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On the constitution and phylogeny of Staphyliniformia (Insecta: Coleoptera)

Michael S. Caterino[a,*], Toby Hunt[b], Alfried P. Vogler[b,c]

[a] Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, 2559 Puesta del Sol Rd., Santa Barbara, CA 93105, USA
[b] Department of Entomology, The Natural History Museum, Cromwell Rd., London SW7 5BD, UK
[c] Department of Biological Sciences, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, UK

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Abstract

The Staphyliniformia is one of the most diverse lineages of Coleoptera, with representatives occupying every conceivable non-marine niche. Phylogenetic relationships among its varied families and lower taxa have defied resolution. The problem has been further complicated by the recent suggestion that another major coleopteran series, Scarabaeiformia, is derived from within it. Here we present the first phylogenetic analyses, based on 18S rDNA sequences and morphological data, to explicitly examine this possibility. Thorough evaluation of alternative alignments and tree construction methods support the contention that Scarabaeiformia is derived from within Staphyliniformia. Though the analyses yielded strong support for few family level groupings within the expanded Staphyliniformia, they conclusively support a close relationship between Hydraenidae and Ptiliidae, which has often been debated. The primary factor hindering additional resolution appears to be the inconsistent rate of divergence in 18S among these taxa.

Keywords: Coleoptera; Staphylinoidea; Scarabaeoidea; Histeroidea; Hydrophiloidea; 18S rDNA; Maximum likelihood; Alignment

1. Introduction

The Coleoptera is one of the most diverse of all organismal lineages, with estimates of numbers of species worldwide ranging to 10 million or higher (Erwin, 1982). This diversity is not, however, evenly distributed among lineages or niches, leading some to explain the remarkable diversity as the result of the success of one or a few groups (e.g., Farrell, 1998). As appealing as such explanations may be, they rest on an as yet inadequate understanding of the actual phylogenetic and ecological distributions of beetle diversity, and must be regarded as tentative.

In the present paper, we explore the diversification of the Staphyliniformia. The Staphyliniformia is one of the major lineages of polyphagan Coleoptera. As presently constituted it contains nearly 20% of described beetle species (Newton and Thayer, 1992), and possibly as much as 35–40% of the actual diversity of the order (Hammond, 1990). Staphyliniformia is an immensely diverse group ecologically, as well, containing significant numbers of predators, algivores, fungivores, detritivores, mammal and social insect inquilines, ectoparasites, and if, as recent studies suggest, this lineage includes the Scarabaeoidea, herbivores as well. Species of Staphyliniformia can be found in nearly all terrestrial and aquatic environments, and even in intertidal marine habitats (Hansen, 1997; Lawrence and Newton, 1982; Lawrence and Britton, 1991; Newton, 1984). The group is an enormous and ecologically important component of global biodiversity and gaining an understanding of its diversification is critical to understanding the role of insects in the Earth's ecological history.

* Corresponding author. Fax: +1 805 563 0574.
E-mail address: mcaterino@sbnature2.org (M.S. Caterino).

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Most recent authors have limited the Staphyliniformia to three main groups, Hydrophiloidea (water scavenger beetles and allies), Histeroidea (clown beetles), and Staphylinoidea (rove beetles and allies), and a group containing these three superfamilies can be easily defined based on adult (absence of intrinsic spring for hind-wing folding) and larval (presence of articulated urogomphi) characters. However, as alluded to above, the position of the Scarabaeoidea (stag beetles, dung beetles, and their allies) with respect to this group is unclear. Recent studies by Kukalová-Peck and Lawrence (1993) and Hansen (1997), have suggested that the Scarabaeoidea may also be staphyliniforms, though they don't exhibit either of the character states cited above. But there is precedent for this larger group in the taxon Haplogastra (Kolbe, 1908), and it finds character support in the structure of the abdominal sterna (Jeannel and Paulian, 1944; Kolbe, 1908), wing venation and folding (Forbes, 1926; Kukalová-Peck and Lawrence, 1993), adult mouthparts (Hansen, 1997), thoracic spiracles (Hansen, 1997), and metacoxae (Hansen, 1997). Crowson, this century's most influential Coleopterist, also initially endorsed this hypothesis (Crowson, 1955) but later rejected the idea in favor of the more restricted Staphyliniformia and a placement of the Scarabaeoidea near the Dascilloidea (i.e., more closely related to Elateriformia than to Staphyliniformia; Crowson, 1960, 1971). Crowson based this later hypothesis on numerous larval similarities shared by Scarabaeoidea and Dascilloidea, though he recognized that little support could be found in adult characters. Grebennikov and Scholtz (2003) have presented larval evidence countering the contention that their similarities are synapomorphic, supporting a placement of Dascilloidea alone in Elateriformia. They did not, however, propose an alternative placement of Scarabaeoidea. At present, there is little consensus beyond agreeing that the scarabs' placement is ambiguous, and ripe for independent assessment.

The majority of species diversity of Staphyliniformia is found within Staphylinoidea, with over 50,000 described species. There has been little question of the monophyly of the superfamily, aside from the question of whether or not to include the Hydraenidae (more on this below) and several synapomorphies support the group (inclusive of Hydraenidae): wing folding with simple concave and convex folds, reduction or loss of medial loop of hindwing, basal piece of aedeagus small and strap-like or absent, and four Malpighian tubules (reduced from the plesiomorphic 6) (Lawrence and Newton, 1982; see Hansen, 1997, for additional potential synapomorphies). Staphylinoids are generally small beetles with secretive habits. The superfamily contains the familiar short-winged rove beetles (Staphylinidae), carrion beetles (Silphidae), small fungus beetles (Leiodidae), feather-winged beetles (Ptiliidae), and several other lesser known groups. Although saprophagy is considered to be the plesiomorphic habit for the superfamily (as well as the entire series Staphyliniformia; Hansen, 1997), mycophagous and predatory species are also numerous. The families of Staphylinoidea are commonly divided into two groups, the leptinid association (Agyrtidae, Leiodidae, Ptiliidae, and Hydraenidae), and the staphylinid group (the remainder of the superfamily), and Hansen's (1997) recent morphological study supports this division. Characters supporting the monophyly of the former group in Hansen's study were considered symplesiomorphies by Lawrence and Newton (1982), and the group was not found in a recent study of staphylinoid larvae (Beutel and Molenda, 1997). However, detailed study of larval Ptiliidae has suggested that a fimbriate galea may be a synapomorphy of the four families (Grebennikov and Beutel, 2002). One of the major phylogenetic questions pertaining to Staphylinoidea is whether the Hydraenidae in fact belong within Staphylinoidea at all; the family has long been considered more closely related to Hydrophiloidea on the basis of numerous aquatic adaptations shared by the adults. By contrast, studies of the larvae strongly suggested a placement in the Staphylinoidea (Böving and Craighead, 1931), and that the aquatic similarities to hydrophiloids are therefore convergent. This view is finding support in an increasing number of adult characters as well and is now generally accepted (Beutel, 1999; Beutel and Molenda, 1997; Hansen, 1991, 1997; Kukalová-Peck and Lawrence, 1993; Lawrence and Newton, 1982). Corroboration based on molecular characters will nonetheless be welcome.

The "staphylinid group" comprises several families, most of which are now considered to have been derived from within Staphylinidae (Ballard et al., 1998; Hansen, 1997; Lawrence and Newton, 1982; Newton and Thayer, 1995). However, their exact placements remain highly controversial. Lawrence and Newton (1982) proposed four major lineages within the "staphylinid group," (omaliine group, tachyporine group, oxyteline group, and staphylinine group) most of which encompass one or more staphylinid subfamilies in addition to previously recognized families. These groups have been variously assumed (tachyporine group: Ashe and Newton, 1993), supported (omaliine group: Thayer, 1985, 1987), or refuted (omaliine group: Beutel and Molenda, 1997). The most comprehensive phylogenetic study on staphyliniforms to date (Hansen, 1997) resolved none as monophyletic. Of particular interest was the wide separation of the families Scydmaenidae, Scaphidiidae, and Silphidae from their placements suggested by Lawrence and Newton's (1982) informal classification. One additional examination of relationships at this level, based on combined molecular and morphological evidence (Ballard et al., 1998), provided limited additional resolution. For the purposes of the present study, our sampling should allow a reasonable assessment of the validity of the informal groups of Lawrence and Newton (1982), and

will hopefully shed some light on the derivation of the more divergent groups usually treated as families.

If the inclusion of the Scarabaeoidea in Staphyliniformia is accepted, its most likely placement seems to be as sister to the Hydrophiloidea + Histeroidea, the three together constituting the sister group of the Staphylinoidea (Hansen, 1997). Despite rather different general appearances in the three superfamilies of this hypothesized clade, a variety of characters would support their monophyly, most obviously the strongly differentiated, three-segmented, pubescent antennal club, long considered a synapomorphy of only Hydrophiloidea + Histeroidea. Hansen (1997) cites as additional possible synapomorphies: larval mandibles without prostheca, larval maxillary palpus with four palpomeres, larval abdomen largely membranous, larval spiracles biforous (among Scarabaeoidea only found in Trogidae, Hansen's lone exemplar), adult antenna with cupuliform segment preceding the antennal club, and hind wings with apical hinge reinforced. The striking differences in habitus of the different superfamilies are easily explained by specialized habits of each. Both larvae and adults of the scarabaeoids have no doubt been strongly modified for a largely subterranean existence. Larvae of Hydrophiloidea and Histeroidea, on the other hand, share modifications related to predation. Adult morphology in Hydrophiloidea has been primarily influenced by the group's aquatic habits, while that of Histeroidea has been profoundly influenced by the evolution of a compact, retractile morphology. Hydrophiloidea and Histeroidea have long been considered sister groups, primarily on the basis of strong larval similarities, especially prognathy, with the labrum and clypeus indistinguishably fused into a toothed nasale (Böving and Craighead, 1931; Hansen, 1997; Lawrence and Newton, 1982; Newton, 1991).

In an attempt to resolve some of these difficult phylogenetic questions, we have obtained complete 18S sequences, and analyze these in combination with existing morphological data for a relatively large sample of Staphyliniformia and Scarabaeoidea. Our primary aim is to examine the hypothetical close relationship between these two major groups. Beyond that this analysis will provide insight into relationships among superfamilies, families, and more divergent subfamilies within these groups.

2. Methods

2.1. Taxa

2.1.1. Ingroup

Our samples have been selected to span the diversity of the Staphyliniformia and Scarabaeiformia. In most cases we have attempted to include representatives of genera examined by Hansen (1997, whose morphological data we incorporate into this analysis; see below). Representatives of most of the families within these lineages are included (see Table 1), for a total of 85 ingroup taxa. While numerous additional representatives of each superfamily could have been included, the present level of sampling allows a more thorough exploration of alternatives based on alternative analytical criteria. Major disagreements among analyses may be taken as an indication that more comprehensive sampling would be beneficial.

2.1.2. Outgroup

Outgroup sampling for this study is of critical importance for several reasons. First, given a taxon whose inclusion in this group is questionable (Scarabaeoidea), a diverse selection of outgroups is needed to provide sufficient alternative placements for this taxon. Resolution of the Scarabaeoidea as either more closely related to one of the outgroup taxa or as sister group to the more conventional Staphyliniformia will be taken as evidence against their being Staphyliniformia. Until global analyses of Coleoptera relationships are carried out with appropriate data, it may be difficult to answer this question conclusively. Our diverse outgroup selection is also intended to lessen the effects of long outgroup branches attracting long branch ingroup taxa (seen in some preliminary analyses). The 25 outgroup taxa include representatives of all beetle suborders (1 Archostemata, 3 Myxophaga, and 6 Adephaga), and among Polyphaga, representatives of the series Elateriformia (13, 5 of them Scirtoidea, increasingly appearing to belong outside of Elateriformia proper; Caterino et al., 2002; Caterino and Vogler, unpub. data) and Bostrichiformia (2) (see Table 1).

2.2. Characters

Our phylogenetic data include both molecular and morphological characters. We have generated complete 18S rDNA (ca. 1850 bp) sequences for all taxa included. Although 18S poses some difficulties for phylogenetic analysis, including length variation and often extreme rate heterogeneity among regions (Hillis and Dixon, 1991; Soltis et al., 1999) as well as among taxa (Abouheif et al., 1998) it has nonetheless proven highly useful for studies of higher level insect relationships (Campbell et al., 1997; Farrell, 1998; Maddison et al., 1999a,b; Vogler and Pearson, 1996; Wheeler et al., 1993; Whiting et al., 1997). Furthermore, continued focus on this popular locus will facilitate synthetic studies of its evolutionary dynamics and hopefully of a global insect phylogeny (see Caterino et al., 2000; Chase and Cox, 1998). GenBank accession numbers for all sequences are given in Table 1.

The core of our non-molecular data set is taken directly from Hansen's (1997) study of the higher phylogeny of Staphyliniformia. The data set comprises 119 characters, including 88 adult characters (7 specific to

Table 1
Taxa used in this analysis, along with higher classification, collection locality, and GenBank accession number

Suborder	Series	Superfamily	Family	Subfamily	Genus/species	Morph[a]	Collection location	GenBank No.
Archostemata			Cupedidae		*Distocupes* sp.	Y	Shull et al. (2001)	AF201421
Adephaga			Carabidae	Carabinae	*Calosoma scrutator*		Maddison et al. (1999b)	AF002800
				Carabinae	*Nebria brevicollis*		Shull et al. (2001)	AF201395
			Gyrinidae		*Gyrinus* sp.	Y	Shull et al. (2001)	AF201412
			Hygrobiidae		*Hygrobia hermanni*		Shull et al. (2001)	AF201414
			Trachypachidae		*Systolosoma lateritium*	Y	Maddison et al. (1999a))	AF012522
					Trachypachus gibbsi		Maddison et al. (1999b)	AF002808
Myxophaga			Hydroscaphidae		*Hydroscapha natans*	Y	Maddison et al. (1999a)	AF012525
			Torridincolidae		*Torridincola rhodesica*	Y	Shull et al. (2001)	AF201420
					Delevea bertrandi		South Africa: W. Cape	AY745554
Polyphaga	Elateriformia	Scirtoidea	Scirtidae		*Cyphon hilaris*		Shull et al. (2001)	AF201419
					Scirtes hemisphericus		UK: Blenheim Palace	AF451937
			Eucinetidae		*Eucinetus* sp.	Y	USA: California	AY745555
			Decliniidae		*Declinia versicolor*		Japan: Honshu	AY745556
			Clambidae	Clambinae	*Clambus arnetti*		Maddison et al. (1999a)	AF012526
		Buprestoidea	Buprestidae	Agrilinae	*Agrilus sinuatus*		UK: Hampshire	AF451934
				Julodinae	*Julodis* sp.		South Africa: Kruger N.P.	AF451935
		Byrrhoidea	Elmidae	Elminae	*Limnius volckmari*		Spain: Albacete	AF451914
			Ptilodactylidae	Ptilodactylinae	*Ptilodactyla serricornis*		USA: Georgia	AF451931
		Dascilloidea	Dascillidae	Dascillinae	*Dascillus cervinus*	Y	UK: Exmoor	AY745558
		Elateroidea	Omalisidae		*Omalisus fontisbellaguei*		France	AF451948
			Drilidae		*Drilus flavescens*		UK: Winchester	AY745559
			Elateridae	Denticollinae	*Stenagostus rhombeus*		UK: Surrey	AF451945
	Bostrichiformia	Derodontoidea	Derodontidae	Laricobiinae	*Laricobius erichsoni*	Y	Caterino et al. (2002)	AF427606
				Derodontinae	*Derodontus esotericus*		USA: Arkansas	AY745560
	Scarabaeiformia	Scarabaeoidea	Geotrupidae	Geotrupinae	*Geotrupes spiniger*		France: Montepelier	AY745561
				Bolboceratinae	*Elephastomus proboscideus*		Australia: Queensland	AY745563
				Lethrinae	*Lethrus karelini*	Y	Kazakhstan: Alma-Ata	AY745564
			Ceratocanthidae		*Philharmostes* sp.		South Africa: Ngome	AY745562
			Glaphyridae		*Lichnanthe rathvoni*		USA: Oregon	AY745565
			Glaresidae		*Glaresis* sp.	Y	South Africa: Pretoria	AY745566
			Hybosoridae		*Liparochrus sculptilis*		Australia: Queensland	AY745567
					Phaeochrous mashunus		South Africa: Skukuza	AY745568
			Lucanidae	Dorcinae	*Dorcus parallelopipedus*		UK: Wales	AY745569
				Nicaginae	*Nicagus obscurus*		USA: Montana	AY745570
				Aesalinae	*Aesalus ulanowskii*	Y	Russia: NW Caucasus	AY745583
			Ochodaeidae	Ochodaeinae	*Ochodaeus* sp.		USA: Arizona	AY745571
			Passalidae	Aulacocyclinae	*Aulacocyclus* sp.		Australia: Queensland	AY745572
				Passalinae	*Odontotaenius disjunctus*		USA: Florida	AY745573
			Pleocomidae		*Pleocoma* sp.		USA: Oregon	AY745574
			Scarabaeidae	Aphodiinae	*Aegialia arenaria*		UK: Norfolk	AY745575
					Chiron cylindricus		Namibia	AY745576
				Scarabaeinae	*Cephalodesmius armiger*		Australia: Queensland	AY745577
					Onthophagus sp.		South Africa: Pretoria	AY745578
				Dynastinae	*Heteronychus arator*		South Africa: Pretoria	AY745579
				Rutelinae	*Plusiotis* sp.		USA: Arizona	AY745580

		Trogidae		*Trox rudebecki*	Y	South Africa: Sani Pass	AY745581
				Omorgus squamosus			AY745582
Staphyliniformia	Hydrophiloidea	Sphaertidae		*Sphaerites glabratus*	Y	Caterino and Vogler (2002)	AY028331
		Synteliidae		*Syntelia histeroides*	Y	Caterino and Vogler (2002)	AY028332
		Histeridae	Dendrophilinae	*Dendrophilus punctatus*	Y	Caterino and Vogler (2002)	AY028351
				Anapleus mexicanus	Y	Caterino and Vogler (2002)	AY028353
			Onthophilinae	*Onthophilus flohri*	Y	Caterino and Vogler (2002)	AY028346
		Hydrophilidae	Hydrochinae	*Hydrochus angustatus*	Y	Caterino and Vogler (2002)	AY028330
			Helophorinae	*Helophorus brevipalpus*	Y	Caterino and Vogler (2002)	AY028329
			Georissinae	*Georissus crenulatus*	Y	UK: Dorset	AY745584
			Hydrophilinae	*Ametor scabrosus*	Y	USA: California	AY745585
				Anacaena limbata		UK: Windsor	AY745586
				Chaetarthria sp.	Y	USA: California	AY745587
				Berosus signaticollis	Y	UK: Hampshire	AY745588
				Helochares lividus		Shull et al. (2001)	AF201418
				Hydrobius fuscipes		UK: Somerset	AY745589
			Sphaeridiinae	*Sphaeridium scarabaeoides*	Y	USA: California	AY745590
				Cercyon litoralis		UK: North Wales	AY745591
	Staphylinoidea	Hydraenidae	Hydraeninae	*Hydraena* sp.	Y		AY745592
			Limnebiinae	*Limnebius* sp.			AY745593
			Ochthebiinae	*Ochthebius minimus*		UK: Wiltshire	AY745594
		Agyrtidae		*Agyrtes bicolor*	Y		AY745595
				Apteroloma tahoecum		USA: California	AY745596
		Leiodidae	Leiodinae	*Leiodes* sp.	Y		AY745597
			Catopocerinae	*Parabathyscia wollastoni*		UK: London	AY745598
			Camiarinae	*Neopelatops*			AY745599
		Ptiliidae	Cephaloplectinae	*Cephaloplectus*		Belize: Cayo	AY745600
			Ptiliinae?	*Ptilium?* sp.	Y		AY745601
			Ptiliinae	Ptinella errabunda		UK: Wimbledon	AY745602
		Scydmaenidae	Scydmaeninae	*Scydmaenus?*	Y	French Guiana: Paracou	AY745603
				Euconnus sp.		UK	AY745604
		Silphidae	Silphinae	*Oxelytrum cayennense*		French Guiana: Paracou	AY745605
				Necrophila prob. *americana*	Y		AY745606
		Staphylinidae	Omaliinae	*Acrolocha sulcula*			AY745607
			Omaliinae	undetermined	Y		AY745608
			Proteininae	*Proteinus* sp.	Y		AY745609
			Proteininae	*Megarthrus martensi*		India: W. Bengal	AY745617
			Proteininae	*Metopsia gallica*			AY745618
			Glypholomatinae	*Glypholoma pustuliferum*		Chile	AY745610
			Micropeplinae	*Micropeplus* sp.	Y		AY745611
			Euaesthetinae	*Euaesthetus ruficapillus*		UK: Surrey	AY745612
			Habrocerinae	*Habrocerus capillaricornis*			AY745613
			Oxyporinae	*Oxyporus maxillosus*	Y	Finland	AY745614
			Steninae	*Stenus providus*	Y	UK: Surrey	AY745615
			Megalopsidiinae	*Megalopinus* sp.			AY745616
			Tachyporinae	undetermined	Y		AY745619
			Aleocharinae	*Myllaena dubia*		UK: Surrey	AY745620
			Aleocharinae	*Aleochara* sp.			AY745621

(*continued on next page*)

Table 1 (*continued*)

Suborder	Series	Superfamily	Family	Subfamily	Genus/species	Morph[a]	Collection location	GenBank No.
				Aleocharinae	*Atheta* sp.			AY745622
				Osoriinae	*Osorius* sp.			AY745623
				Oxytelinae	*Anotylus rugosus*		UK: Windsor	AY745624
				Oxytelinae	*Oxytelus* sp.	Y		AY745625
				Oxytelinae	*Syntomium* sp.		UK	AY745626
				Oxytelinae	*Bledius femoralis*		UK: Windsor	AY745627
				Phloeocharinae	*Phloeocharis subtilissima*	Y	UK: Cumbria	AY745628
				Piestinae	*Piestus* sp.		French Guiana: Paracou	AY745629
				Pseudopsinae	*Pseudopsis sulcata?*	Y	India: W. Bengal	AY745630
				Scaphidiinae	*Scaphidium quadrimaculatum*	Y	UK: Surrey	AY745631
				Paederinae	*Lathrobium brunipes*		UK: London	AY745634
				Paederinae	undetermined			AY745635
				Staphylininae	*Staphylinus olens*	Y		AY745632
				Staphylininae	*Xantholinus linearis*		UK: Surrey	AY745633
				Staphylininae	*Quedius cruentus*		UK: Wiltshire	AY745636
				Staphylininae	*Gyrohypnus* sp.		UK: London	AY745637

[a] The 'morph' column indicates which taxa are represented by morphological data.

males, 5 to females), 1 egg character, 29 larval characters, and 1 behavioral character. Hansen (1997) includes a very thorough analysis and justification of these characters, and we do not repeat it here. We have edited and amended these data slightly either to accommodate the specific taxa that we have sequenced or because, in a few cases, we disagree with his scoring of them. Characters were scored using Browne and Scholtz (1999) for adult morphology, Ritcher (1966) for larval morphology, and directly from specimens where characters were not explicitly dealt with by these authors. Hydrophiloidea had previously been represented by a single hypothetical OTU with those states purportedly present in the common hydrophiloid ancestor. We have replaced this taxon with 7 separate taxa scored using Hansen (1991) for adult morphology and Archangelsky (1998) for larval morphology. Where characters were not explicitly dealt with in either of these above studies, the state assigned in Hansen (1997) was assumed. Histeridae was likewise previously represented by a single OTU which has been replaced by three taxa scored from the senior author's dissections. In *Sphaerites* one character state was changed (fusion of parameres: from separate or nearly so to fused into a nearly complete tube; Caterino and Vogler, 2002; see also Newton, 2000), and states of genitalic characters previously unscored for Synteliidae were scored based on the senior author's dissections. In total, 41 of the 110 total taxa are represented by morphological data. The full data set is available by request from the senior author.

The preparation of material for morphological study followed common procedures (see Caterino, 1998). Dry or alcohol preserved specimens were relaxed in hot water and cleared in KOH, then dissected in glycerin. Genitalic preparations were generally stained in Chlorazol black prior to examination.

2.3. Sequencing methods

Total DNA was isolated from live-frozen or ethanol preserved specimens using either a phenol–chloroform procedure or Qiagen's QIAamp tissue kit. 18S was amplified in four fragments using the primer pairs 18S5′–18Sb5.0, 18Sai–18Sb0.5, 18Sa1.0–18Sbi, and 18Sa2.0–18S3′I, sequences of which are given in Whiting et al. (1997) and Shull et al. (2001). Automated fluorescent sequencing was carried out on either an ABI 377 or an ABI3700 using Perkin–Elmer BigDye sequencing chemistry. Both strands were sequenced for all fragments.

2.4. Phylogenetic analyses

It was first necessary to determine a justifiable alignment of variable positions of the 18S sequences. We implemented sensitivity analyses in conjunction with Clustal X (Thompson et al., 1994) to find the best

alignment. In a slight departure from standard Clustal procedure, we imposed a 'guide tree' for multiple alignments. This is typically calculated by Clustal at the outset, based on pairwise similarity scores obtained by computing all possible pairwise alignments. Inspection of these initial topologies revealed little congruence with well-established relationships, while preliminary results confirmed that many of these initial gross errors were propagated throughout the analysis. Admitting the biasing effect that this starting topology may have, we have specified a guide tree based on parsimony analysis of the conserved regions of the molecule alone (as delimited in Tautz et al., 1988; see also Caterino and Vogler, 2002; Ribera et al., 2002; Shull et al., 2001), assuming that this will help maximize internal congruence across the entire molecule. The strict consensus of 18,045 equally parsimonious trees resulting from this analysis is shown in Fig. 1. Clustal will not accept a non-bifurcating tree as a guide tree, so the first of the most parsimonious trees was arbitrarily selected. For sensitivity analyses we followed a procedure established in Caterino and Vogler (2002). Unaligned sequences were aligned against the conserved regions guide tree under a range of gap opening and extension costs (both ranging from 2 to 10), with the transition:transversion ratio held at one. Each resulting alignment was analyzed by parsimony (500 random addition replicates with TBR branch swapping, keeping no more than 1000 trees at each step), and all most parsimonious trees saved. The most parsimonious trees from each of the five sets of alignment parameters were compared to the topology of the strict consensus tree based on the conserved regions of the molecule alone via quartet (the 'd' statistic; Estabrook et al., 1985) and tree bipartition ('SD'; Penny and Hendy, 1985) methods. Where the alignment produced multiple equally parsimonious trees, the average for all comparisons was used. These values were plotted for all alignments to determine maximum congruence, with the best alignment carried forward to subsequent analyses.

The single best alignment was analyzed, alone and in combination with morphology, by several methods. Five analyses of aligned 18S sequences were undertaken, under three distinct optimality criteria. Standard parsimony analysis (500 random sequence addition replicates, TBR branch swapping) was implemented in PAUP* (4b10; Swofford, 2003). Bayesian analysis utilized MrBayes (Ronquist and Huelsenbeck, 2003), implementing four chains, three heated, one cold. Model settings (see Table 3) were determined using ModelTest (Posada and Crandall, 1998), comparing 56 possible models and selecting that identified as optimal by the Akaike Information Criterion (AIC; Akaike, 1974). Each chain was started from one of the most parsimonious trees, and run for 10^6 generations, sampling every 100th cycle. The first 2000 trees (of 10,000) were discarded as burn-in samples, and majority rule consensus of the remaining trees was used to determine clade posterior probabilities. Maximum likelihood analyses of 18S alone were conducting using three programs differing mainly in tree building procedure, PAUP*, PhyML (Guindon and Gascuel, 2003), and MetaPIGA (Lemmon and Milinkovitch, 2002). The last two of these are relative newcomers, attractive for the increased speed they offer compared to conventional algorithms. Their calculations of likelihood for particular trees should not differ from those in PAUP. However, they may examine novel areas of tree space. The model and parameter values for PAUP ml were those specified by ModelTest (same as for Bayesian analyses, above). For PhyML, a generic $GTR + I + \Gamma$ model was specified and it estimated its own specific parameter values. In MetaPIGA, HKY85 is the most complicated model allowed, and it was similarly allowed to estimate parameter values under this model. To determine whether PhyML or MetaPIGA found better trees than PAUP* for likelihood analyses, we conducted likelihood ratio tests on all resulting trees. These were conducted in PAUP*, implementing the model derived from ModelTest. Likelihood scores were computed, and significance of differences among scores (separately for each model) was assessed using Shimodaira–Hasegawa tests (Shimodaira and Hasegawa, 1999), as implemented in PAUP*.

Combined morphological and 18S data sets were analyzed under parsimony and Bayesian criteria. PAUP was used for parsimony analyses, with all characters weighted equally (500 taxon addition replicates, TBR). Combined analysis under Bayesian criteria used MrBayes, specifying 18S and morphology as separate character partitions. The same $GTR + I + \Gamma$ used for analysis of 18S alone was implemented for the molecular partition, while a simple model of all changes equiprobable was applied to the morphological characters. Other run parameters were as above.

Branch support was assessed for parsimony analyses using decay indices (Bremer, 1994), and partitioned decay indices for combined data. Support indices for branches of Bayesian trees represent the majority rule frequencies, equivalent to posterior probabilities, of each branch among the retained topologies.

3. Results

3.1. The data

Unaligned 18S sequences ranged from 1819 (Hydrophilidae: *Sphaeridium*) to 2103 bases (Trachypachidae: *Systolosoma*) in length. Adephagan sequences were longer on average than Polyphagan sequences (1992 avg. bases vs. 1849), though a handful of polyphagans exhibited much longer sequences, particularly the two Passalidae (1938 and 1956 bases). Nucleotide composition was

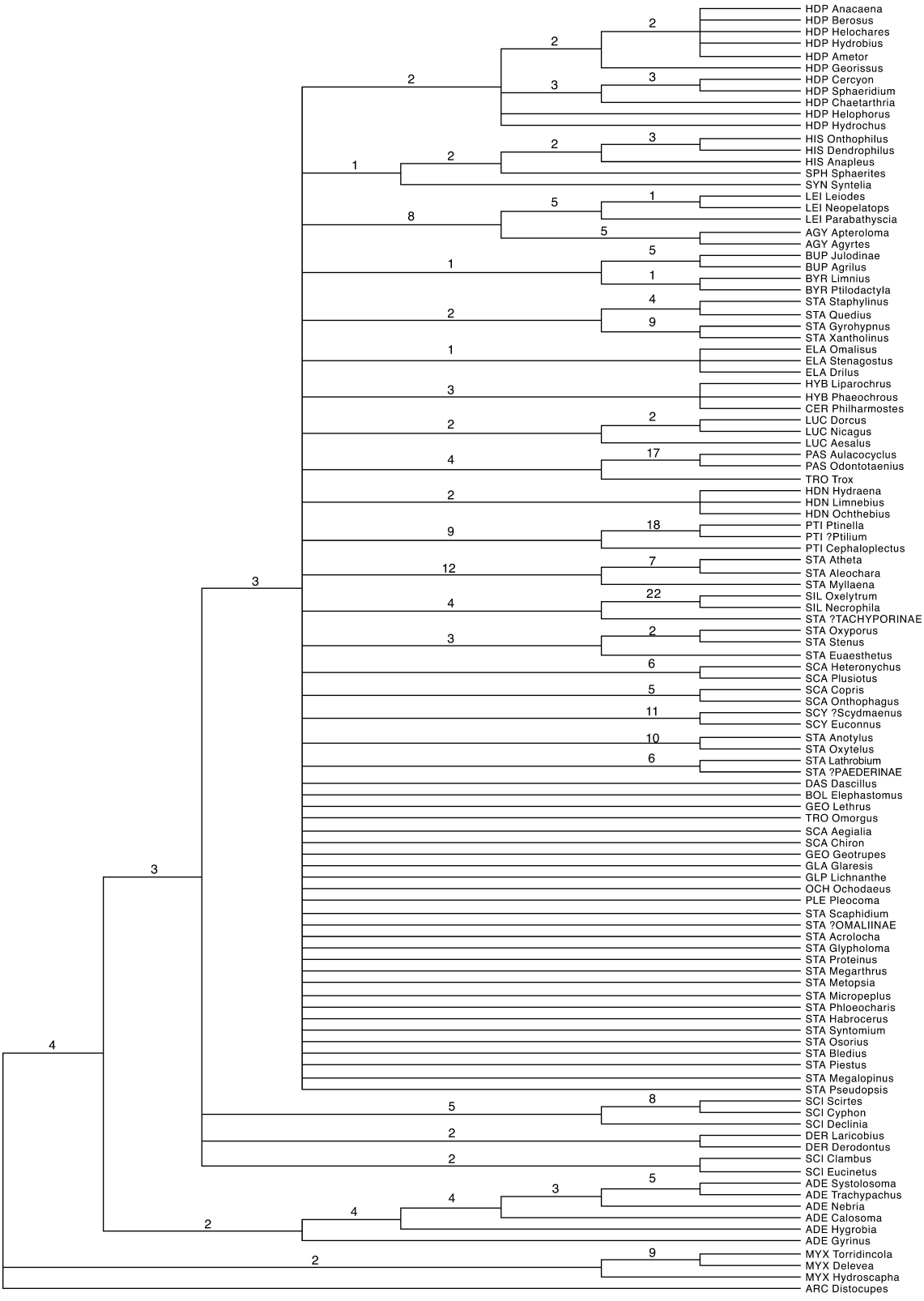

Fig. 1. Strict consensus of 18,045 trees based on conserved regions only data set, with decay indices on branches. For this figure and Figs. 3–6, taxon generic names are preceded by a three letter code (in CAPS) indicating higher taxa as follows: ARC, Archstemata; MYX, Myxophaga; ADE, Adephaga; SCI, Scirtoidea; DER, Derodontoidea; ELA, Elateroidea; BUP, Buprestoidea; BYR, Byrrhoidea; DAS, Dascilloidea; SYN, Synteliidae; SPH, Sphaeritidae; HIS, Histeridae; HDP, Hydrophilidae; LUC, Lucanidae; PAS, Passalidae; GEO, Geotrupidae; CER, Ceratocanthidae; TRO, Trogidae; GLA, Glaresidae; GLP, Glaphyridae; HYB, Hybosoridae; OCH, Ochodaeidae; PLE, Pleocomidae; SCA, Scarabaeidae; HDN, Hydraenidae; AGY, Agyrtidae; LEI, Leiodidae; PTI, Ptiliidae; SCY, Scydmaenidae; SIL, Silphidae; and STA, Staphylinidae.

Table 2
Tree lengths and congruence statistics for alternative alignments

Tree measures	Gap opening	Gap extension	Ti/tv	Alignment size	No. of informative characters	Length	Number found	CI	RI	SD[a]	d-mpts[a]
Data set											
Conserved regions				1744	341	2336	18045	0.3549	0.4583	NA	NA
Clustal1	2	2	1	2284	668	4543	972	0.3273	0.4147	79.109	90.584
Clustal2	5	2	1	2249	667	4727	1113	0.3323	0.4156	76.791	92.029
Clustal3	5	5	1	2212	666	4898	1000	0.3281	0.4143	76.000	92.020
Clustal4	10	5	1	2191	675	5183	242	0.3209	0.4126	**75.826**	**89.257**
Clustal5	10	10	1	2171	663	5291	2	0.3192	0.4126	88.000	92.035

[a] SD and d columns show optimum congruence score in bold.

nearly unbiased, with only a slight overabundance of guanine residues (mean % ACGT: 24.05, 24.07, 27.73, and 24.15). This was not constant across the molecule, however, with frequencies in the variable regions (V2, V4, and V6) showing a markedly lower adenine, and higher CG, content (mean % ACGT: 13.54, 32.03, 31.20, and 23.23).

3.2. Phylogenetic results

Analyses of alignment quality via internal congruence agreed on a single 'best' alignment (Table 2, Fig. 2). The parameters yielding this alignment were relatively restrictive on the insertion and extension of gaps (gap opening = 10; gap extension = 5), giving a total alignment of 2191 bases (about 90 bp longer than longest actual sequence), 675 positions of which were parsimony informative. All subsequent analyses use only this alignment.

The likelihood model identified as best by ModelTest (AIC) was a GTR + I + Γ, with parameter values as given in Table 3. (ModelTest hierarchical likelihood ratio tests selected TrN + I + Γ; analyses conducted under this model resulted in identical ranking of topologies; results not shown.) Parameter values were also similar when

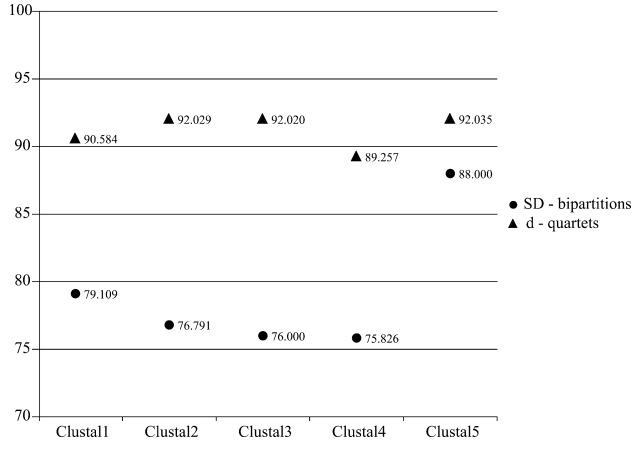

Fig. 2. Graph of congruence of 18S parsimony topologies based on alignments 1–5 vs. conserved regions only strict consensus (see Fig. 1). Clustal parameters generating each alignment are given in Table 2.

Table 4
Likelihood comparison of topologies obtained under various optimality criteria and tree building algorithms

Data set	Analysis	−ln *L*	SH significance
Conserved regions 18S	Parsimony	26948.26772	y
Whole aligned 18S	Parsimony	26743.79900	n
	ml—PAUP	26590.48227	best
	ml—MrBayes	26699.21885	n
	ml—PhyML	26687.51351	n
	ml—MetaPIGA	26740.22713	n
18S + morphology	Parsimony	26788.68656	n
	ml—MrBayes	26872.19052	y

Likelihoods were calculated under the GTR + I + Γ model, parameter values as chosen by AIC test in Modeltest (see Table 3). Shimodaira–Hasegawa significance was calculated from a one-tailed distribution, 'n' indicating no significant difference from best topology.

Table 3
Parameter estimates for all models discussed

Model settings	Nst	rAC	rAG	rAT	rCG	rCT	Ti/tv	Rates	nCAT	α	Pinvar	FreqA	FreqC	FreqG	FreqT
General model type															
ModelTest—TrN + I + Γ	3	1	2.5409	1	1	6.0642	NA	gamma	4	0.5221	0.4477	0.2442	0.2365	0.2566	0.2627
ModelTest—GTR + I + Γ	6	1.6768	3.8047	1.926	1.1298	8.0283	NA	gamma	4	0.5185	0.4511	0.2141	0.2415	0.2783	0.2661
MetaPIGA—HKY85	2	NA	NA	NA	NA	NA	0.5	equal	NA	NA	NA	0.2404	0.2406	0.2772	0.2418
PhyML—GTR + I + Γ	6	1.2228	2.9061	1.5918	1.021	7.6417	NA	gamma	4	0.448	0.399	0.2405	0.2407	0.2773	0.2415

Model Test results reflect findings based on hierarchical likelihood ratio tests (TrN) and Akaike Information Criterion (GTR). For MetaPIGA and PhyML parameter estimates were obtained by those programs after fixing the general model type (HKY85 being the most parameter-rich offered by MetaPIGA).

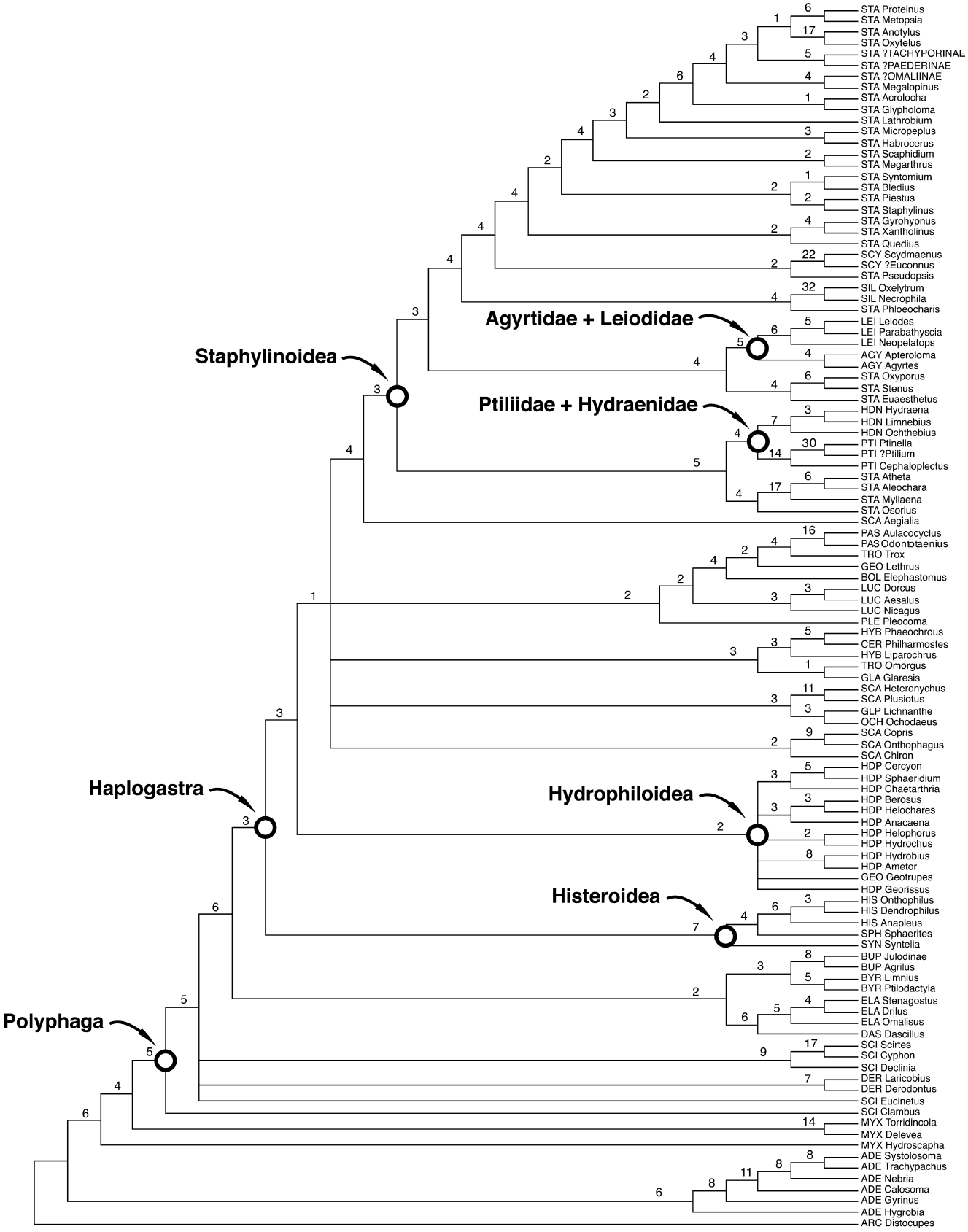

Fig. 3. Strict consensus of 242 trees from parsimony analysis of 18S alone, with decay indices on branches.

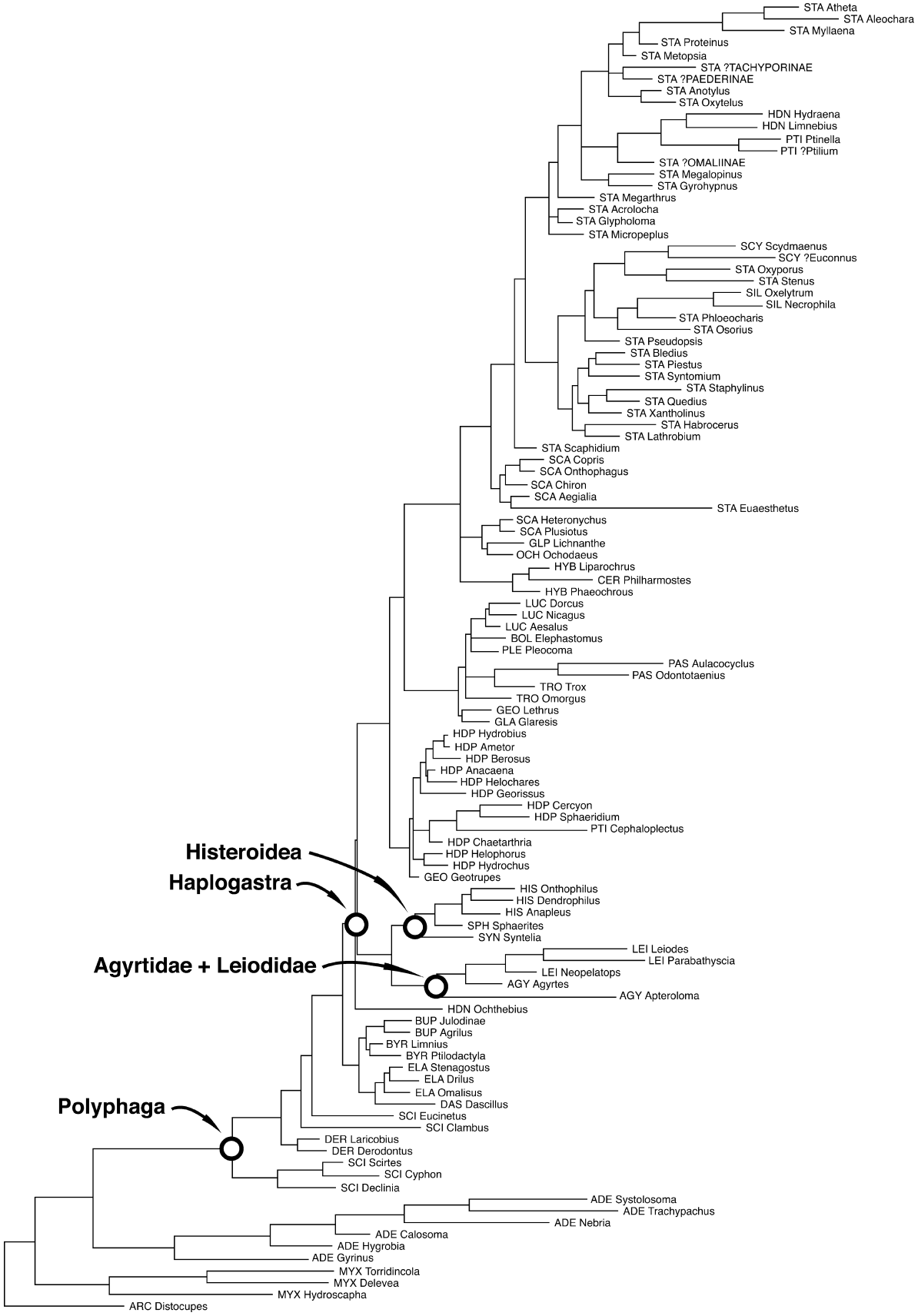

Fig. 4. Most likely topology based on PAUP search.

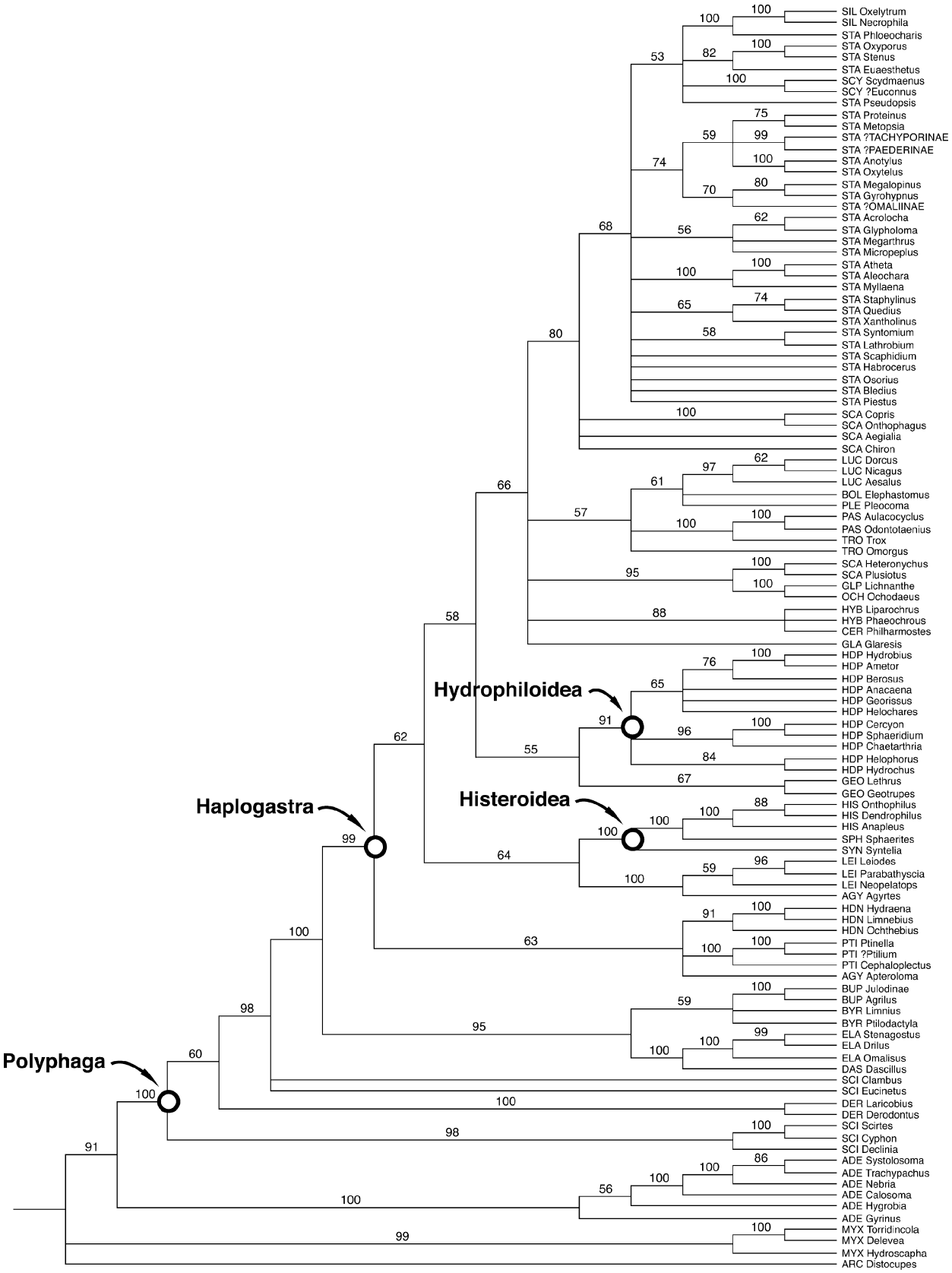

Fig. 5. Result of Bayesian analysis. This tree is a majority rule consensus of 8000 trees (all trees obtained following 200,000 burn-in generations). Numbers on branches are majority rule consensus indices, representing posterior probabilities of branches.

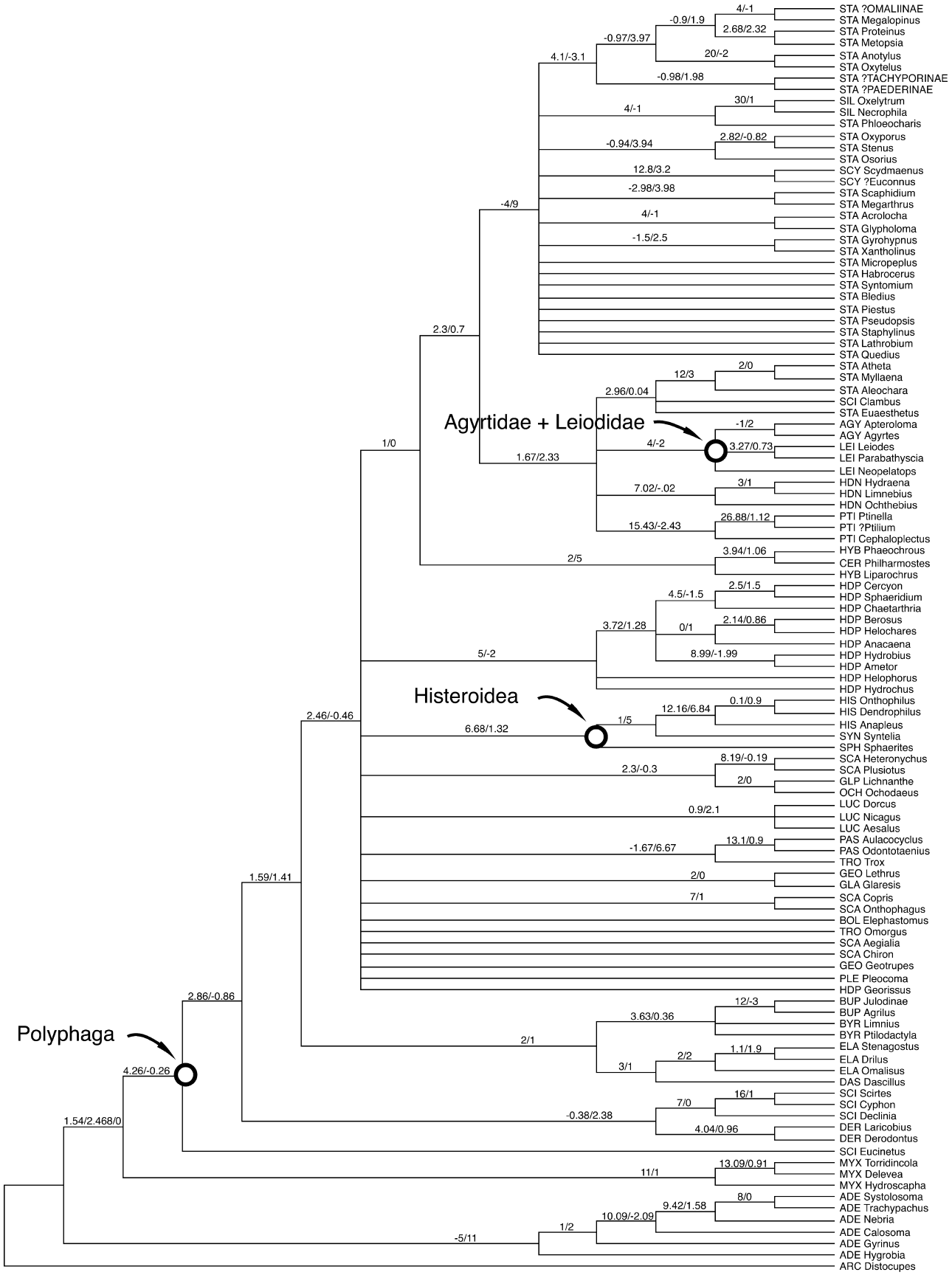

Fig. 6. Parsimony tree including 18S and morphology. Partitioned decay indices are given on branches as 18S/morphology.

Table 5
Support of focal clades under various analyses

	Data set:	Conserved regions	Whole 18S			18S + morphology	
	Optimality criterion:		Parsimony	PAUPml	Bayesian	Parsimony	
	Support measure:	Parsimony Decay	Decay	Observed y/n	Posterior probability	Partitioned decay	Frequency supported
Polyphaga		3	5	y	100	4.26/−0.26	5/5
Haplogastra		0	3	y	99	0	3/5
Staphylinoidea		0	3	n	<50	0	1/5
Hydrophiloidea		2	2	n	91	0	3/5
Histeroidea		1	7	y	100	6.68/1.32	5/5
Scarabaeoidea		0	0	n	<50	0	0/5
Staphylinidae		0	0	n	<50	0	0/5
Scarabaeidae		0	0	n	<50	0	0/5
Lucanidae		2	3	y	97	0.9/2.1	5/5
Agyrtidae		5	4	n	<50	−1/2	3/5
Leiodidae		5	6	y	59	0	4/5
Hydraenidae		2	7	n	91	7.02/−.02	4/5
Ptiliidae		9	14	n	100	15.43/−2.43	4/5
(Hydraenidae, Ptiliidae)		0	4	n	<50	0	1/5
(Histeroidea, Hydrophiloidea)		0	0	n	<50	0	0/5
(Leiodidae, Agyrtidae)		8	5	y	<50	4/−2	4/5
(Hydrophiloidea + Scarabaeoidea + Staphylinoidea)		0	3	n	<50	0	1/5
((Leiodidae + Agyrtidae),Histeroidea)		0	0	y	<50	0	1/5
(Silphidae + Phloeocharinae)		0	4	y	100	4/−1	4/5
(Scydmaenidae + Pseudopsinae)		0	2	n	<50	0	1/5
(Euaesthetinae + Oxyporinae + Steninae)		3	4	n	82	0	3/5
((Rutelinae + Dynastinae) (Glaphyridae + Ochodaeidae))		0	3	y	95	2.3/−0.3	4/5

Decay indices for combined parsimony analysis are partitioned into molecular/morphological components.

estimated by PhyML (Table 3). Under the chosen likelihood model the PAUP ml tree was found to have the best score (of eight trees; Table 4), though this was only significantly better (by one-tailed SH test) than the parsimony conserved regions only tree (Fig. 1) and the Bayesian combined data tree (Fig. 5). Although not significantly worse, PhyML and MetaPIGA results represent suboptimal solutions obtained by the same optimality criterion (by different tree-building methods) as the PAUP ml tree, and are not further discussed here. Thus, four topologies will be considered viable in this discussion: 18S topologies as obtained by parsimony, PAUP ml, and MrBayes, and the combined 18S + morphology tree obtained by parsimony. These trees are presented in Figs. 3–6, respectively.

Support for selected clades of interest in the four viable analyses is compared in Table 5. Few higher level clades find unanimous support. The primary clade of interest in this analysis, Haplogastra, is supported by all analyses of 18S alone, relatively strongly by parsimony and Bayesian criteria, but not by parsimony analysis of combined data. Clades finding moderate to strong support by all analyses include Polyphaga, Histeroidea, Lucanidae, a grouping of Silphidae and Phloeocharinae (Staphylinidae), and a clade comprising Dynastinae, Rutelinae (both Scarabaeidae), Glaphyridae, and Ochodaeidae (both non-scarabaeid Scarabaeoidea). The families Hydraenidae and Ptiliidae are each found to be monophyletic by three of four analyses, with neither observed in the PAUP ml tree. The family Leiodidae is also supported by three of four analyses, not found in the combined analysis, and a grouping of Leiodidae with Agyrtidae is found by all analyses except Bayesian analysis of 18S. Most other groups of interest (above the family level) are found only in one or two analyses, or not at all.

Outgroup relationships are worth brief examination. First, in 3 of 5 trees, the resolution of suborders (Archostemata (Myxophaga (Adephaga, Polyphaga))), as found in Caterino et al. (2002), was obtained. Parsimony analysis of both 18S alone and of combined data (Fig. 6) placed Myxophaga as sister to Polyphaga. All trees put some arrangement of Derodontoidea and Scirtoidea outside of all remaining Polyphaga. Taxon sampling is inadequate to say much more about this, except to note that it conflicts with the placement of Scirtoidea within Elateriformia (e.g., Lawrence and Newton, 1995). Elateriformia otherwise appear monophyletic, including Buprestoidea, Byrrhoidea, and Elateroidea, and most interestingly Dascilloidea. Thus, there is no support whatsoever for a suggested relationship between Dascilloidea and Scarabaeoidea (e.g., Crowson, 1960).

4. Discussion

Our analyses help resolve some relationships among lower Polyphaga. In particular, the grouping of Staphyliniformia with Scarabaeoidea, as the traditional Haplogastra, is supported by most analyses. The conflict with this finding found in the combined data tree (Fig. 6) derives solely from the odd inclusion of the scirtoid *Clambus* within this clade (near aleocharine Staphylinidae), seemingly with good decay support by both 18S and morphology. However, *Clambus* is not represented by morphological data in this study, so 18S must be responsible, despite the fact that the taxon is found in its more conventional placement in all analyses of 18S alone. This clade (including also Agyrtidae, Leiodidae, Hydraenidae, and Ptiliidae) does contain some of the most divergent sequences in the analysis, and it is tempting to infer long branch attraction problems. However, many intervening lineages (Passalidae, Histeroidea) are similarly divergent but not similarly misled (see Fig. 4). Whatever the explanation, it seems clear that this placement of *Clambus* is an anomaly of some sort, and that the monophyly of Haplogastra may be concluded with some confidence.

Within Haplogastra, the only superfamily level group found consistently to be monophyletic with strong support is Histeroidea (Histeridae, Synteliidae, and Sphaeritidae). Hydrophiloidea (sensu stricto) is supported by parsimony and Bayesian analyses of 18S alone (Figs. 3 and 5, respectively), though single lineages prevent its monophyly in the PAUP ml tree or analysis of combined data. In the former case, the ptiliid *Cephaloplectus* appears within an otherwise monophyletic Hydrophiloidea, while in the latter, *Georissus* is separated from the monophyletic remainder (in the strict consensus, though not in all of the most parsimonious trees). These inconsistencies are very minor, and cannot be considered sufficient to overturn a relatively large body of morphological evidence supporting hydrophiloid monophyly (Hansen, 1997). Support for monophyly of the other two superfamilies in this analysis, Staphylinoidea and Scarabaeoidea, is weaker or nonexistent, respectively. Staphylinoidea monophyly is found by parsimony analysis of 18S alone (Fig. 3), with moderate support (three steps). In the combined data analysis (Fig. 6), as discussed above, the scirtoid *Clambus* appears within an otherwise monophyletic Staphylinoidea. Even if this odd result can be safely discounted, these data can be said to offer only ambiguous support for a monophyletic Staphylinoidea. The lack of support for Scarabaeoidea is rather more surprising, as this group's monophyly has never been seriously questioned, and it is well defined by both adult and larval characters. Several families and subfamilies of Scarabaeoidea tend to group together, particularly several families generally considered basal within the superfamily, Pleocomidae, Geotrupidae, Lucanidae, Trogidae, and Passalidae. However, some families that many would include here, such as Glaresidae and Hybosoridae/Ceratocanthidae, never fall out with these. With a couple of extreme exceptions, like Passalidae and to a lesser extent Trogidae, the 18S sequences of these scarabaeoids exhibit relatively little overall divergence and it may be that there is simply inadequate signal to group them.

The trees presented here are as notable for groups not found as for those supported. At the superfamily level, Scarabaeoidea has already been discussed. That some families (Leiodidae, Hydraenidae, and Ptiliidae) were not consistently found to be monophyletic was also surprising. For Leiodidae, the lack of support seems to derive primarily from the morphological data, as its monophyly is supported (at least moderately) by all 18S analyses. Morphological data were only included for one of three leiodid exemplars, so as with *Clambus* above, missing morphological data seem to negatively impact the resolving power of 18S. For both Hydraenidae and Ptiliidae the PAUP ml tree (Fig. 4) separates one of their representatives widely from the others, with the hydraenid *Ochthebius* placed as sister to the entire remaining Haplogastra, and the ptiliid *Cephaloplectus* placed within Hydrophilidae. With strong support for each of these families' monophyly in both parsimony and Bayesian analyses of the same data set, this is difficult to interpret. It must be considered possible that although the PAUP ml tree exhibits the best likelihood of the trees tested here, it may still represent a suboptimal solution. (Simply moving each of these taxa to the base of their respective families and recalculating likelihoods did not improve the tree scores; results not shown.) A sister group relationship between Hydraenidae and Ptiliidae is moderately well supported by these analyses. It is strictly observed only in the 18S parsimony tree (decay support four steps). In the PAUP ml tree the two families form a clade, but each is lacking a member, as described above. The two are joined by the agyrtid *Apteroloma* in a larger clade in the Bayesian tree, and join an unresolved clade including Agyrtidae, Leiodidae, Aleocharinae, *Euaesthetus*, and *Clambus* in the combined analysis. Sequences of all the hydraenids and ptiliids included here are among the more divergent sequences included, so some long branch attraction problems may be partly responsible for difficulties resolving their relationships, though it might just as well be suggested that this underlies their association where observed as well. In any case, the families are generally close in the tree, and no association between any hydraenid and Hydrophiloidea (as has frequently been suggested) is observed in any analysis.

No support for a Hydrophiloidea + Histeroidea clade was found in these analyses. The two groups (with exceptions to monophyly of the former as above) were, however, frequently in close phylogenetic proximity. In parsimony analysis of 18S alone (Fig. 3), they are found

to form a paraphyletic grade with respect to the remaining Haplogastra, with Histeroidea sister to the entire larger group. Support for the clade excluding Histeroidea is decent, at three decay steps, and it represents an interesting hypothesis, worth examining further. Maximum likelihood analysis (Fig. 4) yields a similar result, adding, however, the Agyrtidae + Leiodidae clade as sister to Histeroidea. Bayesian results (Fig. 5) are also similar, with the same basic arrangement of Hydrophiloidea, Histeroidea, and Agyrtidae/Leiodidae, but with the Hydraenidae + Ptiliidae lineage outside of these, and sister to the remaining Haplogastra.

Monophyly of Staphylinidae is not supported by any analysis. This result is not unexpected, as workers have long suspected that several smaller staphylinoid families, including, here, Scydmaenidae and Silphidae (as well as several not treated here) are derived from within Staphylinidae itself (Lawrence and Newton, 1982). For the taxa included here, Hansen's (1997) tree would have supported staphylinid monophyly, with Scydmaenidae and Silphidae branching off prior to the origin of Staphylinidae *s. str.* In all of our trees Staphylinidae is found to be paraphyletic with respect to these taxa. Specific origins within the group are difficult to pinpoint with confidence. Silphidae have a surprisingly strong association with Phloeocharinae, but the two together are not placed consistently with respect to any other staphylinids. A sister group relationship between Scydmaenidae and Pseudopsinae is observed in the parsimony 18S tree (two decay steps) but this is not supported by other analyses. It is also worth noting that two other taxa historically considered to represent families outside of Staphylinidae, *Scaphidium* and *Micropeplus*, invariably resolve within Staphylinidae.

5. Conclusions

The results presented here ultimately yield valuable insights into the phylogenetic relationships of some of the major lineages of polyphagan Coleoptera. While varied in minor details, the broadest patterns are consistent and relatively well supported. First, our primary goal of conclusively testing the monophyly of Haplogastra, comprising Staphyliniformia and Scarabaeiformia, was achieved; the group is well supported by 18S. Within Haplogastra, a Histeroidea, Hydrophiloidea, and possibly Agrytidae + Leiodidae, grade appears likely to be paraphyletic with respect to the remainder of the group. Hydraenidae are conclusively not hydrophiloids, and are resolved virtually invariably (excepting a slightly problematic lineage of each) as sister to Ptiliidae. These conclusions represent important advances in our knowledge of beetle phylogeny. Beyond these, our results are less consistent. In particular, the placement of some of the higher staphylinoid lineages, including Hydraenidae + Ptiliidae and Agyrtidae + Leiodidae, was ambiguous. Despite relatively thorough sampling, Scarabaeoidea were also problematic, never resolving as monophyletic. Though this is not an inconceivable result, the various scarabaeoid families should certainly show more cohesion than found here. Clearly the major factor hindering further resolution is that this is the lower limit of resolving power for 18S. Sequence from additional more rapidly evolving genes would greatly benefit future analyses of these relationships.

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