Basal relationships of Coleoptera inferred from 18S rDNA sequences

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The basal relationships of the hyperdiverse insect order Coleoptera (beetles) have proven difficult to resolve. Examination of beetle suborder relationships using 18S ribosomal DNA reveals a previously unproposed relationship among the four major lineages: [(Archostemata(Myxophaga(Adephaga, Polyphaga)))]. Adding representatives of most other insect orders results in a non-monophyletic Coleoptera. However, constraining Coleoptera and its suborders to be monophyletic, in analyses of beetle and outgroup sequences, also results in the above beetle relationships, with the root placed between Archostemata and the remaining suborders.

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Introduction

The Coleoptera is the most diverse of all organismal lineages, with estimated numbers of species ranging into the several millions (Erwin 1982; Hammond 1992), and with representatives in nearly every conceivable non-marine habitat (Lawrence & Britton 1991). Despite its prominence and ecological importance, the basal phylogeny of the Coleoptera has not yet been convincingly resolved. The group's very diversity has proved a major obstacle to synthesis, in that establishing comprehensive character matrices for important taxa has been difficult or impossible for many character systems. Yet, resolving the basic phylogeny of the order is essential to understanding the causes and consequences of its diversification.

The Coleoptera has been split into four suborders. Of these four, the Adephaga and Polyphaga are the most prominent, containing over 99% of all beetle species. The monophyly of each group is widely accepted (although some doubts concerning that of the Polyphaga, particularly with regard to Micromalthidae [Archostemata] and Strepsiptera, are occasionally expressed; e.g. Arnett 1962; Hammond 1979). However, the distinctness of two additional groups has grown increasingly apparent. The first of these is the Archostemata which, although well represented in the fossil record, contains few modern species. These are currently referred to four families exhibiting clearly relictual distributions. Larvae of most members of this suborder are wood-boring and their monophyly is generally agreed on the basis of larval and adult characters (with the possible exception of Micromalthidae, as mentioned above). The most recently recognized suborder is the Myxophaga, also with four recent families containing mostly minute species with aquatic or semi-aquatic habits (Crowson 1955). Although many distinctive characters of Myxophaga may result from their small body size, rendering the interpretations of some characters difficult, most recent workers agree on their probable monophyly (see Beutel & Haas 2000 for a list of possible synapomorphies; but see also Barlet 1972).

Several previous studies have evaluated the phylogenetic relationships among these four major lineages, and several possible resolutions have been proposed. The most frequently cited hypothesis unites Myxophaga and Polyphaga as sister groups, with Adephaga as their sister group and Archostemata as the most basal suborder (e.g. Crowson 1960, 1975; Beutel & Haas 2000). The primary basis of this hypothesis is the reduced segmentation of the larval legs of Myxophaga and Polyphaga, and major rearrangements of the thorax and its associated musculature in Adephaga, Myxophaga and Polyphaga. In their review, Lawrence & Newton (1982) remained

neutral on relationships among suborders, but they cited possible evidence for a sister group relationship between Polyphaga and a clade comprising the other three suborders. This hypothesis was further supported by Kukalová-Peck & Lawrence (1993) on the basis of detailed studies of the beetle hind wing. The recent study by Beutel & Haas (2000) laudably brought together the largest, most diverse set of characters yet compiled for evaluating beetle phylogeny. Their support of the (Archostemata(Adephaga(Myxophaga + Polyphaga))) hypothesis therefore constitutes the strongest statement on beetle phylogeny to date.

One of the key problems in establishing a hypothesis of subordinal relationships in Coleoptera has been the uncertainty surrounding higher relationships within Holometabola in general. The most likely sister group of Coleoptera is most frequently considered to be the Neuropteroidea. Several possible synapomorphies of the two groups are given in Lawrence & Newton (1982). However, these authors also stress the paucity of data on many such characters for basal coleopteran groups. Kristensen (1991, 1999) has likewise termed the character support for this sister group relationship as '[in]conspicuous' and 'modest', although additional possible synapomorphies have recently been proposed (Hornschemeyer 1998). Kukalová-Peck & Lawrence (1993) have outlined a system of homologies of major venational features of the beetle hind wing, providing useful basic data for examining holometabolan higher relationships (useful particularly in facilitating the interpretation of many fossils). But, they primarily discuss the more controversial position of Strepsiptera with respect to Coleoptera. Their hypothesis that Strepsiptera and Coleoptera are sister groups obviously precludes the Neuropteroidea from occupying this position and would necessitate the re-examination of the polarities of many characters in, for example, Beutel & Haas's (2000) analysis. Thus, even if a convincing tree of beetle suborders was available, it would be difficult to root.

Molecular data have so far provided limited insight into the problems of either beetle relationships or those of the Holometabola as a whole. The only study to focus specifically on reconstructing beetle family relationships using molecular data (Howland & Hewitt 1995) sampled beetle diversity very sparsely and examined a marker (cytochrome oxidase I) which evolves far too rapidly for the problem, and little meaningful resolution was obtained. Recent analyses primarily examining Adephaga phylogeny using 18S ribosomal DNA (rDNA) (Maddison *et al*. 1999; Shull *et al*. 2001) have included members of most or all beetle suborders, in addition to several neuropteroid outgroups, providing intriguing glimpses of higher relationships among major beetle groups. While the results of Maddison *et al*. (1999) favoured a sister group relationship between Myxophaga and Adephaga, consistent with the hypothesis of Kukalová-Peck & Lawrence (1993), Shull *et al*. (2001) suggested a novel resolution with Adephaga and Polyphaga as sister groups. Relationships among holometabolan orders have been explored most comprehensively by Whiting *et al*. (1997) and, more recently, Wheeler *et al*. (2001). Unfortunately, due to apparent contamination problems (M. F. Whiting, unpublished results), their results with respect to beetle sister group relationships are difficult to evaluate. The most consistent and apparently well-supported result of Whiting *et al*. (1997) placed Coleoptera as sister to the remainder of the Holometabola, a possibility that has not received much attention (but see Boudreaux 1979). This is also generally supported in Wheeler *et al*. (2001), although in their analyses of 18S and combined 18S + 28S rRNA, Holometabola is paraphyletic with respect to some hemimetabolan groups.

The primary purpose of the present paper is to analyse the basal relationships in Coleoptera using 18S rDNA. Although the sampling employed in this study overlaps broadly with that of Shull *et al*. (2001), this analysis is the first to explicitly address the question of suborder relationships in detail. In addition, we have assembled existing, complete 18S sequences for all other holometabolous orders in an attempt to reassess beetle outgroup relationships.

Materials and methods

The taxa included in our analysis are listed in Table 1. We have selected the beetle taxa to conform closely to the sampling regime of Beutel & Haas (2000) in order to make comparisons of the results straightforward. Our data set lacks only two of Beutel and Haas's families: Micromalthidae and Ommatidae (Archostemata). We have also included one additional family of Polyphaga (Scirtoidea: Eucinetidae) reflecting the preliminary results of a study of polyphagan phylogeny which resolves Scirtoidea as the sister group of the remaining Polyphaga. Sequences of outgroup taxa were obtained from GenBank. Available sequences included members of all recognized holometabolan orders, as well as many hemimetabolan (Ephemeroptera, Odonata, Orthopteroidea, Hemiptera) and ametabolan (Thysanura) taxa.

The raw data for this study are complete 18S rDNA sequences. Due to difficulties with alignment as well as an evident lack of deep phylogenetic information in highly variable regions of the molecule, we decided to exclude these from the analysis at the outset. An aligned matrix was produced using Clustal w1.7 under default parameters (favouring substitutions to gaps 15-fold, which results in a relatively compact matrix). This matrix contained a total of 3431 aligned positions (the largest included sequence was *Xenos* Rossius with 3316 nucleotides). Two different exclusion sets were defined from this matrix. The first set was based only on ingroup (beetle) sequences, which in general allowed the retention of a larger set of informative and unambiguously

Table 1 The taxa and sequences used for this study. Asterisks indicate sequences newly reported in this study.

Order	Suborder	Family	Species	GenBank no.
Thysanura		Lepismatidae	*Lepisma* sp.	AF005458
Odonata		Aeshnidae	*Aeshna cyanea*	X89481
Ephemeroptera		Ephemeridae	*Ephemera* sp.	X89489
Orthoptera		Gryllidae	*Acheta domestica*	X95741
		Trigonopterygidae	*Trigonopteryx hopei*	AJ011975
		Batrachideidae	[unidentified]	Z97631
Plecoptera		Perlidae	*Mesoperlina pecircai*	U68400
Dermaptera		Forficulidae	*Forficula* sp.	X89490
Hemiptera		Cicadidae	*Okanagana utahensis*	U06478
		Peloridiidae	*Hemiowoodwardia wilsoni*	AF131198
			Hackeriella veitchi	AF004766
		Delphacidae	*Prokelisia marginata*	U09207
		Aphrophoridae (Cercopoidea)	*Philaenus spumarius*	U06480
		Membracidae	*Spissistilus festinus*	U06477
		Miridae	*Lygus hesperus*	U06476
		Pentatomidae	*Rhaphigaster nebulosa*	X89495
Megaloptera		Sialidae	*Sialis* sp.	X89497
Raphidioptera		Raphidiidae	*Phaeostigma notata*	X89494
Neuroptera		Ithonidae	*Oliarces clara*	AF012527
		Chrysopidae	*Anisochrysa plorabunda*	X89482
Mecoptera		Boreidae	*Boreus* sp.	X89487
		Panorpidae	*Panorpa germanica*	X89493
Siphonaptera		Pulicidae	*Archaeopsylla erinacei*	X89486
Diptera	Nematocera	Tipulidae	*Tipula* sp.	X89496
		Culicidae	*Anopheles nr. punctulatus*	AF121063
		Psychodidae	*Phlebotomus papatasi*	AJ244414
		Tabanidae	*Chrysops niger*	AF073889
		Tephritidae	*Ceratitus capitata*	AF096450
		Drosophilidae	*Drosophila melanogaster*	X15707
		Hippoboscidae	*Ornithoica vicina*	AF073888
Hymenoptera		Vespidae	*Polistes dominulus*	X77785
		Braconidae	*Protaphidius wissmannii*	AJ009348
			Aphidius salicis	AJ009326
			Ephedrus persicae	AJ009329
			Trioxys angelicae	AJ009349
		Formicidae	*Leptothorax acervorum*	X89492
Trichoptera		Hydropsychidae	*Hydropsyche* sp.	X89483
		Brachycentridae	*Brachycentrus nigrosoma*	AF136860; AF136880
Lepidoptera		Micropterigidae	*Micropterix calthella*	AF136863; AF136894
		Pyralidae	*Galleria mellonella*	X89491
		Lymantriidae	*Lymantria dispar*	AF136872; AF136892
		Prodoxidae	*Prodoxus quinquepunctellus*	AF136868; AF136888
		Agathiphagidae	*Agathiphaga queenslandensis*	AF136864; AF136884
Strepsiptera		Mengenillidae	*Mengenilla chobauti*	X89441
		Stylopidae	*Stylops melittae*	X89440
		Stylopidae	*Xenos vesparum*	X74763
Coleoptera	Archostemata	Cupedidae	*Distocupes* sp.	AF201420
	Myxophaga	Hydroscaphidae	*Hydroscapha natans*	AF012525
		Microsporidae	*Microsporus* sp.	AF427599*
		Torridincolidae	*Torridincola rhodesica*	AF201420
	Adephaga	Trachypachidae	*Trachypachus gibbsi*	AF002808
		Hygrobiidae	*Hygrobia hermanni*	AF201414
		Amphizoidae	*Amphizoa lecontei*	AJ318678
		Noteridae	*Hydrocanthus oblongus*	AF201415
		Haliplidae	*Haliplus ruficollis*	AF201416
		Gyrinidae	*Spanglerogyrus albiventris*	AF201413
		Gyrinidae	*Gyretes iricolor*	AJ318662/3
		Dytiscidae	*Hydaticus consanguineus*	AJ318711
		Carabidae	*Carabus nemoralis*	AF012507

Table 1 *Continued*

Order	Suborder	Family	Species	GenBank no.
	Polyphaga	Eucinetidae	*Eucinetus* sp.	AF427609*
		Derodontidae	*Laricobius erichsonii*	AF427606*
		Leiodidae	*Leiodes* sp.	AF427607*
		Hydraenidae	*Ochthebius minimus*	AF427608*
		Silphidae	*Silpha* sp.	AF427600*
		Hydrochidae	*Hydrochus angustatus*	AF427601*
		Scarabaeidae	*Osmoderma* sp.	AF427602*
		Elateridae	*Ampedus balteatus*	AF427605*
		Byrrhidae	*Byrrhus pilula*	AF427604*
		Tenebrionidae	*Tenebrio molitor*	X07801
		Coccinellidae	*Coccidula rufa*	AF427603*
		Chrysomelidae	*Chrysolina hyperici*	AF427610*

aligned positions. This set was implemented for a set of unrooted ingroup-only analyses. A second exclusion set was based on all ingroup and outgroup taxa. These data revealed much higher variability in the margins of the 'hypervariable regions'. Therefore, a smaller number of characters was retained for global ingroup + outgroup analyses. The total number of characters included for ingroup analyses was 2797, of which 175 were parsimony informative (many of these positions are merely 'gaps' in the larger alignment), whereas for ingroup + outgroup analyses 1541 positions were included, of which 543 were parsimony informative.

Although a morphological data set for the taxa included here does exist (Beutel & Haas 2000), we do not present an analysis of combined data. Preliminary analyses of coleopteran 18S in concert with other morphological data sets indicated that the topology of the combined analysis is largely dominated by morphology. Our primary goal in this study was to provide an independent assessment and an exploration of the value of 18S for the question of beetle subordinal relationships.

The primary analysis examined only the Coleoptera sequences and the larger nucleotide set, with the goal of identifying the best supported unrooted ingroup-only topology. Secondarily, we sought the position of the root of this topology using all sequences. (Because the more variable regions of the molecule are excluded from all analyses, our data are expected to be minimally informative with respect to family level relationships, and the topology within beetle suborders that we present should be afforded minimal attention.)

Ingroup-only analyses proceeded from a maximum parsimony search (100 random addition replicates with Tree Bisection and Reconnection (TBR), branch swapping, using PAUP*4.0b8; Swofford 1998) with all positions equally weighted. Nucleotides were then reweighted according to their rescaled consistency indices on these initial topologies and an additional maximum parsimony search was undertaken under the same search conditions. On the topologies resulting from this search, likelihoods were calculated while estimating transition/transversion ratios and the value of alpha for a four-category approximation to a gamma distribution. The values of these parameters for the most likely topology were then fixed and that topology was used as the starting point for branch swapping under maximum likelihood. In addition, to ensure that the most plausible ingroup topologies were examined, we constructed trees constituting all possible relationships of the beetle suborders (holding their respective inner relationships to those found by maximum parsimony), and likelihoods were specifically calculated for these topologies under the same model.

Following the identification of the best supported unrooted topology of beetle relationships, two outgroup + ingroup analyses were performed, both of them using the more restricted nucleotide set. First, global maximum parsimony searches were carried out (100 replications of TBR), followed by reweighting according to rescaled consistency indices. As this analysis resulted in a non-monophyletic Coleoptera (see below), we examined the results of constraining the search. We constrained four nodes in this search, that subtending the Coleoptera as a whole and those subtending each of the Coleoptera suborders that were represented by more than one taxon (Myxophaga, Adephaga and Polyphaga). By forcing homoplasious changes to map onto the otherwise well-supported constraint nodes, it is expected that homoplasy may be reduced at other less secure nodes. Although previous molecular studies (e.g. Whiting *et al.* 1997) have similarly found a non-monophyletic Coleoptera, there is no serious doubt that this results from either homoplasy in rapidly evolving taxa or, possibly, as suggested by Whiting *et al.* (1997), from highly symplesiomorphic features in 18S of basal beetles. The number of morphological synapomorphies supporting Coleoptera monophyly is very large (28 cited by Beutel & Haas 2000 in a far from exhaustive list). The only possible exception to this assumption would be the potential inclusion of Strepsiptera in Coleoptera, an old controversy. This question has already been found to be insoluble using 18S sequences (Huelsenbeck 1997; Whiting *et al.* 1997) and our data do not constitute an independent assessment of

the problem. Therefore, although we include Strepsiptera sequences in the analysis, we follow Kukalová-Peck & Lawrence (1993) in assuming that they constitute, at closest, the sister group of Coleoptera. The assumption of suborder monophyly is generally supported by morphological analyses (Kukalová-Peck & Lawrence 1993; Beutel & Haas 2000), at least for the taxa included in the present study.

Results

The number of trees resulting from each search and their basic statistics are reported in Table 2. Analyses of Coleoptera alone support a single tree with each suborder monophyletic and the relationships ((Archostemata Myxophaga) (Polyphaga Adephaga)) (Fig. 1). The same topology was found by reweighted parsimony (the trees differing in only minor rearrangements within suborders). This tree was 5 log likelihood units better than the nearest alternative arrangement of suborders (–12 905.375 vs. –12 910.786). The same suborder relationships were also favoured when variable positions were included and under several alternative likelihood models (results not shown). It is notable that this topology is inconsistent with the hypotheses of either Kukalová-Peck & Lawrence (1993) or of Beutel & Haas (2000).

Rooting this topology was attempted by including a wide range of insect orders, and with a larger portion of hypervariable regions removed. Global unconstrained searches resulted in a non-monophyletic Coleoptera, under all search conditions. Equally weighted characters resulted in 10 911 equally parsimonious trees (2782 steps, CI = 0.4436, RI = 0.6242). The majority of these (79%) show the insertion of a clade comprising Strepsiptera, Diptera, Trichoptera and Lepidoptera essentially in the middle of the myxophagan Coleoptera, with the Archostematan, *Distocupes*, basal to this clade + Adephaga and Polyphaga. Obtaining a monophyletic Coleoptera with unweighted nucleotides requires two additional steps, resulting in a resolution in which a [paraphyletic] Myxophaga + Archostemata clade is sister to an Adephaga + Polyphaga clade, with the Polyphaga also paraphyletic. Searching over

Table 2 Overview of parsimony analyses reported in this paper. The parsimony scores refer to unweighted data. When the search was performed using reweighted data, the score of the tree was recalculated with the weights set to 1 (scores in parentheses), allowing direct comparison of the departure from maximum parsimony of these trees.

	No. of trees	Length	CI	RI
Ingroup only				
All positions equally weighted	1	818	0.6137	0.5291
Rescaled consistency index reweighted	1	(819)	0.6129	0.5276
Ingroup + outgroup				
Unconstrained, equally weighted	10911	2782	0.4436	0.6242
Unconstrained, reweighted	1	(2785)	0.4431	0.6235
Coleoptera constrained, equally weighted	1552	2784	0.4432	0.6237
Coleoptera constrained, reweighted	3	(2787)	0.4428	0.6230
Suborders constrained, equally weighted	> 12000	2788	0.4426	0.6227
Suborders constrained, reweighted	1	(2791)	0.4421	0.6220

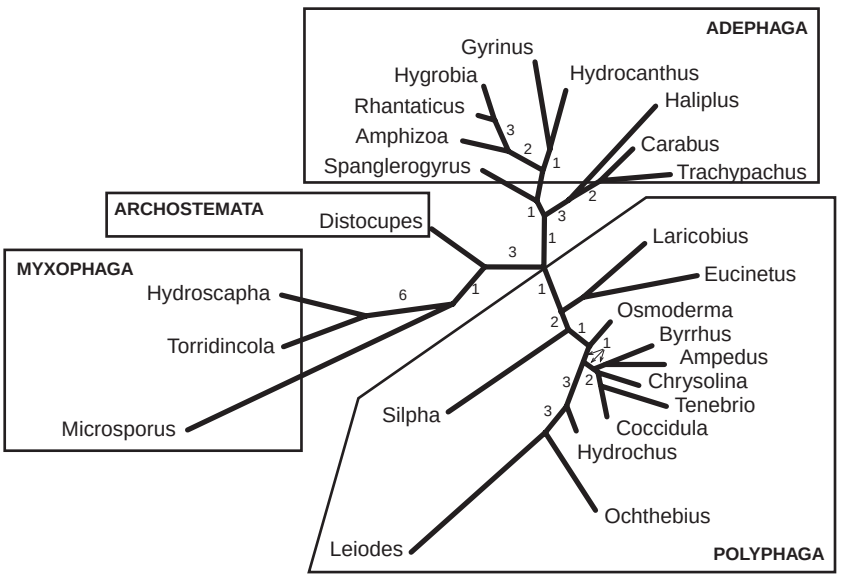

Fig. 1 Unrooted topology of Coleoptera taxa based on conserved nucleotides only. This resolution of suborders was supported by equally weighted and rescaled consistency index (RCI) reweighted parsimony, as well as by maximum likelihood, under a variety of models. Numbers on branches indicate Bremer support values.

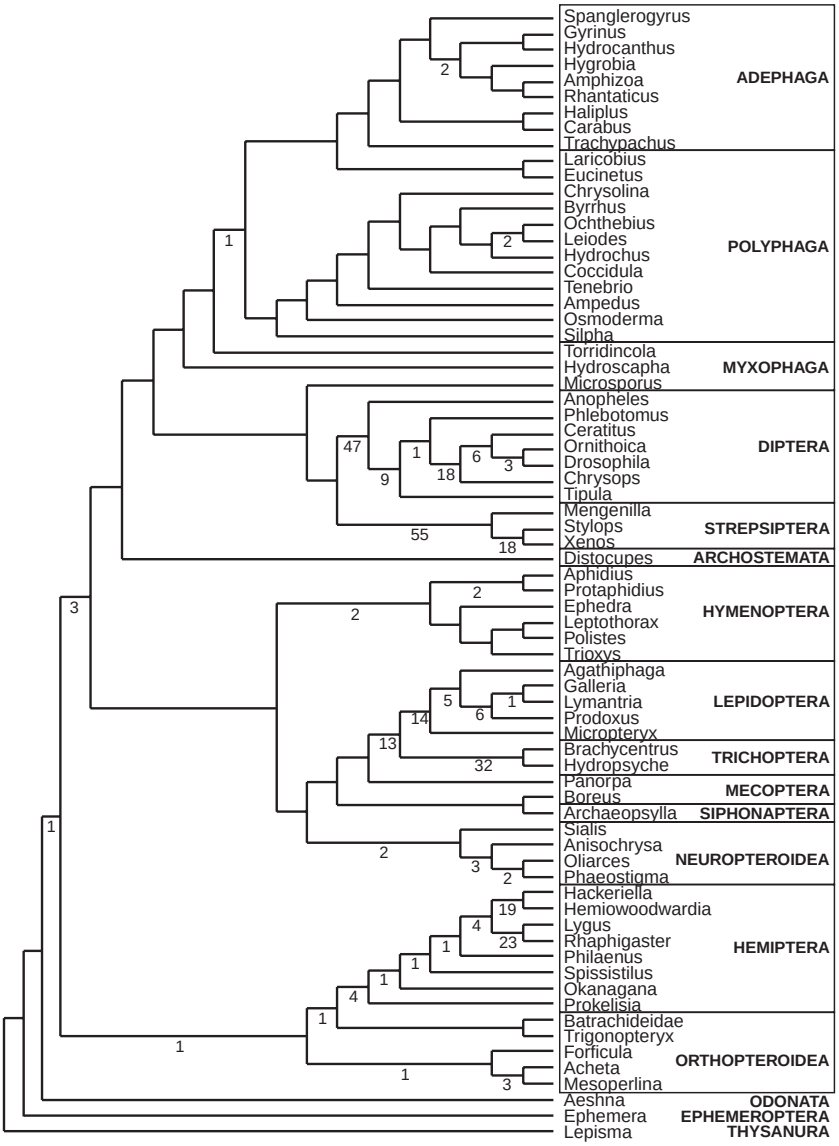

Fig. 2 Single most parsimonious topology found by reweighted parsimony enforcing no topological constraints. Numbers on branches indicate Bremer support values. Note that, although the names of myxophagan taxa are placed in a box together, Microsporus is resolved as more closely related to Diptera + Strepsiptera.

reweighted nucleotides (without constraints) supports a similar resolution (Fig. 2), with a clade comprising only Strepsiptera and Diptera inserting again within the Myxophaga, while the Trichoptera + Lepidoptera clade joins a group containing the remaining Holometabola. This tree also indicates the paraphyly of Polyphaga with respect to Adephaga.

Constraining the search to only those trees in which the Coleoptera and all its suborders are monophyletic required an additional six steps relative to the unconstrained tree (2788 vs. 2782). Over 12 000 trees of this length fulfil this constraint, a very slight majority (51%) of which support a resolution with Archostemata as sister to the remaining Coleoptera and with Myxophaga sister to Adephaga + Polyphaga. The sister group of Coleoptera is resolved to be a clade composed of Neuroptera + Mecoptera/Siphonaptera. Searching over data reweighted according to this tree yielded a single tree (Fig. 3) with the same resolution of Coleoptera suborders [(Archostemata(Myxophaga(Adephaga, Polyphaga)))], with the beetle clade sister to the remaining Holometabola.

Discussion

Our analysis results in a consistent picture of the relationships of beetle suborders. All analyses of ingroup taxa alone agree on a single unrooted resolution of the four lineages (Fig. 1). Furthermore, this resolution is incompatible with what may be considered to be the two prevailing hypotheses (those of Beutel & Haas 2000 and Kukalová-Peck & Lawrence 1993). Determining the relationships of Coleoptera to the

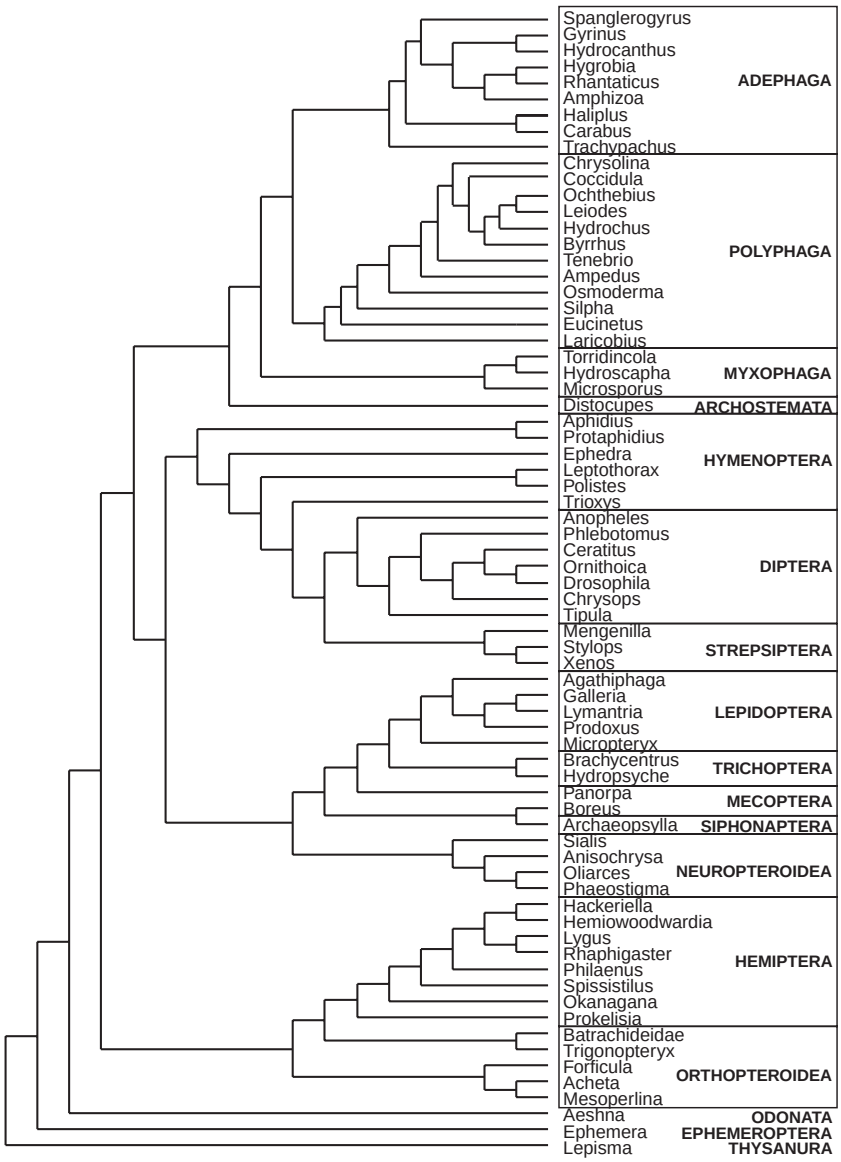

Fig. 3 Single most parsimonious topology found by reweighted parsimony enforcing the monophyly of the Coleoptera and that of each of its suborders.

remaining Holometabola and thus rooting the beetle tree, however, proved to be more difficult with this data set. In global analyses of Coleoptera and other holometabolan taxa, the most parsimonious trees support a paraphyletic Coleoptera (as in previous analyses of 18S; Whiting *et al*. 1997). While on philosophical grounds, hypotheses based on the fewest possible assumptions are certainly to be preferred, this result is at odds with such a large amount of morphological information that it cannot be considered to be viable, and we believe that invoking at least the constraint of Coleoptera monophyly, if not that of its suborders, is a defensible assumption. Enforcing the reconstruction of known branches, and thus permitting homoplasious characters to be mapped to them, might be expected to result in improved character reconstructions over other more tenuous branches.

Imposing only the assumption of beetle monophyly results in trees two steps longer, containing two notable groupings. First Myxophaga is reconstructed as paraphyletic with respect to Archostemata and, second, the Adephaga and Polyphaga group together in all trees, although with Polyphaga paraphyletic. Obtaining all suborders as monophyletic requires an additional four steps, and reweighted nucleotides with this additional constraint then support the resolution of Archostemata as basal to the remaining beetle suborders, with the Coleoptera as a whole sister to the rest of the Holometabola. It is worth noting that this last analysis also

results in generally more conventional resolutions within Adephaga and Polyphaga, despite the fact that much of the data informative at these lower levels has been excluded.

This hypothesis, that Archostemata represents the oldest branch of the Coleoptera tree, accords well with the fossil record. Cupedidae from the Lower Permian (Labandeira 1994) are the oldest fossils definitely attributed to any of the modern suborders. If hypotheses of relationships of modern Myxophaga to the extinct families Catiniidae and Schizophoridae are true (e.g. Ponomarenko 1969; Lawrence & Newton 1982), then Myxophaga also has a history extending back into the Lower Permian. The suborders Adephaga and Polyphaga do not appear as fossils until the Middle and Upper Triassic. Although, according to current interpretations, Adephaga appear much earlier, we would suggest that the Middle Triassic Triaplidae is as eucinetoid-like (Polyphaga) as it is haliplid-like (Adephaga). The beetle fossil record is admittedly fragmentary and difficult to interpret. However, it seems more consistent with the hypothesis of suborder relationships presented here than with previous hypotheses. Among the numerous evolutionary implications of this particular hypothesis, we would especially highlight the parallelisms implied between Myxophaga and Polyphaga. Those characters hypothesized as synapomorphies by Beutel & Haas (2000) all relate to the reduction and/or fusion of various sclerites, both adult and larval. Reinterpreting these characters as parallelisms suggests that the ancestral lineages of both of these suborders went through a phase of extreme size reduction.

Perhaps the most noteworthy outcome of this study is not what particular tree we support, but rather the difficulty of choosing among hypotheses which differ quite substantially in their implications for beetle evolution. It is clear that additional markers must be developed for looking at these questions. Choosing among the few likely resolutions we present requires the evaluation of their respective underlying assumptions. In arriving at the hypothesis we favour, we have invoked assumptions regarding relationships adequately supported on the basis of other data. There can be little argument with the assumption of beetle monophyly, despite the apparent conflict posed by 18S. Assuming the monophyly of all suborders is more tenuous. Recent morphological treatments, however, are unanimous in this hypothesis (at least for the taxa included here), and our ingroup-only analysis is consistent with the monophyly of all suborders.

Although ribosomal genes have had mixed success in reconstructing ancient phylogenetic relationships, they nonetheless offer a glimpse of phylogenetic history available from few other current sources. Given the surprising results of unconstrained analyses, all results presented here should be viewed tentatively. We believe that the primary factor hindering more confident conclusions is minimal sampling density among the smaller suborders. Undoubtedly, with better representation, the 'hypervariable' regions of the molecule will yield important phylogenetic information. The phylogenetic placements of *Micromalthus* and *Lepicerus*, unrepresented in our study, are also extremely important and need to be examined specifically. We are hopeful that, once these sampling gaps are filled, 18S will prove to be an increasingly useful source of information on beetle relationships.

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