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Phylogeny of carabid beetles as inferred from 18S ribosomal DNA (Coleoptera: Carabidae)

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Abstract. The phylogeny of carabid tribes is examined with sequences of 18S ribosomal DNA from eighty-four carabids representing forty-seven tribes, and fifteen outgroup taxa. Parsimony, distance and maximum likelihood methods are used to infer the phylogeny. Although many clades established with morphological evidence are present in all analyses, many of the basal relationships in carabids vary from analysis to analysis. These deeper relationships are also sensitive to variation in the sequence alignment under different alignment conditions. There is moderate evidence against the monophyly of Migadopini + Amarotypini, Scaritini + Clivinini, Bembidiini and Brachinini. Psydrini are not monophyletic, and consist of three distinct lineages (*Psydrus*, *Laccocenus* and a group of austral psydrines, from the Southern Hemisphere consisting of all the subtribes excluding Psydrina). The austral psydrines are related to Harpalinae plus Brachinini. The placements of many lineages, including *Gehringia*, *Apotomus*, *Omophron*, *Psydrus* and *Cymbionotum*, are unclear from these data. One unexpected placement, suggested with moderate support, is *Loricera* as the sister group to *Amarotypus*. Trechitae plus Patrobini form a monophyletic group. Brachinini probably form the sister group to Harpalinae, with the latter containing *Pseudomorpha*, *Morion* and *Cnemalobus*. The most surprising, well supported result is the placement of four lineages (Cicindelinae, Rhysodinae, Paussinae and Scaritini) as near relatives of Harpalinae + Brachinini. Because these four lineages all have divergent 18S rDNA, and thus have long basal branches, parametric bootstrapping was conducted to determine if their association and placement could be the result of long branch attraction. Simulations on model trees indicate that, although their observed association might be due to long branch attraction, there was no evidence that their placement near Harpalinae could be so explained. These simulations also suggest that 18S rDNA might not be sufficient to infer basal carabid relationships.

Introduction

Carabidae, with more than 30 000 described species (Reichardt, 1977), is one of the largest families of organisms, and includes almost all terrestrial members of the suborder Adephaga. Most of these beetles belong to the subfamily Harpalinae (*sensu* Erwin, 1985), a relatively recent radiation (Cretaceous to Recent; Ponomarenko, 1992) which contains the most speciose carabid clades, especially in tropical regions.

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Although most non-harpaline tribes have relatively few species (more than half of the tribes have sixty or fewer species, Kryzhanovskiy, 1976), they represent the breadth of phylogenetic diversity within the family. Many of these tribes appear to be remnants of early radiations in the Triassic and Jurassic (Ponomarenko, 1992). The pattern of these early radiations is the focus of this paper, which examines relationships of carabid tribes outside of Harpalinae.

A suite of exoskeletal characters has traditionally been used to infer phylogenetic structure within carabids (Jeannel, 1941; Ball, 1979; Kavanaugh & Erwin, 1991). The most recent common ancestor of carabids is thought to have had a mandible with a scrobal seta, procoxa open behind (not

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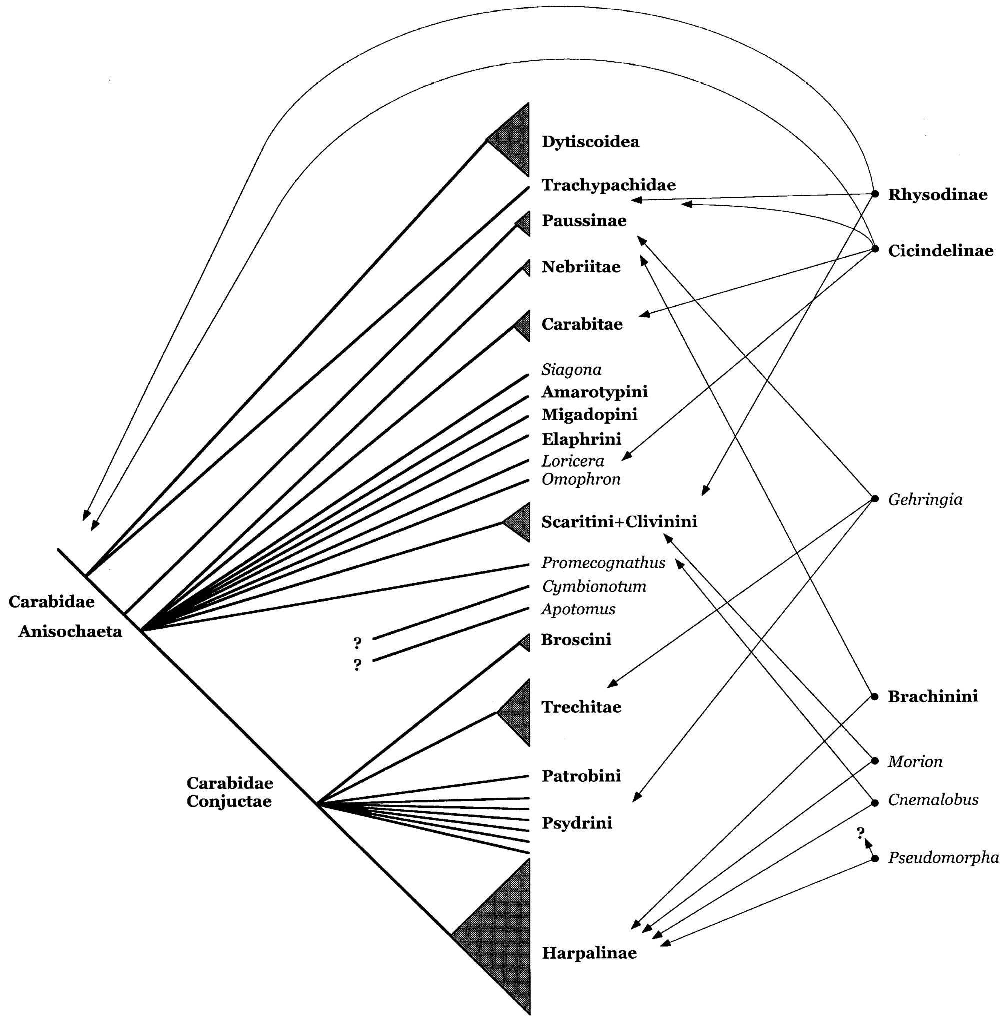

Fig. 1. Relationships of carabid beetles based on morphological evidence, focusing on groups examined in the current study. The taxa on the right are particularly enigmatic; various proposed placements for these are shown.

encircled by prothoracic sclerites); foretibia with two apical spurs, and with a simple, sulcate antenna cleaner; mesocoxal cavity wall composed, in part, of the mesepimeron; hindcoxal cavities conjunct; male parameres setose and symmetrical (Jeannel, 1941; Ball, 1979). Many of these features are present in what are proposed to be 'basal grade' carabids, i.e. carabids branching off early along the path from the most recent common ancestor of Carabidae to Harpalinae. In contrast, Harpalinae exhibit the derived states of mandibular scrobe asetose; procoxal cavities closed; foretibia with one spur displaced proximally, antenna cleaner complex; mesepimeron removed from coxal cavity; hindcoxal cavities disjunct; male parameres asetose and markedly asymmetrical.

Some aspects of carabid phylogeny have been considered reasonably well established based on morphological data (Fig. 1). Paussinae (including *Metrius*) are considered by some authors to be the sister group of remaining carabids (Fig. 1; Beutel, 1993; Liebherr & Will, 1999). Although they possess a number of striking apomorphies (including an explosive chemical defense mechanism in adults and myrmecophilous habits with associated modifications of larval structure), they also possess features considered present in the groundplan of Carabidae. Evidence for the monophyly of remaining carabids ('Anisochaeta') is limited, consisting of a tendency of a proximal shift of one spur of the protibial antenna cleaner (Jeannel, 1941; Hlavac, 1971), characteristics of the preoral filter in larvae (Beutel, 1993) and female genitalia (Liebherr & Will, 1999). Within Anisochaeta, a number of taxa, including the supertribes Nebriitae (Nebriini, Opisthiini, Notiophilini, Notiokasiini) and Carabitae (Carabini, Pamborini, Cychrini), form an old radiation of basal lineages of carabids having, for the most part, exoskeletal characteristics considered primitive within carabids. In contrast, conjunct mesocoxae characterize a large group of carabids sometimes referred to as Carabidae Conjunctae (Fig. 1). These include both 'middle-grade' carabids, Stylifera of Jeannel (1941), and 'higher' carabids or

Harpalinae. The supertribe Trechitae is the most speciose styliferan group, containing most of the smaller carabids (Bembidiini, Trechini, Pogonini, Zolini). The predominately Gondwanan Psydrini include many forms that are very similar to members of the massive radiation of Harpalinae, but that lack a few apomorphies of the latter (including form of parameres and lack of a scrobal seta).

Some taxa do not fit easily into this view of morphological evolution of carabids, with suggested placements varying greatly from author to author (those taxa shown on the right of Fig. 1). Extensive morphological specialization in some of these lineages may have obscured evidence of relationships. Wrinkled bark beetles (Rhysodinae) live in wood, feeding on slime molds; their exoskeleton has become thick and corrugated and their mandibles highly modified (Bell, 1970; Bell, 1994). Tiger beetles (Cicindelinae) are specialized predators, with larvae adapted for life in tubes from which they ambush their prey. Other taxa may have their history hidden because of miniaturization. *Gehringia olympica,* the only member of the tribe Gehringiini, is a minute carabid which lives interstitially in coarse sand of montane creeks. Some groups (e.g. the bombardier beetles, Brachinini; *Morion*; *Cnemalobus*) have characteristics of Harpalinae, but other features that suggest relationships with particular tribes outside of Harpalinae.

The large body of literature on carabid phylogeny has failed to reach a consensus about many aspects of tribal-level relationships (see Ball, 1979; Erwin, 1979; Kavanaugh & Erwin, 1991; Bousquet & Larochelle, 1993). Detailed examination of the antenna cleaner (Hlavac, 1971; Regenfuss, 1975) and thoracic characters (e.g. Kavanaugh & Erwin, 1991) indicates that the structures are not distributed in a pattern of simple transformation from 'basal' to 'higher' carabids. Outside of Harpalinae, these traits conflict with one another, and show a great deal of homoplasy. For this reason these structures have been questioned as key indicators of phylogenetic pattern within carabids (e.g. Erwin & Stork, 1985; Kavanaugh & Erwin, 1991).

In the last few decades, new characters have been examined, ranging from traits of the exoskeleton (e.g. Bell, 1964; Bell, 1967; Hlavac, 1971; Regenfuss, 1975; Hammond, 1979; Evans, 1982; Erwin & Stork, 1985; Nichols, 1985; Deuve, 1988; Kavanaugh & Erwin, 1991; Beutel & Haas, 1996), wing venation (Ward, 1979), female genitalia (Deuve, 1993; Liebherr & Will, 1999), muscles (Bils, 1976; Baehr, 1979; Burmeister, 1980), ventral nerve cord (Heath & Evans, 1990), digestive system (Yahiro, 1990; Yahiro, 1996), defensive glands and secretions (Moore & Wallbank, 1968; Forsyth, 1972; Kanehisa & Murase, 1977; Moore, 1979; Kanehisa & Kawazu, 1985), larvae (e.g. Goulet, 1983; Bousquet & Smetana, 1986; Liebherr & Ball, 1990; Beutel, 1991; Arndt, 1993; Beutel, 1993), as well as chromosomes (Serrano, 1981a,b; Serrano & Yadav, 1984; Serrano, 1992). Although these newly studied characters have adequately addressed the placement of a few tribes, they have failed to resolve many conflicts. Some of the more enigmatic terrestrial adephagans continue to differ dramatically in their phylogenetic placement from study to study, e.g. Rhysodini as basal terrestrial adephagans (Regenfuss, 1975; Deuve, 1988) or as relatives of the scaritine or clivinine carabids (Bell, 1967; Baehr, 1979; Beutel, 1990); Gehringiini as a basal lineage of carabids (Lindroth, 1969; Beutel, 1992) or as a relative of psydrine and trechite carabids (Bell, 1967; Erwin, 1985).

It is likely that morphological data will prove more informative, once a synthesis of available data is made, and once characters are examined from all major lineages, so that a complete matrix of taxa and characters is available. Three recent studies have presented matrices of data, with associated numerical analyses on multiple tribes within carabids. Beutel & Haas (1996) tabulated states in eighty morphological characters of adults and larvae for nineteen carabid tribes and eighteen noncarabids. Their parsimony analysis resulted in a tree with relatively unresolved basal relationships within carabids, but with the few evident clades matching reasonably well traditional hypotheses. Kavanaugh (1996) examined 244 characters of adult structure across eight carabid tribes and two noncarabids, in an effort to examine the relationships of basal grade taxa centred around Nebriitae. The only numerical analysis of most carabid tribes was conducted by Liebherr & Will (1999) on one character system, female genitalia. These papers should serve as models for future work examining morphological data from many character systems across the entire family. But for the moment, the extensive data have not been gathered into a matrix and analysed.

Only recently has molecular sequence data been used to infer aspects of beetle phylogeny. These studies have either focused on levels much broader than Carabidae (Howland & Hewitt, 1995; Whiting *et al*., 1997), or have been restricted to small groups of carabids (Vogler & Desalle, 1992; Vogler & Desalle, 1994; Su *et al*., 1996a,b,c; Vogler & Pearson, 1996). Only our preliminary investigation (Maddison *et al*., 1999) has examined carabid phylogeny as a whole using molecular data. We reported on relationships of several carabid tribes using 18S rDNA sequences, concluding that Trechitae + Patrobini are monophyletic, and *Morion* and *Pseudomorpha* are members of Harpalinae. However, lack of representatives of most of the basal lineages of carabids, and absence of hydradephagan outgroups, precluded firm conclusions about most of the controversial aspects of carabid phylogeny.

In this paper, we examine evolutionary relationships of carabid tribes outside of Harpalinae using 18S rDNA. We extend the preliminary study presented in Maddison *et al.* (1999), adding sequences from eighteen additional tribes, including many whose placement is controversial. Our focus will be on monophyly of major lineages of carabids, including Trechitae and Harpalinae, monophyly of tribes such as Psydrini, Broscini, Migadopini and Elaphrini, as well as relationships of enigmatic groups such as *Gehringia*, *Pseudomorpha*, *Cnemalobus*, Brachinini, Cicindelinae, Rhysodinae, Paussinae and Scaritini.

Taxa examined

Ingroup taxa were sampled to encompass the broadest cross section of carabid lineages possible (Appendix 1). Of the

thirty-two tribes of Carabidae exclusive of Harpalinae listed by Bousquet & Larochelle (1993), we have sampled twenty-seven. The five tribes not sampled are Nototylini, Notiokasiini, Cicindini, Hiletini and Melaenini; the first three combined are known from less than twenty specimens. Sampling within Harpalinae is much less dense, but includes all tribes for which controversy exists about their membership in Harpalinae (with the exception of Peleciini, which was not sampled). We judge that the unsampled tribes of Harpalinae (e.g. Cuneipectini, Idiomorphini, Orthogoniini, Panagaeini) will not affect the deeper splits within carabids studied herein.

Some tribes are represented by more than one species. In these instances, species were chosen, when possible, to represent either side of the basal split within the group. This was most vigorously sought for those clades which have been previously considered very distinctive, long separated from remaining carabids. For example, *Metrius* is the sister group to the remaining Paussinae (Bousquet, 1986); both *Metrius* and two representatives of its sister group were chosen. Some taxa were added during the course of the study to split long branches evident in phylogenies inferred from our 18S rDNA sequences collected to that point. It was not always possible to acquire material of species from either side of the basal splits of key groups. In particular, the following important taxa are missing from the analysis: *Leoglymmius* (Rhysodinae), *Luperca and Enceladus* (Siagonini), *Loricera* (*Elliptosoma*) *wollastoni* (Loricerini), *Diacheila* (Elaphrini), Axinidiina (Promecognathini), *Axonya* or *Broscodes* (Broscini) and *Crepidogaster* (Brachinini).

Distant outgroups in the analysis include five neuropteroid sequences, at least one from each of the three orders, one myxophagan beetle and four polyphagans. These include most of the complete or nearly complete 18S rDNA sequences from these groups available in GenBank. Efforts were made to sequence an archostematan (*Priacma*), and several lineages of Hydradephaga (Gyrinidae, Amphizoidae, Haliplidae, etc.), but with limited success, because 18S rDNA was particularly difficult to sequence in these taxa. The only adephagans other than carabids sampled were *Suphis* (Noteridae) and *Copelatus* (Dytiscidae), in addition to three trachypachids, and two groups of uncertain familial status (cicindelines and rhysodines).

The analysis of 18S rDNA by Whiting *et al*. (1997) indicated that beetles might not be monophyletic, and that more appropriate outgroups for carabids might be found in archostematans (including *Priacma*) and neuropteroids. This would suggest that our lack of inclusion of *Priacma* might be a serious flaw in our taxon sampling. However, although a portion of 18S rDNA reported to be from *Priacma* is available in GenBank (Whiting *et al*., 1997), it was not included, because our preliminary analyses (not shown) indicated that most of the reported sequence was that of a carabid, not *Priacma*. A revised sequence will be submitted to GenBank (M. F. Whiting, personal communication), but it is not yet available for our analysis.

In total, sequences from fifty-five taxa reported in this paper have been added to thirty-nine sequences presented in Maddison *et al*. (1999), along with five sequences available in GenBank, for a total of ninety-nine taxa (five neuropteroids, four polyphagan beetles, one myxophagan beetle, one dytiscid, one noterid, three trachypachids and eighty-four carabids).

DNA sequence data

DNA extraction

DNA samples were prepared from individual insects by extraction of total DNA from fresh or frozen insects, or insects preserved in 95–100% ethanol or silica gel. Voucher specimens have been deposited in the Insect Collection at the University of Arizona. Total nucleic acids were extracted from part or all of the pterothorax of larger specimens (with the digestive system removed), or from the whole insect of very small specimens (*Gehringia, Pericompsus and Hydroscapha*), using one of three methods (CTAB, Lifton buffer and Lysis buffer). In the CTAB method, the tissue was homogenized in 600 µl of 2X CTAB buffer (0.1 M HCL pH 8.0, 1.4 M NaCl, 0.02 M EDTA, 2% CTAB, 0.2% b-mercaptoethanol), followed by addition of 5 µl of 20 mg/ml Proteinase K and incubation for 3 h at 37°C. In the Lifton buffer method, tissue was frozen and homogenized in liquid N_2, 800 ml of Lifton buffer (0.2 M sucrose, 50 mM EDTA, 100 mM Tris, 0.5% SDS) was added to the homogenate and incubated at room temperature for 0.5–2 h, and then incubated on ice with 100 µl of 8 M KOAc for 45 min. In the Lysis buffer method, the tissue was homogenized in 600 µl of lysis buffer (50 mM Tris, 50 mM EDTA, 2% SDS, 50 mM sucrose, 100 mM NaCl) and 5 ml 20 mg/ml Proteinase K, followed by incubation at 52°C overnight. DNA was extracted once with phenol-chloroform and once with chloroform, and then precipitated with ethanol using standard protocols.

DNA amplification

Amplifications were performed in 50 µl volume of a reaction containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3.5 mM $MgCl_2$, 10 pmol of each PCR primer, 2.5 mM dNTPs, one unit of Taq Polymerase (GibcoBRL) and 0.2 µg T4 gene 32 protein (Ambion). Primers used for amplification of all sequences except hydradephagans were 5′18S and 18L. Primer sequences and positions can be found in Table 1. These primers amplify almost all of the 18S rRNA gene, missing only about thirty-five base pairs at either end. Double stranded amplification reactions were performed on a Perkin Elmer DNA Thermal Cycler TC-1 using the following cycling parameters: 30 s at 94°C, 30 s at 56°C or 57°C and 1 min at 72°C, for 30–35 cycles.

Difficulty was encountered when attempting to amplify 18S rDNA from members of Hydradephaga. The hydradephagan sequences included in this paper were amplified and sequenced in four fragments of about 500 base pairs each using the primer pairs 5′18S/519R, 18sai/909R, 515F/18sbi and 1055F/18L; 2 µl DMSO was added to each PCR reaction to help keep the template DNA denatured. We were successful in amplifying

Table 1. Sequences of primers used in this study. The direction of the primers is either forward (F) or reverse (R).

Primer	Direction	Sequence (5′ to 3′)	Location in *Tenebrio* sequence
5′18S	F	GACAACCTGGTTGATCCTGCCAGT	1–21
20F	F	CTGGTTGATCCTGCCAG	4–20
300R	R	TCAGGCTCCCTCTCCGG	399–415
18Sai	F	CCTGAGAAACGGCTACCACATC	411–432
515F	F	GTGCCAGCMGCCGCGG	581–596
519R	R	GWATTACCGCGGCKGCTG	585–602
909R	R	GTCCTGTTCCATTATTCCAT	861–880
1055F	F	GGTGGTGCATGGCCG	1330–1344
18Sbi	R	GAGTCTCGTTCGTTATCGGA	1378–1397
760F	F	ATCAAGAACGAAAGT	1382–1396
1200F	F	CAGGTCTGTGATGCYC	1546–1561
18 L	R	CACCTACGGAAACCTTGTTACGACTT	1876–1901

and sequencing the entire 18S gene from only *Copelatus* and *Suphis* using this method.

DNA sequencing

Prior to sequencing, PCR products were purified and concentrated using Microcon-100 Microconcentrators (Amicon). Sequencing was performed by the DNA Sequencing Service, Laboratory of Molecular Systematics and Evolution, at the University of Arizona, using an ABI automated DNA sequencer. Sequencing of the entire PCR fragment in both directions used the PCR primers (5′18S and 18L) and several internal sequencing primers: 20F, 18Sai, 909R, 18Sbi and 760F. For a few species, in which these primers yielded sequence from only one direction at the 5′ and 3′ ends of the gene, the primers 300R and 1055F were also used for the other direction. Results of individual sequencing reactions were assembled and ambiguous and conflicting bases were corrected using Sequencher 3.0 (Gene Codes Corp.). The 55 DNA sequences introduced in this paper have been deposited in GenBank, accession numbers AF012471, AF012474–AF012527.

Alignment and exclusion of sites

In order to investigate the effect of alignment on the phylogenetic inference, and to allow for a more objective choice of an alignment, several different alignments were produced, most using ClustalW 1.6 (Thompson *et al*., 1996), but with one produced manually.

Eleven alignments were made with ClustalW 1.6 (Thompson *et al*., 1996), using the following as gap opening cost:gap extension cost values: 50:5, 20:5, 15:3, 12:7, 10:5, 10:2, 8:3, 7:2, 5:1, 3:2 and 3:0.5. Three additional alignments were attempted, but the cost ratios used, 100:5, 3:0.1 and 2:0.5, were extreme enough that ClustalW could not complete the alignments. The taxa were randomly reordered before each alignment.

From these eleven alignments, three were chosen using the following method to ensure objectivity, and to remove bias caused by preconceptions about relationships: (1) each matrix was subjected to a neighbour-joining analysis in PAUP*4d55 (Swofford, 1997; with an HKY85 distance measure, and with *Phaeostigma* as the outgroup); (2) the taxa in the matrix were reordered to match the order in the neighbour-joining tree (this caused adjacent taxa in the matrix to be somewhat similar, not randomized, making it easier to judge the quality of the alignment); (3) the alignment was examined in MacClade 4 (Maddison & Maddison, unpublished) and scored for several criteria measuring quality, with the taxon names hidden. The first two steps were performed by DRM, the last step by MDB; this, plus the lack of evident taxon names, and the unfamiliarity of the taxon ordering, made the judgement of the alignment independent of preconceived notions of relationship.

Each alignment was scored for the existence and magnitude of problems (obvious misalignment of blocks of ten to twenty bases; artificial 'pillars', i.e. columns of single nucleotides apparently arbitrarily chosen from each sequence, surrounded by gaps) in each of eleven regions of the sequence, concentrated around the hypervariable region boundaries. One of the alignments (designated Clustal1 herein; 10:2 gap opening:gap extension cost), had only mild problems in four regions; two others (Clustal2, 7:2; Clustal3, 10:5) had mild problems in six regions and mild or moderate problems in a seventh region. All remaining alignments had moderate or severe problems in at least three regions.

For the three matrices judged of highest quality, characters containing internal indels (insertions/deletions) of five or more contiguous gaps for any taxon were excluded from consideration (Chalwatzis *et al*., 1996). In addition, the terminal regions of alignments were excluded, in part because of the lack of data there for many sequences, and in part because of the increased risk of sequencing errors around the amplification primers. The boundaries of these excluded areas were chosen on the basis of sequence length: all sequences except *Chrysoperla* start at position 48 or before; *Clambus* and *Mecylothorax* end fifty-eight sites before the end of the

alignments. The first forty-eight and last fifty-eight sites were excluded from alignments Clustal1–Clustal3.

A fourth matrix was made by merging all eleven Clustal alignments, thus forming an 'elision' matrix (Wheeler *et al*., 1995). Some regions of 18S rDNA show the same pattern of alignment across all eleven matrices. These regions will have their signal repeated eleven times in the merged matrix. Other regions, including the hyper-variable regions, align in different ways under different gap opening:gap extension costs. Sites in these regions will has less effect on analyses conducted with the merged matrix, as the pattern of their states will not be consistent in the eleven matrices. Thus, the merged matrix will naturally downweight regions with an extensive history of insertion and deletion events. For this reason, no characters were excluded from this matrix.

Another alignment, forming the fifth matrix, was made predominately by visual inspection. This alignment is likely to be biased by our preconceived notions about relationship, but it may also lack some of the more obvious flaws found in the ClustalW alignments. The 'Eye' alignment was formed over the months during which sequences were gathered, with new sequences being added and aligned manually in MacClade. Difficult to align regions, as judged by inspection, were excluded from analysis for this matrix.

There were thus five matrices analysed: the top-ranked Clustal1 (or C1), on which most analyses were conducted; two additional Clustal alignments, Clustal2 (or C2) and Clustal3 (or C3); a data matrix formed by merging all eleven Clustal alignments, Merged11; an alignment formed by visual inspection, Eye. These alignments are available on request from D.R.M.

Methods of phylogenetic analysis

In choosing among the many available phylogenetic inference methods (for a review, see Swofford *et al*., 1996), numerous criteria might be considered (Penny *et al*., 1992; Hillis *et al*., 1994; Huelsenbeck, 1995a; Swofford *et al*., 1996): the accuracy of the method with an infinite amount of data (consistency); accuracy with a more limited amount of data (power or efficiency); range of conditions under which the method is accurate (robustness); the ability to implement the method (practicality). Any method repeatedly fails under certain circumstances; it reliably succeeds if it is appropriate for the shape of the phylogeny and mechanism of evolution being studied. For example, maximum likelihood methods are consistent, if their assumptions are met, but inconsistent if they do not appropriately take site-to-site rate variation into account (Gaut & Lewis, 1995), and they are efficient because they consider all of the data (Hillis *et al*., 1994), including characters which are ignored by parsimony methods. In some circumstances likelihood methods are robust to violation of assumptions (Huelsenbeck, 1995b), but they are often not practical because of the complexity of the calculations and subsequent lengthy time required for analyses. Parsimony methods can be inconsistent (e.g. if a few long branches are separated by relatively short branches, the long branches may incorrectly be inferred as related; Felsenstein, 1978; Huelsenbeck, 1997), but they are relatively practical, as calculations are much faster than for likelihood analyses, and they can converge on the correct answer with less data than distance methods (Hillis *et al*., 1994). Distance methods are less efficient than likelihood methods (Hillis *et al*., 1994), presumably as character information is lost in condensation to a distance matrix. They can be consistent under conditions for which parsimony is inconsistent (Huelsenbeck & Hillis, 1993; Huelsenbeck, 1995a). Distance methods (e.g. neighbour-joining) that do not entail a detailed search among alternative trees are significantly faster than parsimony searches, but ones that do involve a thorough search can be much slower (e.g. for the analyses conducted herein, least-squares distance methods are several thousand times slower than parsimony methods).

With ninety-nine 18S rDNA sequences, practicality is a prominent concern. For example, 500 searches for most parsimonious trees required 12–15 h on the fastest computers available (a Power Macintosh 9600/200 and Dell Pentium II/266). To conduct as thorough a search for the least-squares Fitch–Margoliash trees would have required approximately 14 years; for the maximum likelihood trees, more than 300 years. These methods could still be used, but only by reducing the thoroughness of the searches.

Choosing among methods based upon the other criteria is difficult, in part because of our lack of knowledge of the mechanics of evolution of 18S rDNA within beetles. This makes it troublesome to predict the behaviour of different methods for our data. In the absence of this knowledge, we would choose a method that makes maximal use of the data and attempts to avoid long branch attraction. We would prefer to conduct a thorough maximum likelihood analysis, with parameters for the evolutionary model estimated from the data, as maximum likelihood appears to be a relatively robust method (Huelsenbeck, 1995b). This would avoid problems such as parsimony's lack of consideration of multiple changes along a branch, which can lead to long branch attraction (Felsenstein, 1978; Hendy & Penny, 1989; Huelsenbeck, 1997), and the lesser efficiency of distance methods (Hillis *et al*., 1994). However, as it is impractical for us to conduct a thorough examination of our data using likelihood methods, we combine less thorough likelihood methods with more thorough distance and parsimony methods. This also allows us to use presence of a clade from multiple analytical methods as an indication of stronger support for that clade (Kim, 1993).

The top-ranked Clustal1 matrix was analysed using parsimony, distance and maximum likelihood methods. The other alignments were analysed to examine the effect of alignment on results obtained from some parsimony and distance analyses. Unless stated otherwise, all analyses discussed were conducted on the Clustal1 matrix.

Assumptions

For the parsimony, minimum evolution distance, Fitch–Margoliash distance and maximum likelihood analyses, assumptions must be specified, in order to fully define the

Table 2. -ln Likelihoods of one of the most parsimonious trees under sixteen different models, relative to the most parameter rich model, for the Clustal1 data matrix. This tree is the tree of highest likelihood (among the eight trees chosen from parsimony and preliminary distance analyses) for the most parameter rich (GTR + %I + Γ) model. The -ln L of the tree for the most parameter rich model is 21183.16. The values shown are the increase in -ln L with respect to the GTR + %I + Γ model.

Site-to-site rate variation model	Model of nucleotide change			
	JC	F81	HKY85	GTR
Equal rates	+ 5263	+ 5269	+ 4728	+ 4533
%I	+ 2613	+ 2626	+ 2064	+ 1871
Γ	+ 1117	+ 1130	+ 515	+ 333
%I + Γ	+ 815	+ 821	+ 184	0

optimality criteria used to choose trees. Parsimony analyses were conducted for all matrices assuming unordered characters (Fitch, 1971) and equal weighting of the included sites.

Preliminary distance analyses on the Clustal1 matrix used minimum evolution as the objective function, with either LogDet (Lake, 1994; Lockhart *et al*., 1994; Steel, 1994) or HKY85 (Hasegawa *et al*., 1985) distances, assuming the rate of all included characters followed a gamma distribution with shape parameter 0.5.

Maximum likelihood analyses assumed either a simple, F81 model of evolution (base frequencies matching those empirically observed, uniform rate matrix and all sites assumed to evolve at equal rate; Felsenstein, 1981), or a more complex model (base frequencies as empirically observed, symmetrical rate matrix with six rate parameters, a proportion of sites assumed to be invariable, with the remaining sites having rates following a gamma distribution). For the more complex model, the six rate matrix parameters, the proportion of invariable sites and the shape parameter of the gamma distribution were inferred from the data, on various trees (details below). The more complex model corresponds to the General Time Reversible model (Lanave *et al*., 1984; Tavaré, 1986; Rodríguez *et al*., 1990), with the addition of site-to-site variation, and will be refered to as the GTR + %I + Γ model.

The GTR + %I + Γ model was chosen over simpler models because this model fit the data notably better. The most dramatic improvements in fit between the simplest, Jukes–Cantor model and the most complex model appeared to be through the addition of more than one rate matrix parameter, and site-to-site rate variation (Table 2). A likelihood-ratio test (where the statistic is twice the difference in -ln L between two models, the degrees of freedom is the difference in number of parameters between the two models, and the test statistic is expected to be distributed as a χ^2 variable; Goldman, 1993; Yang *et al*., 1995; Sullivan & Swofford, 1997) indicates that addition of almost any parameters yields a significantly better fit. For example, the addition of four rate-matrix parameters in going from the less complex HKY85 + %I + Γ model to the more complex GTR + %I + Γ model decreases -ln L by 184 (Table 2); as the test statistic is 368, significantly greater than the critical value (at $P = 0.005$) of 14.86 for four degrees of freedom, the GTR + %I + Γ model fits the data significantly better. The only models in Table 2 that do not differ significantly in fit are the Jukes–Cantor and F81 models: addition of empirical base frequencies does not significantly improve the likelihood. There is some controversy regarding the accuracy of the χ^2 distribution for this test (Goldman, 1993; Yang *et al*., 1995; Sullivan & Swofford, 1997), but as the difference in -ln L is so much greater than the critical value that use of the more complex, better-fitting models appear worthwhile.

For all maximum likelihood analyses, the parameters determining the accuracy of likelihood scores in PAUP* were set at sdelta = 0.01 delta = 0.01. This notably decreased calculation time, and, given the large values of -ln L (over 20 000), appears unlikely to disrupt choice of optimal trees.

Further distance analyses were then conducted using the GTR + %I + Γ likelihood model as the basis of the distance measure, with parameters estimated using maximum likelihood on a most parsimonious tree, and using either minimum evolution or Fitch–Margoliash least-squares as the objective function. The parameter values used, as estimated by PAUP*, were as follows, using PAUP*′s notation (for all, basefreq = empirical rates = gamma nst = 6 was assumed): Clustal1, lset rmatrix = (0.899 2.60 1.73 0.243 4.07) shape = 0.342 pinvar = 0.495; for Clustal2, lset rmatrix = (0.982 2.72 2.10 0.260 5.02) shape = 0.530 pinvar = 0.546; for Clustal3, lset rmatrix = (0.864 2.34 1.83 0.323 3.96) shape = 0.530 pinvar = 0.525; for Eye, lset rmatrix = (1.11 3.03 2.66 0.308 4.92) shape = 0.459 pinvar = 0.429.

Searching for optimal trees and bootstrapping

For each analysis, the search for optimal trees was conducted using PAUP*4.0d55, d56 or d57 (Swofford, 1997), on a series of Power Macintosh computers, as well as one Dell Dimension XPS 266 MHz Pentium II computer. These searches attempt to discover the trees with the shortest treelength (using parsimony methods), with the lowest summed branch length (minimum evolution distance method), lowest least-squares value (Fitch–Margoliash method) or with lowest value of -ln Likelihood ('-ln L', equivalent to the trees with highest likelihood, for maximum likelihood methods). Preliminary searches were conducted in order to design a search strategy that provided an appropriate balance between thoroughness and speed. For this reason, the exact strategy varied from analysis to analysis. Some analyses were conducted with all ninety-nine taxa, others with only seventy-four taxa (the most divergent twenty-five being removed, chosen as discussed below), and a few with thirty-three (with only a few representatives of major lineages).

Parsimony. Searches for most parsimonious trees entailed multiple replicate searches with the following settings (using PAUP*'s command language for brevity): hsearch addseq = random nreps = [40–2000] nchuck = 2 chuckscore = 2 swap = tbr; hsearch start = current chuckscore = no swap = tbr. The number of replicates varied from forty (for the Merged11 matrix) to 2000 (for the Clustal1, Clustal2, Clustal3 and Eye matrices).

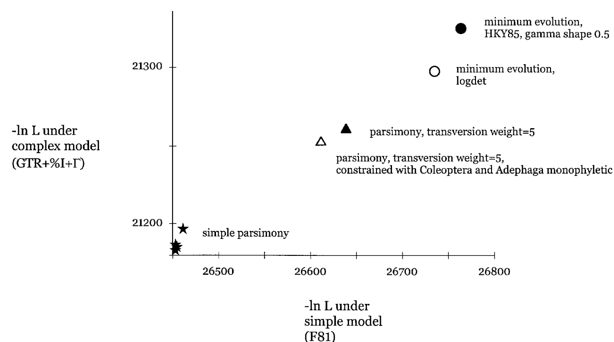

Fig. 2. Comparison of -ln Likelihood values for eight diverse trees found from analysing the Clustal1 matrix using parsimony and distance methods. The *X*-axis shows -ln L under the F81 model; the *Y*-axis shows -ln L under the more complex GTR + %I + Γ model. The trees were chosen from parsimony and distance analyses, in particular: the first and last tree found on each of the two islands of most parsimonious trees, one most parsimonious tree with transversions weighted five times transitions, one most parsimonious tree with transversions weighted five times transitions and Coleoptera and Adephaga constrained to be monophyletic; the minimum evolution tree assuming HKY 85 distances and with site to site rate variation following a gamma distribution with shape parameter 0.5; the minimum evolution tree assuming LogDet distances and no site to site rate variation.

Decay index (Bremer, 1988; Donoghue *et al*., 1992) values, and values for the number of steps added by forcing monophyly of particular groups, were calculated with less thorough searches: hsearch start = stepwise addseq = random nreps = 100 nchuck = 2 chuckscore = 2 swap = tbr enforce converse. Because some searches found most parsimonious trees in only one of the 100 replicates, the decay index values must be viewed as estimates, and may become closer to zero if more thorough searches were conducted. However, as the standard searches for most parsimonious trees are reasonably thorough, it seems likely that the decay indices of one are accurate, because for them to be less (that is, zero) would imply that the standard searches did not find all most parsimonious trees.

To calculate non-parametric bootstrap values for clades, 500 bootstrap replicates were conducted, each using a heuristic search. For analysis of all ninety-nine taxa, settings for the heuristic search were: hsearch addseq = simple nchuck = 2 chuckscore = 2 swap = spr; for analysis of seventy-four taxa, settings were: hsearch addseq = random nreps = 5 nomulpars swap = tbr.

Distance. Because of the length of time required for distance analyses, searches were much less thorough than for parsimony analyses. With minimum evolution as the optimality criterion, two analyses were conducted on the Clustal1 matrix. One began with a starting tree acquired through neighbour joining, followed by NNI branch rearrangement, SPR, and then TBR rearrangement. The other analysis involved four searches, and was of the following form: hsearch start = stepwise addseq = random nreps = 4 swap = spr; hsearch start = current swap = tbr. For the Clustal2, Clustal3 and Eye matrices, only one search was conducted, using the same procedure as for the first Clustal1 analyses. With the least-squares Fitch–Margoliash optimality criterion, the same procedure was used as for the first Clustal1 analysis, except TBR branch rearrangement was not conducted. It is not unlikely that more optimal trees exist than the ones found by these few searches.

Five hundred replicates were conducted for neighbour-joining bootstrap calculations.

Maximum likelihood. The search for maximum likelihood trees could not be conducted in a simple fashion of invoking the GTR + %I + Γ model with PAUP* conducting multiple searches under that assumption. It was not possible to use the GTR + %I + Γ model throughout the maximum likelihood analyses, as calculations involved are excessively time consuming. A suitable surrogate was sought. Eight diverse trees from parsimony and preliminary distance analyses were chosen, and -ln L was calculated for each of these under the F81 model and the GTR + %I + Γ model, with parameters estimated separately for each tree. Resulting -ln L values were correlated (Fig. 2), suggesting that seeking trees with high likelihood under the F81 model would yield trees of high likelihood under the more complex model. Because likelihood calculations for the F81 model are significantly faster than for the GTR + %I + Γ model, the F81 model was employed in early stages of likelihood analyses.

Even with this head start provided by F81 analyses, there was not enough computer time to continue analyses with estimation of GTR + %I + Γ parameters on every tree examined during a search. Instead, the parameters were estimated on a tree of high likelihood, and then fixed for all trees during searches, as suggested by Swofford *et al.* (1996). Either method of calculating likelihood results in virtually identical values for a set of test trees (Fig. 3), suggesting that the time-saving measure of fixing values for all trees has little impact on the relative likelihood of trees.

In addition, constraints were placed on the phylogeny to reduce the number of trees that PAUP* would need to consider. Two constraint trees were used. The mild constraint tree enforced monophyly of the orders, the suborder Adephaga, and taxa that appeared in all most parsimonious and minimum evolution trees (using HKY85 distances with a gamma distribution shape parameter of 0.5, as well as LogDet distances), and taxa with bootstrap values (neighbour joining and parsimony) over 95: *Trachypachus*, Cicindelinae, Rhysodinae, Paussinae, Scaritini, *Loricera*, *Antarctonomus* + *Monolobus*, Cychrini, *Siagona*, *Promecoderus* + *Creobius*, *Cymbionotum*, Zolini, the austral Psydrini and *Brachinus*. These groupings are uncontroversial, with the possible exception of two groups that are strongly supported with our data, Zolini and austral psydrines, but not with female genitalic characters (Liebherr & Will, 1999). A more severe constraint enforced these same taxa as monophyletic, plus several more: Harpalinae, Clivinini, Trechitae, Bembidiina, Patrobini, Nebriitae, Carabitae, Broscini, Elaphrini, Trachypachidae and Polyphaga, all taxa established using morphological features; *Brachinus* + *Pheropsophus*, an association within Brachinini that appeared repeatedly in other analyses of our molecular data.

Two searches for maximum likelihood trees were conducted, the first beginning from one of the most parsimonious

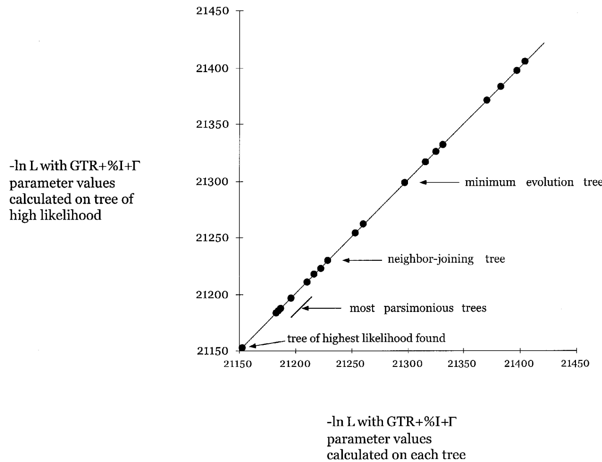

Fig. 3. Comparison of -ln L scores for nineteen trees under the GTR + %I + Γ model, with parameter values calculated on each tree as compared to those determined from one tree of high likelihood and fixed for all trees examined (these latter being the parameter values used in the search that yielded the tree of highest likelihood). Trees examined include the eight trees from Fig. 2, as well as three trees found from limited searches for minimum evolution trees, six trees found from limited searches of parsimonious trees, a neighbour-joining tree and the tree of highest likelihood found. Parameter values calculated using maximum likelihood. Diagonal line indicates line of equality of the two scores.

trees. The tree chosen as the starting point had the highest likelihood (under the GTR + %I + Γ model) from among the eight diverse trees examined in Fig. 2. Parameters for the GTR + %I + Γ model were estimated on this tree using maximum likelihood, and fixed for subsequent searching. Searching was by alternation of NNI and SPR searching with the mild constraint enforced. The search did not run to completion, and was abandoned after 4200 rearrangements.

The second search was conducted entirely without reference to trees found using other optimality criteria, and only used likelihood. This search began invoking the F81 model with likelihood as the optimality criterion, and then conducting a heuristic search under the mild constraint (hsearch enforce constraint = mild start = stepwise addseq = random nreps = 5 swap = nni; hsearch start = current swap = spr nomulpars; the second step was abandoned after 33 000 rearrangements). Parameters for the GTR + %I + Γ model were then estimated on the trees found, and fixed for a subsequent search (hsearch enforce constraint = mild start = current swap = nni nomulpars; hsearch constraint = severe start = current swap = spr; the second step was abandoned after 8200 rearrangements). The tree from this search had a better -ln L score (21158.1) than did that from the first search (21161.7).

The less likely tree from the first search was examined and found to have several differences in taxon placement relative to the tree of higher likelihood from the second search. Several rearrangements of the tree of highest likelihood were made in MacClade, reflecting the different placements seen in the less likely tree. The likelihoods of these modified trees were then calculated in PAUP*, and two rearrangements within Harpalinae + Brachinini were found to improve the likelihood: placement of *Pseudomorpha* as sister to Harpalinae rather than as sister to *Pheropsophus* + *Brachinus*; *Chlaenius* as sister to *Discoderus*. This improved the -ln L value of the tree from the second search to 21153.3. The tree was then subjected to further branch rearrangments under both the mild and severe constraints (hsearch constraint = severe start = current swap = spr; hsearch enforce constraint = mild start = current swap = nni; hsearch noenforce start = current swap = nni; hsearch enforce constraint = mild start = current swap = spr). This search was completed, and thus the tree is of relatively high likelihood, but as only one search was conducted, with mild topological constraints enforced, and only one tree saved, it may not be the maximum likelihood tree. Relationships in this tree outside of Harpalinae + Brachinini were thus determined entirely through likelihood analysis, without reference to parsimony analysis, and thus the tree serves as an independent estimate of the phylogeny. The tree of highest likelihood is preferred over the tree found by rearranging the most parsimonious tree for this reason, in addition to its higher likelihood value.

Analyses with only thirty-three taxa

Parsimony, distance and maximum likelihood analyses were conducted on thirty-three taxa from the Clustal1 matrix, in order to explore results when thoroughness of searches was not of concern. In choosing thirty-three taxa from diverse lineages, the representative of each group chosen was that taxon with the shortest distance to the root on the tree of Fig. 7. The maximum likelihood analysis was conducted as follows: a heuristic search (hsearch addseq = random nreps = 200 swap = nni) was completed under the F81 model; the parameters of the GTR + %I + Γ model were then estimated on the best trees found, and these parameter values were set for a subsequent search under the GTR + %I + Γ model (hsearch start = current swap = nni; hsearch start = current swap = spr; hsearch start = -current swap = tbr nomulpars). Minimum evolution and least-squares Fitch-Margoliash trees were found in a heuristic search with twenty replicates (hsearch addseq = random nreps = 20 swap = tbr), the parsimonious trees with 500 replicates (hsearch addseq = random nreps = 500 swap = tbr).

Simulations on model trees

In some of the inferred trees, Cicindelinae, Rhysodinae, Paussinae and Scaritini form a group (the 'CRPS quartet') near Harpalinae. In order to examine whether or not this pattern could be a result of long branch attraction (Felsenstein, 1978; Hendy & Penny, 1989; Huelsenbeck, 1997), a parametric bootstrapping simulation test (Huelsenbeck *et al.*, 1995; Hillis *et al.*, 1996; Huelsenbeck, 1997) was performed. This approach addressed the question: if the cicindelines, rhysodines, paussines and scaritines are not related to one another, and are not related to

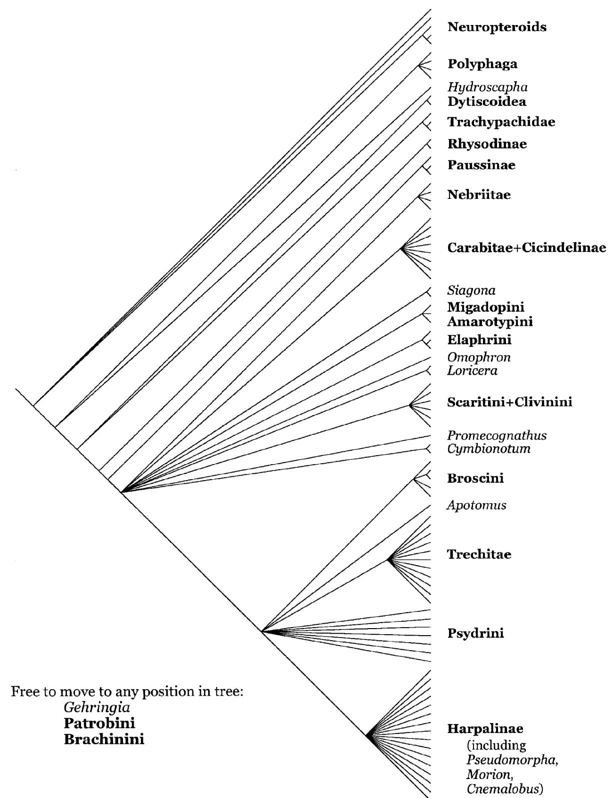

Fig. 4. Backbone constraint tree used during search for the first model tree used in parametric boostrapping simulations.

Harpalinae, but are instead in the more basal positions inferred from morphological data, then how likely would it be that we would incorrectly infer that they are related to each other and to Harpalinae?

Our null hypothesis is a tree with members of the CRPS quartet in basal positions as suggested by morphological data. In order to make predictions about the expected outcome of such a null hypothesis, we expand the hypothesis to include more details about the evolutionary process: details about relationships, branch lengths and parameters of a model of nucleotide evolution. These details are inferred from the 18S rDNA. The expected result under the null hypothesis is then determined by repeatedly simulating evolution of sequences on this model tree, using the estimated model of nucleotide evolution. Trees are inferred from the simulated matrices. If the trees inferred from the matrices simulated under the null hypothesis do not match the trees inferred using the observed 18S rDNA sequences, then the null hypothesis can be rejected. However, if the trees match the observed trees in more than 5% of the simulations, in particular with respect to the placement of members of the CRPS quartet, then the model tree cannot be rejected.

The model tree was chosen as follows. Parsimony and likelihood analyses were performed on the Clustal1 matrix forcing the set of four long branched lineages in question to be separated, and to not be near Harpalinae: rhysodines were placed as sister to Carabidae, paussines as sister to the remaining carabids, cicindelines were placed with Carabitae and scaritines with clivinines. This constrained the tree to match, in general, morphologically based hypotheses of carabid relationships. A depiction of the constraint tree is shown in Fig. 4. A search for parsimonious trees (300 random addition sequence replicates, nchuck = 2 chuckscore = 2 swap = tbr) was conducted, and one of the most parsimonious trees found (of length 3976) was used to estimate the parameters of an HKY85 + %I + Γ model using maximum likelihood estimation. A search for the maximum likelihood tree under this model was then conducted beginning with the chosen most parsimonious tree (hsearch start = current swap = nni nomulpars). Branch lengths and parameters of the HKY85 + %I + Γ model were estimated (tratio = 1.870438 pinvar = 0.286502 rates = gamma shape = 0.191643) on this tree of high likelihood.

The resulting tree with associated branch lengths (Fig. 5) was used as a model tree, and 100 simulated data matrices were made by evolving characters up the tree under the inferred HKY85 + %I + Γ model. The simulations were performed by a module written by DRM for a test version of MacClade 4 (Maddison & Maddison, unpublished), using, in part, code derived from John Huelsenbeck's siminator program (available from ftp://mw511.biol.berkeley.edu/pub/). Each data matrix had 1798 characters, the number of included characters in the Clustal1 matrix. A search for parsimonious trees (100–300 random addition sequence replicates, nchuck = 2 chuckscore = 2 swap = tbr), and a neighbour-joining analysis (using maximum likelihood distances, with the model and parameter values being those used in the simulations) were conducted for each of the 100 matrices.

A second test, with a slightly altered model tree, was conducted in the same fashion. For this second test, a tree of high likelihood for the Clustal1 matrix was found constrained to match that of Fig. 4, with the exception that *Pheropsophus* and *Brachinus* were placed with Harpalinae (*Aptinus* was excluded from the backbone constraint tree, and thus its placement was unconstrained), Clivinini's placement was unconstrained and Scaritini was placed as the sister group to Harpalinae + Brachinini. In placing Scaritini near Harpalinae (a placement that is not as strongly rejected by morphological evidence as is the placement of cicindelines, rhysodines and paussines near Harpalinae), we can test to see if Scaritini are serving as the 'magnet' that attracts the cicindelines, rhysodines and paussines to falsely group near Harpalinae. This model tree was chosen, among trees that are not too unreasonable from morphological evidence, specifically to bias the result so that long branch attraction would lead to the observed placement of the CRPS quartet near Harpalinae. Failure of CRPS to group near Harpalinae in this test would then provide stronger evidence against long branch attraction as the explanation for the observed placement.

In order to explore the role of long branch attraction in the patterns of placement of members of the CRPS quartet in these simulations, three additional tests were conducted on the

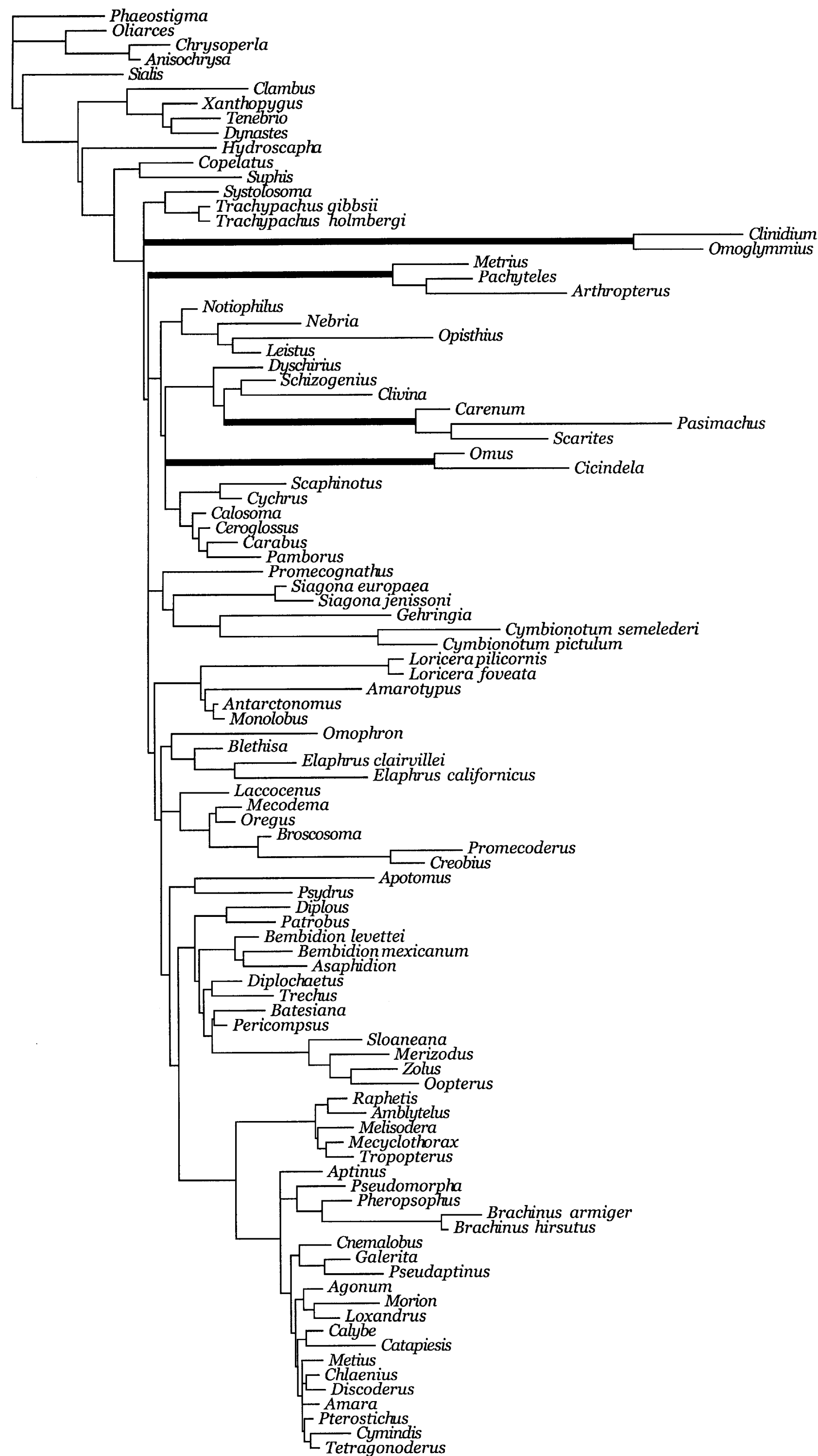

Fig. 5. The model tree used in the first parametric bootstrapping simulations. This is the tree of highest likelihood found constrained to follow the form of Fig. 4. Branch lengths inferred by maximum likelihood using an HKY85 + %I + Γ model, with parameter estimates inferred on this tree with maximum likelihood. The CRPS quartet members are indicated by thicker branches.

second model tree, but with slightly altered branch lengths. Although the estimated branch lengths below each member of the CRPS quartet ranged from 0.14 to 0.42 in the model tree, in the three additional tests they were shortened (to half or quarter their estimated length) or lengthened (to double or triple their estimated length). If grouping of members of the quartet is due to long branch attraction, then we would expect that they would be less frequently grouped when the branches were shortened, and more frequently grouped when the branches were lengthened. Trees for each simulated matrix were estimated using neighbour-joining, with maximum likelihood distances having the same model and parameter values used to simulate the data.

Analyses without long branches

In order to see where individual clades subtending long branches fall if they cannot be influenced by other very long branches, taxa subtending the longest eighteen branches in the tree of Fig. 7 were removed from the Clustal1 matrix (all those of length >0.15). Eighteen was chosen as there was a notable drop in branch length between the eighteenth and nineteenth longest branches. Seventy-four taxa remained after the exclusion. Phylogenetic trees were inferred for this smaller matrix, and then the individual clades on long branches were added. The treelengths of all possible attachment points were determined using MacClade 3.07's All Rerooting and Treelengths Chart features (Maddison & Maddison, 1997), and the most parsimonious attachment point determined.

If the long branches in the tree are artificially grouping together, and if some have several parsimonious places of attachment, then their presence in the analysis may hide the relationships among the remaining taxa by producing conflicting, equally parsimonious trees. In order to explore this possibility, the full suite of analyses was repeated for five reduced matrices (Clustal1, Clustal2, Clustal3, Eye, Merged11) of seventy-four taxa. All searches were at least as thorough as for the full ninety-nine-taxon matrix, with the exception of simple parsimony searches (for which only 500–887 replicate searches were conducted), and maximum likelihood (for which the following search was done, starting from one of the most parsimonious trees: under F81 model: hsearch enforce constraint = mild start = current swap = nni; under GTR + %I + Γ model: hsearch enforce constraint = mild start = current swap=nni; hsearch enforce constraint = mild start = current swap = spr nomulpars; this second search was abandoned after 9400 rearrangements).

Results of phylogenetic analysis

Results for the Clustal1 matrix

Parsimony. One hundred and sixty most parsimonious trees (Fig. 6) of length 3890 were found for the Clustal1 matrix, in two islands (Maddison, 1991). Each island was found seventeen to nineteen times.

Many taxa proposed using morphological evidence appear in the most parsimonious trees (Fig. 6). Coleoptera and its suborders are monophyletic, as are Dytiscoidea, Trachypachidae, Carabidae (including cicindelines, rhysodines and paussines) and several supertribes, tribes and subtribes. In addition to those labelled in Fig. 6, the following groups are monophyletic on the tree: Cychrini (*Scaphinotus* and *Cychrus*), Patrobini (*Diplous* and *Patrobus*), Bembidiina (*Bembidion* and *Asaphidion*) and Zolini (*Sloaneana* through *Oopterus*). *Amarotypus*, previously considered a member of Migadopini, but recently removed from that tribe (Erwin, 1985; Liebherr & Will, 1999), is part of the sister group to the migadopines along with *Loricera*.

There are discrepancies from morphologically based phylogenies, however. A few notable groups are paraphyletic or polyphyletic on the tree. These include Psydrini, Elaphrini, Brachinini and the Scaritini–Clivinini complex. Perhaps the most striking relationship is the presence of four lineages, Cicindelinae, Rhysodinae, Paussinae and Scaritini (the 'CRPS quartet') near Harpalinae.

Support, as indicated by decay index, is very high for some clades near the tips of the phylogeny (for example, a decay index of 20 for the austral Psydrini, 32 for Zolini, 69 for Rhysodinae), but low in the more basal branches of Carabidae, where almost all branches have a decay index of 1 (which means the clade is not present in trees just one step longer).

Maximum likelihood. The tree of highest likelihood found (-ln L of 21151.86, Fig. 7) is similar in many respects to the most parsimonious trees, in that most clades corresponding to named taxa in the most parsimonious trees are also present here. One group, Nebriitae, is not monophyletic in the likelihood tree, even though it was monophyletic in most parsimonious trees, and in the tree of second highest likelihood found. The unexpected placement of the CRPS quartet is similarly near Harpalinae. In addition, Elaphrini is monophyletic and grouped with *Laccocenus* plus a monophyletic Broscini.

Distance. One minimum evolution tree of score 2.40085 was found twice in the five searches. Many of the smaller clades that appear in minimum evolution trees are the same as from the other analyses, but the deeper structure within carabids is dissimilar, as are the details within the clade containing Harpalinae, Brachinini and the CRPS quartet. A strict consensus tree of the minimum evolution tree, the maximum likelihood tree and most parsimonious trees is shown in Fig. 8.

The Fitch–Margoliash tree, with a value of 23.20588, had several unique features: *Loricera* was not associated with the Migadopini or Amarotypini and the latter two tribes were separated from one another. *Cymbionotum* joined the austral psydrines, CRPS quartet and Harpalinae. Although this tree is not illustrated, those clades in the Fitch–Margoliash tree in common with the summary tree in Fig. 8 are indicated by a black Fitch–Margoliash box (the fourth box from the left) on the clade's branch.

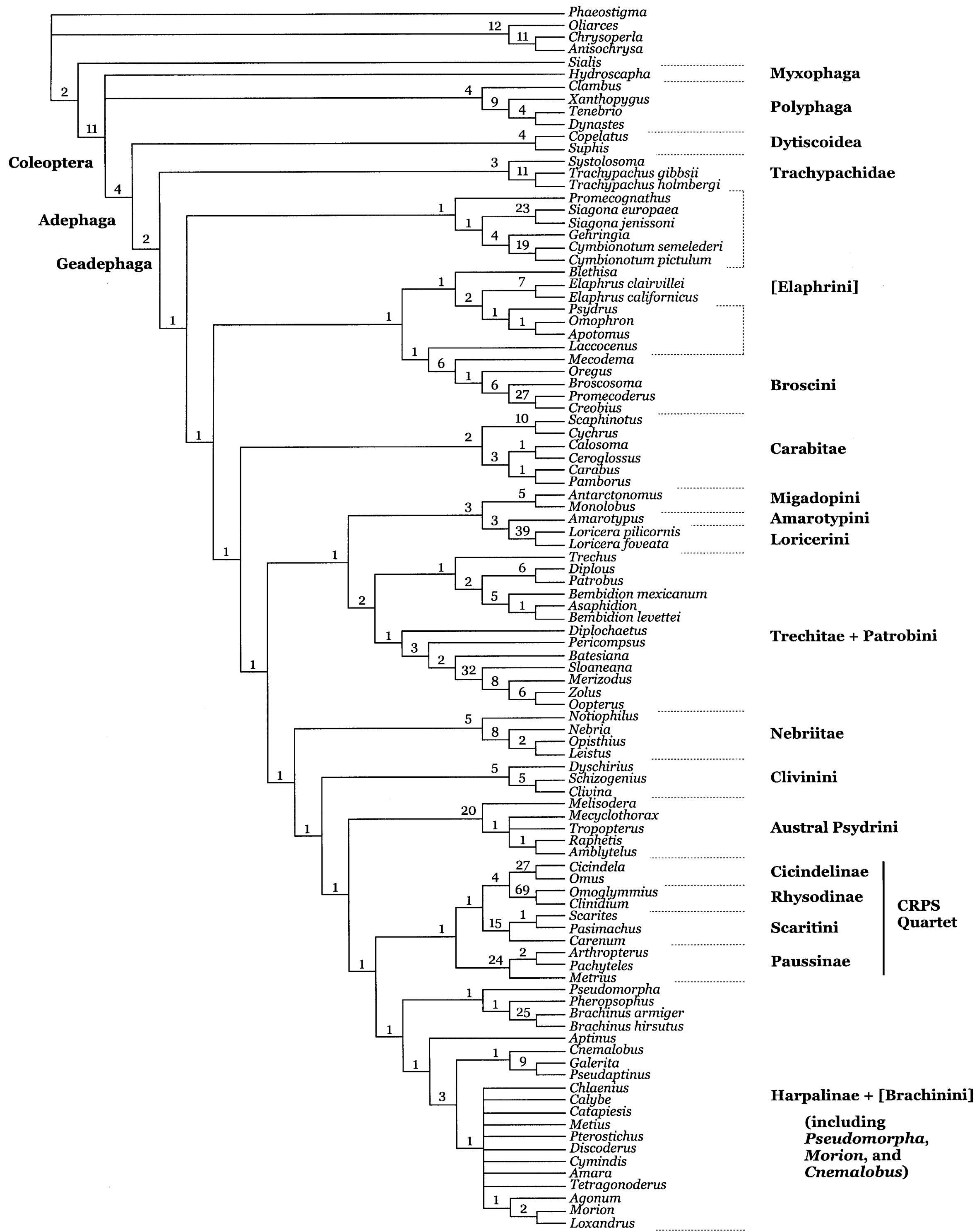

Fig. 6. Strict consensus tree of 160 most parsimonious trees for the Clustal1 matrix, length=3890. Decay index values above branch. Suprageneric taxa indicated on the right; those in brackets appear paraphyletic in this tree.

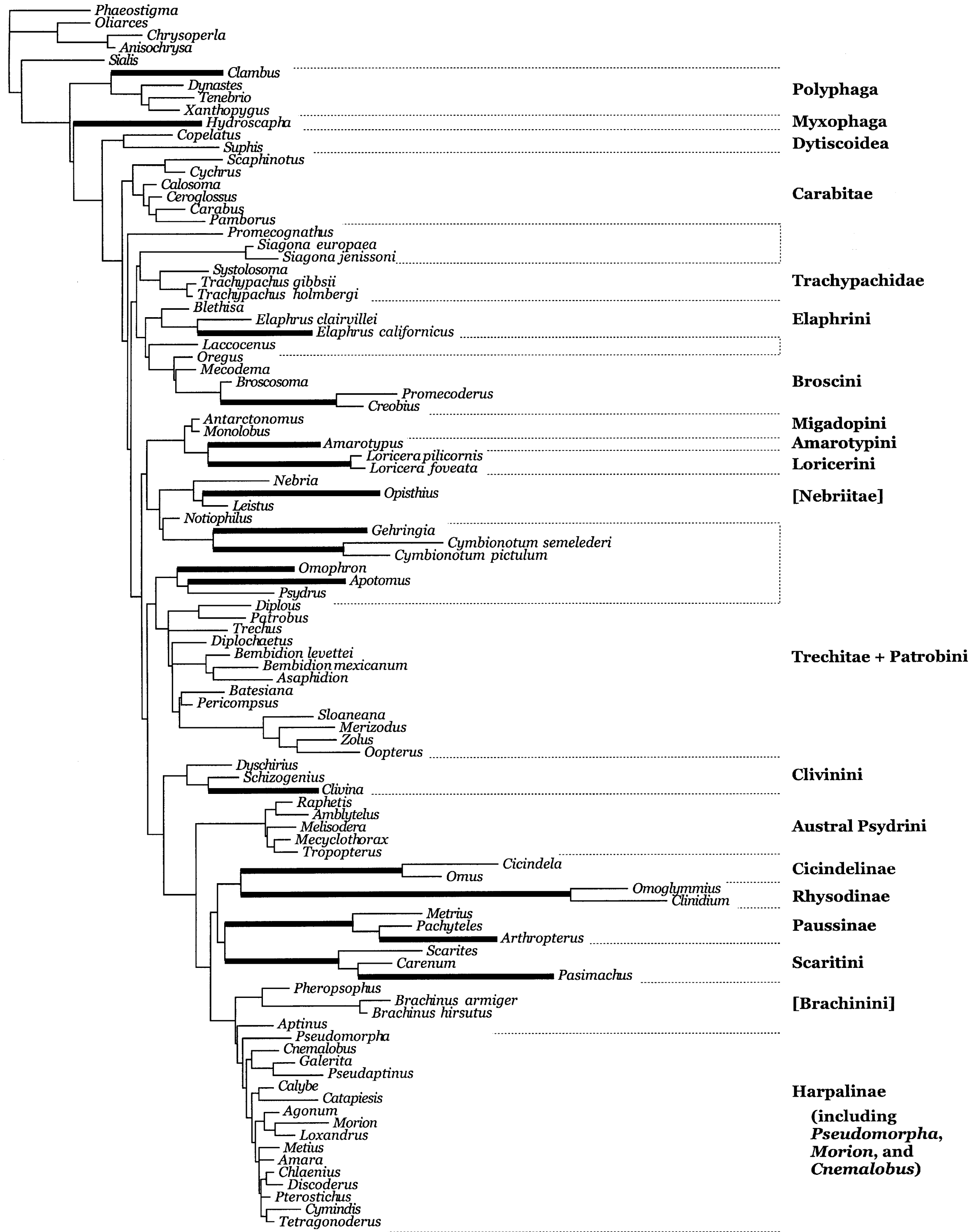

Fig. 7. Tree of highest likelihood found (-ln L of 21151.86), under GTR + %I + Γ model, for the Clustal1 matrix. The longest eighteen branches (all those of length > 0.15) are thickened. Suprageneric taxa indicated on the right; those in brackets appear paraphyletic in this tree. Coleoptera, Adephaga and Geadephaga are monophyletic in this tree.

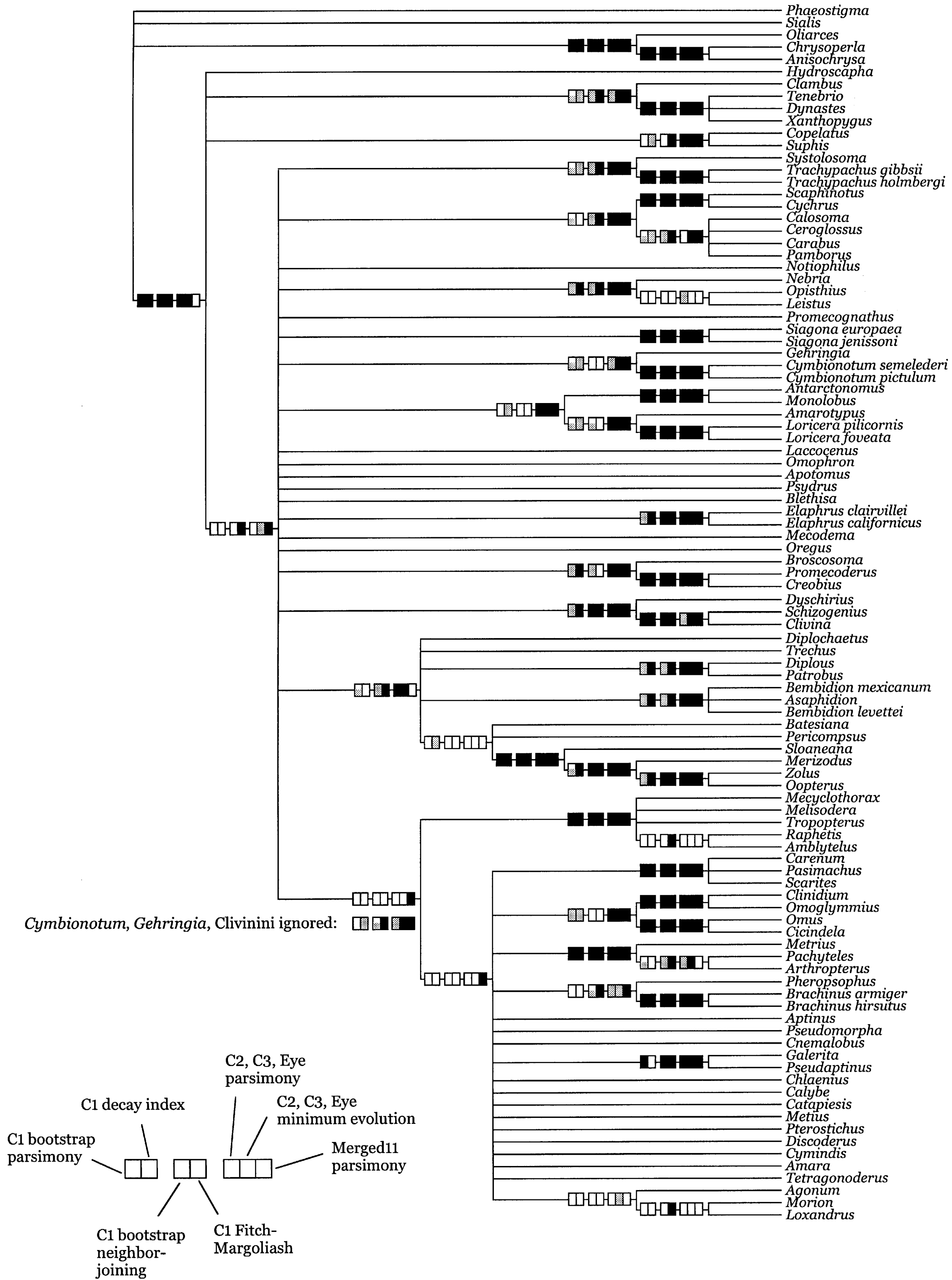

Fig. 8. See legend overleaf.

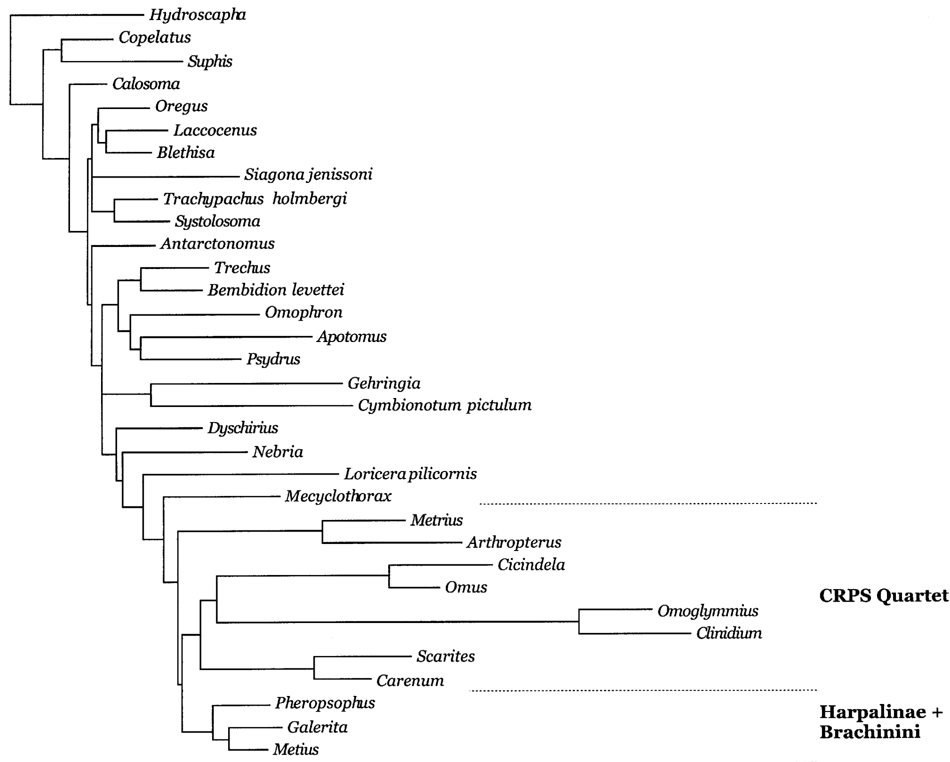

Fig. 9. A tree of highest likelihood found for the Clustal1 matrix with sixty-six taxa removed from the analysis. The other eight equally likely trees differed only in the arrangement of the two trichotomies shown in this tree.

Results for alternative alignments

In general, the clades that are found throughout the analyses of the Clustal1 matrix also appear in the analyses of the other alignments, and almost all clades that appear consistently in the analyses of other alignments are present in Fig. 8.

Parsimony. Two islands with a total of eleven trees of length 3586 were found four to nine times each for the Clustal2 matrix; one island with 120 trees of length 3983 was found eleven times for the Clustal3 matrix; two islands with a total of 2880 trees of length 3511 were found five to seven times each for the Eye matrix. Because the most parsimonious known trees were found relatively few times, it is possible that more parsimonious trees exist. These trees have many of the clades shown in Fig. 8, but not all, indicating that the results are somewhat sensitive to alignment. The Harpalinae + Brachinini + CRPS quartet + austral psydrines clade is not present in the Clustal2 and Eye trees, because *Cymbionotum*, *Gehringia* and Clivinini all move within the complex. Geadephaga is not monophyletic in the Clustal2 and Clustal3 trees, because *Copelatus* and *Suphis* move within Geadephaga as basal lineages within Carabidae.

For the Merged11 matrix, one tree of length 135036 was found twice. Coleoptera is not monophyletic in this tree, as the

Fig. 8. Strict consensus tree of the most parsimonious tree, minimum evolution tree (using distances based on a GTR + %I + Γ with parameters inferred using maximum likelihood), and the tree of highest likelihood found (under the GTR + %I + Γ model), for the Clustal1 matrix. The boxes indicate support for a branch under various analyses, with the darker the box the stronger the support, as follows. The first set of two boxes to the left of each clade illustrates measures of support as judged using parsimony analyses on the Clustal1 matrix, the second set of two boxes indicates results for two different distance analyses analyses with the Clustal1 matrix, and the last set of three boxes indicates presence of the clade under alternative alignments. Box ‘C1 bootstrap parsimony’: parsimony bootstrap values for Clustal1 matrix, half grey, 50–70; grey, 70–90; black, ⩾ 90. Box ‘C1 decay index’: decay index values for Clustal1 matrix, grey, 3–4; black, ⩾ 5. Box ‘C1 bootstrap neighbour-joining’: neighbour-joining bootstrap values for Clustal1 matrix, half grey, 50–70; grey, 70–90; black, ⩾ 90. Box ‘C1 Fitch–Margoliash’: black, clade present in Fitch–Margoliash tree of Clustal1 matrix. Box ‘C2, C3, Eye parsimony’: simple parsimony analysis of alignments Clustal2, Clustal3, grey, clade present for two of the three alignments; black, clade present for all three alignments. Box ‘C2, C3, Eye minimum evolution’: minimum evolution distance analysis of alignments Clustal2, Clustal3, grey, clade present for two of the three alignments; black, clade present for all three alignments. Box ‘Merged11 parsimony’: black, clade present in parsimony analysis of Merged11 matrix, with Coleoptera and Adephaga constrained to be monophyletic.

three Neuroptera are present deep within Carabidae, as sister to *Cymbionotum*. This might be the result of long branch attraction. Constraining Coleoptera to be monophyletic yields a tree with Adephaga not monophyletic (as *Hydroscapha* moves within it); constraining Coleoptera and Adephaga to be monophyletic results in a tree of length 135209, found only once, which shares many elements with that of Fig. 8, with two notable differences: Elaphrini (*Elaphrus* and *Blethisa*) and Nebriini (*Nebria* and *Leistus*) are each monophyletic.

Distance. In the single searches for minimum evolution trees for the other alignments, only a single tree was discovered for each matrix, of the following scores: Clustal2, 2.15992; Clustal3, 2.39625; Eye, 2.02770. These trees all showed the same pattern of having the CRPS quartet associated with Harpalinae, brachinines and austral psydrines; this is not obvious in shading of boxes in Fig. 8 because in some of the trees clivinines and the *Cymbionotum*/*Gehringia* complex are intermingled with brachinines or Harpalinae. In addition, these trees all show Elaphrini as monophyletic. Geadephaga is not monophyletic in the Clustal3 tree, because *Copelatus* and *Suphis* move within Geadephaga as basal lineages within Carabidae.

Analyses with only thirty-three taxa

Likelihood analyses of thirty-three taxa resulted in nine trees very similar in form (Fig. 9) to those from the entire matrix (Fig. 7). The single minimum evolution and Fitch–Margoliash trees were found fifteen to nineteen times; the two most parsimonious trees were found 266 times. The trees from distance and parsimony analyses showed the CRPS next to Harpalinae, although in these trees *Cymbionotum* was associated with the CRPS quartet.

Simulations on model trees

A majority rule consensus tree of the consensus trees from parsimony analysis of the data produced by each of the 100 simulations based on the model tree of Fig. 5 is shown in Fig. 10, and associations between CRPS members in the phylogenies inferred from the simulated data are shown in Table 3. Although the model tree used in the simulations had all four members of the CRPS quartet separated, most parsimonious trees for sixty-two of the 100 simulated matrices had at least two of these clades together (Table 3, first column). Eight of the simulations grouped three members (Paussinae, Cicindelinae, Rhysodinae). Each of the four clades was incorrectly grouped with at least one of the other four in at least four simulations. Oddly enough, neighbour-joining analysis of the simulated matrices shows a similar pattern (Table 3, second column), even though it incorporates the exact model used in the simulations, which should allow it to avoid long branch attraction. Placing Scaritini with Harpalinae + B-rachinini, as in the second model tree, yields similar results (Table 3, third and fourth columns).

In none of the simulated matrices from either model tree were any members of the CRPS falsely inferred to be related to Harpalinae. Even with the second model tree, in which scaritines were placed as sister to Harpalinae + Brachinini, the other CRPS members did not join scaritines near Harpalinae (although in two of the simulations scaritines moved to a more basal position to join the other CRPS members).

The longer the branch length below the CRPS clades, the more likely they are to falsely group together in the analysis of the simulated matrices (Table 3, last four columns); if the branches are shortened less than the estimated values, they have a lesser tendency to group. Both of these observations suggest that long branch attraction is at least partly responsible for the grouping seen in the simulations.

Analyses without long branches

A most parsimonious tree found for the seventy-four-taxon matrix is shown in Fig. 11, with the most parsimonious attachment point for each of the fourteen divergent branches removed from the matrix indicated by arrows. Figure 12 shows other regions in the phylogeny of Fig. 11 to which long branches can be parsimoniously placed.

Most long branches fall in the same place as in the full analysis (Fig. 6). *Gehringia*, which is placed as the sister to *Cymbionotun* and thence to *Siagona* in parsimony analysis of all taxa (Fig. 6), is most parsimoniously placed next to *Siagona*, even though *Cymbionotum* had been removed. *Loricera* also groups near where it does for the full matrix, with the migadopines, even though *Amarotypus*, its sister group in Fig. 6, has been removed. The placements of *Apotomus* and *Omophron* are near *Psydrus* in the reduced tree (Fig. 11), just as they are in the full tree (Fig. 6), although *Psydrus* has moved within Trechitae.

Four of the groups, *Elaphrus californicus*, *Promecoderus* + *Oregus*, *Opisthius* and *Clivina*, are most parsimoniously placed with other members of their respective tribes (or, for *Opisthius*, supertribe), as they were in the analysis of the full matrix (Fig. 6). For all taxa other than *Elaphrus californicus*, the branch to which they attach is not among the ten longest branches in the tree. The fact that only one parsimonious attachment point is indicated for each of these (Fig. 12) confirms their appropriate placement in the tree.

Two groups, Scaritini and *Cymbionotum*, are not placed in the same positions in the reduced tree as in the full tree. Scaritini join to the long branch below brachinines, and *Cymbionotum* moves within Harpalini.

Figure 13 summarises support for some major clades with the reduced matrices. With the CRPS quartet and other long branches removed, Harpalinae + Brachinini are very well supported (parsimony and neighbour-joining bootstrap 98 and 100, respectively, parsimony decay index 16), as is the Harpalinae + Brachinini + austral psydrine clade (94, 98 and 7). These clades are also present in the analyses of the Clustal2, Clustal3, Eye and Merged11 alignments.

Trechitae + Patrobini is also more strongly supported with the long branches removed, with one exception: the placement

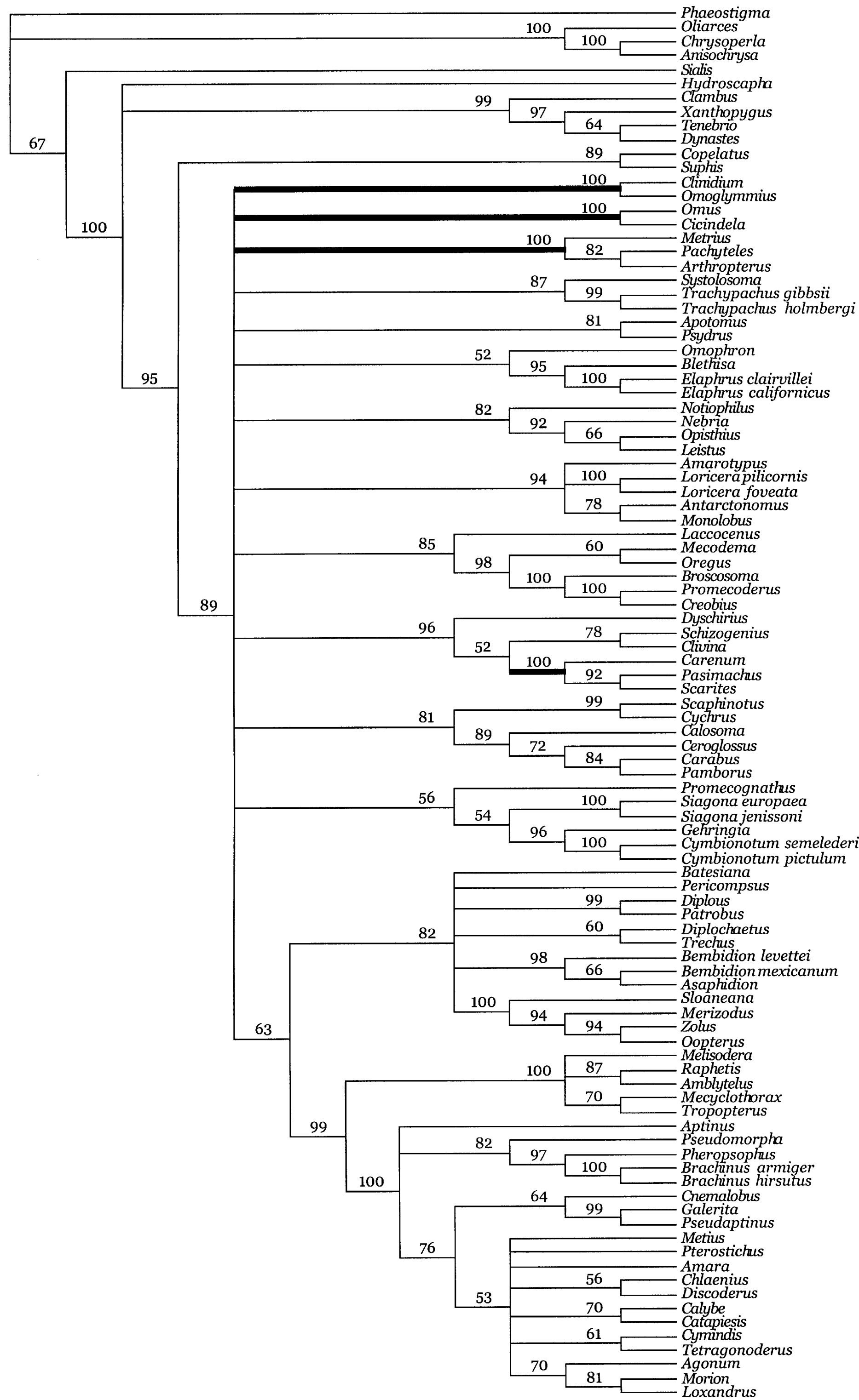

Fig. 10. Majority rule consensus tree of consensus trees from parsimony analyses from the 100 simulated matrices generated from the first model tree (Fig. 5). Numbers above the branches are the percentage of the simulated matrices for which the most parsimonious trees had that branch.

Table 3. Number of simulated matrices which yield a particular grouping of members of the CRPS quartet. A total of 100 simulations were run per column. Model Tree 1 is the tree shown in Fig. 5, with the CRPS quartet members separated, and in basal positions within Carabidae. Model Tree 2 is similar, but with Scaritini as sister to Harpalinae + Brachinini. 'nj' indicates a neighbour-joining analysis. If no value is given, no simulations showed that pattern. Each simulated matrix is counted only once.

Model tree	1		2					
Tree inference	parsimony	nj	parsimony	nj	nj	nj	nj	nj
CRPS branch lengths	estimated	estimated	estimated	estimated	quarter estimated	half estimated	double estimated	triple estimated
Monophyletic groups								
CRPS							1	3
CRP	8	6	13	12	2	4	16	21
CRS								1
CPS							1	2
RPS								4
CR	19	16	22	14	7	12	22	14
CP	9	10	8	11	6	8	16	20
CS	4						1	4
RP	22	20	21	13	6	13	18	11
RS		1	1				1	11
PS							2	2
Total	62	53	65	50	21	37	78	93

of *Psydrus*. In a number of analyses (parsimony analysis of the Clustal1 and Clustal2 matrices, minimum evolution tree for the Clustal3 matrix), *Psydrus* moves within Trechitae (Fig. 11), as sister to the patrobines. In other analyses, it is the sister to Trechitae, *Promecognathus* or Elaphrini. If the placement of *Psydrus* is ignored, the support for Trechitae + Patrobini is higher, with parsimony boostrap value of 81, neighbour-joining bootstrap of 96, and decay index of 4. Trechitae + Patrobini are also monophyletic (ignoring *Psydrus*) in the analyses of all other alignments.

The CRPS quartet: Cicindelinae, Rhysodinae, Paussinae and Scaritini

Before considering the evidence provided by 18S rDNA about relationships of individual carabid taxa, we will consider the most striking general aspect of the trees shown in Figs 6–8: placement of the lineages Rhysodinae, Cicindelinae, Paussinae and Scaritini together near Harpalinae. The first two are considered as separate families by many authors, close to Carabidae (Beutel, 1995), or even the sister group to the remaining Adephaga (Regenfuss, 1975; Bils, 1976; Deuve, 1988). Rhysodines might instead be related to Clivinini (Bell, 1967; Baehr, 1979; Beutel, 1990). Cicindelinae have also been proposed to belong to the Carabite lineage (Yahiro, 1996; Liebherr & Will, 1999), or to be sister to *Loricera* (Arndt, 1993). Paussines are regarded as the sister group to the remaining carabids (Beutel, 1993; Liebherr & Will, 1999). The placement of Scaritini has been less frequently discussed, but they have been regarded as belonging to the grade of carabids outside of Carabidae Conjunctae (Fig. 1; Jeannel, 1941). All of these lineages would be expected to be in more basal positions (i.e. more distant from Harpalinae) than seen in Figs 6–8.

An examination of Beutel & Haas's (1996) matrix indicates that placement of the CRPS quartet near Harpalinae is not parsimonious for morphological data. Constraining the CRPS quartet to be with Harpalinae yields most parsimonious trees of length 145, fourteen steps longer than the most parsimonious trees without the constraint. These longer trees all require more steps in twelve characters than do the most parsimonious trees (as judged by MacClade's Compare Two Treefiles chart), including numerous larval features and aspects of the adult thorax.

This relationship between austral psydrines, the CRPS quartet, Harpalinae and Brachinini is better supported from the 18S rDNA data than might first be apparent from inspection of Fig. 8. This group does not form a clade in many analyses, but not because the group is notably fragmented, rather it is a result of three additional taxa (*Cymbionotum*, with or without *Gehringia* as its sister, and Clivinini) moving within the group. If the placements of *Cymbionotum*, *Gehringia* and Clivinini are ignored, the austral psydrines + CRPS quartet + Harpalinae + Brachinini group is generally present (second set of boxes below the clade's branch in Fig. 8).

Three possible explanations for grouping of these four clades near Harpalinae are apparent: (1) the large number of taxa in the analysis precluded discovery of optimal trees by PAUP*, and optimal trees would have these clades separated and not near Harpalinae; (2) the long branches subtending these clades are subject to long branch attraction, causing them to artifically group together, through unrecognized convergence, and causing them to attach to the relatively long branch

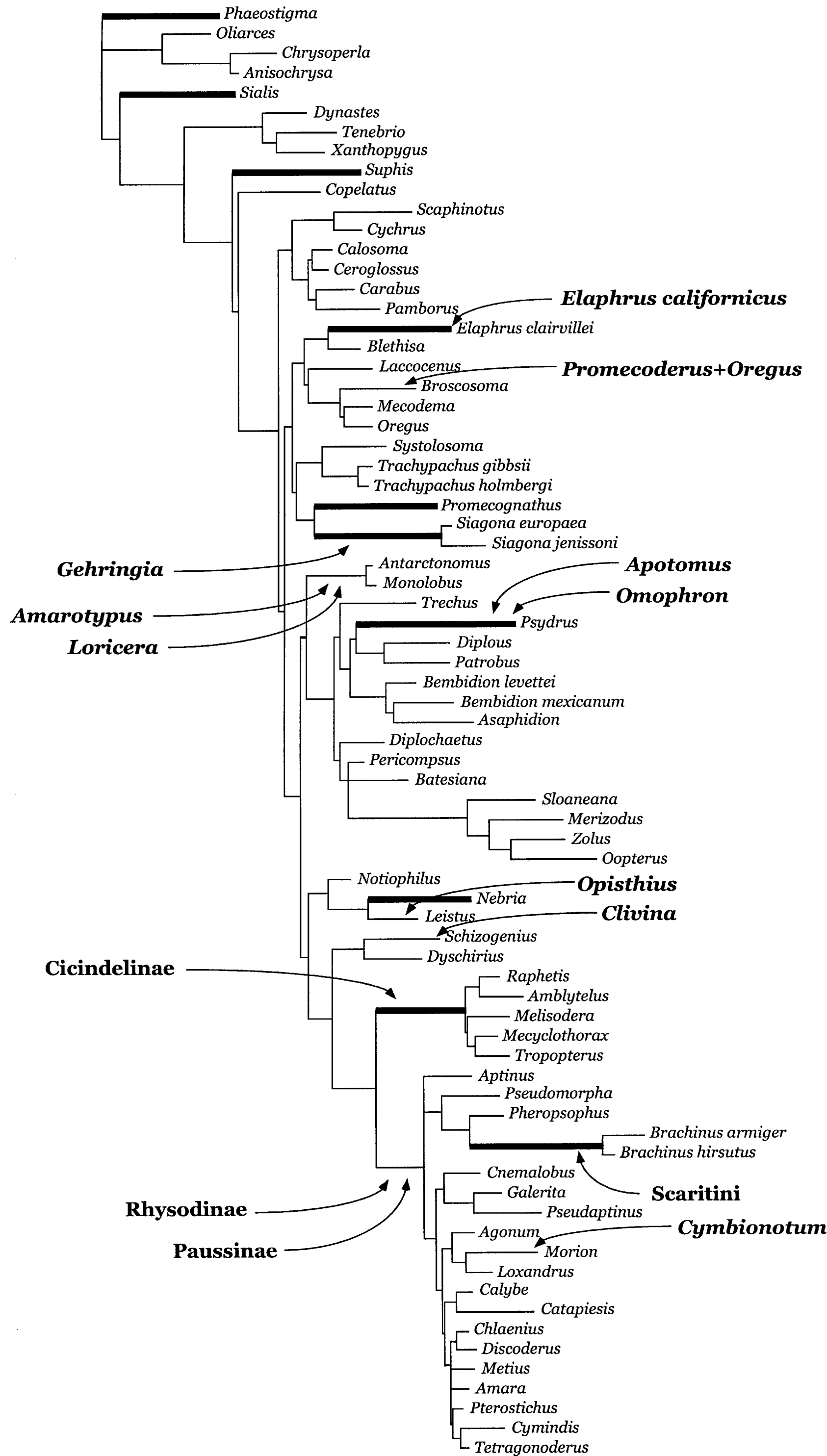

Fig. 11. One of the most parsimonious trees in which the sixteen clades subtending the longest branches in Fig. 7 were removed. The other 713 most parsimonious trees differ only in the arrangement of lineages within Harpalinae, within austral psydrines and within broscines. Branch lengths inferred by maximum likelihood using an HKY85 + %I + Γ model, with parameter estimates inferred on this tree with maximum likelihood. The longest eleven branches are indicated by thick lines; the longest two lead to *Psydrus* and *Brachinus*, the eleventh longest that leading to the austral psydrines. Each of the fourteen adephagan clades were inserted individually onto each branch on this tree, and the treelength calculated. Indicated with arrows is the most parsimonious attachment point for each of the fourteen clades. Relative treelength of other attachment points is shown in Fig. 12.

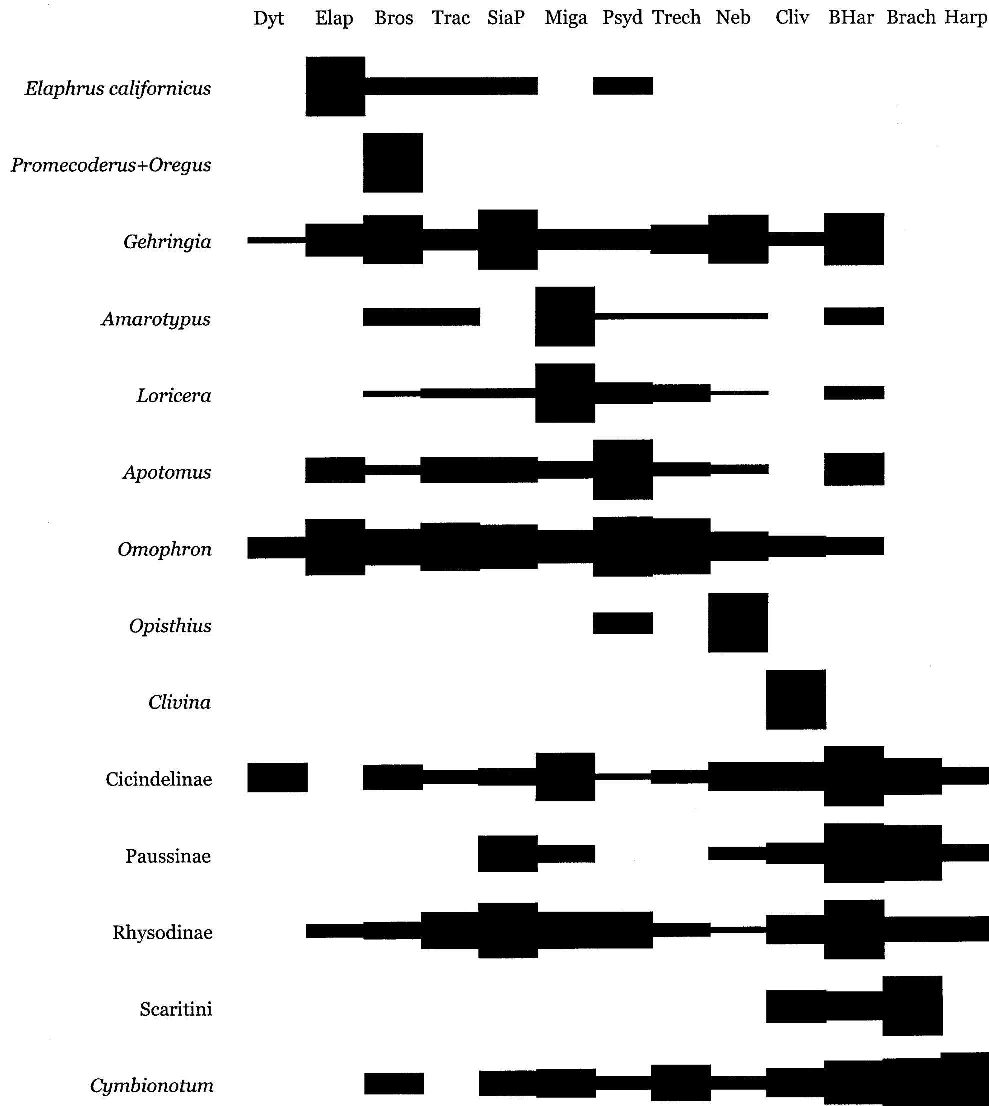

Fig. 12. Chart showing the most parsimonious attachment points for each of the fourteen adephagan clades removed for the analysis of Fig. 11. The fourteen clades are listed on the left, and the regions of the tree across the top (Dyt = Dytiscoidea; Elap = Elaphrini; Bros = Broscini; Trac = Trachypachidae; SiaP = *Siagona-Promecognathus*; Miga = Migadopini; Psyd = *Psydrus*; Trech = Trechitae exclusive of *Psydrus*; Neb = Nebriitae; Cliv = Clivinini; BHar = branch below Harpalinae + Brachinini (from *Aptinus* to *Tetragonoderus* in Fig. 11), or branch below the austral Psydrines or branch below their most recent common ancestor; Brach = *Brachinus* + *Pheropsophus*; Harp = Harpalinae, from *Cnemalobus* to *Tetragonoderus* in Fig. 11. Each of the fourteen clades was reinserted onto each branch on the tree of Fig. 11, and the treelength calculated. The height of the bar indicates the shortness of treelength if the clade is inserted into that region, using the formula: height of bar = 15 – (minimal treelength for attachment of branch in that region – treelength of most parsimonious attachment point). Thus, the most parsimonious regions of attachment have the thickest bars, the least parsimonious the thinnest bars or no bars. Attachment points fifteen or more steps longer than the most-parsimonious attachment point are ignored.

below Harpalinae; (3) these four clades truly are related to one another and to Harpalinae.

Is the matrix too large to find optimal trees?

It might be that the less-than-thorough searches required for the maximum likelihood and distance analyses prevented discovery of the optimal trees, and that, if those trees were to be found, we might discover members of the CRPS quartet separated, and in a position other than that shown in Figs 6–8. Although one might expect that the most parsimonious trees were found for the Clustal1 matrix, as fairly thorough searches were conducted, it would not be surprising if only suboptimal trees were found in the other, less thorough searches.

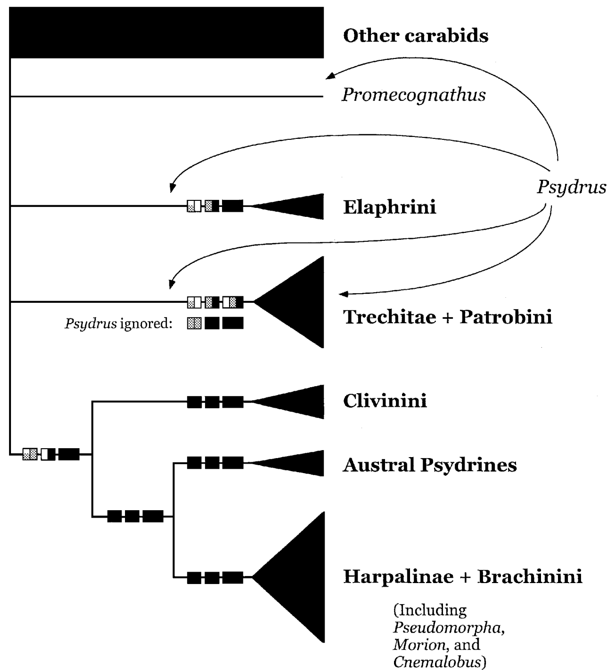

Fig. 13. Summary diagram of analyses in which the sixteen clades subtending the longest branches in Fig. 7 were removed. Symbols for branches as in Fig. 8. *Psydrus* appears in different places in different analyses.

One test of this possibility is to conduct more thorough searches on a subset of taxa, including the CRPS quartet, Harpalinae and a sampling of other diverse taxa. The CRPS quartet is present near Harpalinae in the tree with thirty-three taxa of high likelihood (Fig. 9), as it was in trees from parsimony and distance analyses, and many details of relationships match those seen in Figs 6–8. Although this result does not guarantee that the same pattern observed here and in the best trees found for the complete matrix necessarily holds for the (possibly undiscovered) optimal trees for the complete matrix, it does imply that the result is not simply due to poor searches.

The fact that independent searches using different optimality criteria (parsimony, minimum evolution, maximum likelihood) for the matrix of ninety-nine taxa all yielded the same placement of the CRPS quartet also suggests that inadequate searches are not to blame, as it is unlikely that suboptimal trees would be more similar to one another than to optimal trees.

Are long branches artificially grouping together?

It is possible that the CRPS quartet, containing some of the longest branches on the tree, has artificially formed, and artificially associated with Harpalinae due to long branch attraction. Attraction between two long branches is a result of the many changes along branches of the phylogeny that are temporally long, or that have increased rate of genetic change. The many changes on the two branches yield, by chance, a few similarities, which are incorrectly construed by the inference methods as evidence of relationship, and the method infers the branches as sisters when in fact they are unrelated and their similarities are due to convergence. Long branch attraction can be avoided if an inference method effectively estimates and eliminates those convergent similarities, by compensating for multiple substitutions in characters along a branch. Parsimony methods do not so compensate, and are therefore subject to long branch attraction. Thus, if this phenomenon is the reason for the pattern observed, we might expect to see a different pattern in the trees derived from analyses that are less sensitive to long branch attraction, such as distance and maximum likelihood methods. However, the CRPS quartet association with Harpalinae is evident even from these methods (Figs 7, 8). Although this makes long branch attraction less likely as an explanation, it is still possible, as distance and likelihood methods are also subject to long branch attraction, if the models employed do not appropriately compensate for multiple substitutions along a long branch (Gaut & Lewis, 1995; Swofford *et al.*, 1996).

Inferring parameter values for the parameter-rich $GTR + \%I + \Gamma$ from the data was our effort to make the model as realistic as possible, but it is likely that it still does not capture the full complexity of the evolutionary process. For example, the model assumes that the rate matrix is constant throughout the tree, sites are evolving independently and insertions and deletions do not occur, all assumptions that are false or probably false. Although the success of distance and likelihood methods that employ such inaccurate models has not been investigated, it is possible that they would lead to inconsistency in the methods and long branch attraction.

Expectations from model trees. The grouping of Cicindelinae and Rhysodinae, the most well supported relationship within the CRPS quartet with the observed data, was found in fourteen to twenty-two of the simulations (Table 3). Thus, the null hypothesis that cicindelines and rhysodines are separated (Fig. 5) cannot be rejected, because their grouping is not unexpected ($P = 0.14$–0.22) under the null model. These results suggest that the grouping of these clades observed in the analysis of the original matrix might be artefactual.

However, none of the most parsimonious trees for our simulated matrices showed any of these clades near Harpalinae or the austral psydrines, with ninety-nine of 100 replicates having Harpalinae + Brachinini + austral psydrines monophyletic (Fig. 10). Thus, we reject the null hypothesis reflected in our model trees, because the obtained results are unexpected under the null hypothesis. We can conclude that there is no evidence provided by the simulations that the observed placement of the CRPS quartet is artefactual.

In interpreting these results, it is important to realize that in rejecting the null hypothesis, one is not necessarily rejecting the shape of the model tree (Fig. 5). The problem may be instead with the model of evolution used in the simulations. This model, in assuming independence of sites, lack of insertion and deletion events, and so on, does not capture the full complexity of 18S rDNA evolution. Although it is hoped that inaccuracies in assumptions are not at fault, they may lead to rejection of the null hypothesis, even if the tree matches the true phylogeny.

Placement in trees without long branches. The CRPS quartet members are most parsimoniously attached to the reduced tree in the same general vicinity as in the full tree, near Harpalinae and Brachinini (Fig. 11). Only the scaritines are in a notably different location, as sister to the long branch below *Brachinus*, the second longest branch in the reduced tree, in Brachinini. Although the placement of Cicindelinae and Scaritini might be a result of long branch attraction (as they attach to the eleventh and second longest branches on the tree), those of Rhysodinae and Paussinae cannot be so easily explained, as the branch immediately below Harpalinae + Brachinini to which they attach is the forty-first longest branch in the tree. All of these groups show multiple parsimonious attachment points (Fig. 12), although scaritines cannot be parsimoniously placed outside of the Harpalinae + Brachinini complex except with Clivinini.

Relationships of particular taxa

Given the large number of controversies regarding relationships of particular carabid lineages, it is not possible to address all in the context of this paper, even if our data were relevant to all of them. Some controversial groups (e.g. *Omophron*) are no more clearly placed with our data than with morphological data. For those groups whose monophyly or relationships are addressed by our data, we provide a summary of evidence from 18S rDNA for or against alternative hypotheses.

Cicindelinae and Rhysodinae

Relationships of tiger beetles and wrinkled bark beetles have long been an enigma, in part as a result of the major modifications of body structure associated with the distinctive habits of adults and larvae. All of the previous proposals, as discussed above, place the two groups in a relatively basal position within Adephaga, and certainly not near Harpalinae. To our knowledge they have never been suggested as sister groups.

Most of our analyses of 18S rDNA place the two groups together, as sisters, near Harpalinae. Although the association of Cicindelinae and Rhysodinae might be a result of long branch attraction (see above), the parametric bootstrapping test gives no hint that their placement near Harpalinae could be a result of this. Alternative placements for Cicindelinae and Rhysodinae are less parsimonious, although placing them outside of Carabidae is more parsimonious than among the proposed basal lineages of carabids (Table 4). One difficulty with our data is the relative lack of non-carabid adephagans, which, if present, might more readily allow placement of cicindelines and rhysodines, through shortening of the non-carabid branches in the tree. The placement of cicindelines and rhysodines will be considered more thoroughly in another study (Vogler *et al*., unpublished).

Paussinae

Paussines (including ozaenines and metriines) have generally been considered a basal lineage of carabids, often as the sister group to the remaining carabids. Morphological evidence for the placement of paussines as the sister group of other carabids is not abundant. Ancestral paussines had an apparently primitive antenna cleaner (Jeannel, 1941; Hlavac, 1971), although modifications of the antennae through evolution with ants (Darlington, 1950) resulted in reduction of the antenna cleaner in most paussines (Ball & McCleve, 1990). Paussines lack some traits of the larval preoral filter (Beutel, 1993) present in other carabids, and have female genitalia more primitive in some regards than other carabids (Ball & McCleve, 1990; Liebherr & Will, 1999). When characters of the larvae and antenna cleaner are combined with other morphological data, however, they do not clearly indicate a basal position of Paussinae: Beutel & Haas's (1996) analysis of morphological data for a small collection of adephagan taxa did not suggest that paussines are the sister group of other carabids.

Our results do not support a basal placement of paussines; sixteen steps are added to the most parsimonious trees if paussines are forced to be the sister group of most geadephagans (Table 4). However, this result is not significant with a Kishino–Hasegawa test ($P = 0.12$), and the paussines do display another parsimonious attachment point, near siagonines, in the reduced tree (Fig. 12).

Nebriitae

Morphological evidence for monophyly of Nebriitae has been presented (Kavanaugh & Négre, 1985; Kavanaugh, 1996), and our data provide moderate support for this view (Table 5). Although the supertribe is not monophyletic in the tree of highest likelihood found (Fig. 7), it is present in most other analyses (Table 5).

In parsimony, maximum likelihood and minimum evolution distance analyses of the Clustal1 matrix, as well as parsimony results for other alignments, the Nebriini (*Nebria* + *Leistus*) appear non-monophyletic, as *Opisthius* is the sister to *Leistus*. This is unexpected from morphological studies (Kavanaugh, 1996). However, several analyses of the 18S rDNA data indicate monophyly of Nebriini (Table 5), leaving the issue open. No definitive statement can be made without a more thorough sampling of nebriites.

The sister-group relationship between *Notiophilus* and the remaining Nebriitae (Fig. 6) or its separation from the supertribe (Fig. 7) is contrary to some morphological evidence. Characteristics of larvae of *Notiophilus* suggest that, among the four nebriite genera examined, it should appear as the sister to *Nebria* and *Leistus* (van Emden, 1942; Bousquet & Larochelle, 1993). However, Kavanaugh's (1996) numerical analysis of adult and larval morphological data suggests that the relationship between *Notiophilus* and nebriines might not be this close, as *Notiophilus* is most parsimoniously placed as the sister to

Table 4. Extra steps added to treelength of the most parsimonious tree for the Clustal1 matrix if groups are constrained to be monophyletic, or particular placements are enforced. The second column indicates those taxa that were free to be positioned anywhere in the tree (i.e. they were excluded from a backbone constraint tree), using the following symbols: Tr = Trachypachidae; Rh = Rhysodinae; Ci = Cicindelinae; Pa = Patrobini; Ge = Gehringia; Cl = Clivinini; Br = Brachinini. Asterisks indicate those trees significantly longer than most parsimonious trees as judged by the Kishino–Hasegawa test (Kishino & Hasegawa, 1989): $*P < 0.5$; $**P < 0.001$. Under Templeton's (1983) test, the values marked ** are significant at $P < 0.001$, but only that for Morion + Scaritini is significant at $P < 0.05$. These tests are two-tailed, and thus should be conservative in this context.

Enforced monophyly or relationship	Free	Added steps	
Cicindelinae outside of Carabidae	Tr,Rh	9	
Cicindelinae + *Loricera*		16	
Cicindelinae + Carabitae		23	*
Rhysodinae sister to Adehaga	Tr,Ci	9	
Rhysodinae sister to Geadephaga	Tr,Ci	9	
Rhysodinae + Clivinini		19	
Paussinae at base	Tr,Rh, Ci, Ge	16	
Nebriini		5	
Elaphrini		2	
Elaphrini + Broscini		3	
Elaphrini + Migadopini		14	
Elaphrini + Migadopini + *Amarotypus*		20	
Migadopini + *Amarotypus*		8	
Gehringia + Trachypachidae		10	
Gehringia + Paussinae		12	
Gehringia + Psydrini	Pa	29	*
Gehringia + Trechitae	Pa	14	
Scaritini + Clivinini		10	
Promecognathus + Scaritini		22	*
Promecognathus + Clivinini		7	
Apotomus + Broscini		15	
Trechitae (not including Patrobini)		2	
Bembidiini		8	
Brachinini		6	
Brachinini + Paussinae		9	
Brachinini + Paussinae at base	Tr,Rh, Ci,Ge	49	**
Psydrini		26	*
Psydrina (*Psydrus* + *Laccocenus*)		11	
Morion + Scaritini	Cl	23	*
Cnemalobus + Scaritini	Cl	19	
Consistent with Fig. 4	Pa,Ge,Br	86	**

Opisthiini. Our data support monophyly of the *Opisthius* + *Nebria* + *Leistus* group fairly highly, to the exclusion of *Notiophilus*, with parsimony and neighbour-joining bootstrap values of 81 and 84, respectively, and a decay index of 8, for the Clustal1 matrix.

Elaphrini

Elaphrini consists of three Holarctic genera, and is generally regarded as monophyletic, with *Elaphrus* as the sister to *Blethisa* (Goulet, 1983). The lack of monophyly of elaphrines in several of our analyses is surprising, given the number of derived features of elytra, male genitalia and larvae linking the two genera (Goulet, 1983). However, 18S rDNA does not provide clear evidence against elaphrine monophyly, because only the parsimony analysis suggests non-monophyly. The remaining analyses, including those using other alignments, provide weak support of elaphrine monophyly (Table 5).

Elaphrines have been considered sister to Migadopini (including *Amarotypus*; Jeannel, 1941; Jeannel, 1942; Kryzhanovskiy, 1976), or to Migadopini exclusive of *Amarotypus* (Erwin, 1985). This hypothesis is not parsimonious for our data, as forcing Elaphrini with all three migadopine genera results in a tree twenty steps longer, and forcing it with just *Monolobus* and *Antarctonomus* adds fourteen steps.

Goulet (1983) proposed, in contrast, that elaphrines were related to broscines. Although this is more parsimonious with our data than an association with migadopines, as forcing elaphrines and broscines together adds only three steps to the treelength (Table 4), this is not supported by thoracic (Beutel, 1992) and female genitalic structures (Liebherr & Will, 1999).

Migadopini and Loricera

Erwin (1985) proposed that the migadopines (*sensu* Jeannel, 1938) are not monophyletic, with *Amarotypus* separated from the remainder, a result supported by female genitalic structure (Liebherr & Will, 1999). This conclusion is consistent with all of our analyses. No analyses support monophyly of *Amarotypus* + *Monolobus* + *Antarctonomus* (Table 5), and forcing them together adds eight extra steps in the tree (Table 4). The 18S rDNA analyses suggest that *Amarotypus* is relatively close to migadopines; however, as placement near that tribe is by far the most parsimonious placement with long branches removed (Figs 11, 12).

The placement of *Loricera* within the Migadopini + *Amarotypus* complex has not been previously proposed, but it receives relatively strong support in our analyses. Most compelling is the fact that, even with the long branch of *Amarotypus* removed, *Loricera* still is most parsimoniously placed near migadopines. Placement of *Loricera* is suggested strongly by this result, as the branch to which it attaches in the reduced tree is relatively short (the thirty-sixth longest branch in the tree of Fig. 11), and as there is no indication of any other parsimonious placement for *Loricera* (Fig. 12).

Gehringia, Siagona *and* Cymbionotum

The phylogenetic relationships of *Gehringia olympica* has always been a mystery, with proposals ranging from its being the sister group of Paussinae (R. T. Bell at 1983 Entomological Society of America meetings; Beutel, 1992), to the sister of

Table 5. Status of selected taxa in various analyses. M = those analyses in which the taxon appears monophyletic; E = those in which the clade is present only in the Eye matrix; X = those in which the clade does not appear. Unless stated otherwise, analyses are for the Clustal1 matrix. Abbreviations for taxon names are: Nebrt = Nebriitae; Nebrn = Nebriini; Elaph = Elaphrini; Migad = Migadopini; Amaro = Amarotypini; Scarit = Scaritini; Clivin = Clivinini; Brosc = Broscini; Bemb = Bembidiini; Psyd = Psydrini; Brach = Brachinini.

	Nebrt	Nebrn	Elaph	Migad + Amaro	Scarit + Clivin	Brosc	Bemb	Psyd	Brach
Parsimony	M	X	X	X	X	M	X	X	X
Maximum likelihood	X	X	M	X	X	M	X	X	X
Minimum evolution distance	M	X	M	X	X	X	X	X	X
Fitch–Margoliash distance	M	M	M	X	X	X	X	X	X
C2, C3, Eye parsimony	M	X	M	X	E	M	X	X	X
C2, C3, Eye minimum evolution	M	M	M	X	E	X	X	X	X
M11 parsimony	M	M	M	X	X	M	X	X	X
Parsimony bootstrap value	49	13	28	< 5	< 5	67	< 5	< 5	< 5
Decay index	5	−5	−2	−8	−10	6	−8	−26	−6
Neighbour-joining bootstrap	56	38	33	14	< 5	42	< 5	< 5	< 5

Trachypachidae (Lindroth, 1969; Kryzhanovskiy, 1976), to being a basal carabid (Darlington, 1933; Jeannel, 1941; Bell, 1967); to being a member of Trechitae or a relative of Psydrini (Darlington, 1933; Bell, 1967; Hammond, 1979; Erwin, 1985). All of these placements were based on relatively few morphological characters. None of these proposals are parsimonious for our data, each adding between ten and twenty-nine steps to the treelength (Table 4).

Most of our analyses on 18S rDNA suggest a sister-group relationship between *Gehringia* and *Cymbionotum* (Fig. 8), with these two groups associated with *Siagona* in at least some analyses (e.g. Fig. 7). To our knowledge, a relationship between *Gehringia* and *Cymbionotum* has not been proposed in the literature, perhaps as there appears to be no morphological evidence supporting it. However, *Cymbionotum* and *Siagona* have been considered related by some authors (Kryzhanovskiy, 1976; Erwin, 1978). Others have placed *Cymbionotum* as the sister to *Melaenus* (Erwin, 1985; Liebherr & Will, 1999), near Broscini and *Apotomus* (Erwin, 1978), based upon various morphological features. Thus, the association between *Cymbionotum* and *Siagona* in some of our analyses may not be fallacious, nor might *Gehringia's* inclusion therein. *Gehringia*'s placement with *Siagona* is also supported when the longest branches are removed from the phylogeny (Fig. 11), although there are other placements nearly as parsimonious (Fig. 12). The placement of *Cymbionotum* in analyses with the long branches removed is even less clear (Fig. 12), casting doubt on the association with *Siagona* inferred in some analyses. The addition of *Melaenus*, which could potentially split the long branch below *Cymbionotum*, might allow a test of the possibility that the placement of *Gehringia* near *Cymbionotum* is due to long branch attraction.

Scaritini, Clivinini and Promecognathus

The Scaritini and Clivinini have traditionally been considered related, because of their similar structures associated with fossorial life. The two groups have recently been treated as separate tribes, primarily on the basis of tarsal structure (Erwin, 1985), female genitalic characters (Liebherr & Will, 1999) and defensive secretions (Moore & Wallbank, 1968; Schildknecht *et al.*, 1968; Kanehisa & Murase, 1977; Moore, 1979). Their distinctiveness from one another does not of course necessarily mean they are unrelated, and some classifications show them as sister tribes (Erwin, 1985). In contrast, the analysis of Liebherr & Will (1999) suggests they are unrelated.

In our analyses, they appear related (with Scaritini being derived from within Clivinini or the two groups as sisters) only in the parsimony and distance analyses of the Eye matrix (Table 5). However, for the Clustal1 matrix, ten steps are added to the most parsimonious trees if they are forced together. Their separation in our analyses might be a result of long branch attraction between Scaritinini and the remaining members of the CRPS quartet. In fact, with long branches removed, the second most parsimonious placement of Scaritini is with Clivinini (Fig. 12).

The scaritine-like habitus of *Promecognathus* has led to its placement in Scaritini by some authors (Lindroth, 1969), a placement that is not supported by our data (Table 4). However, our data do not suggest a clear alternative.

Broscini and Apotomus

Broscines and *Apotomus* are typically considered members of Jeannel's Stylifera, a group of carabids intermediate in some respects between basal carabid lineages and Harpalinae. Styliferans are believed to be a grade, characterized by conjunct mesocoxae, styliform, setose parameres (shape changed and setae lost in Harpalinae) and presence of a seta in the mandibular scrobe (lost in Harpalinae). Within Stylifera, broscines and *Apotomus* have been considered related (Kryzhanovskiy, 1976; Erwin, 1985). Liebherr & Will (1999) note, however, that *Apotomus* does not in fact have conjunct mesocoxae, and thus their placement within Carabidae

Conjunctae (Stylifera + Harpalinae) is less likely, as is their proposed association with Broscini. This is consistent with our molecular results, because fifteen steps are added to the most parsimonious tree when *Apotomus* and Broscini are forced together (Table 4).

Although the broscine genera do not appear together in the summary tree (Fig. 8), our data do not clearly support lack of monophyly. Only distance methods suggest broscines are not monophyletic. They are monophyletic in all parsimony and maximum likelihood analyses (Table 5).

Trechitae and Patrobini

The trechites contain most of the small species of carabids. Evidence that the group is monophyletic has come from elytral setal patterns (Jeannel, 1941) and lack of chiasmata in male meiosis (Serrano, 1981b). Inclusion of Patrobini as relatives of Trechitae has received support from male tarsal structure (Müller, 1975), larval characteristics (Müller, 1975; Arndt, 1993) and female abdominal structure (Deuve, 1993). Trechitae + Patrobini are supported as monophyletic with our data, with the possible exception of *Psydrus*, which in some analyses (Figs 11, 13) moved within Trechitae + Patrobini.

Although it is most parsimonious to place patrobines within trechites for the Clustal1 matrix, forcing trechites (exclusive of patrobines) to be monophyletic adds only two steps to the tree. For the Clustal2 and Merged11 matrices the most parsimonious placement of Patrobini is as the sister group of Trechitae. This, combined with the plesiomorphic presence of chiasmatic meiosis in male Patrobini (Galián & Moore, 1994), but its loss in Trechitae, suggests that Patrobini are the sister group to Trechitae.

Within trechites, one tribe generally regarded as monophyletic appears polyphyletic, and one tribe recently proposed as polyphyletic appears clearly monophyletic. Bembidiini, principally characterized by their subulate terminal palpomeres, is separated into two or three pieces in our trees based upon 18S rDNA. Although Bembidiina is monophyletic, members of two of the other subtribes (*Pericompsus*, a member of Tachyina, and *Batesiana*, a member of Xystosomina) are not associated with them. Forcing monophyly of Bembidiini adds eight steps to the tree (Table 5). Possible polyphyly of Bembidiini will need to be examined with a denser sampling of trechites, and a second gene that is evolving at a more appropriate rate to unravel within-trechite divergences. In contrast, the Zolini are strongly supported as monophyletic. All analyses of all matrices support the monophyly of the tribe, with parsimony and neighbour-joining bootstrap values of 100, and a decay index of 32. This contrasts with Liebherr & Will's (1999) recent claim of polyphyly of zolines, based on female genitalia.

Psydrini

The Psydrini are a tribe of styliferan grade that are characterized by few distinctive features. They are effectively defined by what they are not: they do not have the derived features of other groups of Carabidae Conjunctae (Harpalinae, Trechitae, Patrobini, Broscini). Many psydrines are very similar in general form to pterostichines (a group of Harpalinae), differing most clearly by the presence of a seta in the mandibular scrobe, and in the styliform, setose parameres (van Emden, 1936; Moore, 1963; Kryzhanovskiy, 1976). However, some of the Australian forms lack setae on the parameres (e.g. *Melisodera*) or have shortened, more or less conchoid parameres (e.g. *Raphetis*) not too dissimilar in general form to some pterostichines (Moore, 1963), leaving the presence of the scrobal seta the only consistent trait excluding them from Harpalinae. One might expect therefore that psydrines are a grade, not a clade, and that members are fairly close to Harpalinae.

That Psydrini is not a clade seems likely from our data: in no analyses was Psydrini found to be monophyletic (Table 5). In fact, for the Clustal1 matrix, enforcing the monophyly of Psydrini resulted in a most parsimonious tree twenty-six steps longer than the shortest tree in which psydrines were allowed to be non-monophyletic, a result that is significant with the Kishino–Hasegawa test ($P = 0.02$). Our data thus provide strong support for the polyphyletic nature of Psydrini, consistent with studies of female genitalia (Liebherr & Will, 1999).

Female genitalic studies, however, suggest that the austral psydrines (all subtribes other than Psydrina), are themselves paraphyletic or polyphyletic, a view not in accordance with our results. In all of our analyses of all alignments, the austral psydrines are monophyletic, with parsimony and neighbour-joining bootstrap values of 100, and a decay index of 20. The only psydrines not in this clade are the two genera of Psydrina we sampled, *Psydrus* and *Laccocenus*.

Perhaps more surprising than the monophyly of the austral psydrines is the long branch below them (Fig. 7). One might expect that, given the length of the branch as judged by 18S rDNA, there would be a large number of evident morphological synapomorphies for the group, but to date these have been unreported. Perhaps more careful examination of new character systems, including the relatively unstudied larvae, could confirm the clade proposed here.

The monophyly of Psydrina itself (*Psydrus*, *Nomius*, *Laccocenus*) is not supported by our study. Although we have not sampled *Nomius* (a potentially important omission, as it could split the long *Psydrus* or *Laccocenus* branch), *Psydrus* and *Laccocenus* are not associated in any of the analyses, and to force monophyly of this pair results in trees eleven steps longer.

The placement of *Psydrus* itself is not clear from our data. In the various analyses it appears as sister to *Apotomus*, *Apotomus* + *Omophron*, *Omophron*, Elaphrini or *Omophron* + Trechitae. The most common associations are with *Apotomus* and *Omophron*. When the long branches are removed from the tree, *Psydrus* again shows no clear attachment point. In some analyses, it moves within Trechitae as sister to Patrobini, in others as sister to Trechitae as a whole, *Promecognathus* or Elaphrini (Fig. 13). Addition of *Nomius*, should it prove related to *Psydrus*, may be critical, as this may split the *Psydrus*

branch, thereby improving the chance of correctly estimating the position of the group.

Brachinini

Although the austral psydrines are more isolated based on 18S rDNA than one might expect from their lack of morphological distinctiveness, the brachinines are unexpectedly not isolated in characteristics of 18S rDNA. The morphological and chemical distinctiveness of their abdomens, associated with their explosive defensive mechanisms, has resulted in the separation of brachinines into their own subfamily by some workers (Lindroth, 1969). However, brachinines are not only similar to Harpalinae in terms of their 18S rDNA, they do not even appear as a monophyletic group. In none of the analyses are brachinines monophyletic (Table 5; as *Aptinus* is in all cases separated from *Pheropsophus* and *Brachinus*), and forcing brachinines to be monophyletic adds six steps to the most parsimonious tree for the Clustal1 matrix. One would hope that addition of some key members of Brachinini, including crepidogastrines and *Mastax*, and perhaps another species of *Aptinus*, would enable postulation of a monophyletic Brachinini.

The placement of brachinines has proved difficult, because of incompatibilities in the evidence provided by different character systems. The bulk of the evidence, from adult thorax and larval head (summarised by Beutel & Haas, 1996), and female genitalia (Liebherr & Will, 1999), suggests relationships with Harpalinae. A compelling, and apparently contradictory, hypothesis is a sister-group relationship between Brachinini and Paussinae, which is supported by the defensive system: both groups catalyse a reaction between hydrogen peroxide and hydroquinones that results in an explosive discharge of hot quinones (Aneschansley *et al*., 1969; Eisner *et al*., 1977; Roach *et al*., 1979). Our results suggest an intriguing possibility: perhaps the brachinines and paussines are related, and both are related in turn to Harpalinae. The most parsimonious trees in which brachinines and paussines are forced to form a clade are nine steps longer than the most parsimonious unconstrained trees (Table 4), and when so forced, they form the sister group to Harpalinae. Nine steps is not very much, considering that forcing *Aptinus* to join the remaining brachinines, and remove it from its prefered position as sister to Harpalinae itself costs six steps (Table 4). Although this proposal does a reasonably good job of balancing the biochemical evidence of a link between paussines and brachinines and the morphological evidence linking brachinines and harpalines, it fails in one regard: it forces one to presume reversals in the morphological features which suggest that paussines are basal carabids, sister to the rest of the family, as described above. The alternative placement of a possible brachinine plus paussine clade, as sister to most carabids, is quite unparsimonious, adding forty-nine steps to the tree (Table 4). This is mainly a result of forcing brachinines into a basal position in the tree, as forcing just paussines to be basal adds only sixteen steps. It thus seems most likely that brachinines are related to Harpalinae, and probably their sister group, whether or not paussines belong with them.

Harpalinae

When the disruptive effects of the CRPS quartet are removed, Harpalinae is well supported as a monophyletic group, in combination with Brachinini (Fig. 13). This is not surprising, considering the large number of morphological, chemical and cytogenetic features characterizing the group. In addition to the derived morphological features cited earlier, most harpalines use formic acid in their defensive secretions (Moore & Wallbank, 1968; Kanehisa & Murase, 1977; Moore, 1979; Kanehisa & Kawazu, 1985) and most have eighteen pairs of autosomes and an X chromosome in males (Serrano & Yadav, 1984; Serrano, 1992). There are, however, three lineages whose membership in Harpalinae has been the subject of debate: Pseudomorphini, Morionini and Cnemalobini.

Pseudomorphines, with their highly autapomorphic adult body structure and larval habits, due in part to their association with ants (Moore, 1974; Erwin, 1981), have generally been considered a distinctive, unplaced lineage, in some classifications receiving the rank of subfamily (Lindroth, 1969) or family (Notman, 1925). Although many features of male tarsal structure and genitalia suggest relationship with Harpalinae (Erwin, 1981), as do abdominal traits (Deuve, 1988; Deuve, 1993), defensive chemicals (Moore & Wallbank, 1968) and chromosome number (Serrano & Yadav, 1984; Serrano, 1992; Galián & Moore, 1994), their placement within that subfamily is still considered uncertain by some (Baehr, 1994). Our preliminary investigation of 18S rDNA strongly suggested placement in Harpalinae (Maddison *et al*., 1999), a result which is confirmed by the current study (Figs 8, 13).

Morionines have been considered either relatives of Scaritini, as suggested by larval similarities (van Emden, 1953; Lindroth, 1969), or, more commonly, as Harpalinae related to the pterostichines (e.g. Jeannel, 1942; Moore, 1965; Erwin, 1985; Arndt, 1993). Although 18S rDNA is not appropriate to resolve relationships within Harpalinae, and thus a specific relationship with pterostichines cannot be tested, it clearly supports inclusion of *Morion* within the subfamily. Our data reject a placement of *Morion* with scaritines (Table 4).

The Argentinian and Chilean genus *Cnemalobus* was considered by Erwin (1985) to be related to scaritines and clivinines, outside of Harpalinae, but adult abdominal structure, female genitalia, paramere shape, lack of a seta in the mandibular scrobe and larval chaetotaxy indicate relationship with Harpalinae (Arndt, 1993; Roig-Juñent, 1993; Liebherr & Will, 1999). This placement of *Cnemalobus* in Harpalinae is well supported from 18S rDNA (Figs 8, 13), as placement with scaritines added nineteen steps to the tree (Table 4).

Divergence in 18S rDNA within the large harpaline radiation is limited, as indicated by the short branch lengths within that clade in Fig. 7. This is presumably a result in

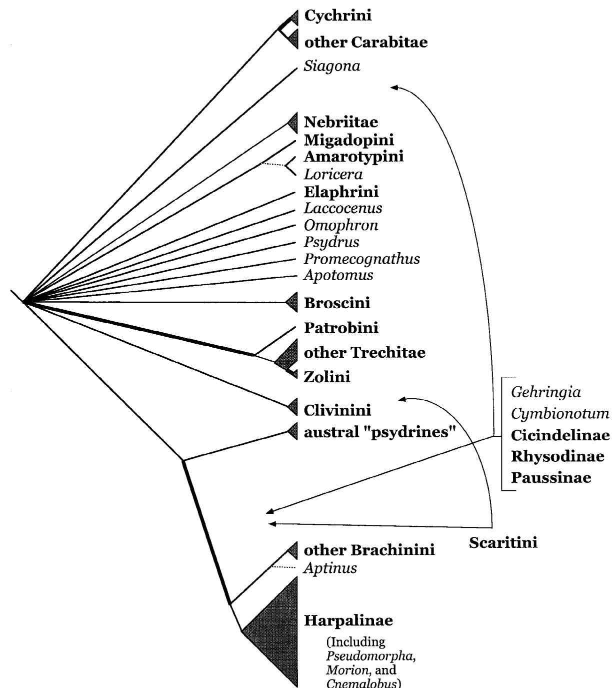

Fig. 14. Summary diagram showing results from our analyses of 18S rDNA. Some aspects of this tree (monophyly of brachinines, placement of patrobines as sister to trechites rather than within them) are based in part on morphological or cytogenetic evidence rather than our molecular data. Thicker branches are better supported with our data.

part of the relatively recent age of the clade (Ponomarenko, 1992).

The sufficiency of 18S rDNA to resolve basal carabid relationships

In conducting a phylogenetic analysis with DNA sequence data, one hopes that the gene chosen is appropriate and sufficient for determining the relationships under investigations. Although 18S rDNA clarified some basic aspects of carabid phylogeny, some of the deepest splits of interest were not resolved by our data (Fig. 8).

We can use the results from the parametric bootstrapping to see if this failure is expected. Although the postulated phylogeny used in the simulations is not likely the true phylogeny, nor does it match the most parsimonious or maximum likelihood tree found from the data, it is presumably relatively similar to the true phylogeny in terms of patterns of branch lengths. The simulated matrices consisted of sequences of equivalent length to the observed data, and were produced under a model of character evolution estimated from the data. If we cannot reliably recover basal carabid relationships from these simulated matrices, then we cannot expect to reliably recover them from the observed matrix (Hillis, 1996). Some elements of the model tree were recovered consistently in the parametric bootstrapping simulations (Fig. 10), suggesting that the amount of sequence and rate of evolution of this molecule is appropriate to recover these divergences. However, the relationships of the basal lineages of carabids were not consistently recoverable (as indicated by the large basal polytomy in Fig. 10), presumably in part because of the short internal branches on the model tree (Fig. 5). It may be that significantly more sequence data, or data from a molecule with different evolutionary properties, is needed to infer these relationships. It is also possible that this is a condemnation of the parsimony and neighbour-joining methods used in the simulation study, and perhaps consistent results would have emerged across simulations if another method of inference was used. It is also possible that if the tree had a denser sampling of taxa, the resulting model tree would have produced simulated matrices yielding more consistent results. Both of these possibilities are worthy of investigation. For the moment, we can say that a molecule of the size of 18S rDNA, that evolves according to the evolutionary model used in the parametric bootstrapping, on a tree as shown in Fig. 5, does not consistently yield data from which the deeper splits in carabids can be recovered using parsimony or neighbour-joining methods.

Conclusions and future directions

Although we might put forward as a bold hypothesis of carabid relationships one of the fully resolved trees (e.g. Fig. 7), we prefer a somewhat more conservative proposal. Figure 14 presents such a conservative view. As is evident, the relationships of basal carabids are not resolved from 18S rDNA. Nonetheless, some conclusions can be reached. In addition to support for the monophyly of various tribes and subtribes, it seems clear that brachinines are related to Harpalinae, probably as their sister group; *Cnemalobus*, *Pseudomorpha* and *Morion* all belong within Harpalinae; all psydrines other than Psydrina are related to Harpalinae + Brachinini; Trechitae is monophyletic, with Patrobini as its likely sister.

Our results also suggest fruitful avenues for future research. Cicindelinae, Rhysodinae and Paussinae, well studied in the past because of the uncertainty about their relationships, are well supported as relatives of Harpalinae. This result is surprising enough from morphological data that an explanation concerning artificial placement due to long branch attraction was explored. As no evidence was found that long branch attraction would result in the placement of these groups near Harpalinae, additional morphological and molecular evidence should be sought to critically examine this novel hypothesis. Other groups proved more enigmatic than they have in the past. Most notable among these are *Laccocenus*, *Psydrus* and *Cymbionotum*, three inadequately studied genera of carabids, which clearly warrant additional attention.

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Appendix 1. Taxa sampled.

Species	GenBank number	Locality of specimen or source of sequence
Neuroptera: Ithonidae		
Oliarces clara Banks	AF012527	USA: Arizona, Mohave Co., Lake Havasu City
Neuroptera: Chrysopidae		
Chrysoperla plorabunda (Fitch)	L10183	Carmean *et al*. (1992)
Anisochrysa carnea (Stephens)	X89482	Chalwatzis *et al*. (1996)
Raphidioptera: Raphidiidae		
Phaeostigma notata (Linné)	X89494	Chalwatzis *et al*. (1996)
Megaloptera: Sialidae		
Sialis sp.	X89497	Chalwatzis *et al*. (1996)
Polyphaga: Clambidae		
Clambus arnetti Endrödy-Younga	AF012526	USA: Arizona, Santa Cruz Co., Tumacocori
Polyphaga: Tenebrionidae		
Tenebrio molitor Linné	X07801	Hendriks *et al*. (1988)
Polyphaga: Scarabaeidae		
Dynastes granti Horn	AF002809	Maddison *et al*. (1999)
Polyphaga: Staphylinidae		
Xanthopygus cacti Horn	AF002810	Maddison *et al*. (1999)
Myxophaga: Hydroscaphidae		
Hydroscapha natans LeConte	AF012525	USA: Arizona, Santa Cruz Co., Sycamore Canyon
Adephaga: Trachypachidae		
Trachypachus holmbergi Mannerheim	AF002807	Maddison *et al*. (1999)
Trachypachus gibbsii LeConte	AF002808	Maddison *et al*. (1999)
Systolosoma lateritium Négre	AF012522	CHILE: Cautin Pr. P.N. Villarrica
Adephaga: Dytiscidae		
Copelatus chevrolati renovatus Guignot	AF012524	USA: Arizona, Santa Cruz Co., Tumacocori
Adephaga: Noteridae		
Suphis inflatus LeConte	AF012523	USA: Mississippi, George Co., Pascagoula River
Adephaga: Carabidae		
Cicindelinae		
Cicindela sedecimpunctata sedecimpuncatata Klug	AF012518	USA: Arizona, Cochise Co., 2.2 km S Willcox
Omus californicus Eschscholtz	AF012519	USA: California, Stanislaus N.F. near Strawberry
Rhysodinae		
Omoglymmius hamatus (LeConte)	AF012520	USA: California, Stanislaus N.F. near Brightman Flat Campground
Clinidium calcaratum LeConte	AF012521	USA: California, Stanislaus N.F. near Boulder Flat Campground
Paussinae		
Metrius contractus Eschscholtz	AF012515	USA: California, Marin Co., Lagunitas Creek, 0.1 miles below spillway of Nicasio Dam
Pachyteles striola species complex	AF012517	ECUADOR: Sucumbios, Reserva Faunistica Cuyabeno
Arthropterus sp.	AF012516	AUSTRALIA: Queensland, Millaa Millaa Falls
Carabini		
Carabus nemoralis O.F. Müller	AF012507	CANADA: Alberta, Edmonton
Calosoma scrutator (Fabricius)	AF002800	Maddison *et al*. (1999)
Ceroglossus chilensis Eschscholtz	AF012509	CHILE: Malleco Prov. 1 km S. of Victoria
Pamborini		
Pamborus guerinii Gory	AF012508	AUSTRALIA: Queensland, Springbrook N.P., Gwongorella
Cychrini		
Scaphinotus petersi catalinae Van Dyke	AF002801	Maddison *et al*. (1999)
Cychrus italicus Bonelli	AF012510	ITALY: Tuscany, Vallombrosa
Notiophilini		
Notiophilus semiopacus Eschscholtz	AF002804	Maddison *et al*. (1999)
Nebriini		
Nebria (Boreonebria) hudsonica LeConte	AF002805	Maddison *et al*. (1999)
Leistus ferruginosus Mannerheim	AF002806	Maddison *et al*. (1999)

Species	GenBank number	Locality of specimen or source of sequence
Opisthiini		
Opisthius richardsoni Kirby	AF012511	CANADA: Alberta, Edmonton
Siagonini		
Siagona europaea Dejean	AF012493	TURKMENISTAN: West slope of Kugitang Mont. Range, Dere-Dere stream
Siagona jenissoni Dejean	AF012494	SPAIN: Cádix, Cortijo Salomón
Loricerini		
Loricera pilicornis pilicornis (Fabricius)	AF002799	Maddison *et al*. (1999)
Loricera foveata LeConte	AF012503	USA: California, Sonoma Co., 10 miles NW Santa Rosa
Elaphrini		
Elaphrus clairvillei Kirby	AF002802	Maddison *et al*. (1999)
Elaphrus californicus Mannerheim	AF012514	CANADA: Alberta, Bow River near Vauxhall
Blethisa multipunctata aurata Fischer von Waldheim	AF002803	Maddison *et al*. (1999)
Migadopini		
Antarctonomus complanatus (Blanchard)	AF012504	CHILE: Chiloé Pr. Miraflores
Monolobus ovalipennis Straneo	AF012505	CHILE: Chiloé Pr. Quemchi
Amarotypini		
Amarotypus edwardsi Bates	AF012506	NEW ZEALAND: South Island, Southland, Mistletoe Creek at Lake Te Anau
Omophronini		
Omophron obliteratum G.H. Horn	AF012513	USA: Arizona, Gila Co., Carrizo
Gehringiini		
Gehringia olympica Darlington	AF012512	CANADA: British Columbia, Alexander Creek just W of Crowsnest Pass
Scaritini		
Pasimachus obsoletus atronitens Casey	AF002794	Maddison *et al*. (1999)
Scarites subterraneus Fabricius	AF002795	Maddison *et al*. (1999)
Carenum interruptum Macleay	AF012491	AUSTRALIA: N.S.W., 'Ooyella', Collector Hill
Clivinini		
Clivina ferrea LeConte	AF002796	Maddison *et al*. (1999)
Schizogenius falli Whitehead	AF002797	Maddison *et al*. (1999)
Dyschirius sphaericollis (Say)	AF002798	Maddison *et al*. (1999)
Promecognathini		
Promecognathus crassus LeConte	AF012492	USA: California, Marin Co., Lagunitas Creek, 0.1 miles below spillway of Nicasio Dam
Broscini		
Mecodema fulgidum Broun	AF012501	NEW ZEALAND: Canterbury, Porters Pass, Springfield
Oregus aereus White	AF012500	NEW ZEALAND: Canterbury, Porters Pass, Springfield, streamside rocks
Broscosoma relictum Weissmandl	AF012502	ITALY: Lombadia, Val di Scalve (BG)
Creobius eydouxi (Guérin-Ménéville)	AF012498	CHILE: Cautin Pr. P.N. Villarrica
Promecoderus near *brunnicornis* Dejean	AF012499	AUSTRALIA: Tasmania, Mt. Field N.P.
Cymbionotini		
Cymbionotum semelederi (Chaudoir)	AF012495	TURKMENISTAN: West slope of Kugitang Mont. Range, Dere-Dere stream
Cymbionotum pictulum H.W. Bates	AF012496	TURKMENISTAN: West slope of Kugitang Mont. Range, Dere-Dere stream
Apotomini		
Apotomus rufithorax Pecchioli	AF012497	TURKMENISTAN: West part of Kopetdag Mont. Range, Sumbar River
Patrobini		
Diplous californicus (Motschulsky)	AF002785	Maddison *et al*. (1999)
Patrobus longicornis (Say)	AF002786	Maddison *et al*. (1999)
Zolini		
Zolus helmsi Sharp	AF002787	Maddison *et al*. (1999)
Oopterus sp.	AF012488	NEW ZEALAND: South Island, Canterbury Province: Arthur's Pass National Park, Klondyke Corner, 700 m

Species	GenBank number	Locality of specimen or source of sequence
Merizodus angusticollis Solier	AF012487	CHILE: Valdivia Pr. Rincón de la Piedra
Sloaneana tasmaniae (Sloane)	AF002788	Maddison *et al*. (1999)
Pogonini		
Diplochaetus planatus G.H. Horn	AF002789	Maddison *et al*. (1999)
Bembidiini		
Pericompsus laetulus LeConte	AF002790	Maddison *et al*. (1999)
Batesiana hilaris (Bates)	AF012489	ECUADOR: Sucumbios, Reserva Faunistica Cuyabeno
Bembidion levettei carrianum Casey	AF002791	Maddison *et al*. (1999)
Bembidion mexicanum Dejean	AF012490	USA: Arizona, Cochise Co., Chiricahua Mtns., Turkey Creek
Asaphidion curtum (Heyden)	AF002792	Maddison *et al*. (1999)
Trechini		
Trechus chalybeus species group	AF002793	Maddison *et al*. (1999)
Psydrini		
Psydrus piceus LeConte	AF002784	Maddison *et al*. (1999)
Laccocenus ambiguus Sloane	AF012486	AUSTRALIA: Queensland, Springbrook
Amblytelus curtus (Fabricius)	AF012484	AUSTRALIA: New South Wales, Kosciusko N.P., Wilsons Valley
Melisodera picipennis Westwood	AF012481	AUSTRALIA: Victoria, Errinundra Plateau
Raphetis sp.	AF012485	AUSTRALIA: Queensland, Springbrook
Mecyclothorax vulcanus (Blackburn)	AF012482	USA: Hawaii, Island of Hawaii: Pu'u Makahala Natural Area
Tropopterus sp.	AF012483	CHILE: Osorno Pr. P.N. Puyehue
Brachinini		
Brachinus (Neobrachinus) hirsutus Bates	AF012478	USA: Arizona, Pima Co., Arivaca Creek near Arivaca
Brachinus (Metabrachinus) armiger Dejean	AF012479	SOUTH AFRICA: East Cape, Graaff Reinet, 32°13′S 24°30′E
Pheropsophus aequinoctialis Linné	AF012477	BOLIVIA: near Santa Cruz de la Sierra
Aptinus displosor Dufour	AF012480	SPAIN: Cádix, Cortijo Salomón
Catapieseini		
Catapiesis brasiliensis (Gray)	AF012476	ECUADOR: Napo. Res. Ethnica Waorani, Onkone Gare Station, 220 m, 00°10′ S 76°26′ W
Metiini		
Metius sp.	AF012475	CHILE: Reg. Metropolitana, road to Farellones, curve 18
Loxandrini		
Loxandrus sp.n., nr *amplithorax* Straneo	AF002778	Maddison *et al*. (1999)
Pterostichini		
Pterostichus melanarius (Illiger)	AF002779	Maddison *et al*. (1999)
Morionini		
Morion aridus Allen	AF002783	Maddison *et al*. (1999)
Cnemalobini		
Cnemalobus sulciferus Philippi	AF012474	CHILE: Talca Pr. Area de Protección Vilches
Platynini		
Agonum extensicolle (Say)	AF002775	Maddison *et al*. (1999)
Pseudomorphini		
Pseudomorpha nr *angustata* Horn	AF002782	Maddison *et al*. (1999)
Zabrini		
Amara apricaria Paykull	AF002774	Maddison *et al*. (1999)
Harpalini		
Discoderus cordicollis Horn	AF002776	Maddison *et al*. (1999)
Chlaeniini		
Chlaenius ruficauda Chaudoir	AF002777	Maddison *et al*. (1999)
Galeritini		
Galerita lecontei lecontei Dejean	AF002780	Maddison *et al*. (1999)
Zuphiini		
Pseudaptinus (Thalpius) cf. *rufulus* LeConte	AF002781	Maddison *et al*. (1999)

Species	GenBank number	Locality of specimen or source of sequence
Lachnophorini		
Calybe laetula (LeConte)	AF002772	Maddison *et al*. (1999)
Lebiini		
Cymindis (Pinacodera) punctigera LeConte	AF002773	Maddison *et al*. (1999)
Cyclosomini		
Tetragonoderus latipennis LeConte	AF012471	USA: Arizona, Pima Co., Tucson