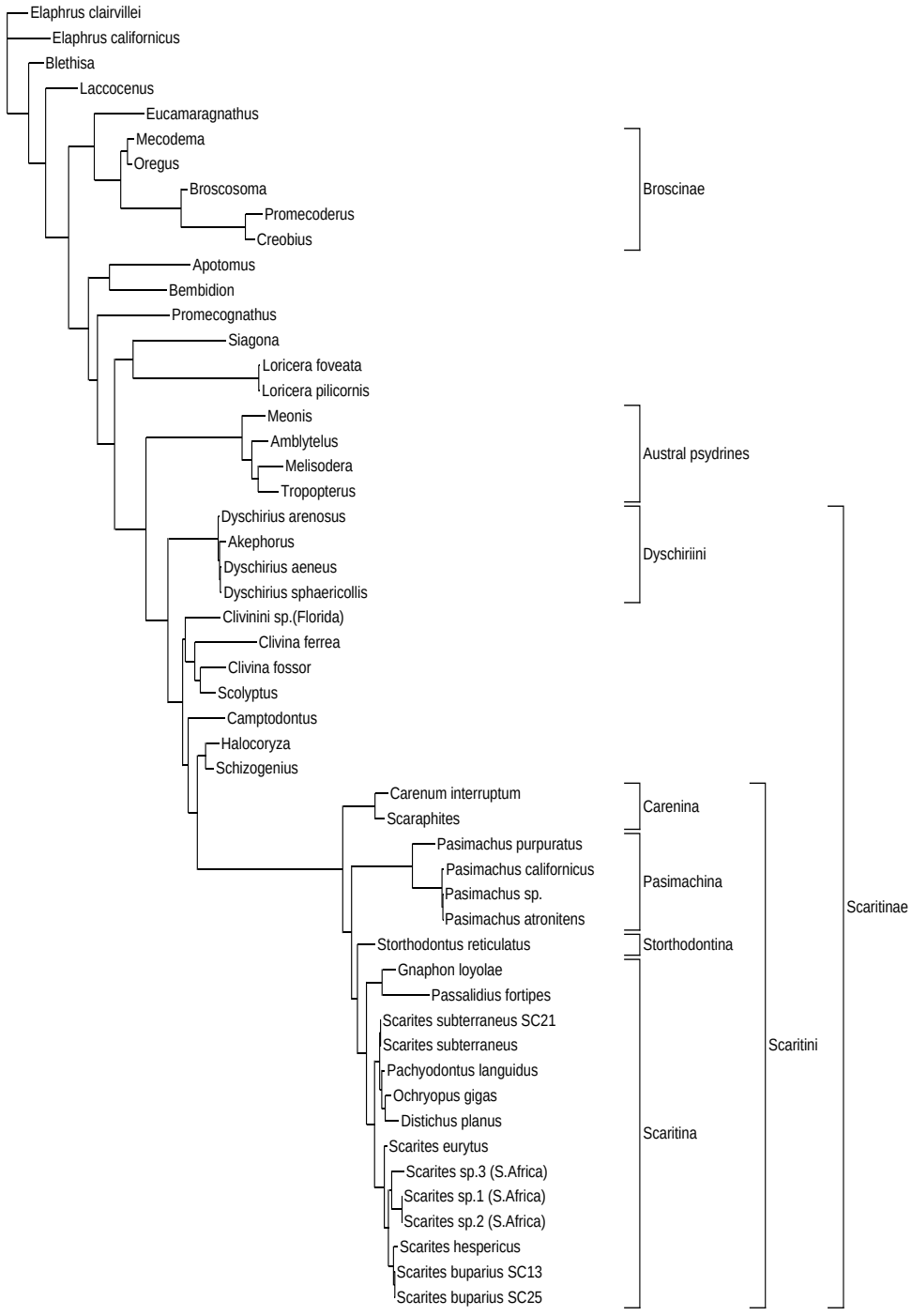

Figure 4.18. Bayesian 50% majority rule consensus phylogram of 752 trees resulting from analysis of the full 18S sequence aligned using the default ClustalX gap penalty 15:6.66.

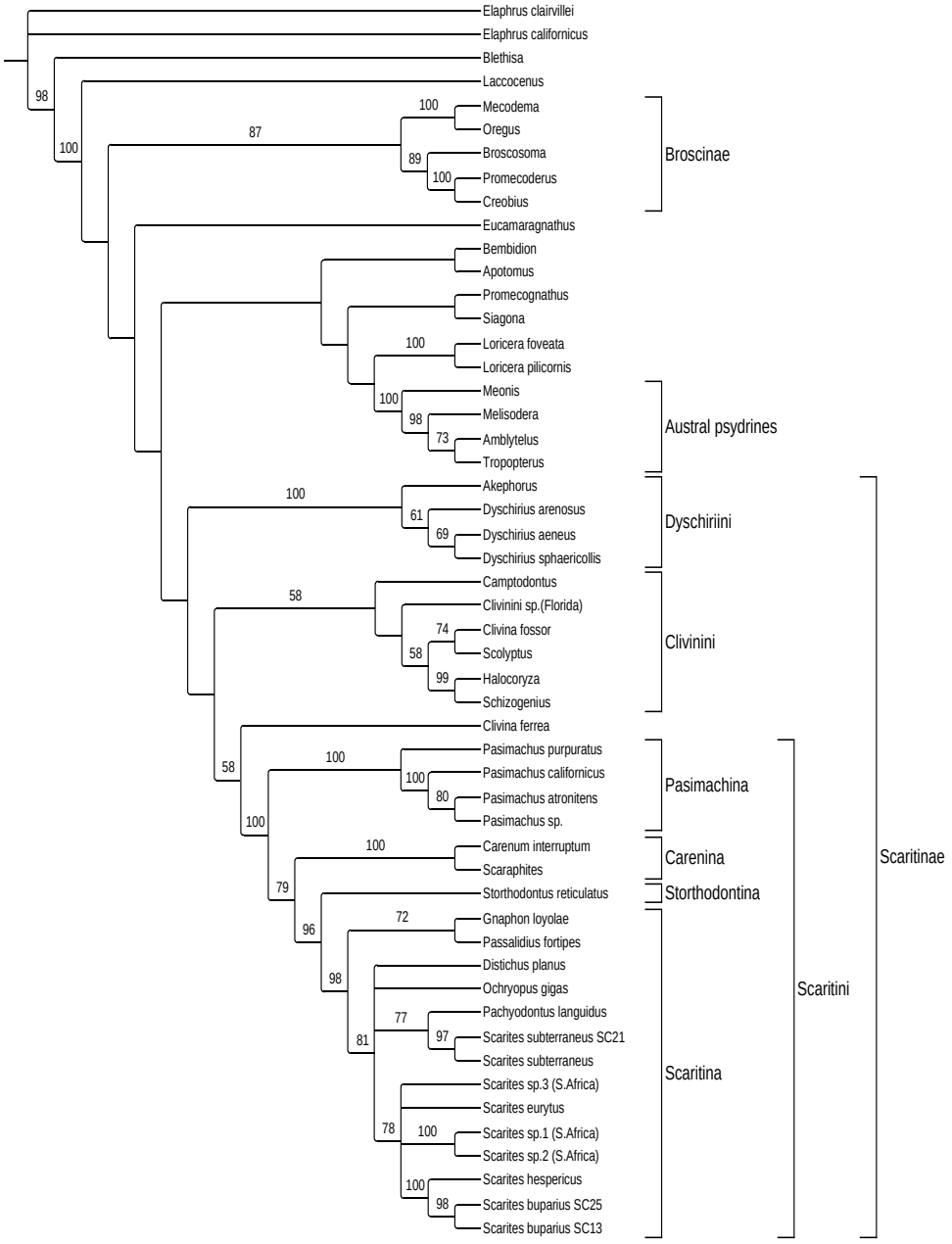

Figure 4.19. Strict consensus of 5 equally parsimonious trees resulting from analysis of the full 18S sequence aligned using the default MAFFT gap penalty 1.53:0. Numbers to the left of nodes are percentage bootstrap support values.

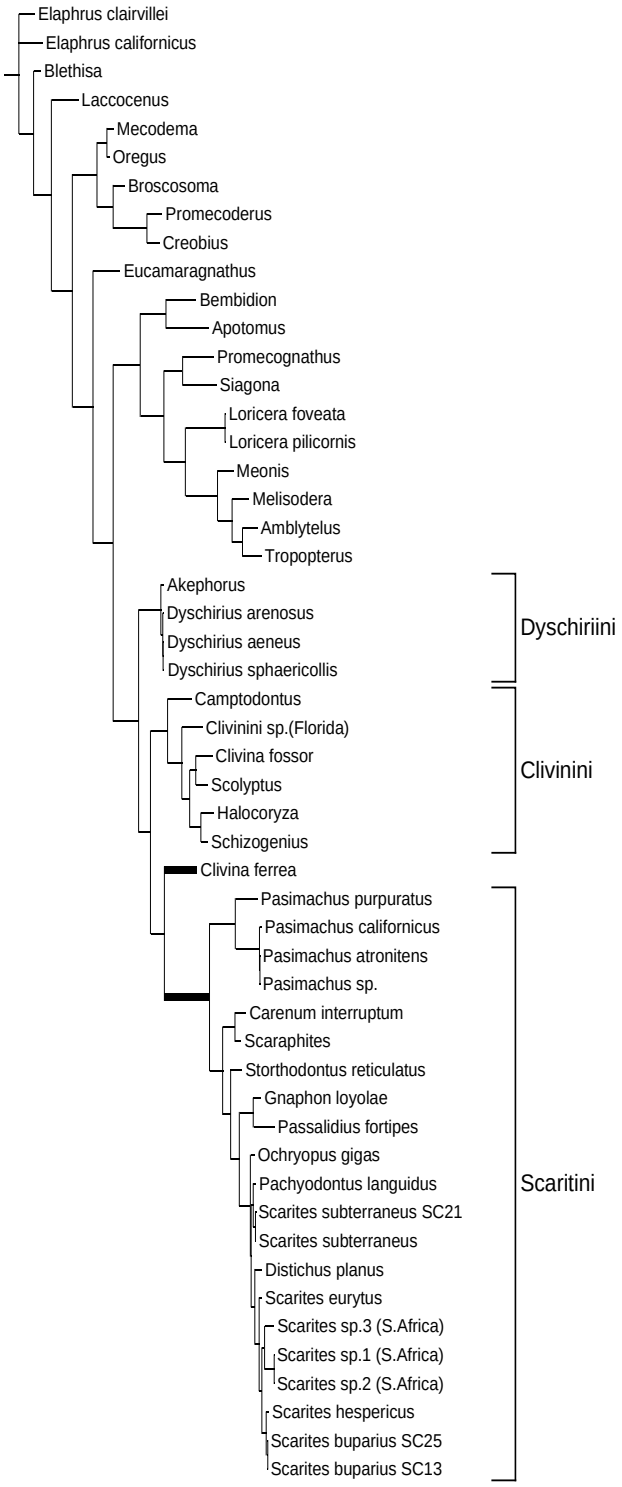

Figure 4.20. 1 of 5 equally parsimonious phylograms resulting from analysis of the full 18S sequence aligned using the default MAFFT gap penalty 1.53:0. Long branches leading to *Clivina ferrea* and Scaritini are highlighted in bold.

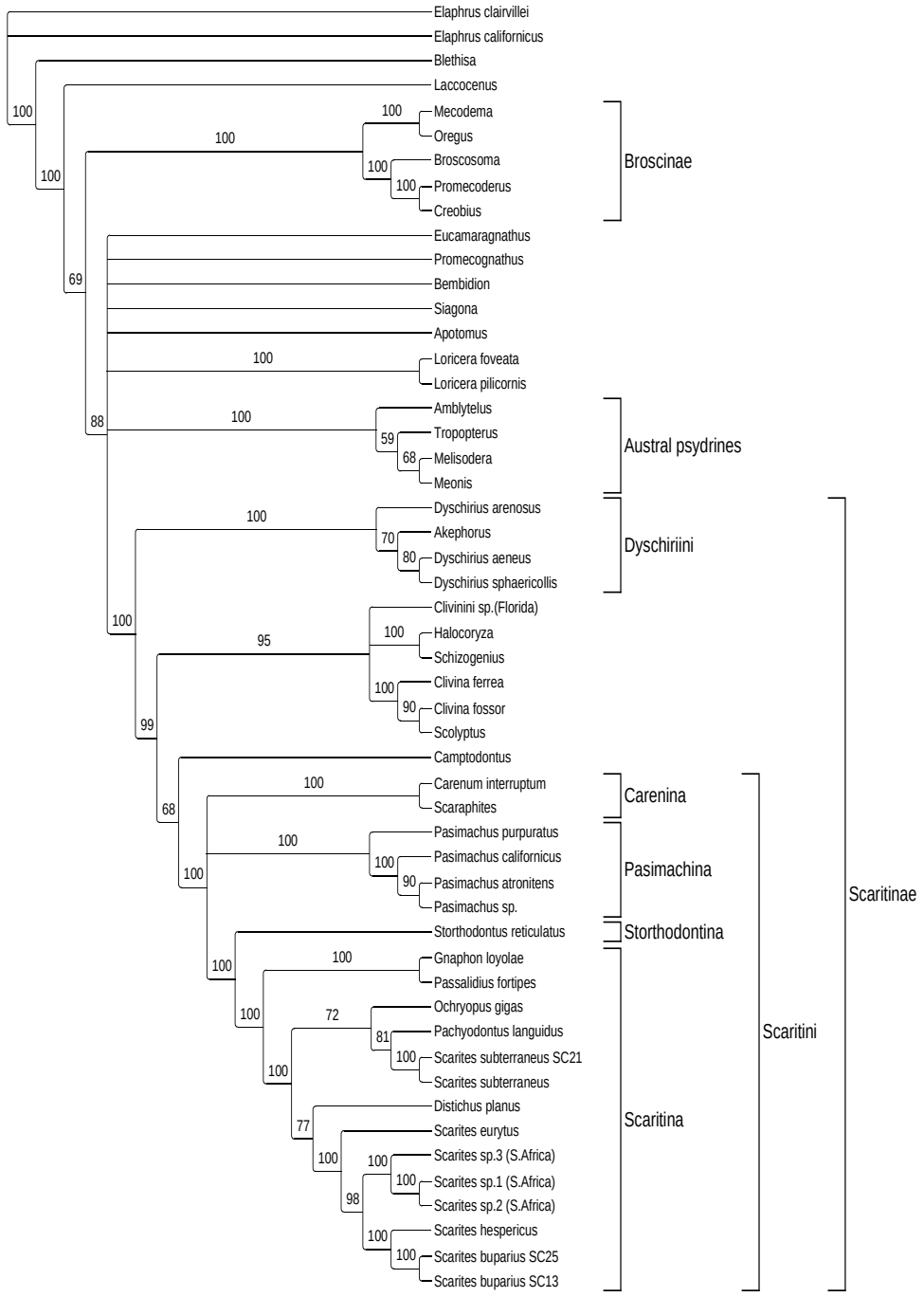

Figure 4.21. Bayesian 50% majority rule consensus cladogram of 948 trees resulting from analysis of the full 18S sequence aligned using the default MAFFT gap penalty 1.53:0. Numbers above nodes indicate clade credibility values greater than 50%.

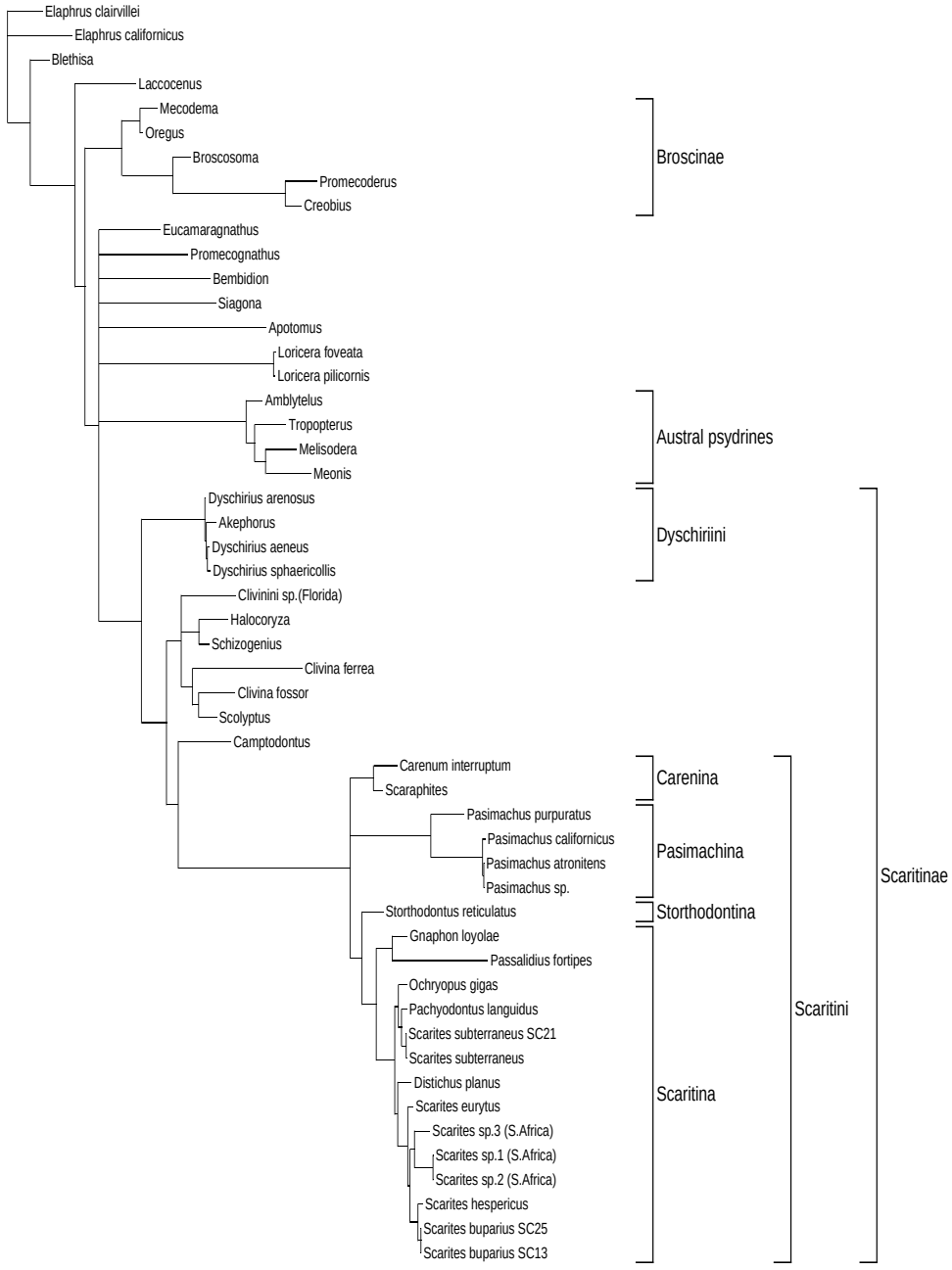

Figure 4.22 Bayesian 50% majority rule consensus phylogram of 948 trees resulting from analysis of the full 18S sequence aligned using the default MAFFT gap penalty 1.53:0.

4.3.5 Reversible jump MCMC and substitution models.

In all analyses the substitution model with the highest posterior probability was the general time reversible (GTR) submodel 123421. The GTR model has separate substitution rates for each of the six substitutions AC, AG, AT, CG, CT, GT respectively (123456) (Huelsenbeck and Ronquist, 2005). GTR submodel 123421 has only four separate substitution rates as the rates are the same for AG + CT and AC + GT.

4.3.6 Phylogenetic relationships.

In common with the separate data partitions, analysis of the full length 18S sequence resulted in many alternative topologies. The placement of the outgroup taxa was particularly sensitive to user-defined variation of the gap penalties, while the effect on the ingroup topology was considerably less. The performance of ClustalX and MAFFT and the results obtained with parsimony and Bayesian inference are discussed in the context of the inferred relationships of the mid-grade tribes and Scaritinae.

4.3.6.1 The mid-grade Carabidae outgroups.

The Austral psydrines are a disparate group of mid-grade Carabidae confined to the southern hemisphere. The morphological evidence provided by Baehr (1998) strongly supports monophyly of this group.

The Austral psydrines are recovered as a monophyletic group in all analyses (table 4.8) and with high bootstrap support or clade credibility values (for example, figures 4.16 and 4.17). However, the relationship between this clade and other mid-grade Carabidae could not be resolved with the 18S data.

The subfamily Broscinae is another mid-grade carabid group well-founded on morphological grounds (Roig-Juñent, 2000). Five members of this subfamily were included in this analysis, but their recovery as a clade was dependent on alignment method.

A monophyletic Broscinae was inferred from only two of the ClustalX alignments; 15:6.66 using parsimony (although unsupported, figure 4.16.) and Bayesian inference (figure 4.17) and 1:0.5 with Bayesian inference only (tree not shown). The opposite situation occurred with MAFFT and all parsimony and Bayesian analyses of the MAFFT alignments infer a well-supported Broscinae (figures 4.19 and 4.21).

Promecognathini is a group sometimes classified as part of Scaritinae, most recently by Bouchard et al. (2011). The placement of Promecognathus within the scaritines, either within Dyschiriini, Clivinini or Scaritini, is not supported by any of the analyses.

Erwin & Stork (1985) proposed a possible sister relationship of Hiletinae to Scaritini and this relationship is evident in at least some of the analyses.

Eucamaragnathus was the single representative of Hiletinae included in the analysis. The placement of *Eucamaragnathus* was particularly unstable and in the consensus trees it variously occupied a basal position or as sister to Scaritinae s.l. or Dyschiriini, but never Scaritini *sensu stricto*.

This sister relationship to Scaritinae s.l. or Dyschiriini was obtained without any clear pattern, with high and low gap costs from a proportion of the ClustalX and MAFFT alignments and both with maximum parsimony and Bayesian inference (table 4.8.). When inferred by maximum parsimony, support for this sister relationship was generally low (data not shown) and in only one case (ClustalX 10:2) received bootstrap support greater than 50%. Bayesian inference also returned low clade credibility values for this relationship from the MAFFT alignments, but values of 99-100% were obtained from five of the ClustalX alignments (1:0.25, 4:2, 5:1, 7:2 and 10:2).

4.3.6.2 Scaritine relationships.

Scaritinae *sensu lato*, meaning a clade containing Dyschiriini + Clivinini + Scaritini was obtained from all Bayesian analyses and with good support (for example figure 4.21). A monophyletic Scaritinae was also evident from all parsimony analyses except with ClustalX alignments 1:0.5 and 15:6.66 and MAFFT alignments 2:0.5 and 3:1 (table 4.8). Scaritine relationships were unresolved with a further two ClustalX alignments (1:0.25 and 2:1) but still compatible with monophyly.

Dyschiriini is recovered as a well-supported monophyletic group in all analyses. The relationship between Dyschiriini and the other scaritines varied according to the method of phylogenetic inference employed, and consistent results were only obtained with Bayesian inference.

With maximum parsimony the exact placement of Dyschiriini is inconsistent. Dyschiriini either has a sister relationship to Clivinini (table 4.6, figure 4.16) or the tribe occupies a basal position within the Scaritinae clade (figure 4.19). In contrast, Bayesian analysis always places Dyschiriini at the base of the Scaritinae clade, for example figure 4.21.

Across all the analyses undertaken members of Clivinini were recovered as a number of alternative topologies. A consistent result was only obtained from Bayesian analysis of the MAFFT alignments.

Parsimony analysis of the ClustalX (4:2, 7:2) and MAFFT (1:0, 1.53:0, 1.53:0.5 and 3:0) alignments in particular produced an unexpected result where *Clivina ferrea* (LeConte) was placed outside of Clivinini as sister to Scaritini *sensu stricto*. This result must be considered an artefact as the morphological evidence supporting a close relationship of *C.ferrea* to *C.fossor* (L.) (the other species of *Clivina* included in the analysis) or indeed any other species of *Clivina*, is compelling (Ball, 2001). Furthermore, Bayesian analysis of all the ClustalX and MAFFT alignments consistently places *C.ferrea* in the 'correct' position, in a clade with *C.fossor* and *Scolyptus* (a genus which should probably be placed in synonymy with *Clivina*). The fact that this artefact only occurs with parsimony, and that

C.ferrea has the longest branch among the Clivinini (figures 4.20 and 4.22) would suggest long-branch attraction is the cause of this odd placement. While parsimony analysis sometimes produced spurious relationships, Bayesian analysis of all the MAFFT alignments produced an identical result. Clivinini was recovered as a clade with the exception of *Camptodontus*, which was placed as sister to Scaritini *sensu stricto*. An example of this relationship is shown in figure 4.21. *Camptodontus* is currently classified within the Forcipatorina, an exclusively Neotropical subtribe somewhat isolated morphologically from other clivinines.

Scaritini is recovered as a well-supported monophyletic group in all analyses of the full length 18S sequence and separate analyses of the LC and V regions.

Within Scaritini the two subtribes Carenina and Pasimachina (Pasimachina in this analysis only represented by the genus *Pasimachus*) consistently occupy a basal position in the Scaritini clade (figures 4.13 and 4.21).

The inferred relationship of Carenina and Pasimachina to the remaining Scaritini is somewhat unstable. Either subtribe can be placed as sister to the rest of the Scaritini depending on alignment and inference method, but no combination of alignment and inference methods provides an absolutely consistent result (table 4.8.).

From this analysis the accepted relationship is that Carenina is sister to the remaining Scaritini and is based on weight of evidence rather than a single consistent result.

Evidence supporting this relationship (Carenina sister to all other Scaritini) is recovered in the following analyses:

- All parsimony analyses of the LC regions aligned by ClustalX. This relationship is less resolved but overall also not contradicted by parsimony analysis of the LC region aligned by MAFFT (figure 4.5).
- All Bayesian analyses of the full length 18S sequences aligned by ClustalX (table 4.8 and example in figure 4.17)
- All parsimony analyses of the full length 18S sequence aligned by ClustalX, except with alignments 8:3 and 15:6.66.
- Bayesian analysis of the full length 18S sequence aligned by MAFFT in six out of nine alignments. In addition, with the remaining alignment (1.53:0) the relationship in is unresolved but not contradictory (table 4.8 and figure 4.21).

Evidence against this relationship (*Pasimachus* and not Carenina sister to the remaining Scaritini) is evident from the following analyses:

- Parsimony analysis of the V regions aligned by ClustalX and MAFFT (figures 4.10. and 4.11)
- Most (eight of ten) of the parsimony analyses of the full length MAFFT alignments (table 4.8 and figure 4.15.).

This analysis includes two genera of Carenina; *Carenum* and *Scaraphites*. Based on adult and larval morphological characters, Moore and Lawrence (1994) instead hypothesised the classification of *Scaraphites* within Scaritina. The results provide no evidence for this and in every case *Carenum* and *Scaraphites* are always placed as sister taxa.

With the exception of two ClustalX alignments, the Madagascan scaritine *Storthodontus reticulatus* always has a sister relationship to Scaritina (table 4.8). This placement is consistent with a hypothesis that the Madagascan scaritines (subtribes Dyscherina + Storthodontina) comprise the sister group to Scaritina. However, as only one storthodontine was included in the analysis (*Storthodontus reticulatus*) this hypothesis can only be tentative.

Lastly, the large and diverse subtribe Scaritina, containing the bulk of the species and generic level diversity in the Scaritini, is recovered as a clade from all analyses. The genus *Passalidius* is also included in Scaritina, contrary to its traditional placement in the Scapterina, a subtribe not sampled in this analysis.

Scarites sensu lato is the most speciose genus of Scaritini. This genus is always recovered as a paraphyletic clade by the inclusion of the genera *Distichus*, *Ochryopus* and *Pachyodontus*. Based on morphological characters, *Distichus* is evidently closely related to Scarites. However, in overall appearance *Ochryopus* appears very different to species of *Scarites*, and in some classifications has been placed in the separate subtribe

Ochryopina (Basilewsky, 1973b). Scoring of the morphological characters in chapter 2 reveals that *Ochryopus* is defined only by autapomorphies (for example the form of the labial palps and the elytral setae) and that its generic status may not be justified in a strict cladistic classification.

The curious monobasic genus *Pachyodontus* (figure 2.2) has historically either been classified as a subgenus of *Scarites* or as a separate genus, again based on autapomorphies. In all analyses *Pachyodontus* is placed in the *Scarites* clade close to the North American species *Scarites subterraneus*. This provides good evidence that *Pachyodontus* belongs within the genus *Scarites*, either as a separate subgenus or within another of the established subgenera. The exact placement of *Pachyodontus* will undoubtedly become clearer with further sampling of *Scarites*.

The inclusion of these other genera within *Scarites* is significant. Assuming the 18S gene evolves at an appropriate rate to resolve these closer relationships, the analyses conducted here indicate that the current classification of Scaritina does not reflect evolutionary history.

4.4 Discussion.

4.4.1 The data partitions.

Separate analysis of the V regions indicates they contain a phylogenetic signal at least partly consistent with that of the LC regions. This provides sufficient justification for including these regions in the analysis. When the

LC and V regions were analysed together, fewer alternative MP trees were produced, presumably because weak phylogenetic signals from each partition combined to give a stronger signal.

If different alignments are examined, for example figure 4.4, it shows that different gap costs produce unambiguous but different homology statements. These homologies can then potentially give rise to conflicting but well supported relationships. This highlights the need to explore a range of gap costs.

This analysis demonstrates that for 18S sequences, alignment method, gap cost and method of inference have a considerable influence on the resulting phylogenetic hypothesis.

4.4.2 Alignment methods.

Variation in the total length of the alignments (tables 4.6 and 4.7) suggests that a reasonable coverage of alignment space was achieved for each method.

The performance of ClustalX and MAFFT is not directly comparable since they both operate over a different range of parameter values, but some general comparisons can nonetheless be made.

Judged by the criteria of difference in length of the alignments, number of informative characters and overall variation in the resulting topologies,

MAFFT produced more consistent results than ClustalX over the range of chosen parameter values.

The ClustalX alignments produced with low gap costs (GOP:GEP 1:0.25, 1:0.5 and 2:1) performed especially poorly, producing large numbers of equally parsimonious trees or failing to resolve particular nodes recovered in most of the other analyses (table 4.8). In fact, recovery of key nodes well supported by morphological data is another criterion which could be used to assess the quality of alignments. In this regard ClustalX also proved inferior by inferring a monophyletic Broscinae in only 3 out of 20 analyses, while analysis of all the MAFFT alignments recovered this clade irrespective of gap cost.

4.4.3 Phylogenetic methods.

Both parsimony and Bayesian inference methods produced broadly similar results, but these results also differed in some important details.In some instances parsimony or Bayesian inference led to particular relationships being consistently favoured over others. For example, Bayesian analysis of the MAFFT alignments usually recovered Carenina as sister to the remaining Scaritini, whereas parsimony analysis of the same alignments favoured *Pasimachus* as the sister group.

The only relationship uniquely derived from one particular inference method was a monophyletic clade placing Dyschiriini and Clivinini as sister groups. This was recovered from some of the ClustalX and MAFFT

alignments only by maximum parsimony (table 4.8), while Bayesian analysis always placed these tribes as paraphyletic to Scaritini.

The results of a comprehensive literature review by Rindal and Brower (2011) questioned the need for using multiple inference methods. They argued that with real (not simulated) molecular data, parsimony and model-based methods such as Bayesian inference produce very similar results. This was found to be true to some extent with this analysis, but the differences which these authors could have interpreted as minor, such as alternative placements of one particular taxon, can be important. This was certainly true in the case of the clivinine *Camptodontus*, which was consistently placed as the sister taxon to Scaritini by Bayesian inference but in a number of other positions by parsimony. In a broader context, the analyses presented here clearly show that different methods can sometimes produce inconsistent results. This is important as these inconsistencies may reveal areas of uncertainty in the inferred phylogenies, which require testing with further data.

4.4.4 Relationships of the Scaritinae.

The diagram in figure 4.23 provides an overall summary of the results of the analysis.

This diagram is a synthesis of all the well supported and consistently obtained relationships across all the analyses. However, despite considering the effects of ClustalX and parsimony on tree topology in some detail, in the

end this summary tree is very similar to the consensus tree obtained by Bayesian analysis of the MAFFT alignment under the default gap costs (figure 4.21).

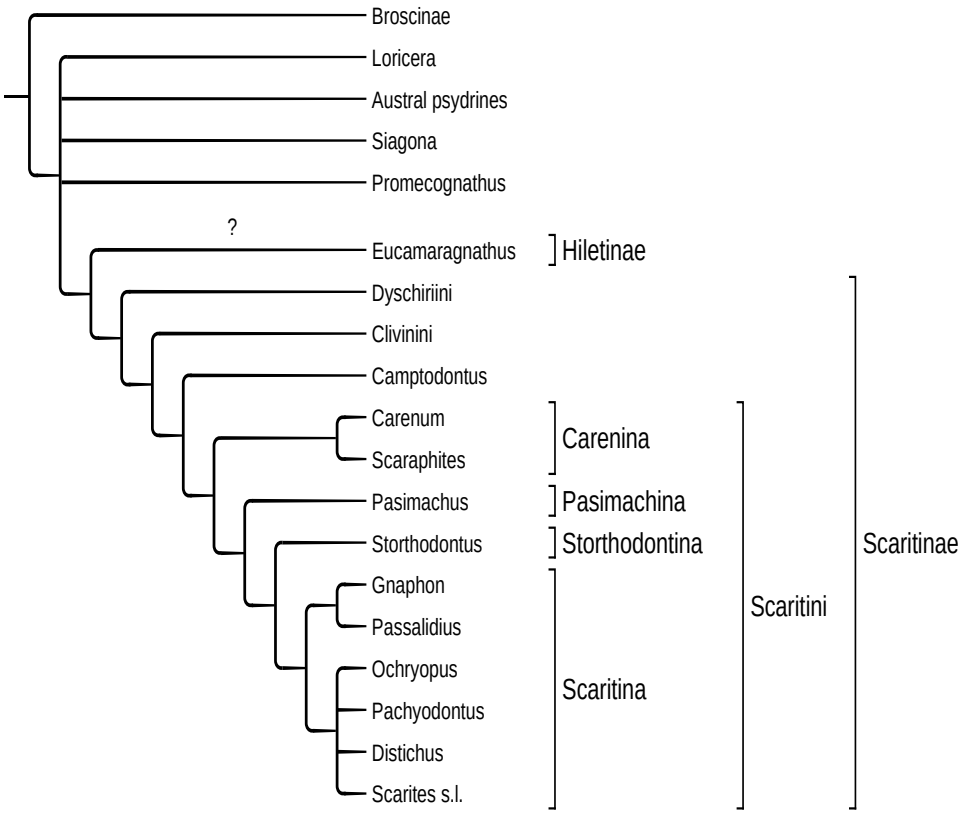

Figure 4.23. Diagram summarising the results of the analysis of 18S rRNA.

4.5 Conclusions and future directions.

The 18S data used in this study was suitable to resolve relationships within Scaritini, and most clades were recovered consistently and with good statistical support. The relationships within Scaritinae *sensu lato* were less clear, but consistent results were still obtained, particularly by Bayesian analysis of the MAFFT alignments. However, deeper relationships between the scaritines and other mid-grade carabid groups could not be consistently recovered.

Several important conclusions about the evolution of the scaritines can be drawn from this analysis.

- Among the mid-grade tribes the most likely sister group to the Scaritinae is Hiletinae.
- Scaritinae *sensu lato* and the individual tribes Dyschiriini, Clivinini (excluding Forcipatorina) and Scaritini are all monophyletic groups.
- The sister group to Scaritini is a forcipatorine, either the genus *Camptodontus* or another of the genera not sampled.
- The Australian Scaritini (subtribe Carenina) are sister to all the remaining groups of Scaritini.
- The Australian genus *Scaraphites* should be classified in the Carenina and not the Scaritina.
- The subtribe Scaritina is a monophyletic group but the genus *Scarites sensu lato* (as currently defined) is probably a paraphyletic group.

The addition of more sequence data is necessary to confirm the results obtained from this study. As the 18S rRNA gene has proved unsuitable for resolution of mid-grade carabid relationships, data from new markers is required, although there does not appear to be a suitable candidate at present. The nuclear protein-coding gene *wingless* has been used in a number of recent phylogenetic studies of Carabidae (Ribera et al., 2005; Ober and Maddison, 2008; Maddison et al., 2009) but as with 18S, has failed to provide support for deeper carabid relationships.

Efforts were made to acquire the broadest range of taxa possible but significant gaps nonetheless remain. The Indian subtribe Oxylobina (containing the single genus *Oxylobus*) would be desirable to include as this was the only main group of Scaritini missing from the analysis, but representation of Australian and Madagascan species could also be improved. Attempts to circumvent this problem by amplifying DNA from pinned specimens unfortunately failed, but as practical considerations remain a significant obstacle, this may be the only way to sample some taxa.

Chapter 5

Biogeography of the Scaritini

5.1 The present distribution of Scaritini.

In common with many large insects, Scaritini are absent from the colder temperate regions of the northern and southern hemispheres.
However, climatic factors cannot be solely responsible for the distribution of Scaritini as different groups have different distribution patterns on different continents. Members of the subtribe Carenina, for example, are exclusively Australian and are not found in similar habitats in Africa or South America, whilst members of the subtribe Scapterina as currently defined (Lorenz, 2005) occur in Southern Africa, Australia and Indo-China.

Figure 5.1 shows the world distribution of Scaritini at subtribal level. Scaritina are found worldwide between latitudes 50'N and 40'S and are mapped separately in figure 5.2. Each of these subtribes represents a monophyletic group recovered in the phylogenetic analysis (chapters 2 and 4).
The subtribe Scapterina are not mapped as the monophyly of this subtribe is questionable, based on the results of the 18S rRNA analysis.
The subtribes Ochryopina (containing the single genus and species *Ochryopus gigas* Schiödte) and Acanthoscelitina (containing the single

species *Acanthoscelis ruficornis* (F.)) are included here within Scaritina, based on results of the phylogenetic analysis.

Two genera of Scaritina contain numerous species (Lorenz, 2005) and are therefore not mapped. These are *Distichus sensu lato* (38 species, Palearctic and Pantropical excluding Australia) and *Scarites sensu lato* (189 species, Nearctic, Palearctic and Pantropical excluding Australia).

These maps clearly demonstrate the highly structured distribution of the main groups (subtribes) of Scaritini and individual genera of Scaritina. Some taxa are widespread, others are disjunct whilst some are restricted to isolated locations. The current juxtaposition of the continents differs from the past due to tectonic movement, and the appearance of Coleoptera in the fossil record occurred at times when land masses were in different configurations to now. The key issues addressed in this chapter focus on reconciling phylogeny with current distribution, taking into account geological history and potential dispersal abilities.

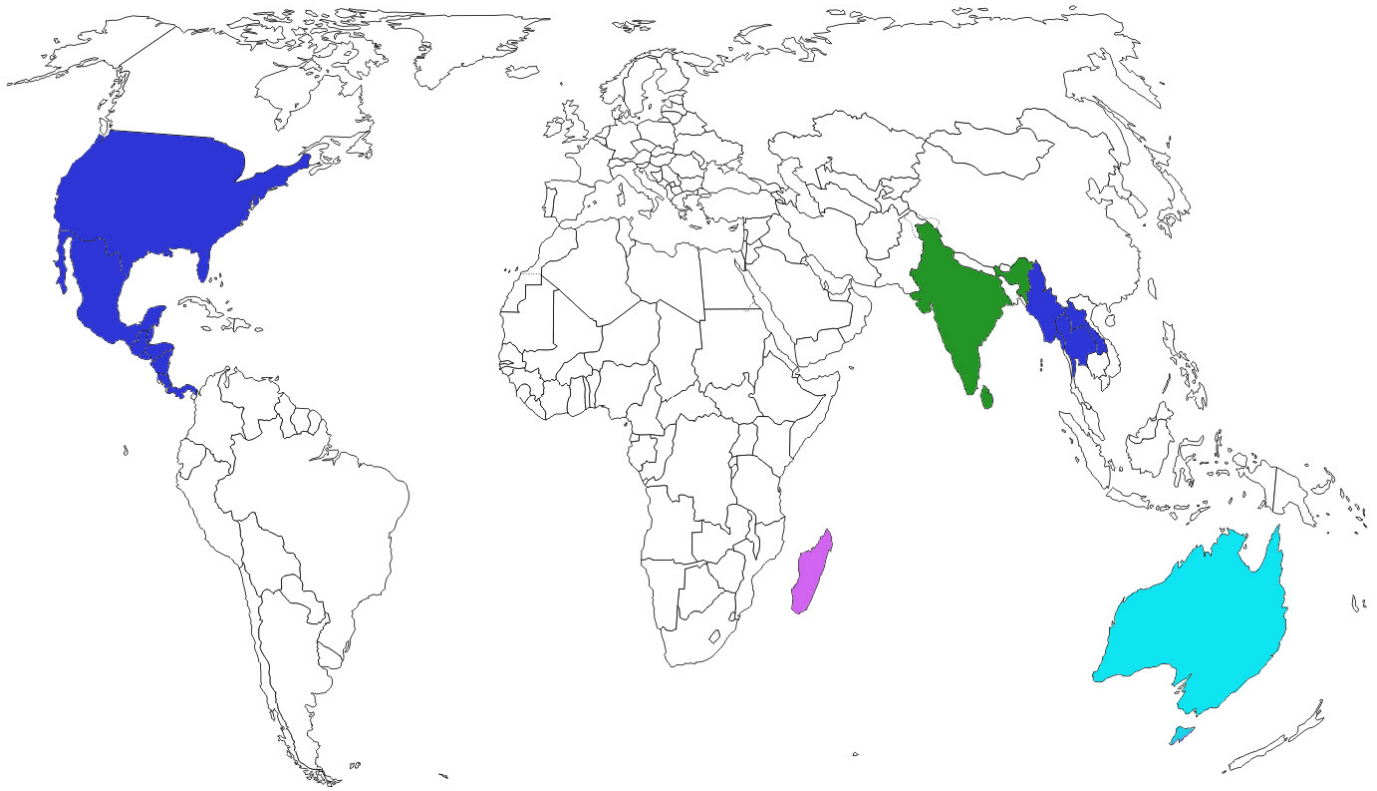

Figure 5.1. World distribution of the subtribes of Scaritini (excluding Scaritina). Dark blue = Pasimachina, green = Oxylobina, turquoise = Carenina, purple = Dyscherina + Storthodontina.

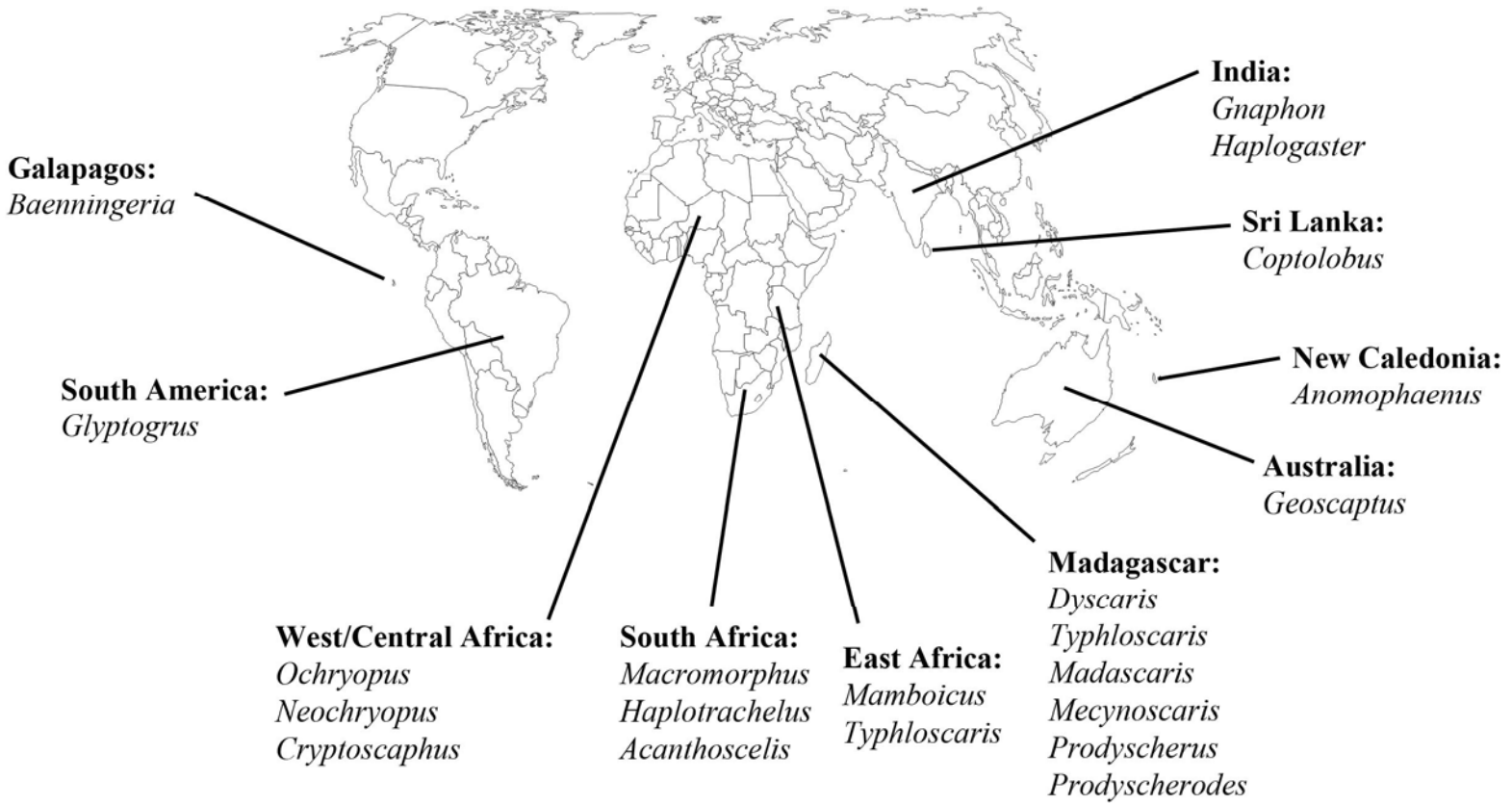

Figure 5.2. The distribution of genera of Subtribe Scaritina, excluding *Distichus* s.l. and *Scarites* s.l.

5.2 The fossil record of Carabidae and Scaritinae.

Fossils provide direct evidence of where and when organisms once existed. However, for most organisms the record is usually incomplete, being dependent on preservation of remains and the finding of these remains. Unlike many insects, the fossil and sub-fossil remains of Coleoptera, which are heavily chitinised, tend to preserve relatively well, but even then the record is relatively incomplete considering the extant diversity of the order (Smith and Marcot, 2012).

The first fossil Coleoptera are known from the Permian (290 Ma ago) belonging to the suborder Archostemata. By the Late Triassic (200Ma ago) Carabidae begin to appear in the fossil record (Arnol'di et al., 1991).

A fossil of a single fore-tibia from the Upper Cretaceous (140 Ma ago) exhibits a well-developed antennal cleaner characteristic of advanced Carabidae in the subfamily Harpalinae (*sensu* Crowson, 1955) and by the Early Cretaceous (140 Ma ago) a number of extant carabid subfamilies are recognised.

The fossil record of Scaritinae is extremely poor and those that are known only date back as far as the Middle Eocene (47 Ma ago) (Lutz, 1990). *Scarites haldingeri* Heer was described from Lower Miocene rocks (Heer, 1861) and the genus *Glenopterus* was established by Heer (1847) for a scaritine fossil from Miocene deposits in Germany. Any Scaritinae fossils require re-evaluation before any firm conclusions can be drawn from them.

Although scaritine fossils are rare, fossils of other mid-grade Carabidae can provide an indication of the timing of scaritine evolution.

An important fossil promecognathine from 80 - 100 Ma old volcanic deposits in Botswana was described by McKay (1991). If it is reasonably assumed Promecognathinae are contemporary with Scaritinae, it can be inferred that the Scaritinae were established by the Late Cretaceous (100 Ma ago).

Exceptionally well preserved dyschiriine fossils from Baltic amber (45 Ma old) closely resemble extant species of *Dyschirius* and *Dyschiriodes* (figure 5.3) and it is likely that many of the modern scaritine genera were in existence by this time.

Figure 5.3. An undescribed fossil species of Scaritinae, Dyschiriini from Baltic amber (44 Ma old). © A.L.Damgaard, with permission.

5.3 Vicariance and the distribution of Scaritini.

The diversity of scaritines is concentrated in the Southern Hemisphere, with genera endemic to areas formerly united as the southern continent of Gondwana. The areas of South America, Australia, New Caledonia, Africa, Madagascar and India are land masses resulting from the fragmentation of Gondwana beginning in the Early Jurassic (180 Ma ago).

Figure 5.4 shows the position of the continents during the Early Cretaceous showing the fragmentation of Gondwana. If the Scaritinae originated in Gondwana at the time when this supercontinent was fragmenting, it is likely that the current distribution of scaritine groups will reflect a complex history of radiation and isolation events occurring during the fragmentation process.

Figure 5.4. Palaeomap of 105Ma ago showing fragmentation of Gondwana. By this time Africa and Madagascar + India have separated from the rest of Gondwana leaving a connected land mass made up of South America, Antarctica and Australia. © R.Blakey, with permission.

Theories regarding the fragmentation of Gondwana can be summarised with area cladograms (Sanmartín and Ronquist, 2004), facilitating comparison with phylogenetic trees. One such area cladogram is shown in figure 5.5.

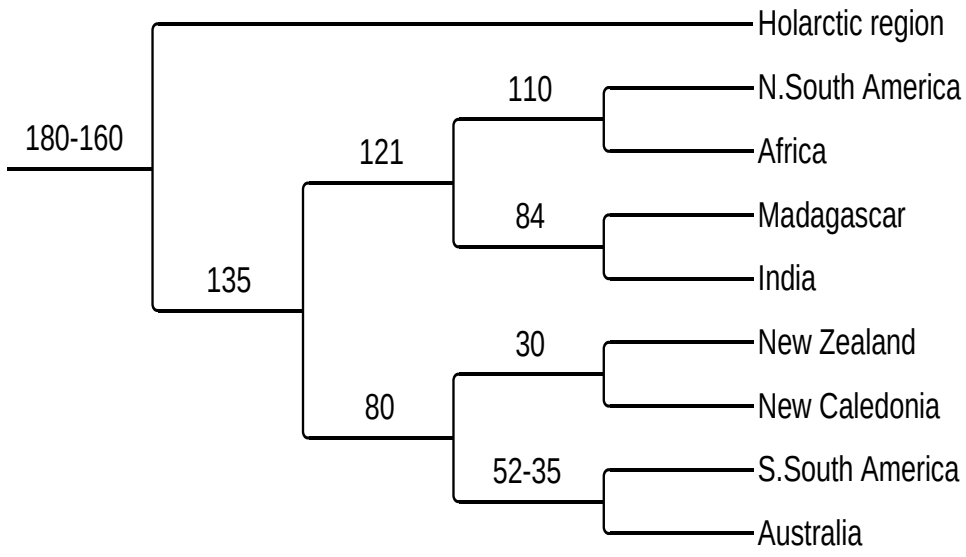

Figure 5.5. Area cladogram showing events during fragmentation of Gondwana. Numbers to the left of nodes indicate time in Ma ago. Adapted from Sanmartín and Ronquist (2004).

A summary diagram showing the phylogenetic relationships established by this study and the geographical distribution of each clade is shown in figure 5.6. Clearly, the relationships of the tribes and subtribes do not match perfectly to the area cladogram of Sammartín and Ronquist (2004). The interpretation of this therefore requires a careful historical reconstruction.

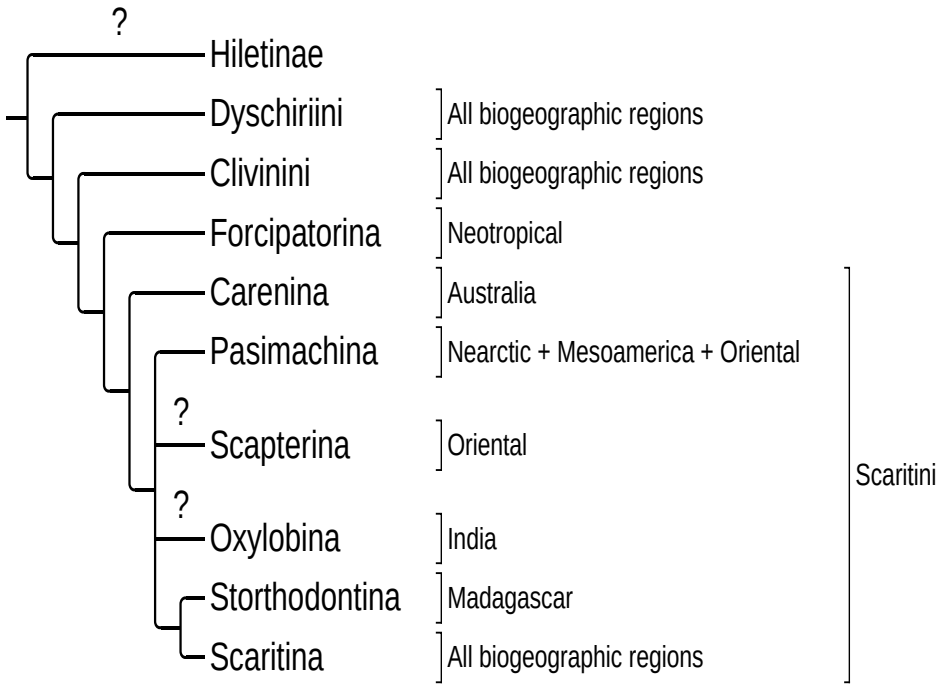

Figure 5.6. Diagram incorporating the relationships of the tribes and subtribes of Scaritinae obtained from the phylogenetic analysis with distribution data.

5.4 Biogeography of the basal subtribes of Scaritini: Carenina and Pasimachina.

The phylogenetic analysis has shown that Carenina is sister to Pasimachina and the remaining Scaritini. Carenina and Pasimachina have very different distributions; Carenina in Australia and Pasimachina in the Northern Hemisphere (figure 5.1). There are a number of possible historical processes to account for this.

Before the fragmentation of Gondwana, plate tectonics caused a major North-South division of landmasses. This event was the separation of

Laurasia in the Northern hemisphere from Gondwana in the Southern Hemisphere. It is possible that this separation isolated Carenina in the South and Pasimachina in the North.

However, the timing of this major geological event does not match the fossil record of Carabidae. Laurasia and Gondwana are thought to have separated in the late Triassic to early Jurassic (ca. 200 Ma ago) at the time when the first carabid fossils begin to appear. The early fossil record of Carabidae is very incomplete, but a bold proposal by Erwin and Stork (1985) nonetheless gave a Jurassic origin of the mid-grade tribe Hiletinae, a group contemporary with Scaritinae. Until Jurassic fossils of Scaritini or other mid-grade tribes are discovered, the hypothesis of vicariance caused by the separation of Laurasia and Gondwana must remain speculative.

Another possibility is that the extant groups of Scaritini arose some time before the fragmentation of Gondwana, with subsequent vicariance of these established clades by plate tectonics. If this occurred there is an expectation that members of the same monophyletic group (genus, subtribe) would be distributed in two or more Gondwanan areas.

For example, an origin of the carabid tribe Galeritini in Gondwana, followed by vicariance by plate tectonics was proposed by Ball (1985) as a mechanism to account for the extant distribution of the genus *Galerita* in S.America, Africa and S.E.Asia. The same mechanism was also proposed for Hiletinae (Erwin and Stork, 1985), where the present distribution of the genus *Eucamaragnathus* includes South America, Africa, Madagascar and

Southeast Asia. This hypothesis is falsified by the proposed phylogeny in figure 5.6, because members of the basal subtribes of Scaritini exist in either a single Gondwanan area (Carenina in Australia and Storthodontina in Madagascar) or in multiple areas including those which were not part of Gondwana (Pasimachina in North and Central America and Indo-China). Despite this, the possibility still exists that the basal subtribes had non-overlapping, relictual distributions in areas that were later separated by the fragmentation of Gondwana, such as Australia, India and Madagascar. This possibility was also considered by Ball (1985) for Galeritini, but without fossil evidence this theory cannot be tested. If fossils of Carenina were found outside of Australia for example, this theory could be disproved.

Rather than causing the separation of previously established clades, the fragmentation of Gondwana may have driven the evolution of certain groups. New clades arise because they become isolated on Gondwanan fragments and the sequence of disconnection of the continents is reflected in the phylogeny. Examples of groups where this is thought to have occurred include dinosaurs (Upchurch et al., 2001) and frogs (Bocxlaer, 2006).
If the evolution of Scaritini was driven by the fragmentation of Gondwana, sister groups would be expected in different Gondwanan areas, and relationships should match the sequential disconnection of the continents (see area cladogram figure 5.5).
The summary phylogeny obtained in this work (figure 5.6) shows a connection between S.America and Australia (Forcipatorina-Carenina)

typical of Gondwanan taxa due to a connection via Antarctica. But, as the Australian Carenina are the sister group to all other Scaritini, there is no congruence between the phylogeny and the sequence of dis-connection of the continents. In addition, the relationship of Carenina to Pasimachina and their Northern - Southern hemisphere distribution does not show a typical Gondwanan pattern. Erwin (1985) considered this distribution an example of an 'amphiantarctic' spatial pattern. The amphiantarctic pattern is displayed as a distribution from the Neotropics south through Southern South America across Antarctica to Australia and northwards from the Neotropics to the Nearctic. In this case the absence of Pasimachina in South America is then accounted for by extinction. Dispersal across Antarctica is presumed to be possible because warmer conditions in the past left Antarctica ice-free. This explanation is plausible, but lacking in supporting data.

At this time, there is insufficient data to explain the biogeographical connections between the basal lineages Carenina, Pasimachina and Storthodontina, only that the fragmentation of Gondwana does appear to have played a significant role. It may be that the biogeographical history of the basal lineages has become blurred by a mixture of vicariance, dispersal and extinction and may now be difficult to reconstruct.

Extinction will erase biogeographic patterns and the precise signals of previous occurrences are absent without fossils. The distribution of Pasimachina (North and Central America and Indo-China, figure 5.1.) could

be accounted for by extinction. This group may once have had a much wider range, with extinction in intermediate areas leading to the extant relictual distribution. Other groups with similar relictual distributions occur, for example the coleopteran family Amphizoidae (Dettner, 2005) and the cockroach genus *Cryptocercus* (Nalepa and Bandi, 1999), both of which are only found now in North America and China. This extinction hypothesis is speculative, and in reality insects are such a diverse group that further examples of any distribution can be found to support any particular scenario.

5.5 Dispersal and the distribution of Scaritina.

So far this provisional analysis has concentrated on the basal subtribes of Scaritini, but the biogeography of scaritines is further complicated because scaritine diversification appears to have occurred in at least 2 separate phases (chapters 2 and 4).

The faunas of Australia, North America and Madagascar are all composed of at least two elements, an older basal lineage and more recently derived species or genera, mostly resulting from the Scaritina radiation (figures 5.1 and 5.2).

The scaritine fauna of Australia for example is composed of three discrete groups:

- The endemic subtribe Carenina comprising 11 genera and 204 species, all of which are flightless.

- The endemic genus *Steganomma* belonging to the African, Oriental and Australasian subtribe Scapterina, composed of 3 flightless species.

- *Geoscaptus*, a genus of winged species belonging to subtribe Scaritina also occurring in New Guinea.

The distribution of the genera of Scaritina is given in figure 5.2.
A well-supported generic-level phylogeny of this subtribe does not yet exist, but in the absence of this data it is proposed that the global distribution of Scaritina is due to recent and current dispersal.
The dispersal ability of Scaritina can be inferred by examining island faunas (table 5.1.). Unlike members of the subtribes Carenina, Pasimachina and Storthodontina which are all flightless, many Scaritina are capable of flight.

Table 5.1. The island faunas of Scaritina.

Island	Origin	Island group age	Distance to continental landmass	Taxon
Canaries	Volcanic	Oldest - Fuerteventura 20 Ma (1)	100 km to Africa (Fuerteventura)	1 widespread N.African/Mediterranean species *Scarites buparius* Forster (1).
São Tomé	Volcanic	15.7 Ma (2)	250 km to Africa	1 endemic species *Scarites fatuus* Karsch (6).
Madeira	Volcanic	5.2 Ma (3)	520 km to Africa	1 endemic species *Scarites abbreviatus* Dejean (7)
Galapagos	Volcanic	Oldest - Espanola 3.5 Ma (4)	972 km to Ecuador	1 Endemic genus *Baenningeria* (2 species) (8).
New Caledonia	Continental	Separation from Australia 80-65 Ma ago (5)	1500 km to Australia	1 Endemic genus *Anomophaenus* (8 species) (9).

(1) Machado and Oromí, 2000. (2) Schlüter, 2008. (3) Moores and Fairbridge, 1997. (4) Grehan, 2001. (5) Sanmartín and Ronquist, 2004. (6) Serrano, 1995. (7) Boieiro et al., 2010. (8) Van Dyke, 1953. (9) Heller, 1916.

Dispersal, whether actively by flight or passively by other means such as rafting, is the only method by which Scaritina could have reached the volcanic islands of the Canaries, Madeira and Galapagos. This is because these islands have recently arisen in geological time and have never been connected to a continental landmass. The furthest definite dispersal of almost 1000 km is indicated by the endemic Genus *Baenningeria* of the Galapagos Islands.

To invoke dispersal to account for the presence of *Anomophaenus* (figure 5.7) on New Caledonia is less certain because this landmass is the result of the fragmentation of Gondwana. Therefore trans-oceanic dispersal and vicariance by plate tectonics are both credible mechanisms to explain the presence of this genus, especially as New Caledonia contains a spectacular

relict Gondwanan fauna and flora. Since Scaritina can definitely disperse up to 1000 km in a 3.5 Ma timespan, as indicated by *Baenningeria*, dispersal of about 1500 km from mainland Australia to New Caledonia over the much longer time period of 65 Ma is conceivable. In addition, very long distance dispersals of other large Carabidae are evidently possible, a good example being *Aplothorax burchelli* Waterhouse of the isolated volcanic island of Saint Helena, almost 2000 km from the African mainland (Basilewsky, 1985).

This dispersal hypothesis could be falsified by the discovery of scaritine fossils of Cretaceous age or earlier on New Caledonia.

Figure 5.7. *Anomophaenus costatogranulatus* (Chaudoir) (Scaritini: Scaritina). New Caledonia. Scale bar = 10 mm.

The generic and species-level diversity of Scaritina is greatest in Africa, while the Nearctic and Neotropical regions have a more marginal fauna. An origin of Scaritina on the African landmass is therefore proposed, followed by dispersals to Asia and Australia and to the Nearctic and Neotropical regions via Beringia.

5.6 Biogeography and taxonomy.

Biogeography and taxonomy are inter-related disciplines and in some cases biogeographical data can highlight potential taxonomic problems.

An example of this is provided by the genus *Typhloscaris*. All species of *Typhloscaris* are flightless and have highly restricted distributions in the mountains of East Africa (figure 5.8) and Madagascar. The presence of the genus in similar habitats in both Africa and Madagascar is difficult to account for on a biogeographical basis, as both dispersal and vicariant hypotheses are unlikely. Vicariance would require *Typhloscaris* to be a very old genus, as the Madagascar-India landmass split from mainland Africa during the Early Cretaceous (120 Ma ago). To account for dispersal in either direction would require a flightless high-altitude species to cross lowlands and 400 km of ocean.

These biogeographic problems raise the question as to whether *Typhloscaris* represents a monophyletic group or whether the East African and Madagascan groups are independently derived. An independent origin is possible, as the scoring of morphological characters (chapter 2) shows that

Typhloscaris are essentially flightless forms of the widespread winged genus *Scarites*. Minor but consistent morphological differences initially led to the Madagascan '*Typhloscaris*' being assigned to the genus *Oroscaris*. Later, Basilewsky (1973b) synonymized *Oroscaris* with *Typhloscaris* because of their close similarity. It is clear that this taxonomic hypothesis should be critically re-examined, especially as *Typhloscaris sensu lato* may provide insights into the effect of convergent adaptations linked to flightlessness and high-altitude environments.

Figure 5.8. *Typhloscaris gracilis* Bänninger (Scaritini: Scaritina). Tanzania. Scale bar = 5 mm.

5.7 Conclusions and future directions.

The difficulty of biogeographic reconstruction is that there is usually no direct evidence of past events. Instead reliance must be placed on extant

distributions and reconstructions of phylogeny and past landmass distributions.

The various biogeographical hypotheses given here for the Scaritini are based wherever possible on phylogenetic relationships. Data regarding these relationships allows certain biogeographic hypotheses to be falsified, but if further data and analyses reveal errors in the phylogeny these biogeographical scenarios must be re-examined.

Additional phylogenetic data would certainly shed more light on the biogeography of the basal scaritine lineages. The relationship between the pasimachine genus *Mouhotia* with Carenina is especially important to resolve as it should provide more information on the early evolution of Scaritini.

Phylogenetic data is also necessary to test the proposed dispersal hypothesis for the Scaritina, but even without this data it is shown that long distance dispersal is a plausible mechanism to account for the presence of taxa on Gondwanan landmasses such as New Caledonia.

A molecular clock approach could also be used to provide valuable dating evidence for clades, although the lack of fossil Scaritinae poses serious problems of calibration. Despite this, more reliable estimates of dates could be obtained for the Scaritina by using the maximum geological age of islands as calibration points.

Chapter 6

Conclusions and directions for further work

This chapter provides a synthesis of the results of the phylogenetic and biogeographical analysis.

6.1 Morphological versus molecular data.

A major component of this study concerns the phylogenetic analysis of morphological data. This is contrary to the current trend in systematics, where molecular data has greatly superseded the use of morphology. There are good reasons for this, the most important being that molecular data can reveal relationships that are impossible to reconstruct with morphology. This is because with molecular data, large numbers of unambiguous characters can be easily generated and analysed using explicit substitution models (Wortley and Scotland, 2006). Despite the obvious advantages of molecular data and its rightful prominence, the use of morphology continues to play an essential role in phylogenetics.

In this study, morphological data provide the basis by which the molecular results were interpreted. This is especially important in Scaritini because it is likely that not all currently defined genera represent monophyletic groups. For example, in the 18S analysis *Scarites* was recovered as a paraphyletic group by the inclusion of other genera of Scaritina such as *Pachyodontus* and *Ochryopus*. However, scoring of morphological characters reveals that

Pachyodontus and *Ochryopus* are defined mainly by autapomorphies, and in other respects are morphologically close to *Scarites*. Therefore despite their generic placement, their inclusion within the *Scarites* clade can be interpreted as correct.

A problem encountered with the phylogenetic analysis of the morphological data (chapter 2) was that many clades had bootstrap support values of less than 50%. This is a due to the low number of characters per taxon in the data matrix (Bremer et al., 1999). As a single morphological synapomorphy represents a number of underlying molecular changes, bootstrap support values should perhaps be interpreted less stringently that those from molecular trees.

With further work new morphological characters will no doubt be discovered. Scotland et al. (2003) argued that for many groups the supply of unambiguous morphological characters is exhausted and adding further characters only increases homoplasy and decreases accuracy. For the Scaritinae this is certainly not the case, and although challenges of homology assessment remain, there are a number of additional character systems that deserve further investigation, including male (Roig-Juñent, 2011) and female (Liebherr and Will, 1998) genitalia and mandibles (Ball, et al., 2011).

Despite low node support, especially with the parsimony analyses, the morphological results are in general accordance with those obtained from

the 18S data (figure 6.1). In particular, Bayesian analysis of the morphological data supports a monophyletic Scaritinae, Scaritini, Carenina and Storthodontina and highlights a relationship of Forcipatorina with Scaritini *sensu stricto*. This congruence between the morphological and molecular results strongly suggests that the main conclusions reached by this study about the evolution of the scaritines are correct.

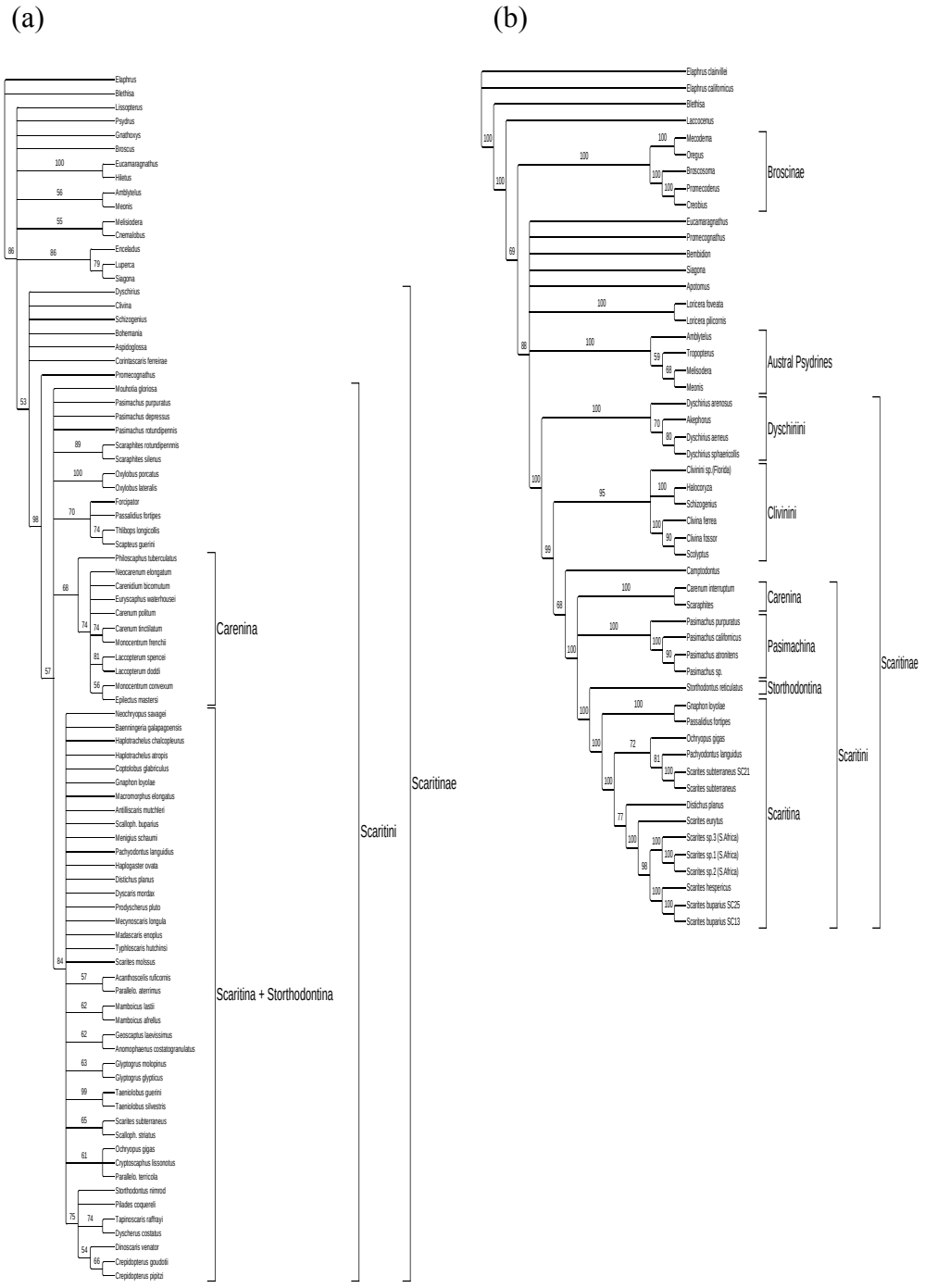

Figure 6.1. Comparison of trees resulting from analysis of the morphological and molecular datasets. (a) Bayesian 50% majority rule consensus cladogram from analysis of the unordered morphological data (reproduction of figure 2.15). (b) Bayesian 50% majority rule consensus cladogram from analysis of the full 18S sequence aligned with the default MAFFT gap penalty 1.53:0 (reproduction of figure 4.21). Numbers above nodes indicate clade credibility values greater than 50%.

6.2 Evolution of the mid-grade Carabidae and Scaritinae.

One of the main aims of this study was to reconstruct the phylogeny of the Scaritinae. Due to the homoplasy of morphological characters and limited taxon sampling for the molecular data, this was not fully realised. Even so, important new conclusions and directions for further work have been reached and are as follows:

- Scaritinae are shown conclusively to be a monophyletic group. This relationship is supported by many of the parsimony analyses of the 18S data and all Bayesian analyses of the morphological and molecular data.

- The tribes Dyschiriini, Clivinini (excluding Forcipatorina) and Scaritini are monophyletic groups and their current classification as separate tribes is justified.

- The molecular data provides evidence for Forcipatorina as the sister group to Scaritini *sensu stricto*. The morphological data also suggest a relationship of Forcipatorina outside of Clivinini, in various positions at the base of the Scaritini clade. This relationship has not been reported previously and warrants further investigation.

- Within Scaritini *sensu stricto* the subtribes Carenina, Pasimachina, Storthodontina and Scaritina are monophyletic groups.

- Based on the 18S analysis and structure of the male genitalia, the Australian subtribe Carenina is the sister group to all other Scaritini. This relationship was also proposed by Moore and Lawrence (1996) on the basis of larval morphology, and this analysis supports their recommendation to elevate this group to the taxonomic rank of tribe. However, contrary to the opinion of Moore and Lawrence (1996) the molecular data consistently places the genus *Scaraphites* (figure 2.17) with the Carenina and not Scaritina. Assuming this placement is correct, and on the basis of morphological characters scored in this study, *Scaraphites* represents the most plesiotypic lineage of Scaritini.

- Relationships among Scaritina are not well defined. This subtribe has several genera that lack unique synapomorphies and the species have short branch lengths on the 18S trees, both suggesting this group is the result of a recent radiation.

- A generic-level taxonomic revision founded on evolutionary relationships is required for the Scaritina. The results of this study already show that the two subtribes Ochryopina and Acanthoscelitina included by Basilewsky (1973b) for the

Afrotropical fauna are based based on autapomorphies and should be synonymized with Scaritina. In addition, Subtribe Scapterina should be re-defined by moving *Passalidius* to Scaritina.

The relationships of the mid-grade tribes Hiletinae and Promecognathinae could not be resolved with either the morphological or molecular data, reflecting the general problem of resolving deeper nodes of the phylogenies. The 18S sequence data provides weak evidence for a sister relationship between Scaritinae *sensu lato* and Hiletinae and deserves further research. The promecognathines (figure 1.6) continue to resist all attempts at classification, despite evidence from adult morphology (this study), larval morphology (Bousquet, 1986), fossils (McKay, 1991) and 18S sequences (Maddison et al., 1999; this study). A more confident placement of these groups should be possible with additional sequence data.

6.3 Classification of the Scaritinae.

The phylogenetic results obtained so far justify some changes to the classification of the scaritines. To accommodate the sister group relationship of Carenini to Scaritini an additional rank of supertribe is required. A new classification scheme is proposed as follows:

Subfamily Scaritinae
- Supertribe Dyschiriitae
- Supertribe Clivinitae
  - Subtribe Forcipatorina *incertae sedis*
- Supertribe Scarititae
  - Tribe Carenini
    - Subtribe Carenina
  - Tribe Scaritini
    - Subtribe Pasimachina
    - Subtribe Oxylobina
    - Subtribe Scapterina
    - Subtribe Storthodontina
    - Subtribe Dyscherina
    - Subtribe Scaritina
      - *Corintascaris incertae sedis*

6.4 Biogeography.

The lack of Jurassic or Cretaceous fossils poses serious problems for biogeographic reconstructions of Scaritinae and until these fossils are found any such reconstructions remain speculative. There is no direct evidence for past distributions during the important geological events that occurred in the Southern Hemisphere 180-50 Ma ago. In addition, fossils are also required as calibration points for future work using molecular clocks, although maximum ages of some clades may be inferred by island distributions. Further work to re-appraise the scaritine fossils that do exist may yet provide useful information, especially now as for the first time external morphological characters have been scored for most genera of Scaritini.

The historical biogeography of the basal lineages of Scaritini has proved difficult to reconstruct, perhaps due to a combination of extinction, dispersal and vicariance occurring over a long time period. On the other hand, by

analysing the extant distribution of members of the subtribe Scaritina on volcanic islands it is possible to deduce their considerable dispersal powers. From this it can be reasonably hypothesised that scaritine faunas in areas such as Australia represent several elements with different biogeographical histories; an older vicariant or relictual pattern overlaid with a more recent dispersal pattern.

6.5 Concluding remarks.

The results of each separate analysis presented in this work (morphological, molecular and biogeographical) provide a certain degree of reciprocal illumination.

The morphological data have enabled interpretation of the molecular results, particularly the problems in the definition of genera within Scaritina.

The molecular data have revealed several new relationships within the scaritines (the groups Forcipatorina and Carenina for example) opening up interesting avenues for further morphological work. Lastly, an analysis of an important component of the evolutionary history of the scaritines, that of historical biogeography, has led to the generation of new hypotheses to be further tested by phylogenetic data.

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Appendix

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