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The phylogeny of the Histeroidea (Coleoptera: Staphyliniformia)

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Abstract

For its size (ca. 4000 species) the Histeridae is one of the most ecologically and morphologically diverse families of beetles. Its mostly predaceous members occupy a wide variety of habitats for which their morphologies may be highly modified. Previous attempts to resolve the phylogeny of the family based on morphological data have left many difficult issues unresolved. This study is the first to utilize either larval or molecular (18S rDNA) data in combination with adult morphology in an attempt to resolve these issues. We compare the performance of optimization alignment with a fixed positional homology approach, over a range of parameter space. Optimizing alignment parameters for combined analyses of 18S and morphology for both approaches resulted in very similar topologies. Contrary to previous hypotheses which held the cylindrical, subcortical forms of the family (e.g., *Niponius*, *Trypanaeus*, *Trypeticus*) to be the most primitive, our analyses find these to be highly specialized forms derived from within other more generalized taxa. Basal lineages within the family instead include *Onthophilus*, *Anapleus*, and *Dendrophilus*, all of which are ovoid, mainly generalist forms.

Keywords: Ecomorphology; Canalization; Myrmecophily; Optimization alignment; Partitioned Bremer support; POY

Introduction

Beetles have featured prominently in the quest to understand patterns and underlying causes of organismal diversity. They have long been recognized as one of evolution's greatest success stories and have motivated many of our greatest evolutionary thinkers, with Darwin, Wallace, and Bates all beginning their naturalist lives as Coleopterists. While the numerical diversity of Coleoptera has been widely cited, their unrivalled ecological diversity is less generally appreciated. However, linking studies of the two promises to reveal many fundamental evolutionary patterns. Beetles in the family Histeridae offer a particularly interesting system with which to examine the interaction between ecological differentiation and diversity. Their potential value in this respect was first pointed out by Henry Walter Bates (1863), reflecting on his explorations in Amazonia:

> It is curious to observe how some small groups of insects exhibit the most diversified forms and habits... Thus the Histeridae are most diversified in structure and habits in the Amazons region; nevertheless all the forms preserve in a remarkable degree the essential characters of the family. Several families of insects show similar diversities of adaptation amongst their species; but none, I think, to the same extent as the Histeridae... The facts presented by such groups... must be taken into account in any explanation of the way the almost infinite diversity of the forms of life has been brought about on this wonderful earth.

Histerid beetles, although generalist predators, have widely varied habits. Common habitats include dung, carrion, fungi, dead and dying trees, leaf litter or other decomposing vegetation, and symbioses with other animals, most spectacularly with social insects. This ecological diversity is often paralleled by their morphological diversity, with many lineages exhibiting extreme modifications in body form (Fig. 1). Many species that inhabit the subcortical spaces under the bark of dead or dying trees are perfectly flat, while those associated with the galleries of wood-boring beetles may be perfectly cylindrical. These morphologies contrast sharply with the widespread, ovoid morphology seen in many common species. Within ecomorphological

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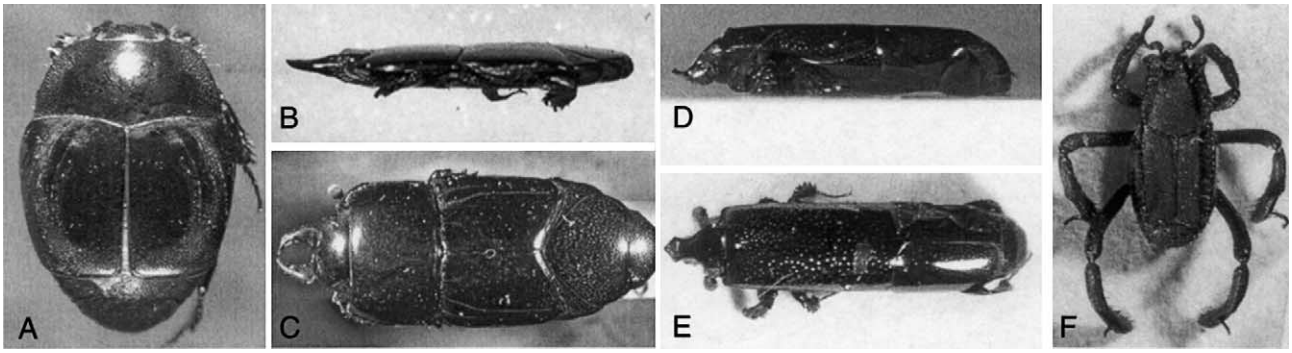

Fig. 1. Some of the more common body forms in Histeridae. (A) *Saprinus discoidalis* LeConte, exhibiting the widespread, convex ovoid body form. (B) Lateral and (C) dorsal views of a species of *Hololepta* (Histerinae), illustrating the flattened, subcortical morphology. More extremely flattened species than this are known. (D) Lateral and (E) dorsal views of *Trypanaeus junceus* Lewis (Trypanaeinae) illustrating the cylindrical morphology found in many bark and ambrosia beetle predators. (F) *Euxenister caroli* Reichensperger, a myrmecophile, showing one of an almost endless array of unusual morphologies associated with this life history.

groups it is notable that morphology is typically highly conserved, with numerous species showing only superficial variation. There are, however, a few significant exceptions to this morphological conservatism in the family, particularly in the inquilinous groups, where genera and species may differ tremendously from one another (Helava et al., 1985; Fig. 1F). Thus histerids present an array of interesting ecomorphological problems. Using a phylogeny of Histeridae, based on morphological and molecular data, it is the purpose of this paper to examine the relationships among the major lineages of Histeridae. This framework then provides the basis for a preliminary exploration of evolutionary patterns in the group.

Systematics of the Histeroidea

Histerid beetles are members of the Hydrophiloidea (sensu Lawrence and Newton, 1995), all of which share, in the larval instars, desclerotized abdomens, articulated urogomphi, prognathy, large, falcate mandibles (lacking molar region), and fusion of the labrum and clypeus to the head capsule forming a toothed "nasale." Furthermore, all known larvae are carnivorous. Adults of Hydrophiloidea are more varied in morphology, but generally possess an antenna with an elongate scape and compact, three-segmented club. Recent works (e.g., Archangelsky, 1998; Hansen, 1997; Ohara, 1994) have tended to recognize two separate superfamilies within this group: the Hydrophiloidea, composed primarily of aquatic taxa, and the Histeroidea, which all have terrestrial habits. However, these groups are widely accepted to be monophyletic sister taxa (Hansen, 1991; Hansen, 1997; Lawrence and Newton, 1995). Monophyly of the histeroid lineage is supported by several features of adults, including truncate elytra, elbowed antennae, and ovipositor with dentate, scoop-like gonocoxites, and at least one character of the larvae, reduced number of stemmata (0 or 1) (Lawrence and Newton, 1982; Newton, 1991). This lineage contains three families: Sphaeritidae, Synteliidae, and Histeridae. Sphaeritidae and Synteliidae contain single genera with 4 and 5 species, respectively. *Sphaerites* appears to be saprophagous in the adult but predaceous during larval instars (Löbl, 1996; Newton, 2000; Nikitsky, 1976b; Barclay, personal communication), while *Syntelia* are probably predaceous in all stages (Mamayev, 1974; Newton, 1991). The predaceous Histeridae contains the majority of the diversity in the lineage, with around 330 genera and 3900 described species (Mazur, 1997).

The phylogeny of Histeridae is receiving increasing attention. Wenzel (1944) implemented the first explicitly evolutionary classification of Histeridae, separating several groups previously lumped on the basis of gross similarity and combining some others whose autapomorphies had led to taxonomic recognition at inappropriately high levels. Crowson (1955, 1974) discussed putatively basal histerids in the interest of shedding light on higher level relationships. While the earlier of these papers espoused the hypothesis that the most basal forms would be found among the cylindrical species associated with dead wood (*Niponius*, Trypanaeinae, Teretriini), in the later paper he suggested that *Onthophilus* might be "among the most primitive" Histeridae. Unfortunately he did not elaborate on the apparent conflict of these hypotheses. The first cladistic examination of histerid relationships was Ohara's (1994). Among his conclusions was the suggestion that Wenzel's two main lineages of Histeridae, Saprinomorphae, and Histeromorphae, were likely to be artificial (see Fig. 2A). However, his analysis was based on a limited character set (16 characters, only 9 of which were phylogenetically informative), which, combined with ad hoc decisions of character polarity, yielded few other unambiguous results. More recently Ślipiński and Mazur (1999) presented a more thorough analysis, with representatives of all histerid subfamilies and tribes, and 29

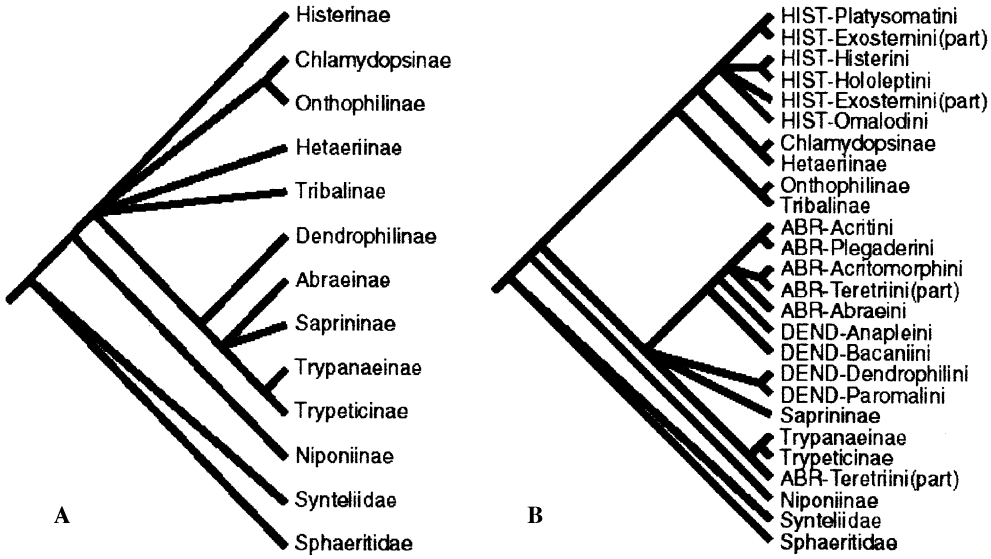

Fig. 2. Previous hypotheses of histerid relationships. (A) The hypothesis of Ohara (1994). (B) The hypothesis of Ślipiński and Mazur (1999). Abbreviations follow Table 1.

characters of adult morphology. Their results (Fig. 2B) agreed with several aspects of Ohara's phylogeny, most notably the artificiality of the Saprinomorphae/Histeromorphae division and a basal position for the cylindrical bark-beetle predator *Niponius*, supporting Crowson's (1955) hypothesis. Yet their work leaves many issues unresolved. Their character set retains many characters that change only on terminal or uncontested branches, with branches of real interest either unresolved or supported by few changes. Despite this fact the authors used their phylogeny as the basis for a major reclassification of the family. While some of these changes are justified (and long anticipated), others are poorly supported and seem likely to be short-lived.

Analytical issues

We utilize 18S rDNA sequences as our molecular marker. It is known that variation in 18S is not distributed evenly through the molecule. Due to incompletely understood functional constraints related to secondary and higher level structure, four more or less "conserved" regions are separated by more variable segments (Hancock et al., 1988; Tautz et al., 1988). Substitution dynamics may vary greatly among these regions, particularly in the frequencies of indels, which may be high in the variable segments. Thus closely related sequences may differ substantially in length, and positional homologies may be difficult to determine. A variety of methods have been developed for analyzing such length-variable data (reviewed in Phillips et al., 2000). Perhaps the most philosophically promising of those yet developed is optimization alignment (Wheeler, 1996). Whereas most approaches attempt to designate positional homologies in length-variable regions a priori (assuming there are homologies in these regions, which may be questionable (Hancock and Vogler, 2000)), optimization alignment optimizes indels and substitutions, via parsimony, onto topologies being compared and assigns positional homologies only during tree building. Hence, indels are treated as character state observations (Wheeler, 1996). This method also overcomes the bias of constructing a tree from what is essentially a tree-based alignment (as in Clustal). However, despite the intellectual appeal of the optimization alignment approach, it has not yet been widely applied and it is unclear how to best parameterize the algorithm. In this paper we examine the behavior of optimization alignment (as implemented in the computer program POY) alongside a more traditional multiple alignment approach, over similar parameter space, in the reconstruction of histerid relationships.

Materials and methods

Taxa

Outgroup. We have included four taxa as outgroups, including two presumably basal hydrophiloids (Archangelsky, 1998; Hansen, 1991), *Hydrochus* and *Helophorus*, and representatives of both other families of Histeroidea, Sphaeritidae the Palearctic *Sphaerites glabratus* Fabricius, and Synteliidae, the Asian *Syntelia histeroides* Lewis.

Ingroup. We have included one or more members of all generally recognized tribes and subfamilies of Histeridae (listed in Table 1), with the exception of Acritomorphini, for a total of 37 ingroup taxa. This selection

Table 1
Taxa sampled for this study

Family	Subfamily	Tribe	Species	Locality	GenBank Accession No.
Helophoridae			*Helophorus brevipalpus*	UK: Wiltshire	AY028329
Hydrochidae			*Hydrochus angustatus*	Spain: Girona	AY028330
Sphaeritidae			*Sphaerites glabratus*	Russia: Priosko-Terrazny Reserve, Moscow District	AY028331
Synteliidae			*Syntelia histeroides*	Japan: Kyushu: Ohita	AY028332
Histeridae	Niponiinae (NIP)		*Niponius andrewsi*	India: Kumaon	Morphology only
	Trypanaeinae (TRYP)		*Xylonaeus* sp.	Ecuador: Napo	AY028333
			Coptotrophis proboscidea	French Guiana: Paracou	AY028334
	Trypeticinae (TRYP)		*Trypeticus* sp.	Indonesia: Sulawesi	Morphology only
	Abraeinae (ABR)	Teretriini (Ter)	*Teretrius* sp.	USA: NV: Reno	AY028335
		Plegaderini	*Plegaderus* prob. *nitidus*	USA: CA: Contra Costa	AY028336
		Acritini	*Acritus* sp.	USA: GA: Cobb	AY028337
		Abraeini	*Abraeus globosus*	UK: Berkshire: Silwood Park	AY028338
	Saprininae (SAP)		*Xerosaprinus* sp.	USA: CA: Stanislaus	AY028339
			Aphelosternus interstitialis	USA: CA: San Diego	AY028340
			Saprinus lugens	USA: CA: Mendocino	AY028341
			Neopachylopus sulcifrons	USA: CA: Sonoma	AY028342
	Tribalinae (TRIB)		*Epierus* sp.	Costa Rica: Guanacaste	AY028343
			Plagiogramma sp.	Ecuador: Napo; Yasuni Station	AY028344
			Idolia sp.	Belize: Orange Walk	AY028345
	Onthophilinae (ONTH)		*Onthophilus flohri*	Mexico: Durango	AY028346
	Dendrophilinae (DEND)	Paromalini (Paro)	*Xestipyge geminatum*	USA: NY: Suffolk	AY028347
			Platylomalus aequalis	USA: GA: Cobb	AY028348
			Paromalus flavicornis	UK: Richmond Park	AY028349
		Bacaniini (Bac)	*Bacanius punctiformis*	USA: GA: Cobb	AY028350
		Dendrophilini	*Dendrophilus punctatus*	USA: WI: Wood	AY028351
			Kissister minima	Morroco: Atlas Mts.	AY028352
		Anapleini (Anap)	*Anapleus* sp.	USA: TX: Brewster	AY028353
	Histerinae (HIST)	Exosternini (Exo)	*Phelister nr. williamsi*	Belize: Cayo	AY028354
			Baconia sp.	Belize: Cayo	AY028355
			Operclipygus sp.	Belize: Cayo	AY028356
		Omalodini (Oma)	*Omalodes grossus*	Belize: Cayo	AY028357
		Platysomatini (Pla)	*Platysoma punctigerum*	USA: AZ: Apache	AY028358
		Hololeptini (Hol)	*Hololepta* sp.	Belize: Cayo	AY028359
		Histerini (Hist)	*Hister unicolor*	UK: London	AY028360
			Macrolister gigas	South Africa	AY028361
			Margarinotus graecus	Morocco: Atlas Mts.	AY028362
			Spilodiscus sellatus	USA: CA: Marin	AY028363
	Hetaeriinae (HET)		*Psalidister* sp.	Belize: Cayo	AY028364
			Synoditulus sp.	Ecuador: Napo, Yasuni Station	AY028365
			Scapicoelis sp.	Belize: Cayo	AY028366
	Chlamydopsinae (CHL)		*Orectoscelis brendelli*	Indonesia: Sulawesi	Morphology only

Note. Abbreviations given after names in tribe and subfamily columns are applied in subsequent figures to facilitate association.

includes three taxa for which we have only been able to obtain morphological data (*Trypeticus*, *Niponius*, and Chlamydopsinae [*Orectoscelis*]).

DNA extraction and sequencing

Total DNA was isolated from live-frozen or ethanol-preserved specimens using either a phenol–chloroform procedure or Qiagen's QIAamp Tissue Kit. It was found in the course of the study that in the latter procedure it was possible to digest sufficient tissue from specimens without grinding them. Specimens were broken apart between the pro- and mesothorax and bathed in warm lysis buffer and proteinase K for 2–3 h; the two parts were then removed, rinsed in ethanol, and mounted as voucher specimens (which are in the collection of the senior author). 18S was amplified in four fragments using the primer pairs 18S5′–18Sb5.0, 18Sai–18Sb0.5,

18Sa1.0–18Sbi, and 18Sa2.0–18S3′I, the sequences of which are given in Shull et al. (2001) and Whiting et al. (1997). Amplification or sequencing was unsuccessful for a few segments as follows: *Xestipyge geminatum* (18Sa2.0–18S3′), *Baconia* (18Sbi (so the penultimate fragment is incomplete) and 18Sa2.0–18S3′). Automated fluorescence sequencing was carried out on either an ABI 377 or an ABI 3700 using Perkin–Elmer BigDye sequencing chemistry. Fragments were sequenced in both directions.

Morphological characters

The morphological data used in this study include 37 adult characters and 15 larval characters. These characters were developed and scored using the collections of the senior author and specimens in The Natural History Museum, London. All characters are analyzed as unordered and polarities are determined solely by outgroup comparison. These data are shown in Table 2. See Appendix A for discussion of these characters and their respective states. Most of these are illustrated in Figs. 7–10.

Molecular characters

The molecular data for this study consist of complete sequences of the 18S ribosomal RNA gene. This gene has a long history of application to higher level phylogenetic studies, in insects as well as nearly all other major groups (e.g., Abouheif et al., 1998; Campbell et al., 1997; Caterino et al., 2002; Soltis et al., 1999; Whiting et al., 1997). The 18S gene generally evolves very slowly and most problems to which it has been applied have focused on relationships among taxa at the family level and higher. However, preliminary indications from larger studies in progress were that Histeroidea 18S showed greater than normal variation that might prove useful at the intrafamily level.

Phylogenetic analysis

It is our goal to determine those aspects of the phylogeny best supported by simultaneous analysis of available data, on the basis that this maximizes the overall explanatory power of the resulting hypothesis (Kluge, 1998). However, given the variety of data sources, parameterizing the simultaneous analysis would benefit from preliminary examinations of certain data subsets. In particular, the varied dynamics of 18S, with regions of differing length and nucleotide composition, constitute a major challenge for phylogenetic reconstruction. Some authors have assumed these hypervariable regions to constitute little more than "noise," discarding them a priori. This can only properly be determined via phylogenetic analysis. There is no reason to believe that these regions have experienced a different phylogenetic history than the conserved regions. Their information is at worst obscured by multiple changes and frequent insertion/deletion events. Our initial set of analyses examines the question under which parameter sets homology in the variable regions may be maximized. In the absence of objective criteria for independent establishment of these parameters it is possible to arbitrate among parameter values by testing for congruence with data not affected by alignment ambiguity (sensitivity analysis *sensu* Wheeler, 1995). Thus, we begin with separate analyses of morphology and conserved regions of 18S and use the resulting trees to compare results of POY and Clustal analyses of full 18S sequences. These comparisons then guide parameterization of combined analyses of 18S and morphology.

Preliminary examinations included analyses of separate and combined larval and adult morphological data. These separate analyses do not bear on our final phylogenetic conclusions, but the information content of histerid morphology has been debated, and their behavior apart from 18S is worth examining. The combined morphology tree is utilized below for congruence analyses. Morphological data were analyzed using PAUP* (Swofford, 1998): 100 random addition replicates, TBR, followed by an additional search using weights based on RCI reweighting on these initial trees. Reweighting helped to reduce the very large number of equally parsimonious trees found in some analyses (Carpenter, 1988), mainly a problem for larval and, by extension, complete morphological data.

18S sequences were first divided into 4 conserved and 3 variable regions (designated V(ariable)1–7; following Shull et al., 2001 and Tautz et al., 1988). These regions are delimited somewhat arbitrarily based on primary structure variation. With very few exceptions in Coleoptera, regions of substantial alignment ambiguity are narrowly bound by conserved regions. These boundaries are generally sharp and readily recognizable in aligned data matrices. Hence, the delimitation of the variable regions leaves little ambiguity, and even if the boundaries were drawn slightly differently the associated "error" from constraining which bases can be homologized to each other would be small. The subdivision of the sequence into seven region is conservative, as regions with substantial sequence similarity can be detected within the variable regions and could be used to subdivide sequences further and hence constrain the homologization of bases more narrowly. (Previous workers have recognized as many as 47 regions within 18S; e.g., Giribet et al., 2000.)

For Clustal (Clustal X1.81; Thompson et al., 1994) alignments, a preliminary tree was computed based on the conserved regions of the molecule alone (V1, V3, V5, and V7). This was constructed with PAUP*: 100 random addition replicates, TBR, 100 trees held at each

Table 2
Morphological data for the taxa included in this study

	Larvae															Adults																																				
Character:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Helophorus	2	3	3	2	1	1	2	1	2	2	1	1	1	1	2	3	1	1	1	1	1	2	2	1	1	2	3	2	1	1	2	1	2	1	2	1	1	1	1	1	1	1	1	2	1	1	1	1	2	3	2	1
Hydrochus	2	2	2	2	1	2	1	1	2	2	1	1	1	1	4	3	1	1	1	1	1	2	2	1	1	2	3	2	1	1	2	1	2	1	2	1	1	1	2	1	1	1	1	2	1	2	1	1	1	3	2	1
Sphaerites	1	1	2	2	1	1	1	1	?	2	1	1	1	?	2	2	1	a	1	1	1	2	1	1	1	1	3	2	1	1	2	1	2	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1
Syntelia	1	1	2	2	2	1	2	2	?	2	1	1	1	1	2	1	1	2	1	1	1	2	1	1	1	2	3	2	2	1	2	1	2	1	2	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1
Onthophilus	1	1	3	2	2	1	2	2	1	2	2	1	1	1	1	2	1	2	3	1	1	2	1	2	1	2	1	1	2	1	2	1	1	1	1	2	2	2	1	1	1	1	1	2	1	2	1	1	1	1	2	1
Dendrophilus	1	2	2	2	2	1	2	2	1	2	2	1	1	2	1	2	1	2	1	1	3	2	2	1	1	2	1	2	2	2	1	2	1	1	1	2	2	2	1	1	1	2	2	2	1	2	1	1	1	2	2	1
Kissister	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	2	2	3	1	1	2	2	2	1	2	1	2	2	3	1	2	1	1	1	2	2	2	1	1	1	2	2	2	2	2	1	2	2	2	2	1
Platylomalus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	1	2	1	1	2	a	1	2	3	2	1	2	2	1	1	2	1	a	1	2	2	2	1	1	1	1	2	2	2	2	2	2	2	2	2	1
Xestipyge	1	1	2	2	2	1	2	2	1	2	2	1	1	2	1	2	1	2	1	1	2	2	2	2	1	2	1	2	2	1	1	2	1	1	1	2	2	2	1	1	1	1	2	2	2	2	1	2	2	2	2	1
Paromalus	1	1	2	2	2	1	1	2	1	2	2	1	1	1	1	2	1	2	1	1	2	1	1	2	3	2	1	2	2	1	1	2	1	a	1	2	2	2	1	1	1	1	2	2	2	2	2	2	2	2	2	1
Bacanius	1	1	2	2	2	1	2	2	1	2	1	1	1	1	4	3	1	1	2	1	4	2	2	2	1	2	1	2	1	2	2	2	1	2	1	2	2	2	2	2	2	2	2	2	1	2	1	1	1	2	1	1
Abraeus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	1	2	3	1	4	2	2	2	1	2	1	2	1	2	2	2	a	a	a	2	2	4	2	1	1	1	1	2	1	2	2	3	?	2	2	1
Acritus	1	1	2	2	2	2	2	2	1	2	?	2	1	1	4	c	1	2	2	1	5	2	2	2	2	2	1	2	2	2	2	2	1	a	1	2	2	2	2	1	1	1	2	2	1	2	1	3	?	2	1	1
Plegaderus	1	1	1	2	2	2	2	2	1	2	2	2	1	1	1	3	1	2	2	1	4	2	2	2	1	2	1	2	1	1	2	2	1	1	1	2	2	2	2	1	1	1	2	2	1	2	2	3	?	2	1	1
Teretrius	1	1	a	2	2	2	2	2	1	2	?	2	a	1	b	3	1	2	2	1	5	2	2	2	2	2	1	2	1	1	2	2	1	1	a	2	2	2	1	1	1	1	2	2	1	2	1	3	?	2	2	1
Coptotrophis	1	1	2	1	2	2	2	2	1	2	2	2	1	1	3	3	1	2	1	1	5	2	2	2	1	2	1	2	1	1	2	2	1	1	2	2	2	2	2	2	2	1	2	2	1	2	3	3	?	2	1	1
Xylonaeus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	3	1	2	1	1	5	2	2	2	1	2	1	2	1	1	2	2	1	1	2	2	2	2	2	2	2	1	2	2	1	2	3	3	?	2	1	1
Saprinus	1	1	3	2	2	1	2	2	2	1	1	1	1	2	1	2	1	2	1	1	4	1	1	2	1	2	2	2	1	1	2	1	1	1	1	2	2	2	1	1	1	1	2	2	1	2	1	2	1	2	2	1
Neopachylopus	1	1	3	2	2	1	2	2	2	1	1	1	1	2	1	2	1	2	1	1	4	1	1	2	1	2	2	2	1	1	2	1	1	1	1	2	2	2	1	1	1	1	2	2	1	2	1	2	1	2	2	1
Aphelosternus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	1	2	1	1	4	2	1	2	1	2	2	2	1	1	2	1	1	1	1	2	2	2	1	1	1	1	2	2	1	2	1	2	1	2	2	1
Xerosaprinus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	1	2	1	1	4	a	1	2	1	2	2	2	1	1	2	1	1	1	1	2	2	2	1	1	1	1	2	2	1	2	1	2	a	2	2	1
Margarinotus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	2	1	1	1	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	2	2	1	2	3	2
Macrolister	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	2	1	1	1	a	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	2	2	1	2	3	2
Hister	1	2	2	2	2	1	2	2	2	2	1	1	2	3	1	1	1	2	1	1	1	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	2	2	1	2	3	2
Spilodiscus	1	1	2	2	2	1	?	2	2	2	1	1	2	3	1	1	1	2	1	1	1	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	2	2	1	2	3	2
Idolia	1	1	3	2	2	1	2	2	?	2	2	1	1	1	1	2	1	1	3	1	1	2	2	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	2	2	?	2	2	1
Epierus	1	a	2	2	2	1	2	2	1	2	2	1	1	1	1	4	1	1	1	1	1	2	2	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	1	2	1	2	1	1
Plagiogramma	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	4	1	1	1	1	1	2	2	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	2	2	?	2	2	1
Synoditulus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	2	1	2	6	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	2	1	1	1	2	2	1	2	3	2	1	2	?	1
Psalidister	1	1	2	2	2	1	2	2	2	2	1	1	1	2	4	1	2	2	1	2	6	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	2	2	2	2	2	2	1	2	3	2	2	2	?	1
Scapicoelis	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	2	2	1	2	6	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	2	2	2	2	2	2	1	2	1	2	1	2	?	1
Operclipygus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	2	1	1	3	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	3	1	1	1	1	2	2	1	2	2	2	1	2	1	1
Baconia	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	2	1	1	3	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	3	1	1	1	1	2	2	1	2	1	2	1	2	1	1
Phelister	1	1	2	2	2	1	2	2	2	2	1	1	1	?	?	1	1	2	1	1	3	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	1	2	2	2	1	2	1	1
Omalodes	1	2	2	2	2	1	2	2	2	2	1	1	1	2	1	1	1	2	1	1	3	a	1	2	3	2	1	1	2	1	2	2	1	1	a	2	2	3	1	1	1	1	2	2	1	2	2	2	1	2	1	1
Platysoma	1	1	3	2	2	1	1	2	2	2	1	1	1	1	1	1	1	2	1	1	3	1	1	2	3	2	1	1	2	1	2	2	1	1	1	2	2	3	1	1	1	1	2	2	1	2	2	2	1	2	1	1
Hololepta	1	1	3	2	2	1	2	2	2	2	1	1	1	3	1	1	1	2	1	1	3	a	1	2	3	2	1	1	a	1	2	2	1	1	a	2	2	3	1	1	1	1	2	2	1	2	2	2	1	2	1	1
Anapleus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	1	2	3	1	3	2	2	2	1	2	1	1	2	2	2	1	1	1	1	2	2	4	2	2	2	1	2	2	1	2	1	1	1	1	1	1
Trypeticus	1	1	2	1	2	2	2	2	1	2	?	2	1	1	3	3	1	2	1	1	5	2	2	2	1	2	1	2	1	1	2	2	1	1	2	2	2	2	2	2	2	1	2	2	1	2	3	3	?	2	1	1
Niponius	1	1	2	2	2	1	2	2	?	2	1	1	2	?	1	2	1	2	1	1	2	2	1	2	1	2	3	2	2	1	2	2	1	1	1	2	2	2	1	1	1	1	2	2	2	2	1	2	2	2	2	1
Orectoscelis	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	2	4	2	4	2	1	2	2	2	1	1	2	1	2	2	1	1	1	2	2	2	2	2	2	2	2	2	1	2	2	2	1	2	?	1

Note. Polymorphism codes: a = (1,2); b = (1,3); c = (2,3).

step, followed by an additional search using weights based on RCI reweighting on these initial trees. (Reweighting allowed a single fully resolved topology, required by Clustal, to be obtained.) We designated this tree as a "guide tree" for Clustal's alignment of the variable regions. (Examination of the default dendrograms, which Clustal bases on pairwise alignment scores, revealed little meaningful resolution.) Complete, unaligned sequences were then aligned under a range of gap opening costs (see Table 3 for specific parameters used; gap extension costs were held equal to the gap opening costs so as to be comparable to the results from POY, which does not differentiate the two costs). Transition/transversion ratios were kept at one for all Clustal alignments. The four resulting alignments (CL1-4) were analyzed under parsimony using PAUP* (all positions equally weighted, gaps coded as missing data, 100 random addition replicates, TBR). Two of these (designated CL1w and CL3w) were also analyzed with conserved regions of the molecule weighted at twice the variable regions.

The program POY (Gladstein and Wheeler, 1999) was used for tree alignments. For these analyses, the seven regions of 18S were constrained such that the algorithm could only designate homologies within and not across regions. Because the behavior of the POY algorithm is less well understood, unaligned sequences were analyzed for a slightly broader, and more finely divided, range of gap costs than for Clustal alignments (see Table 3). The analyses were run on a parallel processor (running PVM software), maintained by The Natural History Museum, invoking the following options: -parallel -onan -maxtrees 5 -multibuild 50 -random 10 -tbr. While limiting the number of trees retained to 5 might appear to limit search efficiency, in fact for no optimizations were even 5 trees found. We also carried out optimizations in which the conserved regions of 18S were weighted at 2, 5, and 10 times changes in the variable regions (for these gap cost was set at 2). After completion of searches, the topologies obtained were resubmitted to POY with the "implied alignment" option, resulting in matrices which could be inspected directly.

Few standard metrics allow direct comparison between Clustal/PAUP and POY parameterizations. A variety of possible internal congruence measures, such as consistency indices, numbers of informative characters, can be utilized in such sensitivity analyses. But because POY optimizations do not utilize a fixed size matrix during tree construction (implied alignments are inferred after tree construction), these numbers are not strictly comparable, and do not permit unambiguous comparisons across platforms. We have instead chosen to utilize measures of external congruence, based on topological comparisons. We have adopted as standards of comparison two trees, one based on the full morphological data (Fig. 3C) and one based on the con-

Table 3
Variability across data partitions and alignment schemes

	Morphology		18S only																
				Clustal				POY											
	Larvae	Adult	Constant	CL1	CL2	CL3	CL4	PO1	PO2	PO3	PO4	PO5	PO6	PO7	PO8	PO9	PO10	PO11	PO12
Gap opening penalty	—	—		2	5	10	20	0.33	0.5	1	2	3	4	5	10	20	2	2	2
Gap extension penalty	—	—	—	2	5	10	20	0.33	0.5	1	2	3	4	5	10	20	2	2	2
Weight: conserved regs.	—	—	—	1	1	1	1	1	1	1	1	1	1	1	1	1	2	5	10
Total No. chars.	15	37	1739	2454	2253	2232	2220	3983	2762	2551	2422	2385	2343	2336	2328	2317	2422	2499	2502
Total informative chars.	15	34	152	262	319	314	343	58	205	243	266	276	283	286	310	320	270	255	255
Informative chars. (V1)	—	—	—	22	31	33	38	6	27	31	31	31	31	31	32	33	31	32	32
Informative chars. (V2)	—	—	—	29	37	35	35	10	18	27	29	29	34	34	33	36	30	30	31
Informative chars. (V3)	—	—	—	35	38	39	43	10	36	38	37	37	37	38	39	40	37	38	38
Informative chars. (V4)	—	—	—	50	80	81	75	6	28	35	54	60	62	65	72	73	56	41	40
Informative chars. (V5)	—	—	—	37	44	43	49	5	41	42	42	42	43	43	42	43	42	43	43
Informative chars. (V6)	—	—	—	44	47	45	53	10	26	32	35	38	37	36	51	54	36	33	33
Informative chars. (V7)	—	—	—	37	42	38	50	11	29	38	38	39	39	39	41	41	38	38	38

Note. Parameter values for gap coding and weighting schemes implemented during alignment are given across the top of the table. The values for the "conserved regions" are calculated from the CL3 alignment. The numbers of informative characters for POY implied alignments were calculated using PAUP* in a "gaps as missing data" context. Although this is misleading to the extent that informativeness in POY is not assessed in these terms, it does serve to allow conventional comparison of the two programs' output.

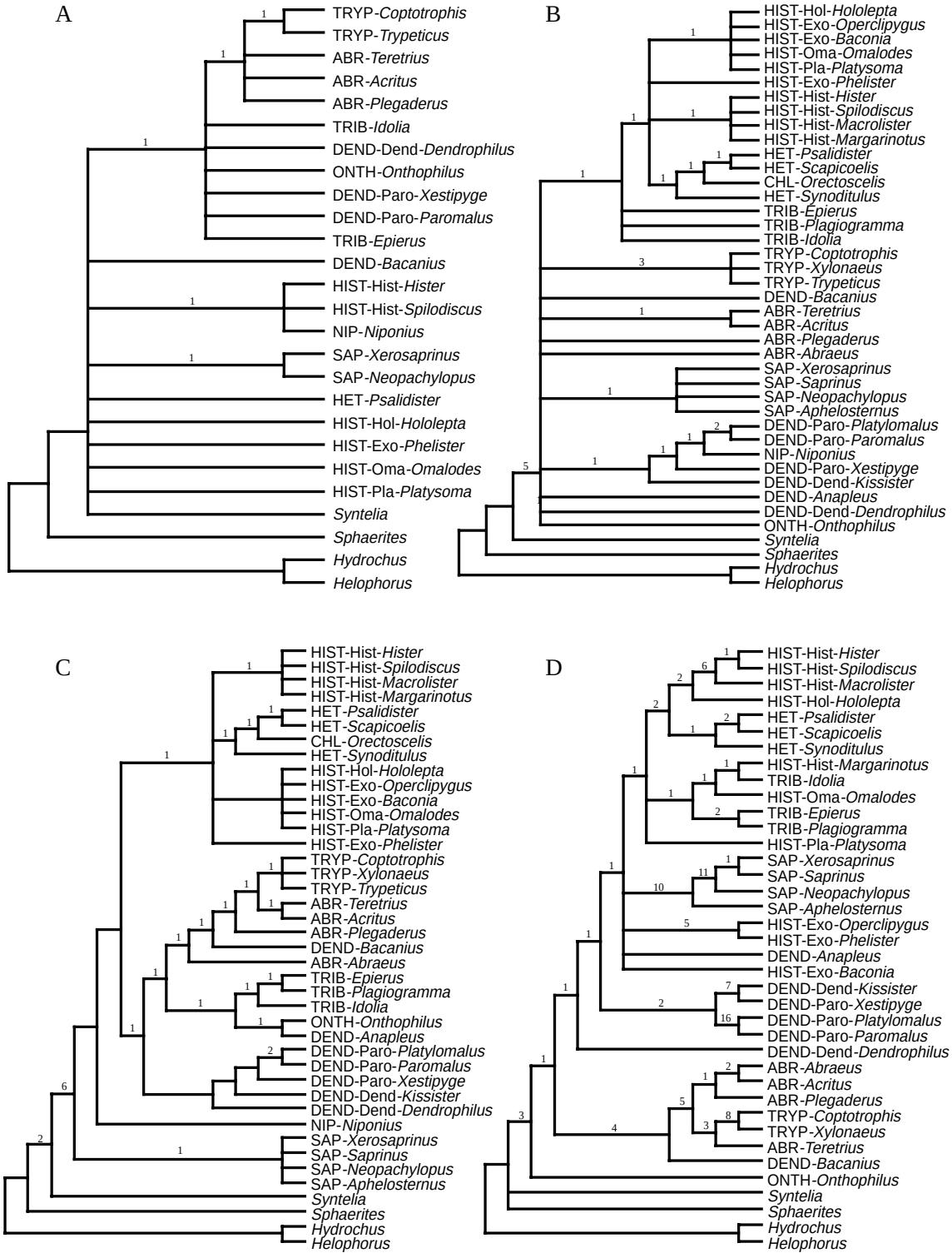

Fig. 3. Trees based on separate and combined morphology and on conserved 18S regions. Abbreviations designating higher taxa refer to those presented in Table 1. Numbers on branches indicate Bremer support. (A) Strict consensus of trees based on larval morphology (total number of most-parsimonious trees unknown; the search was terminated with over 150,000 in memory). (B) Strict consensus of 840 equally parsimonious trees based on adult morphology. (C) Strict consensus of 5780 equally parsimonious trees based on complete morphology. Here, while the Bremer support values derive from unweighted searches, the topology results from one round of rescaled consistency index reweighting (thus some branches have no support value shown). (D) Strict consensus of eight equally parsimonious trees based on conserved regions alone.

served regions (Fig. 3D) of 18S. All complete 18S trees produced by Clustal/PAUP and POY were compared to each of these. While we do not suggest that either the morphology tree or the conserved regions tree represent accurate phylogenetic histories, if the alignment results are sufficiently variable (and preliminary analyses suggested they were), then the "standard" trees should serve as appropriate guides to the approximate quality of alternative alignments. The argument could be raised that comparisons to the conserved regions tree will be biased by their inclusion at various stages of the actual alignments. However, in our implementation, the signal in the conserved regions is invoked in both Clustal and POY procedures, and the exact degree of bias is difficult to assess. Use of the morphology tree as an independent standard serves in part to test the assumption that this bias is roughly similar across analyses. The tree comparisons were implemented using PAUP*. We report results based on both the standard "d" statistic, which measures those quartets which are resolved and different between trees (Estabrook et al., 1985), and the "symmetric difference" (SD), which is based on the proportion of different bipartitions between trees (Penny and Hendy, 1985). Where more than a single tree was obtained for any analysis a strict consensus was used for comparison. (These two measures were chosen in part because they can accommodate trees that are not strictly dichotomous.)

Simultaneous analyses of full 18S data together with the morphological data were carried out on one alignment each from Clustal/PAUP and POY analyses (the best of each as identified above). For the Clustal matrix, the 52 morphological characters were appended to the aligned data and analyzed in PAUP* (100 random addition replicates, TBR, all characters equally weighted; no reweighting). For POY analysis, an additional optimization was run, under the same search parameters as those specified for the best 18S-only analysis, with morphological data included as a separate character partition. Thus the morphological and molecular data were allowed to interact during the assessment of alignment costs. Where the variable regions of 18S were downweighted, the morphological characters were weighted the same as the conserved regions.

Branch support for all analyses was estimated using decay indices (Bremer support; Bremer, 1994). For combined analyses partitioned bremer support (PBS) was used to determine the support derived from each data set. Partitioned Bremer indices also served to assess levels of congruence between data partitions. Initially, estimates of support based on bootstrapping were also calculated. However, levels of support and groups supported differed very little from those derived from decay index calculations and are therefore not presented.

Results

The data

The 18S sequences included in this analysis presented surprising levels of variability, with uncorrected distances ranging from <1% (between two Hetaeriinae) to >15% (between HIST-Exo-*Baconia* and TRYP-*Xylonaeus*). Although there is slight variation depending on which alignment they are based on, consistently about 90% of pairwise divergences are below 7%, with virtually all those greater than 7% divergent involving *Baconia* or *Xylonaeus*. These two are clearly implicated in some long-branch attraction problems (discussed further below). The latter of these two has the longest 18S so far known from polyphagan Coleoptera (2180 bases). While most sequences differ little from the approximately modal (for polyphagan Coleoptera in general) 1830 bases, obvious departures from typical lengths occur in nearly all major groups.

The alignments derived from different methods under different parameter values differ greatly in size and informativeness (see Table 3). Unsurprisingly, as gap costs increased, the total size of the alignment decreased. The rate of decrease is more rapid in Clustal than in the POY alignments, with Clustal alignments near an apparent (and nearer an absolute, based on the length of these sequences) minimum at a gap cost of 10, whereas the size of POY implied alignments continues a slow decrease above gap costs of 10. As alignment length decreases, the number of informative characters increases (as nucleotide changes gain favor over unique insertions) and the overall consistency indices consequently decrease (not shown). Regardless of alignment, informative variation is clearly distributed throughout the molecule, with both conserved and "variable" regions contributing similar numbers of informative sites to the analysis (Table 3). The alignments on which the analyses presented here are based are available on The Willi Hennig Society Homepage.

The topologies

Trees based on the larval and adult data are presented in Figs. 3A and B, respectively. Separately, neither provided strong resolving power, with few groups supported beyond a single decay step. In the combined morphology tree (Fig. 3C), however, although levels of support are still uniformly low, several groups emerge that are not observed in either separate tree, and the topology makes considerable sense, with most subfamilies and tribes appearing monophyletic. This resolution is discussed further with respect to histerid relationships below.

Analysis of the conserved regions of 18S alone resulted in a relatively well-resolved topology (Fig. 3D).

This tree furthermore contains considerable Bremer support for some groups, especially in comparison with levels of support for those groups in the morphological data. Although a few groups in this tree are clearly questionable (e.g., TRIB-*Idolia* + HIST-Hist-*Margarinotus*) it generally accords well with the classification (except in a few, expected cases). Therefore, this topology is also expected to serve as a useful basis for measuring topological congruence with varied alignment topologies.

The results of comparisons of trees derived from alternative alignments to the morphology and conserved region trees are presented in Fig. 4. It is immediately obvious that all measures, to either topology and according to both statistics (d and SD), agree quite closely on the relative performance of various analyses. Within both Clustal and POY-analyzed data, there is a general trend toward poorer performance with increasing gap cost. In Clustal aligned data, this effect is small and the results of various analyses are all quite similar, despite varied gap costs and weighting schemes. The CL1 matrix (gap cost 2), with conserved regions either unweighted or weighted, gave the highest congruence scores (determined by summing ranks of each analysis across the four measures) among Clustal/PAUP analyses, and we use the unweighted CL1 matrix in combined analyses. This 18S-only topology is shown in Fig. 5A. Over a comparable range of POY parameter space, a larger range of phylogenetic congruence scores is seen, corresponding to a greater range in the size and informativeness of the resulting alignments (see Table 3).

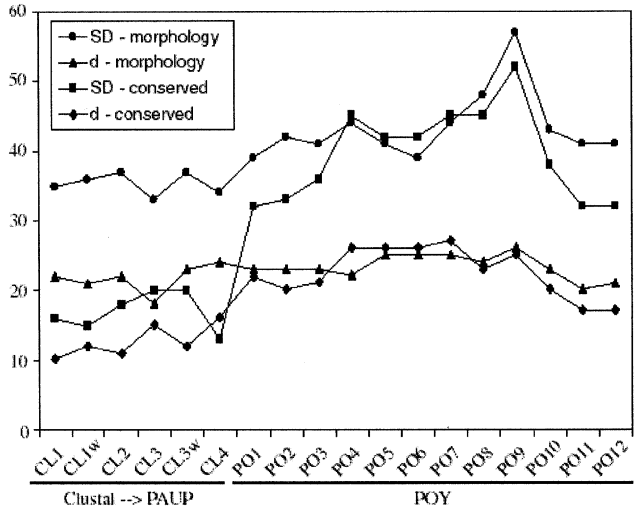

Fig. 4. Results of comparisons of most parsimonious tree (or strict consensus thereof) resulting from alternative alignment/analysis schemes with trees based on morphology alone and on the conserved regions of 18S alone (trees in Figs. 3C and D, repsectively). An increase in value of either statistic indicates increasing dissimilarity from the trees used as standards. The basis of each statistic, d and SD, is described in the text.

Congruence with either morphology or with the conserved regions alone decreases markedly with high gap costs. This appears to result from a proportionate increase in "long-branch attraction" with gap cost (discussed in more detail below). Weighting the conserved regions during POY analyses (analyses PO10–12) made a greater difference to these results than seen when these regions were weighted in PAUP analyses of Clustal matrices. Regardless of the weight applied (2, 5, or 10) improvements were seen in all cases (though of course this is expected in comparisons to the conserved regions tree), and it is these weighted analyses that show the best performance overall (as above, determined by summing the ranks of each analysis across the four measures). Analyses PO11 and PO12 were both ranked first or second (among POY analyses) for all comparisons. In fact, the two implied alignments were very similar (see Table 3). For the purposes of combined analyses we have selected the (more similarly weighted) PO11 conditions as preferred. The 18S-only topology produced by this analysis is shown in Fig. 5B.

The topologies of these 18S trees (Figs. 5A and B) conflict in many respects, although both offer interesting and reasonably well-supported resolutions. The CL1 tree is largely a more fully resolved, and more highly supported, version of the conserved-regions-only tree (on which its alignment was based). There are a couple of significant exceptions to this, however, including the more basal position of *Dendrophilus* and substantial shuffling of the Tribalinae/Exosternini/Platysomatini taxa. The resolution of the latter groups is particularly noteworthy, resulting in a monophyletic Histerinae with Tribalinae as its sister group. Perhaps the most interesting difference seen in the PO11 18S tree (Fig. 5B) is the nearly monophyletic Dendrophilinae, with only *Anapleus* not included. The position of *Anapleus* as basal to the remaining Histeridae is also striking (though not wholly unexpected). In agreement with the CL1 tree, the POY analysis generally supports the relationship of Trypanaeinae to Abraeinae. However, that the histerine *Baconia* is also found within this group (with stunning Bremer support) is unexpected and peculiar. As is obvious from the phylogram (and as discussed above), *Xylonaeus* and *Baconia* 18S sequences (and to a lesser extent *Coptotrophis* and a few others) are highly divergent from those of other histerids. This is manifested both in more divergent conserved regions, and in unusually long insertions in most variable regions (though not all; interestingly *Xylonaeus* has the shortest of all histerid V2 regions). *Xylonaeus* and *Baconia* both have over 100 V4 bases unmatched by other members of their respective subfamilies or tribes, and the attraction of the two was not overcome under any parameter combination examined in POY.

The two combined data topologies based on PAUP analysis of the CL1 matrix with morphology and on

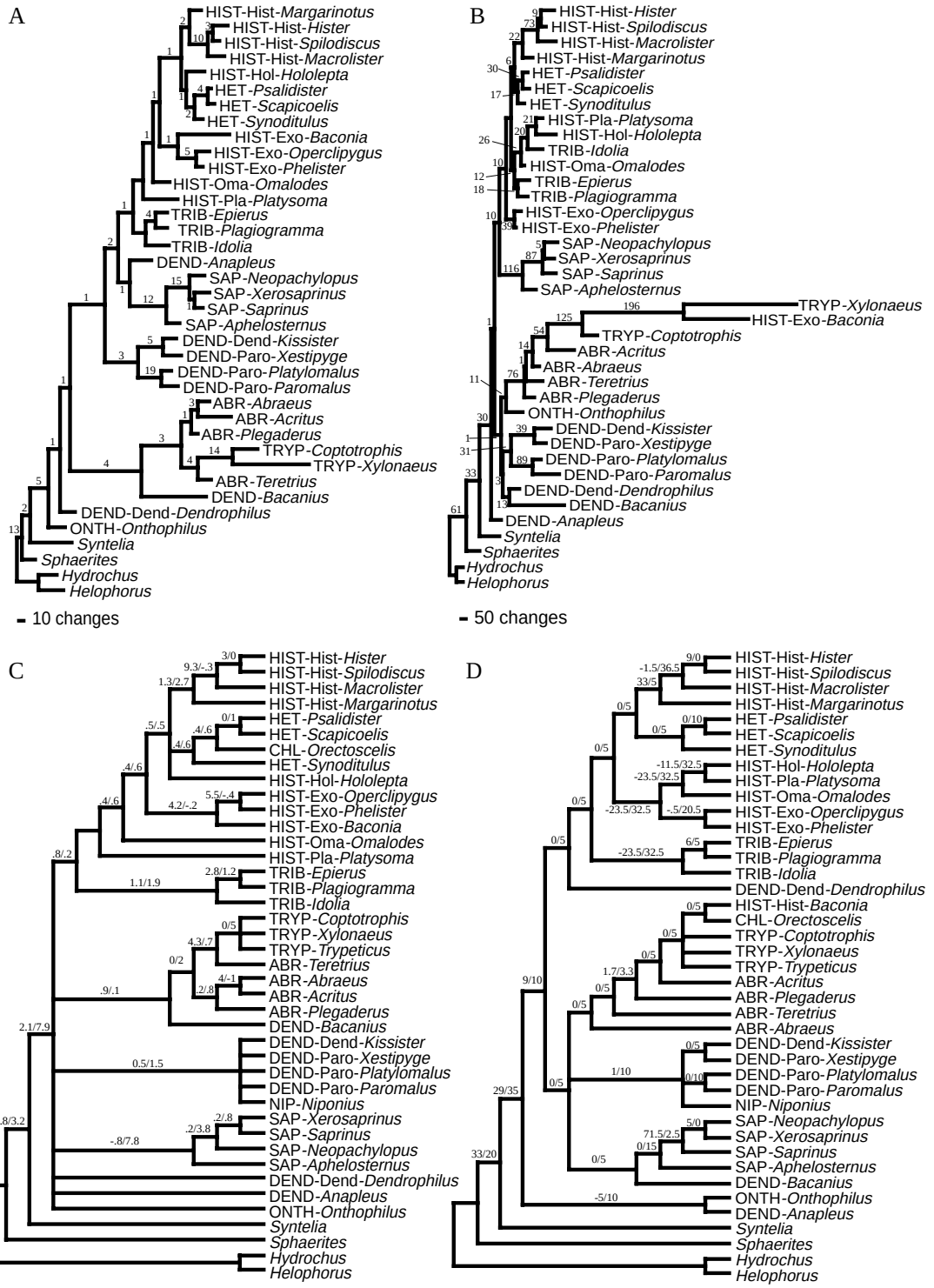

Fig. 5. Results of molecular and combined data analyses with Bremer support shown on branches. In B and D, Bremer supports were calculated under the weighting and gap cost scheme imposed in POY; therefore these support indices are not directly comparable to those resulting from analysis of the Clustal matrices. In C and D Bremer support is partitioned into that support derived from 18S/morphology. (A) One of two equally parsimonious trees based on analysis of the CL1 matrix (18S alone, gap cost 2; the other tree differed in placing *Platysoma* at the base of the Tribalinae). (B) The single most-parsimonious tree resulting from the PO11 matrix (18S alone, gap cost 2, weight of conserved:variable regions, 5:1). (C) Strict consensus of 36 equally parsimonious trees based on analysis of the CL1 matrix in combination with morphology. (D) Strict consensus of 21 equally parsimonious trees based on POY analysis of 18S and complete morphology under the conditions used for PO11 analysis (gap cost 2, conserved regions and morphology weighted 5 times the variable regions).

POY analysis of the complete 18S data with morphology are shown in Figs. 5C and D, respectively. Apart from differences in degree of resolution, the only significant differences in these topologies are the positions of HIST-Hist-*Baconia* (within Abraeinae + Trypanaeinae), observed in all POY analyses, and CHL-*Orectoscelis* within Abraeinae + Trypanaeinae, also a general result of POY analyses. Levels of support for those groups found in both trees are similar overall (note that the weighting scheme (5:1) and gap cost (2:1) utilized in the POY analysis inflate tree lengths and Bremer support values accordingly), although it is striking how little of the support is derived from the molecular partition in the POY tree. Many branches with single step Bremer support find that step evenly divided between molecular and morphological partitions in the CL1 + morphology tree, whereas this support is provided exclusively by the morphology partition in the PO11 + morphology tree. The additional resolution found in the PO11 + morphology tree is mostly supported by unit Bremer support (e.g., *Dendrophilus* added to Tribalinae + Histerinae; Saprininae, Paromalini/Niponiinae, and Abraeinae/Trypanaeinae forming a clade). However, interestingly, a clade comprising all taxa except *Onthophilus* and *Anapleus* finds several steps Bremer support derived from both data partitions. The Adams consensus of the CL1 + morphology and PO11 + morphology trees (Fig. 6) graphically reveals the extent of their compatibility. Only a few branches are either conflicting or unresolved in both, mainly at the base of the tree. The implications of this topology with regard to life history and morphological evolution are discussed below.

Discussion

Long-branch attraction and the analysis of length-variable data

Comparing the alignments and resulting trees from the two types of analyses carried out here provides insights into the behaviors of both. The effect of gap cost on POY results was particularly interesting. Alignment size remains much larger and number of informative positions (in a ''gaps as missing data'' context) smaller in higher gap cost POY matrices than in corresponding Clustal alignments. (Although an ''alignment'' matrix is not strictly compatible with the optimization alignment approach, implied alignment size is indicative of the amount of homology POY hypothesizes in the data.) It seems that POY remains more reluctant to force homology on mismatched bases in the variable regions. It would seem to follow that homoplasy in the variable regions would thus be effectively minimized. However, apparent severe long-branch attraction problems would question this conclusion. When a few sequences have especially long insertions (e.g., *Baconia* and *Xylonaeus*, especially), it is less costly to consider their shared insertions as homologies (regardless of base identity) rather than as separately autapomorphic. It is difficult to determine the exact circumstances when this would be expected to occur, although there will be some relationship with the numbers of synapomorphies linking each long insertion taxon with its proper relatives. For example, if two unrelated taxa have unique and nonhomologous insertions of 10 bases, and the cost of gaps for the analysis is 2, the 20 steps it would take to force these insertions to be nonhomologous would have to be outweighed by more than 20 synapomorphies for the proper relationship in the unambiguous segments of the alignment. Clearly the effect becomes worse with increasing gap cost. Indeed in some high gap cost trees (not shown), the clustering of taxa was dictated largely by the absolute lengths of sequences. Enforcing some kinds of topological constraints would seem to be the only real solution; treating gaps as missing data and the use of a guide tree in Clustal are probably the main factors preventing similar effects.

Histerid phylogeny

The reasonably well-resolved consensus of POY and Clustal/PAUP combined analyses offers a number of novel and interesting hypotheses of relationships among major histerid groups. Perhaps the only major disappointment is that no analysis strongly supports a basalmost resolution of Histeridae proper. Nonetheless, several candidate taxa for most basal histerid have come to light that had not been previously considered. Furthermore, our results in this respect cast serious doubt on previous workers conclusions that Niponiinae occupy this position. Although we cannot be overconfident in this result until molecular data for *Niponius* can be analyzed, *Niponius* clearly lacks several genitalic plesiomorphies shared by *Onthophilus*, *Anapleus*, and *Dendrophilus* (see character discussion in Appendix A). There is a tendency for *Dendrophilus* to move further up into the tree, frequently near the nominally dendrophiline Paromalini (e.g., conserved regions tree (Fig. 3D), combined morphology (Fig. 3C)), and thus as Fig. 6 suggests, *Onthophilus* and *Anapleus* (possibly the two together) are the best contenders for most basal status. There is no support for relationships to the subfamilies with which they are commonly allied, Tribalinae and Dendrophilinae, respectively. The broad resolution of the remainder of the family into two large clades is intriguing. Although their limits have differed, there has generally been a sense among workers that two large clades existed, the Histeromorphae and Abraeomorphae (Saprinomorphae of earlier publications; Wenzel, 1944). Yet, if this is indeed the case, it must also be recognized

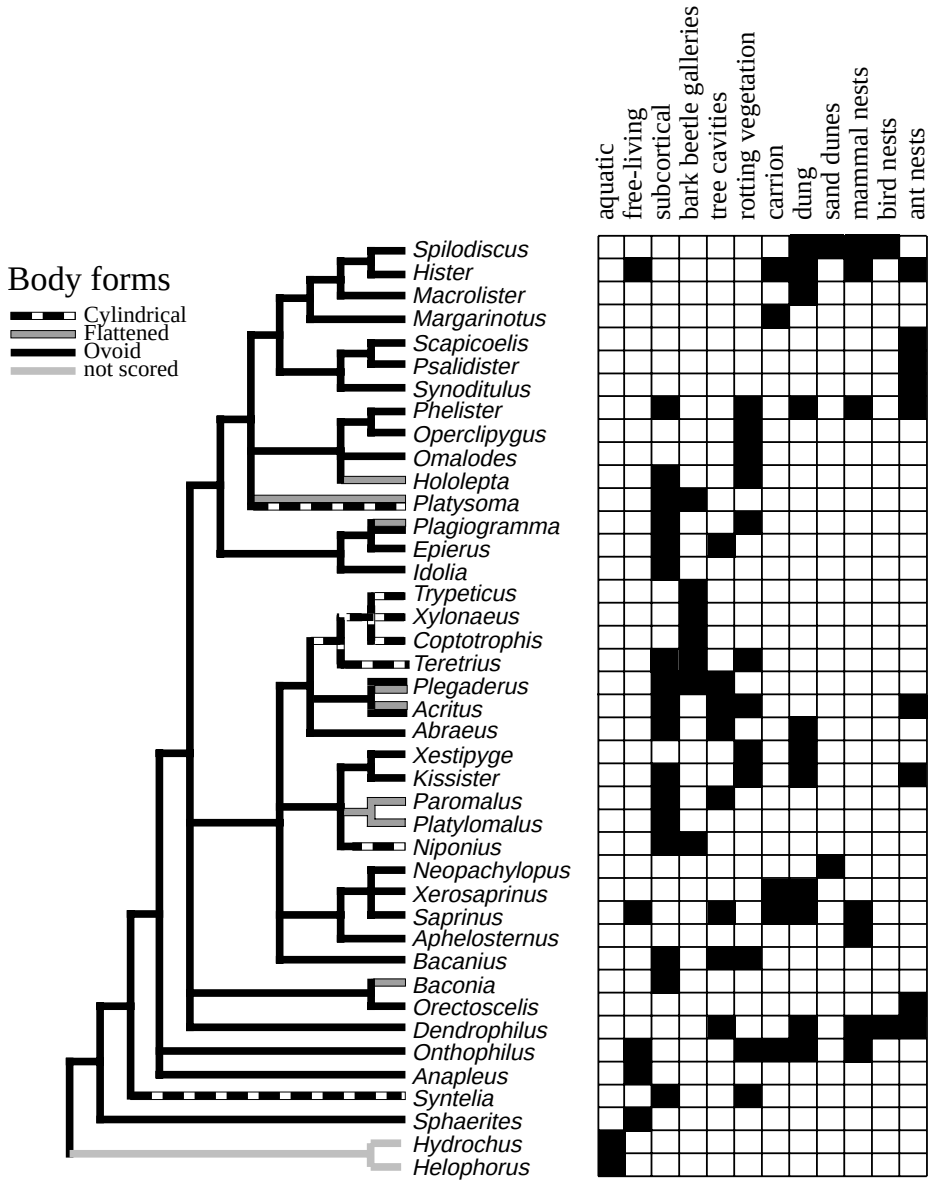

Fig. 6. Adams consensus of PO11 + morphology and CL1 + morphology trees. Body shape is mapped onto this tree using parsimony with variable lineages indicated by multiple branches (e.g., *Platysoma*, *Baconia*, *Plagiogramma*, *Plegaderus*, *Acritus*). The outgroups *Helophorus* and *Hydrochus* were considered not to fall into any histerid shape category and thus uninformative with respect to shape evolution. The table to the right of the tree indicates habits shown by the exemplar taxon and/or known close relatives.

that several taxa would probably not fit into such a scenario.

With regard to finer scale relationships, our combined analyses support Ślipiński and Mazur's (1999) conclusion that the Dendrophilinae (as traditionally comprising Anapleini, Bacaniini, Dendrophilini, and Paromalini) is polyphyletic (also suggested by Lawrence and Newton, 1995). Morphological data (Fig. 3C) support a group containing only two of these four tribes (Dendrophilini and Paromalini), based on a single character, albeit a seemingly strong one—the prosternum grooved for receiving the protibial spur (however, depending on resolution, this state could still be considered homologous in other topologies, with a loss occurring in one or more other groups). The PO11 18S tree suggests monophyly of all of these tribes except Anapleini, which it places at the base of the histerid tree. With respect to *Anapleus*, this result is relatively consistent. However, given quite different results in combined analyses the monophyly of the other three tribes does not seem likely. *Bacanius*, in particular, seems more likely to be related to Abraeinae, as seen in analyses of morphology alone, conserved regions alone, and in CL1 separate and combined analyses, although the PO11 + morphology tree favors a position basal to Saprininae. Paromalini

shows a weak relationship to either Abraeinae (PO11) or a larger Abraeinae/Saprininae lineage (PO11 + morphology). The relationship of *Niponius* to Paromalini, supported by morphology and all combined analyses, is surprising. This enigmatic genus has been considered highly isolated (even its own family at one point), and no hypothesis of relationship to any other histerid lineage has been proposed. It is worth highlighting the fact that adult morphology places *Niponius* within Paromalini, as sister group only of the *Paromalus*/*Platylomalus* group. Several additional similarities, including subcortical habits, might be cited in support of this hypothesis. Lacking molecular data for *Niponius*, this group needs additional study.

Among the most interesting and well-supported results of these analyses is the position of the Trypanaeine/Trypeticine lineage within the Abraeinae. Essentially all data and analyses agree that these large cylindrical bark-beetle predators are derived from well within the (generally minute and ovoid) Abraeinae (as first suggested by Lawrence and Newton, 1995). The two combined analyses differ on their position within the subfamily, with CL1 favoring a sister group relationship to Teretriini and PO11 favoring one to *Acritus*. Given similar shape and habits, as well as moderately strong support (three steps) from the conserved regions of 18S alone, their relationship to *Teretrius* must be considered the most likely alternative. Sampling is not sufficiently dense for relationships among the remaining abraeine tribes to be very meaningful.

The monophyly of Tribalinae + Histerinae (including Hetaeriinae) emerges as fairly well-supported by most analyses. The monophyly of the former (represented by *Idolia*, *Epierus*, *Plagiogramma*) presents little ambiguity, although the three exemplars here are relatively closely related and others might be expected to give a rather different picture. Monophyly of the Histerinae, although seemingly obvious, has always found relatively modest character support. Here its monophyly (generally including Chlamydopsinae, more on this below) is generally supported except in those cases where Tribalinae is included within Histerinae (both CL1 (one of two trees) and PO11 analyses of 18S alone), although support indices for either alternative are never more than moderate. Nonetheless, several morphological characters would support the Histerinae to the exclusion of Tribalinae, including loss of teeth of prementum in the larval labium, loss of adult labral setae, the possession of a frontal stria, the prominent incisor of mandible (and possibly correlated loss of molar region; Ślipiński and Mazur, 1999), and insertion of spermatheca near the apex of the bursa (though this undergoes a reversal in Histerini).

Relationships among subgroups of Histerinae cannot be addressed with much confidence given fairly sparse sampling within the group, although a few comments are justified. PO11 analysis of 18S alone and in combination with morphology reveal a *Platysoma* + *Hololepta* clade (the traditional Platysomatini) with good Bremer support. Where *Platysoma* is separate from *Hololepta*, it may join a clade containing *Omalodes* and the three Exosternini (*Phelister*, *Operclipygus*, and *Baconia*). It is somewhat surprising that the Hetaeriinae do not group with this Platysomatine/Exosternine group; a possible close relationship to Exosternini was suggested by Helava et al. (1985) and would probably be the general consensus of contemporary worker's opinions. Instead they invariably group with Histerini (in some cases along with *Hololepta*). This would be fairly easy to discount as insufficient hetaeriine sampling were it not for the inclusion of *Synoditulus*, which is perhaps the most exosternine-like hetaeriine. Thus, a closer relationship between Histerini and Hetaeriinae should be considered. Finally, as yet, little can be said about the Chlamydopsinae. Although our morphological data continue to place it close to (within!) the Hetaeriinae, we still consider this the result of convergent morphological responses to myrmecophily. Settling the question of these groups' relationship will require molecular data for Chlamydopsinae.

The consensus tree based on both combined analyses is sufficiently resolved and robust to justify a preliminary investigation of life history and morphological evolution of the histerids. Looking first at the relatively straightforward reconstruction of body shape on this tree (as well as on most other supportable basal reconstructions), the basal histerid is unambiguously hypothesized to have had an ovoid form. Crowson's hypothesis of the plesiomorphic nature of the cylindrical, dead wood-associated taxa was presumably based largely on the similarity of these forms (*Niponius*, *Trypanaeus*, and *Trypeticus*) and habits with those of *Syntelia*. However, this tree indicates that these characteristics are autapomorphic for *Syntelia*, as well as multiply derived within Histeridae. Regarding possible ancestral habits, varied habits for many basal lineages (*Onthophilus*, *Dendrophilus*, *Bacanius*) preclude unambiguous parsimony reconstructions of habit at the family's basal node. But the diverse habits of these groups suggest that ancestral histerids were ecologically flexible. Although few individual species in these groups could be considered true generalists, it does seem that their preferences are relatively plastic. Specialization from this putatively ancestral generalist habit appears to have progressed to different degrees in different lineages. In Abraeinae (including Trypanaeinae), by far the most common associations are with dead wood, and subcortical habits map unambiguously as ancestral in the lineage. The Teretriini/Trypanaeinae lineage has taken this specialization a step further into the transverse galleries of bark and ambrosia beetles. In the tribaline/histerine lineage, it is also possible that subcortical habits are plesiomorphic.

At least insofar as indicated by the taxa included here, the Tribalinae and a couple of more or less basal Histerinae (*Hololepta* and *Platysoma*) are all largely subcortical. Species within each of these groups have clearly followed the path of increasing specialization to produce highly adapted flat (some *Epierus*, many Platysomatini and Exosternini, most hololeptines) or cylindrical (many Platysomatini, some Exosternini) forms. However, these lineages also contain many generalist groups. In the Tribalinae, *Tribalus* in particular, which is not examined here, is ecologically diverse and may prove to be basal within Tribalinae. In Histerinae, on the other hand, the wide diversity of habits seen in Histerini would represent a secondarily evolved flexibility if the reconstruction of subcortical habits as basal in the subfamily is accurate. Although the preliminary evolutionary scenarios presented here will undoubtedly be revised as new data on relationships and natural history are produced, the broadest outlines of this scenario are both novel and relatively robust.

Conclusions

The data sets produced for this study vary substantially in their variability and resolving power. The larval data, with one significant exception, provided limited information (although we do not doubt that additional study would be beneficial). Adult morphology, alone and in combination with the larval data, offers much better resolution, and most widely recognized taxa are recovered. However, it is also apparent that the recovery of some of these widely recognized groups is due to the inclusion of several widely recognized taxonomic characters. With low levels of support for most of these groups it is difficult either to defend the morphology tree as confirming earlier ideas or to deride it as biased by our preconceptions. Given the ambiguities of interpreting the morphological data, the 18S gene proved an extremely valuable counterpart for this analysis. The number of groups with moderate to high levels of support in the molecular and combined data trees is much greater than by morphology alone.

How to best extract phylogenetic information from a diverse and, especially, length-variable data set remains a difficult question. Our analysis has relied on maximizing both internal (with conserved regions) and external (morphology) congruence. And certainly some such synthetic approach is essential to deriving straightforward hypotheses from multiple data sets. However, even with such criteria as we have utilized, there is substantial room for flexibility, and many areas of parameter space may provide quite different, yet equally defensible results. This is further complicated when comparing approaches with very different underlying bases (fixed positional homology alignments vs optimization alignment vs others perhaps yet to be developed). We would generally appeal to arguments of philosophical consistency and assumption minimization, and on these grounds the optimization alignment approach is clearly superior. Yet its behavior is poorly understood, and it suffers some clear difficulties with highly length-variable data. These can probably be overcome with appropriate parameterization, but it needs to be further explored.

With respect to histerid phylogeny, our results cast serious doubt on many of the recent taxonomic changes proposed by Ślipiński and Mazur (1999) and we would urge that major changes not be adopted until a firmer phylogenetic framework is established. In particular, we find no support for their "Divisions" as proposed and we believe establishing a high ranking taxon for *Niponius* alone to be entirely unjustified. The monophyly of a taxon containing Abraeinae, Saprininae, Paromalini, Anapleini, and Dendrophilini (their Abraeomorphae) is very doubtful, particularly with regard to the last two. On the other hand, Trypanaeinae (including Trypeticinae) clearly merits no higher than tribal ranking within Abraeinae, and even with this change Teretriini would probably remain paraphyletic. We find no support for the combination of Onthophilinae and Tribalinae nor any support for the inclusion of the former in a taxon comprising Tribalinae + Histerinae. With regard to the myrmecophilous subfamilies Hetaeriinae and Chlamydopsinae, our results clearly show the former to be subordinate within Histerinae, while the latter continues to defy attempts at placement. We do not propose any specific taxonomic changes at present, but would recommend that the more traditional (more highly split) taxonomy of Mazur (1997) be followed until a truly comprehensive phylogeny can be established.

Our preliminary exploration of life history evolution in the family outlines an interesting evolutionary scenario. If indeed *Onthophilus* and *Anapleus* are among the most basal histerids, the general trend of evolution for the family would be one of primitive generalists, in body shape and in habits, giving rise in parallel to numerous specialist lineages. Subcortical habits have arisen multiple times and have perhaps been basal to several large radiations. Within the subcortical environment, it appears that certain highly specialized morphologies (e.g., strongly flattened and perfectly cylindrical) are almost inevitable; both have arisen in parallel in at least three separate lineages (Abraeinae, Paromalini (including *Niponius*), and Histerinae). Myrmecophily has likewise arisen in at least five different lineages. Even the habits considered most widespread, the predation on maggots in dung and carrion, have evolved multiple times, in at least Saprininae and Histerinae.

The bottom line is that there is yet significant work to be done in understanding the evolution of Histeridae. Satisfactorily resolving the phylogeny of the group will

require data, especially molecular, for numerous additional taxa. Many groups have assumed much greater importance in the problem as this study has progressed, particularly Onthophilinae and Anapleini, and these groups need to be better represented. Buried within otherwise well-delimited taxa, there are several genera and species which might prove particularly valuable. The tribaline genus *Stictostix* is phylogenetically interesting, and some of its Australian representatives may hold the key to the relationship of Chlamydopsinae to the remainder of the family (this might also be said of the myrmecophile *Peploglyptus* in the Onthophilinae). *Sphaericosoma*, a Malagasy genus currently placed in Tribalinae, appears to represent a transition from open to closed antennal cavities (or vice versa) in the family and will likely fall out somewhere "between" the groups delimited by these relatively distinctive states. And numerous phylogenetically interesting forms will be found among the smaller, seemingly nondescript histerids. For example, one small species of *Abraeus* (New Zealand's *A. vividulus* Broun) was suggested to be an anapleine (Wenzel, unpublished data). We do not agree with this particular idea but neither can we offer a better suggestion beyond agreeing that it is likely not an abraeine. It is also obvious that resolving relationships within the more diverse subfamilies (Abraeinae and Histerinae, especially) will require much denser sampling. The very diverse Exosternini might well prove to subtend all of the other groups of Histerinae when adequately represented. Looking beyond the phylogenetic problems, resolving the many fascinating questions of life history evolution will require much new data on the natural history of histerids, data which are difficult and time-consuming to gather. However, if, as Bates so eloquently suggested, histerids could serve as a valuable model for large-scale ecomorphological evolution, the rewards would certainly repay the effort.

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Appendix A. Morphological characters

The 52 morphological characters used in this study result from detailed studies of adult and larval specimens, including several larvae which remain to be formally described. While many of our characters represent "classic" histerid characters, none have been included solely on that basis. Adult characters were all scored by the senior author from direct study of cleared and dissected specimens. Larval states of *Hydrochus*, *Sphaerites*, *Syntelia*, *Niponius*, *Teretrius*, *Onthophilus*, *Plegaderus*, and *Paromalus* were scored principally from published descriptions and illustrations (Gardner, 1930; Böving and Craighead, 1931; Mateu, 1972; Mamayev, 1974; Nikitsky, 1976a,b; Kovarik and Passoa, 1993; Beutel, 1994, 1999). Larval data given for *Xestipyge* were obtained from specimens of the closely related paromaline, *Carcinops*. The full morphological matrix is presented in Table 2. Most characters and their respective states are illustrated in Figs. 7–10. For additional illustrations see Newton (1991), Ohara (1994), and Ślipiński and Mazur (1999). It is worth noting that several taxa are identical in morphological scoring (e.g., within Histerini, Saprininae). Although characters could have been included to resolve relationships within these groups, characters informative at these lower levels would have added significantly to the homoplasy at higher levels, where our main present questions lie. Several polymorphisms are indicated in the matrix where the state differs in close relatives of the included examplars.

Some characters not included in this analysis should be mentioned. Prosternal structure, particularly the means of concealing the antennal club, has always been of primary importance in histerid systematics. This character system has been subject to quite varied interpretation due to difficulty of parsing the considerable variation into discrete characters. While Ślipiński and Mazur (1999) have successfully eliminated most inter-character correlations in their data set, problems remain with their interpretation of the prosternal grooves or "notches" through which the antennal funicle passes when the antenna is retracted. As they have scored this character it remains dependent on the presence of prosternal alae; when these latter are present the prosternal notches (which are concealed by the alae) are scored as absent. In our opinion the prosternal notches are present in virtually all histerids, including those with "alae." The prosternal notches are only truly absent in forms which have undergone radical body modifications; e.g., most hololeptini, Niponiinae, and Trypanaeinae. These cases clearly represent independent losses. We have therefore excluded the character from our analysis, as it would only function as a synapomorphy of Histeridae, which is not in question. The desclerotization of the coxites of the ovipositor was

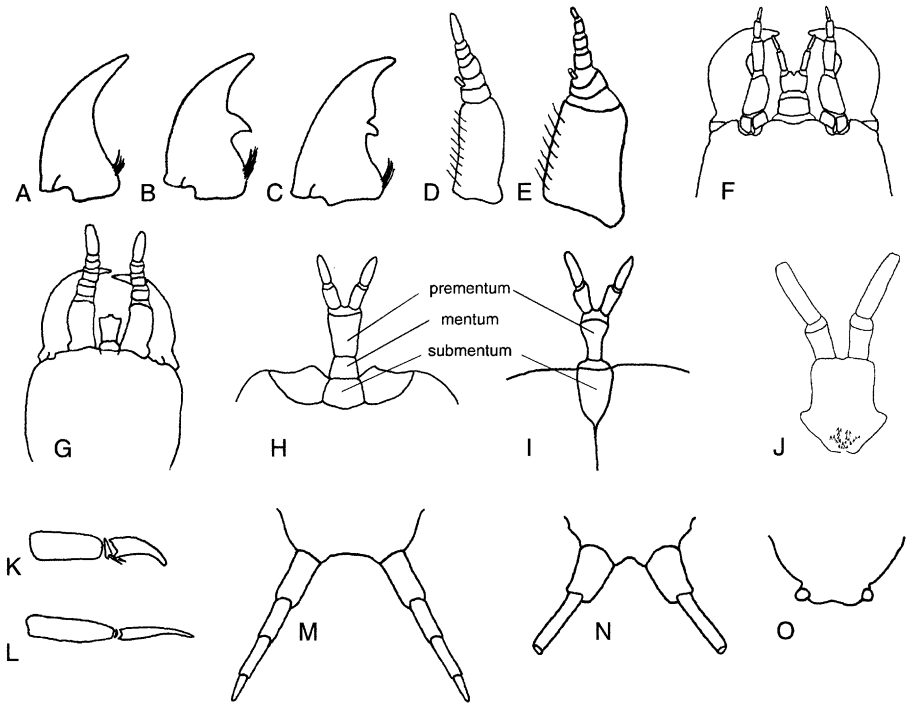

Fig. 7. Larval characters used in phylogenetic analysis. (A–C) Dorsal view of left mandible. (D,E) Dorsal view of right maxilla. (F,G) Ventral view of head. (H,I) Ventral view of labium. (J) Dorsal (internal) view of labium. (K,L) Tarsungulus. (M–O) Urogomphi.

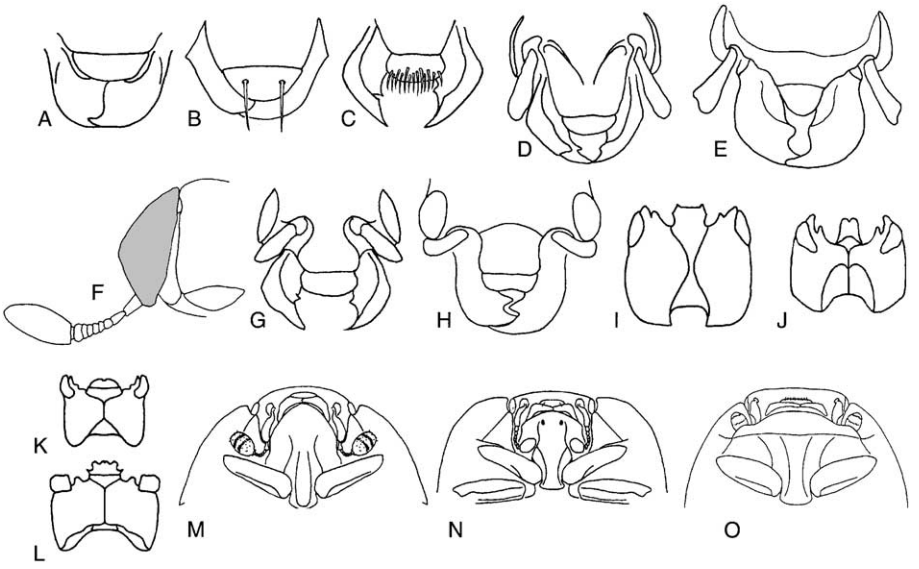

Fig. 8. Adult characters used in phylogenetic analysis. (A–H) Anterior view of head. (I–L) Ventral view of head. (M–O) Ventral view of prothorax.

given as a synapomorphy of Trypanaeinae + Trypeticinae by Ślipiński and Mazur (1999). This state was also scored for Hetaeriinae. We have seen an ovipositor in several Trypeticinae and Hetaeriinae (although much reduced in the latter) and do not think that the character is informative at the intersubfamily level.

Larval characters

1. *Antennal foramen of head*: (1) separated from base of mandible by only a membranous strip; (2) separated from mandible by sclerotized band of cuticle.The close association of the mandible and antennal base

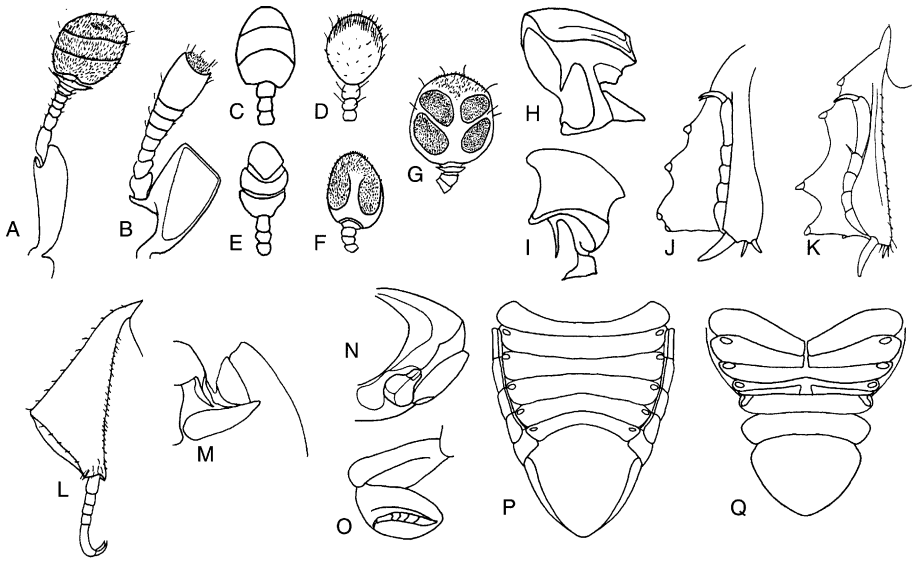

Fig. 9. Adult characters used in phylogenetic analysis. (A–G) Antennae. (H, I) 56, 57. Lateral view of prothorax. (J–L) 58–60. Anterior view of protibia. (M) Ventral view of prothorax. (N) Anterior view of mesothorax and mesocoxa. (O) Anterior view of protibia. (P,Q) Dorsal view of abdomen.

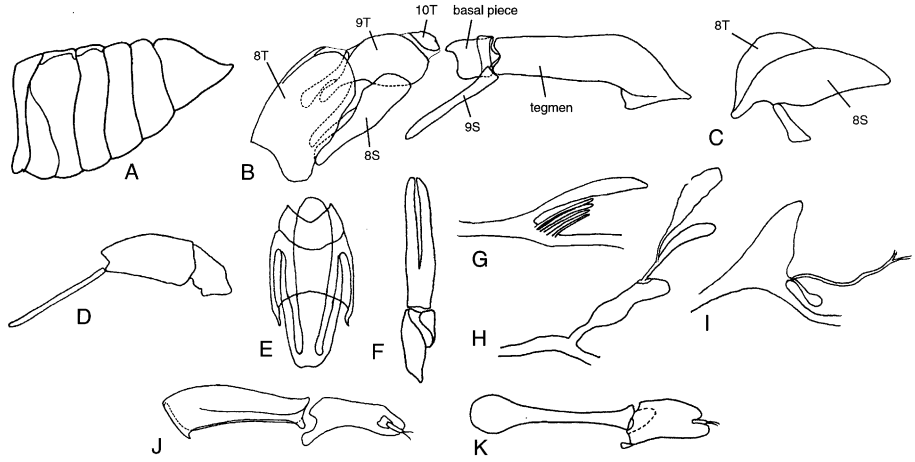

Fig. 10. Genitalic characters used in phylogenetic analysis. (A) Lateral view of abdomen. (B) Lateral view of abdominal segments 8–10 (genital tube or capsule) and the aedeagus, showing nomenclature used in this paper. (C) Lateral view of abdominal segment 8. (D) Lateral view of abdominal segment 9. (E) Dorsal view of abdominal segments 9 and 10 in *Sphaerites*. (F) Dorsal view of aedeagus of *Sphaerites*. (G-I) Lateral view of internal female genitalia, including vagina (at left), bursa copulatrix, spermatheca(e), and common oviduct. (J,K) Dorsal view of right half of ovipositor, showing valvifer and gonocoxite (with gonostyle).

appears to be a synapomorphy of Sphaeritidae + Synteliidae + Histeridae.

2. *Stemmata*: (1) absent; (2) one stemma on each side; (3) six stemmata on each side.
3. *Mandible*: (1) without teeth on mesal margin (Fig. 7A); (2) with one mesal tooth (Fig. 7B); (3) with two mesal teeth (Fig. 7C).
4. *Maxilla*, *articulation*: (1) eversible; (2) normally articulated to head.The eversible maxilla was first reported by Newton (1991, 1995) and is shared by all known Trypanaeinae and Trypeticinae.
5. *Maxilla*, *cardines*: (1) distinct, free (Fig. 7F); (2) fused to head capsule (Fig. 7G).Previous workers have proposed several possible origins of state 2, including fusion of the cardines to the mentum (in *Syntelia*; Newton, 1991) and fusion to the stipes (in histerids; Beutel, 1999). As partitioned here, state 2 is one of the principal possible synapomorphies of Synteliidae + Histeridae.
6. *Maxilla*, *palpus*: (1) with four palpomeres (Fig. 7D); (2) with five palpomeres (Fig. 7E).A five-segmented maxillary palpus (state 2) is shared by Trypanaeinae and Trypeticinae, as well as several genera in the Abraeinae. This state may constitute the best morphological evidence that the last subfamily is paraphyletic with respect to the first two. In all taxa

examined to date, the distribution of these states corresponds exactly to that of the number of labial palpal segments and the two characters may turn out to be nonindependent. As the maxillary and labial structures are serial homologues, single mutations affecting both would not be unlikely. A more thorough survey of taxa is necessary, however, before this correlation can be established. Beutel (1999) has referred to these states as three- and four-segmented, respectively, not counting the palpifer as a palpomere.

7. *Labium*, *submentum*: (1) submentum distinct (Fig. 7H); (2) fused to head capsule (Fig. 7I).Surprisingly, the presence of a distinct submentum delimited by sutures is figured by Nikitsky (1976a) for *Paromalus* and *Platysoma* and by Gardner (1930) for *Niponius*. Within the Histeridae, these are the only taxa in which it is scored as present on the basis of these previous studies. However, these sutures have not been observed by the authors in related species of the first two genera. In *Omalodes* the anteroventral portion of the head is impressed in such a way as to imply the presence of gular sutures and even a submentum but no actual sutures are present. It is likely that in *Platysoma*, at least (which may be closely related to *Omalodes*), this is the condition that has previously been interpreted as these structures' presence. It is doubtful that a true, separate submentum is present in any histerid larvae.
8. *Labium*, *mentum*: (1) distinct (Fig. 7H); (2) not present as a distinct sclerite (Fig. 7I).The mentum in Histeridae and Synteliidae is not represented by an obvious sclerite. It is unclear whether this is due to fusion with some other sclerite or simply to desclerotization.
9. *Labium*, *prementum*: (1) with several small dorsobasal teeth (Fig. 7J); (2) without such teeth.A group of dorsal labral denticles is found in most Abraeinae, Tribalinae, Onthophilinae, and Dendrophilinae (Hinton, 1944; Newton, 1991).
10. *Labium*, *prementum*: (1) with membranous setiferous area dorsobasally (see figures in Newton, 1991); (2) without membranous setiferous area on dorsum.
11. *Labium*, *prementum*: (1) without lateral projections (Fig. 7I); (2) with lateral projections (Fig. 7J).
12. *Labium*, *palpus*: (1) with two palpomeres; (2) with three palpomeres.As mentioned above under character 6, it is possible that this character is not evolving independently of the number of maxillary palpomeres.
13. *Tarsungulus*: (1) claw-like (Fig. 7K); (2) filiform (Fig. 7L).
14. *Tarsungulus*: (1) bisetose (Fig. 7K); (2) unisetose; (3) without setae (Fig. 7L).Crowson (1974) regarded this as a particularly valuable character.
15. *Urogomphi*: (1) two-segmented (Fig. 7N); (2) four-segmented (Fig. 7M); (3) absent; (4) one-segmented (Fig. 7O).

Adult characters

16. *Dorsal labral setae*: (1) zero (Fig. 8A); (2) two (Fig. 8B); (3) many (Fig. 8C); (4) four.
17. *Labrum*: (1) free; (2) fused to clypeus (with or without an impression indicating its location).
    The primary historical importance of this character has been in uniting the myrmecophilous Hetaeriinae and Chlamydopsinae. However, in both groups the character can be very difficult to score. A transverse impression or line between the clypeus and labrum can be seen in most taxa whether they are truly fused or not. It appears to us that there are species in both superfamilies with the labrum free (including the Chlamydopsinae scored herein) and it seems likely that fusion has occurred separately in the two subfamilies.
18. *Epicranial suture*: (1) present (Fig. 8H); (2) absent.
    The presence of an inverted v- or y-shaped suture on the frons (presumptive frontoclypeal suture) has been considered to be a primitive character in histeroids, which has been lost in synteliids and histerids. An apparent suture in this location in some histerids (e.g., *Bacanius* and *Epierus*) has thus been assumed to be a secondary acquisition (e.g., Hansen, 1997). We see little justification for this assumption and do not make it here.
19. *Antennal insertion*: (1) lateral, between eye and mandible (Fig. 8E); (2) frontal, insertion anteriorly open (Fig. 8G); (3) frontal, beneath protuberances (Fig. 8D); (4) above the eye, on the vertex (Fig. 8F).
20. *Antennal scape*: (1) more or less cylindrical, at most weakly expanded apically (e.g., Figs. 8D, E, and G); (2) expanded toward apex, approximately pyramidal in shape (Fig. 9B); (3) strongly expanded, covering the eye and antennal cavities when retracted (Fig. 8F).
21. *Annuli of antennal club*: (1) distinct, straight (Fig. 9A); (2) outwardly arcuate (Fig. 9C); (3) inwardly arcuate (interrupted medially in some) (Fig. 9E); (4) obsolete, club entirely pubescent; (5) obsolete, club sclerotized on bases of upper and lower surfaces (Figs. 9D and F); (6) obsolete, club sclerotized otherwise (Fig. 9B).
    Ślipiński and Mazur (1999) accurately point out the problem with interpreting the annuli of the antennal club as true sutures separating the terminal antennomeres. However, the annuli themselves nonetheless appear informative (and probably do represent former segmental junctions). It is possible that some taxa exhibiting true sutures and some merely exhibiting annuli will have been lumped together in state 1 (annuli distinct and straight). However, since this seems to be the plesiomorphic state for the family, this error should not introduce any inaccurate groupings.

22. *Frontal stria*: (1) present (Fig. 8E); (2) absent.
23. *Incisor portion of mandible*: (1) exposed beyond the labrum when closed (Figs. 8A, E, and H); (2) incisor hidden beneath labrum when mandibles closed, only apices visible (Figs. 8C, D, and G).
    State 1 corresponds to, and is related to the degree of development of the incisor in some histerids. In most taxa exhibiting state 2, the incisor is poorly developed.
24. *Gular sutures*: (1) separate throughout their length (Fig. 8I); (2) confluent medially (Figs. 8J–L).
    Separate gular sutures are clearly plesiomorphic within Coleoptera. Ślipiński and Mazur (1999) were the first to make the important observation that some histerids show this condition. However, while we agree that the gular sutures in *Dendrophilus* are separate, we have not yet seen this state in any species of *Bacanius*, and state 2 is scored for that taxon. *Bacanius* is a diverse and variable taxon and it may be that this character varies among species.
25. *Gula*: (1) gular sutures posteriorly divergent, triangular portion of gula visible at base of head (Fig. 8K); (2) gular sutures diverging at right angles posteriorly, exposing rectangular portion of gula (Fig. 8L); (3) medial gular suture reaching posterior margin of head capsule, gula entirely hidden (Fig. 8J).
26. *Protrochantin*: (1) visible; (2) hidden by prosternum.
    The exposed protrochantin is clearly a Coleoptera plesiomorphy, retained among Histeroidea only in *Sphaerites*. Thus, its concealment should constitute a synapomorphy of Synteliidae + Histeridae, although in both of the included hydrophiloid outgroups it is also concealed.
27. *Antennal club*: (1) received under anterior corners of pronotum in repose (Figs. 8M and O); (2) received medially, alongside prosternum (Fig. 8N); (3) not received under prosternum in repose.
    While the mode of concealment of the antenna in repose is a phylogenetically important feature of histerids, it has been historically difficult to describe the variation in terms of discrete characters. We have represented this system by two characters: simply the position of the club and whether or not the prosternum is developed into alae or lateral extensions.
28. *Antennal cavities* (*if lateral*): (1) at least partly covered by lateral extensions of the prosternal lobe (prosternal alae) (Fig. 8); (2) cavities completely exposed from beneath (Figs. 8M and N).
    The development of the prosternal alae may vary considerably in taxa possessing them. Although in *Hololepta* and many Histerini these are much reduced, such that the antennal club is largely exposed, there is little question that this represents secondary reduction (Ślipiński and Mazur, 1999) and they are scored as present.
29. *Presternum*: (1) absent; (2) present.
    This character is the prosternal lobe of Wenzel (1944), separated from other prosternal specializations. The presternum is considered present if a transverse suture is present across the anterior portion of prosternum. If the suture is not detectable, despite apparent anterior production of the prosternum, state 1 is scored.
30. *Prosternal keel*: (1) excavate laterally for reception of the protibial spur (Fig. 9M; Ślipiński and Mazur, 1999: Fig. 36); (2) without shallow excavation for reception of protibial spur.
    This character is one of the few characters supporting the association of Dendrophilini and Paromalini.
31. *Lateral pronotal margin*: (1) simple, a single lateral carina (Fig. 9H); (2) double, with a submarginal carina which is either parallel to or anteriorly divergent from the lateral (Fig. 9I).
    This character is difficult to interpret and score unambiguously in some taxa (especially in some not included in the present study.) In some groups, such as *Anapleus* and *Abraeus*, especially, this submarginal carina is prominent and the overall difference from the common simple lateral pronotal margin quite distinct.
32. *Mesotrochantin*: (1) visible (Fig. 9N); (2) hidden.
    That this character varied within Histeridae was first reported by Ślipiński and Mazur (1999).
33. *Metasternum*: (1) with femoral lines; (2) without femoral lines.
34. *Metepisternum*: (1) visible; (2) hidden or fused to metasternum.
    The metepisternum is not visible externally in several taxa, apparently due to fusion with the metasternum (or possibly concealment under the elytral epipleuron.) The fact that all genera exhibiting this also have members in which the metepisternum is normally exposed might argue that it has occurred independently, possibly in connection with small body size. However, generic limits in most of these taxa are poorly defined and this character may prove informative.
35. *First visible abdominal sternite*: (1) with femoral lines; (2) without femoral lines.
36. (*True*) *Second abdominal sternite*: (1) Visible (with elytra removed), sclerotized at sides (Fig. 10A); (2) not visible at sides, either desclerotized or lost.
37. *Propygidium* (*abdominal tergite 6*): (1) with spiracle (Fig. 9P); (2) propygidium lacking spiracle (Fig. 9Q).
38. *Protibia*: (1) without a groove on the anterior surface for reception of the tarsus; (2) with a straight tarsal groove on anterior surface (Fig. 9J); (3) with an S-shaped tarsal groove on anterior surface (Fig. 9K); (4) with tarsal groove along apicolateral margin (Fig. 9L).

39. *Protibial spurs*: (1) present; (2) absent.
40. *Mesotibial spurs*: (1) present; (2) absent.
41. *Metatibial spurs*: (1) present; (2) absent.
    This and the previous character (No. 40) are obviously serial homologues, their distribution is identical in the present examplars, and it may be that they are not independent.
42. *Meso- and metatibiae*: (1) not grooved for reception of tarsi; (2) grooved for reception of tarsi (Fig. 9O).
43. *Pretarsal empodium*: (1) present, bisetose; (2) absent.
44. *Male genitalic capsule*: (1) with continuous ventral sclerite connecting lateral sclerites of the eighth abdominal segment (Fig. 10C); (2) lateral sclerites not connected by a ventral sclerite.
    Characters 44–47 refer to the segments of the abdomen that have been to varying degrees incorporated into the male genitalia. The numbering of these with respect to their "true" identity has not been agreed upon by histerid workers (Helava et al., 1985; Ohara, 1994). Our interpretations are labeled in Fig. 10B. Some distinct sclerites (such as that indicated by state 1 of character 44) have not been previously dealt with in the literature. The lateral portions of the eighth sternite (as identified by Lawrence and Britton (1991)) are generally separated by a ventral longitudinal division in Coleoptera, although they may be approximate and occasionally fuse (e.g., in some *Saprinus*; see Ohara, 1994, p. 140). In *Sphaerites*, however, although they are approximate ventrally, there is an additional transverse sclerite present.
45. *Proximal apodemes of ninth tergite*: (1) gradually expanded posteriorly to unarticulated junction with tergal apices (Fig. 10B); (2) slender, distinctly more strongly sclerotized than apical portions of the tergite; in some cases, articulated with the apical portions of the ninth tergite (Fig. 10D).
    State 2, in the articulated form, is mainly characteristic of Paromalini. However, the condition in *Niponius*, although not fully articulated, is very similar, and I have interpreted it as potentially homologous.
46. *Ninth abdominal sternite* (*spiculum gastrale*): (1) nested within a v-shaped sclerite, to which it is basally (anteriorly) fused (Fig. 10E); (2) free, not fused basally to another sclerite (Fig. 10B).
    This unusual configuration (state 1) of genital sclerites is apparently limited to *Sphaerites* and *Syntelia* and is the only known character that suggests that they might be more closely related to each other than either is to Histeridae.
47. *Tenth tergite*: (1) entire; (2) divided; (3) absent.
    The undivided 10th tergite clearly represents the plesiomorphic condition for the Hydrophiloidea/Histeroidea. It has likely become divided or lost multiple times, particularly in small-bodied lineages.
48. *Basal piece of aedeagus*: (1) not fused into a complete ring (Fig. 10F); (2) complete sclerotized ring; (3) not visible, either fused with tegmen or lost.
    The almost certainly plesiomorphic state 1 (found in the histerids *Anapleus*, *Onthophilus*, and *Dendrophilus*) is also illustrated for *Sphaerites* in Löbl (1996). It is unclear whether the absence of basal piece observed in most Abraeinae results from loss or fusion.
49. *Basal piece, if present*: (1) shorter than tegmen, usually considerably so; (2) as long or longer than tegmen.
50. *Valvifers of ovipositor*: (1) semicylindrical, open medially, not strongly sclerotized (Fig. 10J); (2) valvifers sclerotized, paddle-like (Fig. 10K); (3) Ninth tergites not specialized to form elongate valvifers (see figures in Lawrence and Britton, 1991, and Hansen, 1997).
    Although the scoop-like gonocoxites of the Histeroidea ovipositor are generally well conserved, significant variation in the form of the valvifers has not been previously reported. In *Sphaerites*, *Syntelia*, and several basal Histeridae (*Anapleus*, some *Onthophilus*, *Dendrophilus*), the valvifer is a medially open semitubular structure. If the paddle-like valvifers have evolved only once, they will prove one of the strongest characters for the internal phylogeny of Histeridae.
51. *Spermathecal insertion*: (1) at or near apex of bursa (Fig. 1H); (2) on and near base of bursa (Fig. 10I); (3) inserted on common oviduct (off bursa entirely) (Fig. 10G).
52. Spermatheca, number: (1) single; (2) multiple (see Ohara, 1994).
    The discovery of significant variability in the spermatheca was an important advance in histerid phylogenetics (Ohara, 1994). Useful information is found in the form, position, and the number (this last, principally in Histerini) of spermathecae. The form of glands attached to the spermatheca may also be very useful for phylogenetics but the difficulty of preparing specimens has precluded a comprehensive survey for this study.

References

Abouheif, E., Zardoya, R., Meyer, A., 1998. Limitations of metazoan 18S rDNA sequence data: Implications for reconstructing a phylogeny of the animal kingdom and inferring the reality of the Cambrian explosion. J. Mol. Evol. 47, 394–405.

Archangelsky, M., 1998. Phylogeny of Hydrophiloidea using characters from adult and preimaginal stages. Syst. Entomol. 23, 9–24.

Bates, H.W., 1863. The Naturalist on the River Amazons. John Murray, London.

Beutel, R.G., 1994. Phylogenetic analysis of Hydrophiloidea based on characters of the head of adults and larvae. Koleopt. Rund. 64, 103–131.

Beutel, R.G., 1999. Morphology and evolution of the larval head of Hydrophiloidea and Histeroidea. Tijds. Entomol. 142, 9–30.
Böving, A.G., Craighead, F.C., 1931. An illustrated synopsis of the principle larval forms of the order Coleoptera. Entomol. Am. (N.S.) 11, 1–351.
Bremer, K., 1994. Branch support and tree stability. Cladistics 10, 295–304.
Campbell, B.C., Steffen-Campbell, J.D., Sorensen, J.T., Gill, R.J., 1997. Paraphyly of Homoptera and Auchenorrhyncha inferred from 18S rDNA nucleotide sequences. Syst. Entomol. 20, 175–194.
Carpenter, J.M., 1988. Choosing among equally parsimonious cladograms. Cladistics 4, 291–296.
Caterino, M.S., Shull, V.L., Hammond, P.M., Vogler, A.P., 2002. The basal phylogeny of the Coleoptera based on 18S rDNA sequences. Zool. Scr. 31, 41–49.
Crowson, R.A., 1955. The Natural Classification of the Families of Coleoptera. Nathaniel Lloyd, London.
Crowson, R.A., 1974. Observations on Histeroidea, with descriptions of an apterous larviform male and of the internal anatomy of a male Sphaerites. J. Entomol. (B) 42, 133–140.
Estabrook, G.F., McMorris, F.R., Meacham, C.A., 1985. Comparison of undirected phylogenetic trees based on subtrees of four evolutionary units. Syst. Zool. 34, 193–200.
Gardner, J.C.M., 1930. The early stages of *Niponius andrewsi*. Bull. Entomol. Res. 21, 15–17 [+1 plate].
Giribet, G., Distel, D.L., Polz, M., Sterrer, W., Wheeler, W.C., 2000. Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cycliophora, Plathelminthes, and Chaetognatha: A combined approach of 18S rDNA sequences and morphology. Syst. Biol. 49, 539–562.
Gladstein, D., Wheeler, W.C., 1999. POY: Phylogeny Reconstruction via Direct Optimization Alignment. Version 2.0. American Museum of Natural History.
Hancock, J.M., Tautz, D., Dover, G.A., 1988. Evolution of the secondary structures and compensatory mutations of the ribosomal RNAs of *Drosophila melanogaster*. Mol. Biol. Evol. 5, 393–414.
Hancock, J.M., Vogler, A.P., 2000. How slippage-derived sequences are incorporated into rRNA variable-region secondary structure: Implications for phylogeny reconstruction. Mol. Phylogenet. Evol. 14, 366–374.
Hansen, M., 1991. The hydrophiloid beetles. Phylogeny, classification, and a revision of the genera. Biol. Skr. 40, 1–368.
Hansen, M., 1997. Phylogeny and classification of the staphyliniform beetle families. Biol. Skr. 48, 1–339.
Helava, J.V.T., Howden, H.F., Ritchie, A.J., 1985. A review of the New World genera of the myrmecophilous and termitophilous subfamily Hetaeriinae. Sociobiology 10, 127–386.
Kluge, A., 1998. Total evidence or taxonomic congruence: Cladistics or consensus classification. Cladistics 14, 151–158.
Kovarik, P.W., Passoa, S., 1993. Chaetotaxy of larval Histeridae based on a description of *Onthophilus nodatus* LeConte. Ann. Entomol. Soc. Am. 86, 560–576.
Lawrence, J.F., Britton, E.B., 1991. Australian Beetles. Melbourne University Press, Carleton.
Lawrence, J.F., Newton, A.F., 1982. Evolution and classification of beetles. Ann. Rev. Entomol. 13, 261–290.
Lawrence, J.F., Newton, A.F., 1995. Families and subfamilies of Coleoptera (with selected genera, notes, references, and data on family-group names). In: Pakaluk, J., Ślipiński, S.A. (Eds.), Biology, Phylogeny, and Classification of the Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson. Muzeum i Instytut Zoologii PAN, Warsaw, pp. 779–1006.
Löbl, I., 1996. A new species of *Sphaerites* from China. Bull. Soc. Entomol. Suisse 69, 195–200.
Mamayev, B.M., 1974. The immature stages of the beetle *Syntelia histeroides* Lewis in comparison with certain Histeridae. Entomol. Rev. 53, 98–101.
Mateu, J., 1972. Les insectes xylophages des Acacia dans les regions sahariennes. Pub. Inst. Zool. Dr. Augusto Nombre 116, 1–714.
Mazur, S., 1997. A world catalogue of Histeridae. Genus (Suppl.), 1–373.
Newton, A.F., 1991. Agyrtidae–Silphidae (pp. 324–341); Pselaphidae (pp. 353–355); Sphaeritidae–Histeridae (pp. 341–352). In: Stehr, F.W. (Ed.), Immature Insects. Kendall/Hunt, Dubuque.
Newton, A.F., 2000. Sphaeritidae. In: Arnett, R.H., Thomas, M.C. (Eds.), American Beetles. CRC Press, Gainesville, FL, pp. 209–211.
Nikitsky, N.B., 1976a. Larval morphology and mode of life of Histeridae in bark beetle passages. Entomol. Rev. 55, 102–111.
Nikitsky, N.B., 1976b. On the morphology of a larva *Sphaerites glabratus* and the phylogeny of Histeroidea. Zool. Z. 55, 531–537.
Ohara, M., 1994. A revision of the superfamily Histeroidea of Japan. Insecta Mastumurana 51, 1–283.
Penny, D., Hendy, M.D., 1985. The use of tree comparison metrics. Syst. Zool. 34, 75–82.
Phillips, A., Janies, D., Wheeler, W., 2000. Multiple sequence alignment in phylogenetic analysis. Mol. Phylogenet. Evol. 16, 317–330.
Shull, V.L., Vogler, A.P., Baker, M.D., Maddison, D.R., Hammond, P.M., 2001. Sequence alignment of 18S ribosomal RNA and the basal relationships of adephagan beetles: Evidence for monophyly of aquatic families and the placement of Trachypachidae. Syst. Biol. 30, 945–969.
Ślipiński, S.A., Mazur, S., 1999. *Epuraeosoma*, a new genus of Histerinae and phylogeny of the family Histeridae. Ann. Zool. 49, 209–230.
Soltis, P.S., Soltis, D.E., Wolf, P.G., Nickrent, D.L., Chaw, S.-M., Chapman, R.L., 1999. The phylogeny of land plants inferred from 18S rDNA sequences: Pushing the limits of rDNA signal? Mol. Biol. Evol. 16, 1774–1784.
Swofford, D.L., 1998. PAUP*: Phylogenetic Analysis Using Parsimony. Version 4.0b8. Sinauer, Sunderland, MA.
Tautz, D., Hancock, J.M., Webb, D.A., Tautz, C., Dover, A., 1988. Compete sequence of the rRNA genes of *Drosophila melanogaster*. Mol. Biol. Evol. 5, 366–376.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties, and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Wenzel, R.L., 1944. On the classification of histerid beetles. Fieldiana Zool. 28, 51–151.
Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst. Biol. 44, 321–331.
Wheeler, W.C., 1996. Optimization alignment: The end of multiple sequence alignment in phylogenetics? Cladistics 12, 1–9.
Whiting, M.F., Carpenter, J.C., Wheeler, Q., Wheeler, W.C., 1997. The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.