

Phylogeny of Hydradephagan Water Beetles Inferred from 18S rRNA Sequences

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Several families in the beetle suborder Adephaga have an aquatic life style and are commonly grouped in the “Hydradephaga,” but their monophyly is contentious and relationships between and within these families are poorly understood. Here we present full-length 18S rRNA sequence for 84 species of Hydradephaga, including representatives of most major groups down to the tribal level, and a total of 68 species of the largest family, Dytiscidae. Using a direct optimization method for the alignment of length-variable regions, the preferred tree topology was obtained when the cost of gaps and the cost of nucleotide changes were equal, and three hypervariable regions of 18S rRNA were downweighted by a factor of five. Confirming recent molecular studies, the Hydradephaga were found to be monophyletic, indicating a single colonization of the aquatic medium. The most basal group within Hydradephaga is Gyrinidae, followed in a comb-like arrangement by families Haliplidae, Noteridae, Amphizoidae, and Hygrobiidae plus Dytiscidae. Under most alignment parameters, Hygrobiidae is placed amid Dytiscidae in an unstable position, suggesting a possible data artifact. Basal relationships within Dytiscidae are not well established, nor is the monophyly of subfamilies Hydroporinae and Colymbetinae. In contrast, relationships at the genus level appear generally well supported. Despite the great differences in the rates of change and the significant incongruence of the phylogenetic signal in conserved vs hypervariable regions of the 18S rRNA gene, both contribute to establish relationships at all taxonomic levels. © 2002 Elsevier Science (USA)

INTRODUCTION

Several groups of Coleoptera (beetles) have acquired an aquatic lifestyle, but the most conspicuous aquatic radiations are the diving beetles in the suborder Adephaga. The group includes some 5000 species in

more than 200 genera and has been subdivided into six families, summarily referred to as Hydradephaga. This includes the true diving beetles (Dytiscidae), the largest group, with more than 3500 species and nine subfamilies, plus several smaller families including the Gyrinidae (whirligig beetles, approx. 1000 species), Noteridae (burrowing water beetles, 270 species), Haliplidae (crawling water beetles, 220 species), and two monogeneric families, Hygrobiidae (squeak beetles, 6 species) and Amphizoidae (troutstream beetles, 6 species). Diving beetles spend most of their life cycle in the water, with only the pupae terrestrial, and they are generally characterized by a flattened hydrodynamic body shape and modified hind legs used as paddles. They have diversified extensively in morphological design, habitat requirements, and underwater habits (Guignot, 1933; Crowson, 1981). Variation in morphological features affects swimming performance, such as velocity and maneuverability, and provides a study system for functional morphology (Ribera and Nilsson, 1995; Ribera *et al.*, 1997).

The great diversity of aquatic life styles and associated morphologies raises the question whether or not the hydradephagan families are likely to be derived from a single aquatic ancestor. The suborder Adephaga includes three further families with terrestrial life style (ground beetles, commonly referred as “Gadephaga”): Rhysodidae, Carabidae (including tiger beetles, Cicindelinae), and Trachypachidae (Lawrence and Newton, 1995). The latter has some morphological characters linking it to the aquatic families, despite fully terrestrial habits (Crowson, 1981; Beutel, 1993, 1998). It is commonly accepted that the Adephaga were ancestrally terrestrial (Lawrence and Newton, 1982), but the basal relationships necessary for such conclusions have been contentious. Based on a set of morphological characters the monophyly of the Hydradephaga (usually excluding Trachypachidae) has been proposed by several authors (e.g., Crowson, 1960; Ponomarenko, 1973; Roughley, 1981; Lawrence and Newton, 1982). However, the more recent literature favors their polyphyly and postulates three independent transi-

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TABLE 1
Taxonomic Coverage of the Families, Subfamilies, and Tribes of Aquatic Aedeopoda

Family ^a	Subfamily	Tribe	No. genera	No. genera sampled	No sequences ^b
Gyrinidae	Spanglerogyrinae		1	1	1
	Gyrininae	Gyrinini	4	2	1 (1)
		Enhydrini	5	2	2
		Orectochilini	3	2	2
Haliplidae	Noterinae		5	2	4
		Hydrocanthini	3	2	2 (1)
Noteridae		Noterini	4	1	1
		Notomicrini	6	1	1
Amphizoidae	Phreatodytinae	Suphisini	1	1	1
			1	—	—
Hygrobiidae			1	1	1 (1)
Dytiscidae	Agabetinae		1	1	3
	Aubehydrinae		1	1	(1)
	Colymbetinae	Agabini	11	3	1
		Anisomerini	2	—	6
		Colymbetini	8	3	4
		Matini	3	—	—
	Copelatinae		4	2	5
	Coptotominae		1	1	2
	Dytiscinae	Aciliini	7	3	3
		Cybistrini	6	2	5
		Dytiscini	2	1	1
		Eretini	1	1	2
	Hydroporinae	Hydaticini	2	1	3
		Bidessini	38	6	7
		Carabhydrini	1	—	—
		Hydroporini	40	15	16
		Hydrovatini	2	1	2
		Hyphydrini	13	1	(2)
		Laccornini	1	1	1
		Methlini	2	1	(1)
		Pachydrini	2	1	1
		Vatellini	4	—	—
		Incertae sedis	7	—	—
		Laccophilinae	11	2	3
	Lancetinae		1	1	2
Total	13	26	206	66	83 (7)

^a Classification follows Nilsson and Roughley (1997), with the addition of tribe Pachydrini (Biström *et al.*, 1997) and the suppression of tribe Hydronebriini (Nilsson, 2000).

^b In parentheses, number of incomplete sequences (i.e., with at least one region missing, see Material and Methods).

tions to the aquatic environment (Beutel and Roughley, 1988; Beutel, 1993, 1995, 1997, 1998; Beutel and Haas, 1996; initially suggested by Bell, 1966). According to this hypothesis, the first invasion of the aquatic environment led to the Gyrinidae, with two further invasions by ancestors of Haliplidae (Hammond, 1979; Kavanaugh, 1986) and, independently, Dytiscoidea (= Dytiscidae, Hygrobiidae, Noteridae, and Amphizoidae). However, the hypothesis of a multiple origin is rejected by recent molecular studies (Shull *et al.*, 2001), confirming the traditional view of a monophyletic Hydradeopoda.

Relationships are reasonably well understood for the smaller hydradeopodan families, which have been treated thoroughly in Noteridae (Beutel and Roughley,

1987; Belkaceme, 1991; Beutel, 1997), Haliplidae (Beutel, 1997), and Gyrinidae (Beutel and Roughley, 1988, 1994). Within the Dytiscidae, however, the relationships among main groups are largely unknown, with the possibility that some of the currently accepted subfamilies and tribes (Table 1) may not reflect monophyletic lineages. The most recent comprehensive classification separates Dytiscidae into nine subfamilies (Nilsson and Roughley, 1997), based on all phylogenetic information available at the time. In a recent analysis based mostly on characters of the female reproductive system, Miller (2001) proposed a new arrangement, raising Matini and Agabini to the rank of subfamilies (the former being sister to the remaining Dytiscidae) and creating a further subfamily for the

new genus *Hydrodytes*. No sound analysis of relationships has been attempted for Hydroporinae, the largest subfamily, which is also the most diverse morphologically.

Here we attempt a comprehensive phylogenetic analysis of basal relationships in Hydradephaga by including a wide representation of all major lineages. Our study builds on a data set to determine relationships in the wider Adephaga based on the nuclear 18S rRNA gene initiated by Shull *et al.* (2001), which provided only a very limited representation of the Dytiscidae and other aquatic families. The 18S rRNA gene has been used with some success in phylogenetics of Coleoptera (Vogler and Pearson, 1996; Farrell, 1998; Maddison *et al.*, 1999; Shull *et al.*, 2001). It contains both slow- and fast-evolving sections and is potentially useful for resolving relationships over a wide hierarchical range.

However, sensitivity to the choice of alignment parameters in 18S rRNA compounds the problem of optimal-tree search and adds uncertainty to phylogenetic conclusions. The analyses conducted here involve the implementation of simultaneous procedures of aligning and tree building in a parsimony framework, as implemented in the POY software (Gladstein and Wheeler, 1997). The results from alignments can frequently be assessed in the context of congruence with other data (Wheeler, 1995), but information about hydradephagan relationships is too scarce for this approach. We therefore explored a new method to select the preferred trees based on internal characteristics of the 18S rRNA data. Specifically, we used the resilience of nodes to variation in alignment conditions as a criterion for assessing the quality of trees and selected those alignment parameters which produced the largest number of nodes consistently found across the parameter space.

MATERIAL AND METHODS

Sampling

Sequences of the 18S rRNA gene were obtained for all families of aquatic Adephaga (Tables 1 and 2). All recognized subfamilies of Dytiscidae (following Nilsson and Roughley, 1997) were included in the analysis. Of the 26 currently recognized tribes within Hydradephaga, 22 were represented (Table 1). No specimens of the stygobiont noterid subfamily Phreatodytinae could be obtained. The tribes missing from our study represent only a small fraction of the total species diversity of the lineage (Table 1). Outgroups were obtained from Shull *et al.* (2001) and include representatives of all suborders of Coleoptera (Table 2).

DNA Extraction, PCR, and Sequencing

Specimens were collected in the field or obtained from colleagues and preserved in ethanol (Table 2).

Voucher specimens are kept in the Department of Entomology, The Natural History Museum. Total DNA was extracted from single specimens as described in Vogler *et al.* (1993). For most of the DNA amplification Ready-To-Go PCR beads (Amersham Pharmacia Biotech) were used, which contain 1.5 U *Taq* DNA polymerase, 10 mM Tris-HCl, pH 9.0, at 25°C, 50 mM KCl, 1.5 mM MgCl₂, 200 μM each dNTP, and stabilizers including bovine serum albumin. Each 18S rDNA sequence was amplified as four overlapping fragments of 500–800 bp (for details, see Shull *et al.*, 2001).

The following cycling conditions were generally used: 1 min at 95°C; 30 s at 94°C, 30 s at 45–55°C (depending on the melting temperatures of the primer pair used), and 1–2 min at 72°C (repeated for 30 to 40 cycles); 10 min at 72°C. Amplification products were purified using a GeneClean II kit (Bio 101, Inc.). Automated DNA sequencing reagents were supplied by either Perkin Elmer Applied BioSystems Ltd. (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit) or Amersham Life Science, Inc. (Thermo Sequenase Dye Terminator Cycle Sequencing Pre-Mix Kit). Sequencing reactions were purified by ethanol precipitation and electrophoresed at the DNA Sequencing Facility of the Natural History Museum. Sequences were edited and contigs were constructed using the Sequencher 3.0 software package (Gene Codes Corp.).

Difficulties with PCR amplification were encountered for the V4 and V6 regions for some Gyrinidae, Haliplidae, Amphizoidae, and *Deronectes* and relatives (Dytiscidae) (see also Maddison *et al.*, 1999 and Shull *et al.*, 2001) and had to be omitted from the tree searches in a few cases (see Table 2 for details). EMBL accession numbers are listed in Table 2.

Phylogenetic Analysis

The establishment of character homologies in genes with extensive length variation is critical for phylogenetic reconstruction. We follow a one-step tree alignment procedure (Sankoff, 1975; Wheeler, 1996), using POY software (Gladstein and Wheeler, 1997). The method assesses directly the number of character transformations (nucleotide changes plus implied insertions/deletions), given a particular tree topology (Wheeler, 1996). The preferred tree is the one for which character optimization is the least costly under the specific alignment parameters.

In the 18S rRNA gene highly conserved regions can be used to delimit gene regions of obvious homology which bracket length-variable regions. We defined seven fragments prior to the analyses, according to the degree of length variation across the sampled taxa. Regions 1, 3, 5, and 7 have almost no variation in length ("conserved" regions C1, C3, C5, and C7), while regions 2, 4, and 6 have large length differences ("variable" regions V2, V4, and V6 of Tautz *et al.*, 1988) (Table 3).

TABLE 2
Studied Taxa, with Sampling Localities, Collector, and Sequence Accession Numbers

Taxa ^a	Tribe	Species	Code ^b	Locality ^c	Collector	Accession No.
Archostemata						
Cupedidae		<i>Discotupes</i> sp.	RCupDiscot	Shull <i>et al.</i> , 2001		AF201421
Myxophaga						
Hydroscaphidae		<i>Hydroscapha natans</i> LeConte	MHydHydros	Maddison <i>et al.</i> , 1999		AF012525
Torridincolidae		<i>Torridincola rhodesica</i> Steffan	MTorTorrid	Shull <i>et al.</i> , 2001		AF201420
Polyphaga						
Staphyliniformia						
Hydrophiloidea						
Hydrophilidae		<i>Helochares lividus</i> (Forster)	PHydHeloch	Shull <i>et al.</i> , 2001		AF201418
Scarabeiformia						
Scarabaeoidea						
Scarabaeidae		<i>Dynastes granti</i> Horn	PScaDynast	Maddison <i>et al.</i> , 1999		AF002809
Elateriformia						
Scirtoidea						
Clambidae		<i>Clambus arnetti</i> Endrödy-Younga	PClaClambu	Maddison <i>et al.</i> , 1999		AF012526
Scirtidae		<i>Cyphon hilaris</i> Nyholm	PSciCyphon	Shull <i>et al.</i> , 2001		AF201419
Cucujiformia						
Tenebrionidea						
Tenebrionidae		<i>Tenebrio molitor</i> Linnaeus	PTenTenebr	Hendriks <i>et al.</i> , 1988		X07801
Curculionoidea						
Anthribidae		<i>Bruchela conformis</i> (Suffriani)	PAntBruche	Shull <i>et al.</i> , 2001		AF201417
Curculionidae		<i>Brachycerus muricatus</i> (Fabricius)	PCurBrachy	France	M. Barclay	AJ318658
Adephaga						
Trachypachidae		<i>Systolosoma lateritium</i> Negre	ATraSystol	Maddison <i>et al.</i> , 1999		AF012522
		<i>Trachypachus holmbergi</i> Mannerheim	ATraTrachy	Maddison <i>et al.</i> , 1999		AF201394
Carabidae		<i>Agonum albipes</i> (F.)/ <i>marginatum</i> (L.)	ACarAgonum	Shull <i>et al.</i> , 2001		AF201403
		<i>Bembidium tetracolum</i> Say	ACarBembid	Shull <i>et al.</i> , 2001		AF201402
		<i>Chlaenius cupreus</i> (Paykull)	ACarChlaen	Shull <i>et al.</i> , 2001		AF201404
		<i>Creobius eydouxi</i> (Guérin-Ménéville)	ACarCreobi	Maddison <i>et al.</i> , 1999		AF012498
		<i>Dyschirius aeneus</i> (Dejean)	ACarDischi	Shull <i>et al.</i> , 2001		AF201401
		<i>Elaphrus cupreus</i> Duftschmid	ACarElaphr	Shull <i>et al.</i> , 2001		AF201397
		<i>Loricera pilicornis</i> (Fabricius)	ACarLoric	Shull <i>et al.</i> , 2001		AF201396
		<i>Mecyclothorax vulcanus</i> (Blackburn)	ACarMecycl	Maddison <i>et al.</i> , 1999		AF012482
		<i>Nebria brevicollis</i> (Fabricius)	ACarNebria	Shull <i>et al.</i> , 2001		AF201395
		<i>Omophron americanum</i> Dejean	ACarOmophr	Shull <i>et al.</i> , 2001		AF201398
		<i>Psydrus piceus</i> LeConte	ACarPsydru	Maddison <i>et al.</i> , 1999		AF002784
Gyrinidae						
Spanglerogyrinae		<i>Spanglerogyrus albiventris</i> Folkerts	HGyrSpaalv	Shull <i>et al.</i> , 2001		AF201413
Gyrininae	Gyrinini	<i>Aulonogyrus striatus</i> (Fabricius)	HGyrAulstr*	Spain	I. Ribera	AJ318660/1 ^d
		<i>Gyrinus</i> sp.	HGyrGyrsp1	Shull <i>et al.</i> , 2001		AF201412
	Enhydrini	<i>Andogyrus ellipticus</i> (Brulle)	HGyrAndell	Chile	I. Ribera	AJ318659
		<i>Macrogyrus</i> sp.	HGyrMacsp1	Australia	J. Mate	AJ318664
	Orectochilini	<i>Gyretes iricolor</i> Young	HGyrGytiri	U.S.	S. Oygur	AJ318662/3 ^d
		<i>Orectochilus villosus</i> (Muller)	HGyrOrevil	Spain	I. Ribera	AJ318665
Haliplidae		<i>Haliplus (Haliplus) ruficollis</i> (De Geer)	HHalHalruf	Shull <i>et al.</i> , 2001		AF201406
		<i>Haliplus (Liaphlus) mucronatus</i> Stephens	HHalHalmuc	Spain	I. Ribera	AJ318667
		<i>H. (Neohaliplus) lineatocollis</i> (Marshall)	HHalHallin	Spain	I. Ribera	AJ318666
		<i>Peltodytes rotundatus</i> (Aubé)	HHalPelrot	Spain	I. Ribera	AJ318668
Noteridae						
Noterinae	Notomicrini	<i>Notomicrus tenellus</i> Clark	HNotNomten	Australia	C. Watts	AJ318671
	Hydrocanthini	<i>Suphisellus</i> sp.	HNotSulsp1	Venezuela	D. Bilton	AJ318669
		<i>Hydrocanthus oblongus</i> Sharp	HNotHctobl*	Shull <i>et al.</i> , 2001		AF201415
		<i>Hydrocanthus</i> sp.	HNotHctSp1	Venezuela	D. Bilton	AJ318670
	Noterini	<i>Noterus clavicornis</i> De Geer	HNotNotcla	Shull <i>et al.</i> , 2001		AF201416
	Suphisini	<i>Suphis inflatus</i> Leconte	HNotSupinf	Maddison <i>et al.</i> , 1999		AF012523
Amphizoidea		<i>Amphizoia insolens</i> LeConte	HAmppAmpins*	U.S.	A. Cognato	AJ318675/6/7 ^d
		<i>Amphizoia lecontei</i> Matthews	HAmppAmple	Canada	NHM	AJ318678
Hygrobiidae		<i>Hygrobia australasiae</i> Clark	HHygHygaus	Australia	C. Watts	AJ318672
		<i>Hygrobia hermanni</i> (Fabricius)	HHygHygher	Spain	I. Ribera	AJ318673
		<i>Hygrobia maculata</i> Britton	HHygHygmac	Australia	D. Norton	AJ318674
Dytiscidae						
Agabetinae		<i>Agabetes acuductus</i> Harris	HAgAgaacu*	U.S.	C. Hernando	AJ318697
Aubehydrinae		<i>Notaticus fasciatus</i> Zimmermann	HAubNotfas	Venezuela	D. Bilton	AJ318698
Colymbetinae	Agabini	<i>Agabus bipustulatus</i> (Linnaeus)	HColAgabip	Spain	I. Ribera	AJ318687
		<i>Agabus brunneus</i> (Fabricius)	HColAgabru	Morocco	I. Ribera	AJ318688
		<i>Agabus heydeni</i> Wehncke	HColAgahey	Morocco	I. Ribera	AJ318689
		<i>Ilybius hozgargantae</i> (Burmeister)	HColIlyhoz	Spain	I. Ribera	AJ318690
		<i>Ilybius meridionalis</i> Aubé	HColIlymer	Portugal	I. Ribera	AJ318692
		<i>Platynectes decempunctatus</i> (Fabricius)	HColPladec	Australia	C. Watts	AJ318694
	Colymbetini	<i>Colymbetes schilcknechti</i> Dettner	HColColsch	Spain	I. Ribera	AJ318691
		<i>Meladema coriacea</i> Castelnau	HColMelcor	Morocco	I. Ribera	AJ318693
		<i>Rhantus (Nartus) grapii</i> (Gyllenhal)	HColRhagra	U.K.	I. Ribera	AJ318695
		<i>Rhantus (Rhantus) suturalis</i> (McLeay)	HColRhasut	Spain	I. Ribera	AJ318696

TABLE 2—Continued

Taxa ^a	Tribe	Species	Code ^b	Locality ^c	Collector	Accession No.	
Copelatinae		<i>Copelatus (Copelatus) angustatus</i> gr	HCopCopang	Brazil	I. Ribera	AJ318746	
		<i>C. (Copelatus) haemorrhoidalis</i> (F.)	HCopCophae	U.K.	I. Ribera	AJ318679	
		<i>Copelatus (Papuadytes) utowaensis</i> Balke	HCopCoputo	New Guinea	M. Balke	AJ318682	
		<i>Lacconectus peguensis</i> Brancucci	HCopLacpeg	Myanmar	Schillhammer	AJ318680	
Coptotominae		<i>Lacconectus</i> sp.	HCopLacsp1	India	D. Boukal	AJ318681	
		<i>Coptotomus interrogatus</i> (Fabricius)	HCotCotint	U.S.	NHM	AJ318685	
		<i>Coptotomus lenticus</i> Hilsenhoff	HCotCotlen	U.S.	K. Miller	AJ318686	
Dytiscinae	Acilini	<i>Acilius sulcatus</i> (Linnaeus)	HDytAcisul	U.K.	I. Ribera	AJ318699	
		<i>Graphoderus cinereus</i> (Linnaeus)	HDytGrhcin	Spain	I. Ribera	AJ318705	
		<i>Thermonectus</i> sp.	HDytThesp1	Venezuela	D. Bilton	AJ318712	
	Cybistrini	<i>Cybister (Scaphinectes) lateralimarginalis</i> (De Geer)	HDytCyblat	Spain	I. Ribera	AJ318700	
		<i>Cybister (Cybister) tripunctatus</i> (Olivier)	HDytCybtri	Australia	C. Watts	AJ318702	
		<i>Megadytes</i> sp.	HDytMegsp1	Peru	M. Barclay	AJ318701	
		<i>Megadytes (Megadytes)</i> sp.	HDytMegMeg	Venezuela	D. Bilton	AJ318710	
		<i>Megadytes (Bifurcius)</i> sp.	HDytMegBif	Venezuela	D. Bilton	AJ318709	
		<i>Hyderodes schuckardi</i> Hope	HDytHyrsch	Australia	C. Watts	AJ318708	
	Eretini	<i>Eretes australis</i> (Erichson)	HDytEreaus	Australia	C. Watts	AJ318703	
		<i>Eretes sticticus</i> (Linnaeus)	HDytEresti	Iran	H. Fery	AJ318704	
	Hydaticini	<i>Hydaticus (Guignotites) leander</i> (Rossi)	HDytHyclea	Spain	I. Ribera	AJ318706	
		<i>H. (Guignotites) consanguineus</i> Aubé	HDytHycco	Australia	C. Watts	AJ318711	
		<i>H. (Hydaticus) transversalis</i> (Pontoppidan)	HDytHyctra	U.K.	I. Ribera	AJ318707	
	Hydroporinae	Bidessini	<i>Bidessodes mjobergi</i> (Zimmermann)	HBidBismjo	Australia	C. Watts	AJ318725
<i>Bidessus calabricus</i> Guignot			HBidBiscal	Iran	H. Fery	AJ318723	
<i>Bidessus goudoti</i> (Castelnau)			HBidBidgou	Spain	I. Ribera	AJ318724	
<i>Clypeodytes bifasciatus</i> (Zimmermann)			HBidClybif	Australia	C. Watts	AJ318726	
<i>Hydroglyphus pusillus</i> (Fabricius)			HBidHylpus	Portugal	I. Ribera	AJ318727	
<i>Liodessus</i> sp.			HBidLiosp1	Australia	C. Watts	AJ318728	
<i>Yola bicarinata</i> (Latreille)			HBidYolbic	Spain	I. Ribera	AJ318729	
Hydroporini			<i>Graptodytes flavipes</i> (Olivier)	HHydGrtfla	Spain	I. Ribera	AJ318730
			<i>Herophydrus musicus</i> (Klug)	HHydHermus	Spain	I. Ribera	AJ318731
			<i>Heterosternuta pulcher</i> (LeConte)	HHydHetpul	Canada	Y. Alarie	AJ318732
			<i>Hydroporus planus</i> (Fabricius)	HHydHydpla	Spain	I. Ribera	AJ318734
			<i>Hydrotarsus pilosus</i> Guignot	HHydHydpl	Tenerife	D. Bilton	AJ318733
		<i>Hygrotus confluens</i> (Fabricius)	HHydHytcon	Portugal	I. Ribera	AJ318735	
		<i>Hygrotus impressopunctatus</i> (Schaller)	HHydHytimp	Spain	I. Ribera	AJ318736	
		<i>Hygrotus inaequalis</i> (Fabricius)	HHydHytina	Spain	I. Ribera	AJ318737	
		<i>Laccornellus copelatoides</i> (Sharp)	HHydLlccop	Chile	I. Ribera	AJ318738	
		<i>Metaporus meridionalis</i> (Aubé)	HHydMetmer	Spain	I. Ribera	AJ318739	
		<i>Necterosoma dispar</i> (Germar)	HHydNecdis	Australia	C. Watts	AJ318740	
		<i>Neoporus undulatus</i> (Say)	HHydNeound	Canada	Y. Alarie	AJ318741	
Hydrovatini		<i>Paroster gibbi</i> Watts	HHydPargib	Australia	C. Watts	AJ318742	
		<i>Porhydrus lineatus</i> (Fabricius)	HHydPorlin	U.K.	I. Ribera	AJ318743	
		<i>Stictonectes epipleuricus</i> (Seidlitz)	HHydStnepi	Spain	I. Ribera	AJ318744	
		<i>Suphrodytes dorsalis</i> (Fabricius)	HHydSupdor	U.K.	D. Bilton	AJ318745	
		<i>Hydrovatus clypealis</i> Sharp	HHyvHyvcl	Spain	I. Ribera	AJ318716	
		<i>Hydrovatus nigrata</i> Sharp	HHyvHyvnig	Australia	D. Norton	AJ318717	
		Hyphydrini	<i>Hyphydrus aubei</i> Ganglbauer	HHypHypaub*	Spain	I. Ribera	AJ318721
			<i>Hyphydrus ovatus</i> (Linnaeus)	HHypHypova*	U.K.	I. Ribera	AJ318722
		Laccotmini	<i>Laccornis oblongus</i> (Stephens)	HLallLacobl	U.K.	D. Bilton	AJ318715
		Mehltini	<i>Celina</i> sp.	HMetCelsp1*	Brazil	I. Ribera	AJ318718/9 ^d
			<i>Pachydrus globosus</i> (Aubé)	HPacPacglo	Brazil	I. Ribera	AJ318720
	Laccophilinae	<i>Australphilus montanus</i> Watts	HLacAusmon	Australia	C. Watts	AJ318713	
<i>Laccophilus hyalinus</i> (De Geer)		HLacLaphya	Shull <i>et al.</i> , 2001		AF201410		
<i>Laccophilus poecilus</i> Klug		HLacLappoe	Spain	I. Ribera	AJ318714		
Lancetinae	<i>Lancetes nigriceps</i> (Erichson)	HLanLannig	Chile	I. Ribera	AJ318683		
	<i>Lancetes varius</i> (Fabricius)	HLanLanvar	Chile	I. Ribera	AJ318684		

^a Family classification follows Lawrence and Newton (1995); Dytiscidae classification follows Nilsson and Roughley (1997), with the addition of tribe Pachydrini (Biström *et al.*, 1997).

^b Codes are those used in Figs. 2–4. Asterisks mark incomplete sequences. Region V4 was omitted from the analyses in *Aulonogyrus striatus*, *Amphizoa insolens*, and *Celina* sp. and region V6 in *Hydrocanthus oblongus*, *Amphizoa insolens*, *Agabetes aceductus*, *Hyphydrus aubei*, and *H. ovatus*. Smaller fragments were missing in some species at the end of region C3, the beginning of regions C5 and C7, and the end of region C7.

^c Full details available on request. The original reference is given for the sequences obtained from GenBank.

^d Noncontiguous fragments of the sequence were submitted separately.

Sensitivity Analysis

Sensitivity to the inclusion and exclusion of taxa or regions, and to different gap costs and weighting schemes (Wheeler, 1995; Phillips *et al.*, 2000), was assessed in three sets of analyses. First, all data (in-

cluding outgroups) and full-length sequences were included (Analysis 1). Second, the variable regions V2, V4, and V6 of Archostemata, Myxophaga, and Polyphaga were excluded to avoid alignment problems resulting from the great divergence and length differ-

TABLE 3
Length Variation in Individual Regions of 18S rRNA

		C1	V2	C3	V4	C5	V6	C7	All
Raw sequences									
All taxa	Min.	201	24	467	28	654	62	385	1844
	Max.	207	45	472	210	663	219	393	2162
	Average	203.2	34.4	470.7	63.5	661.0	123.0	388.5	1944.2
	Std. dev.	0.74	4.05	0.76	38.14	1.14	31.55	1.43	59.53
Hydradephaga only	Min.	203	35	471	50	661	115	388	1870
	Max.	205	43	472	210	663	186	390	2169
	Average	203.1	34.6	470.8	57.3	660.9	122.7	388.3	1937.8
	Std. dev.	0.5	3.6	0.5	33.3	1.8	27.4	1.2	47.8
Aligned									
Analysis 2									
V4,V6 excluded	gap 2	214	108	483	—	678	—	430	1913
Weight co 5: va 1	gap 1	219	103	482	771	689	546	442	3252
Analysis 3									
Weight co 1: va 1	gap 1	209	86	475	390	679	357	419	2615
	gap 2	208	73	476	315	670	334	414	2490
	gap 5	208	76	475	255	670	290	410	2384
Weight co 5: va 1	gap 1	209	81	476	537	671	370	415	2759
	gap 2	208	77	474	353	668	340	413	2533
	gap 5	208	76	474	280	668	318	410	2434
V4, V6 excluded	gap 1	209	74	475	—	670	—	415	1843
	gap 2	208	67	475	—	668	—	413	1831
	gap 5	208	60	473	—	668	—	404	1813
Std. dev.	(Hydradephaga)	0.5	7.1	0.9	92.7	3.3	26.0	4.0	348.2

Note. C1, C3, C5, and C7, conserved regions; V2, V4, and V6, variable regions. Weight co 5: va 1, conserved regions weighted 5, variable regions 1, etc. (see Material and Methods).

ence between beetle suborders in these regions (Analysis 2). Third, only the ingroup (Hydradephaga) sequences were analyzed, as their monophyly was considered to be well established based on the first two sets of analyses (see Results) and previous results of Shull *et al.* (2001) (Analysis 3).

For each of the three data sets (Analyses 1, 2, and 3), phylogenetic analyses were carried out, as follows. A parameter space of two variables was defined, varying gap cost (gap cost = 1, 2, or 5) and relative weight of the conserved vs variable regions (equal weight, conserved regions 5:variable 1, and excluding variable regions V4 and V6) (Table 4). In total, six different parameter combinations were tested, for each of the three data sets, resulting in a total of 24 searches. POY searches consisted of 20 random-addition replicates (command *-multibuild 10*) and retained no more than five shortest trees (*-maxtrees 5*). All searches were performed with POY version 2.0 on a Fujitsu AP3000 Parallel Server using 16 UltraSPARC 300-MHz single processors running in parallel using PVM software.

From these primary analyses, we computed the strict consensus of all trees obtained when varying only one parameter value, i.e., the consensus of all trees obtained under the same gap cost for the three weighting schemes applied (the consensus of a row in the parameter space) and the consensus of all trees with

the same weighting scheme for the three gap costs (the consensus of a column in the parameter space) (Table 4). All nodes recovered in these “marginal” consensus trees (the consensus of the rows and columns of the parameter space matrix) were listed. (The term “marginal” consensus tree is chosen according to terminology from statistics, as this consensus is derived from a

TABLE 4
Number of Trees Obtained in the Sensitivity Analysis

		Gap cost			Marginal consensus trees (weight)
		1	2	5	
Weight	none	1/2/1	1/5/1	5/5/5	7/12/7
	co 5: va 1	1/1/1	1/1/1	5/2/5	7/4/7
	excl. V4,V6	1/-/5	5/-/5	5/-/5	11/-/15
Marginal consensus trees (gap cost)		3/3/7	7/6/7	15/7/15	Total number of trees 25/16/29

Note. Entries refer to the number of trees obtained, respectively, in POY searches in Analyses 1, 2, and 3 (separated by slashes), varying the gap cost and the relative weight of conserved and variable regions. “Marginal consensus trees” refer to the strict consensus of all trees obtained with each of the six parameter values (three gap costs and three weighting schemes). Weight co 5: va 1, conserved regions weighted 5, variable regions 1 (see Material and Methods); excl. V4,V6, variable regions V4 and V6 excluded.

single row or column in a two-entry table representing the values of the parameter space). The nodes resolved in the marginal consensus trees, with the exception of those linking two species of the same genus or two closely related genera present in all trees, were considered “key nodes.” The key nodes were used to assess the stability of the tree under different parameter combinations, as they reflect congruence of tree topologies in a portion of the parameter space (a measure of internal topological congruence). Trees considered optimal were those obtained with the parameter combinations that recovered the maximum number of key nodes. When the marginal consensus trees of two parameter values had the same number of key nodes, the parameter combinations that produced the lowest number of nodes contradicting a key node were chosen.

Bremer Support for trees can be established using the *-bremer* option in POY, but for further tree diagnostics an aligned data matrix would be useful. POY can generate an aligned matrix invoking the *-implied-alignment* option. This alignment is not built before or during the tree search as it is simply an alignment associated with a given tree, computed *a posteriori* from the states of hypothetical ancestors (W. Wheeler, personal communication). It establishes correspondences between bases, given the tree obtained from the initial search. This matrix can be used to calculate standard measures of node support and character congruence by applying standard parsimony procedures. PAUP4.0b2 (Swofford, 1999) was used for these operations and all parsimony trees reported here were obtained after 100 random replicates of TBR branch swapping. The same weighting scheme as applied in the original POY search was used, with gaps coded as a fifth character state (Giribet and Wheeler, 1999; Phillips *et al.*, 2000). Parsimony searches on this alignment may result in topologies slightly different from those of the original POY output.

Constraint trees for determining Bremer Support values (Bremer, 1994) and Partitioned Bremer Support (PBS) were generated with Treerot (Sorenson, 1996). Relationships among PBS values for the different regions were assessed with the nonparametric Spearman's rank correlation (Sokal and Rohlf, 1995). The significance of the Incongruence Length Difference (ILD) (Farris *et al.*, 1994) was assessed with the Partition Homogeneity Test as implemented in PAUP (using 100 replicates of a heuristic search with 10 random addition replicates each). Bootstrap values were computed as implemented in PAUP, with 100 random addition replicates.

Results

Sequence Data and Length Variability

PCR was successful for a total of 84 specimens (Table 2). Total sequence length for Hydradephaga varied

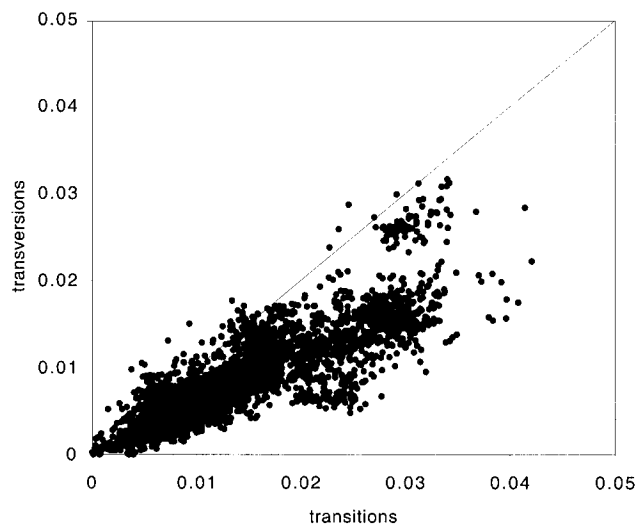


FIG. 1. Pairwise uncorrected distances (“p”) based on transitions vs transversions (only Hydradephaga; alignment using gap cost 1 and weight of conserved vs variable regions 5:1).

from 1870 (*Gyretes iricolor*) to 2169 (*Andogyrus ellipticus*) nucleotides (Table 3). Length variation was significantly higher in the variable regions ($P < 0.05$, ANOVA on the standard deviation of sequence length in conserved vs variable regions), even if only the ingroup (Hydradephaga) is considered and irrespective of the alignment parameters (Table 3). Pairwise sequence divergence among any two taxa ranged from 0.01% (corresponding to 1 bp, between *Hygrotus inaequalis* and *Herophydrus musicus*) to 8.9% (corresponding to 174 bp, between *Peltodytes rotundatus* and *Andogyrus ellipticus*), given the aligned matrix obtained with the preferred parameter values using the ingroup only (Analysis 3; see below). Using this same alignment, pairwise uncorrected divergence for the combined conserved regions was at maximum 6%, while the divergence for the variable regions was roughly an order of magnitude higher (maximum distance 48%, between *Hydrocoptus oblongus* and *Peltodytes rotundatus*). Overall variation was predominantly due to transitions, as apparent from a plot of uncorrected pairwise distances based on transitions vs transversions (Fig. 1), although the ratio tends to be closer to unity for the smaller distances.

Sensitivity Analysis

In total, 28 “key nodes” were recovered in the marginal consensus trees of the sensitivity analysis (Table 5). In comparing the marginal consensus trees obtained either by varying the gap cost or by varying the relative weight of conserved and variable regions, it is remarkable that in most cases a given node was either present or unresolved, but few contradictory topologies were found among them. This applies in particular to Analysis 1 (all species and all regions included), where

TABLE 5
Recovery of Key Nodes in the Sensitivity Analysis

Node	No.	Analysis 1: including outgroups					Analysis 2: only Adephaga V2, V4, V6					Analysis 3: only ingroup (Hydradephaga)					Totals			
		Weight			Gap cost		Weight			Gap cost		Weight			Gap cost					
		co 1:	co 5:	excl.	1	2	5	co 1:	co 5:	1	2	5	co 1:	co 5:	excl.	1	2	5	+	×
		va 1	va 1	V4,V6				va 1	va 1				va 1	va 1	V4,V6					
Adephaga + Polyphaga	1	+	+	× ^a	+	+	—	+	—	—	+	—	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6	1
Polyphaga	2	—	+	+	+	+	—	—	—	+	+	—	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6	0
Adephaga	3	—	—	—	—	—	—	—	—	+	×	—	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1	1
Geadephaga +																				
Trachypachidae	4	—	—	—	—	—	—	—	—	+	—	×	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1	1
Hydradephaga	5	—	—	—	—	—	—	—	—	+	×	×	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1	2
Hydradephaga excluded																				
Gyrinidae	6	—	—	—	—	—	—	—	—	×	×	×	—	—	+	—	—	—	1	3
Gyrinidae	7	—	—	+	—	—	—	—	—	—	×	×	—	—	+	—	—	—	2	2
Gyrininae	8	—	—	+	—	—	—	—	—	—	×	—	—	—	+	—	—	—	2	1
Noteridae	9	—	—	+	+	—	—	—	—	—	×	—	—	—	+	—	—	—	3	1
Noteridae excluded																				
<i>Notomicrus</i>	10	—	+	+	+	+	—	+	+	+	—	—	+	+	+	+	—	—	11	0
Haliplidae	11	—	—	—	+	—	—	—	—	+	—	×	—	+	+	+	—	—	5	1
Dytiscidae +																				
Hygrobiidae +																				
Amphizoidea	12	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	—	—	2	0
Dytiscidae +																				
Hygrobiidae	13	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	+	—	3	0
Dytis. + Hygrobiidae																				
excl. Hydroporinae	14	—	—	—	+	—	—	—	—	×	—	—	—	—	—	—	—	—	1	1
Cybistrini	15	—	+	—	+	+	—	—	+	+	—	+	+	—	+	—	—	—	9	0
Colymbetini	16	—	—	—	—	—	—	—	—	+	—	—	—	×	×	—	—	—	1	2
Aciliini + Eretini	17	—	+	+	+	+	—	—	+	+	—	—	+	+	+	—	—	—	10	0
Aciliini	18	—	×	—	—	—	—	—	—	+	×	—	—	—	—	—	—	—	1	2
Dytiscinae + <i>Notaticus</i>																				
(excl. Cybistrini)	19	—	—	+	+	+	—	—	+	+	—	—	+	+	+	+	—	—	10	0
<i>Hydaticus</i> (<i>Guignotites</i>)																				
+ <i>Notaticus</i>	20	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—	15	0
Hydaticini (inc. <i>Notaticus</i>)	21	—	×	—	—	—	—	—	—	×	—	—	+	+	—	+	+	—	4	2
Hydaticini (inc. <i>Notaticus</i>) +																				
<i>Hyderodes</i>	22	—	×	—	—	—	—	—	—	+	—	—	+	—	—	+	—	—	3	1
Laccophilinae	23	—	+	—	+	+	—	—	+	+	—	+	+	—	+	+	—	—	10	0
Bidessini	24	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	16	0
Methlini + Bidessini	25	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	1	0
<i>Graptodytes</i> group	26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	17	0
<i>Hygrotus</i> group	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	17	0
<i>Hydroporus</i> group	28	—	—	—	—	—	—	—	—	+	—	—	—	—	+	—	—	—	2	0
Totals		+	5	10	10*	14*	11	4	5	9*	21*	10	5	8	11*	10	14*	11	3	
		×	0	3	1	0	0	0	0	0	3	7	5	0	0	1	1	0	0	

Note. +, Node supported; —, node unresolved but compatible with node supported; ×, node not supported; n.a., not applicable; weight co 5: va 1, conserved regions weighted 5, variable regions 1, etc. (see Material and Methods); excl. V4, V6, excluding variable regions V4 and V6. Optimal parameter combinations marked with asterisks (see text). Key nodes listed as recovered are those present in the strict consensus of all trees obtained with the parameter on top of the column kept constant (e.g., the first column includes the nodes recovered in the strict consensus of all trees obtained with gap costs 1, 2, or 5 when conserved and variable regions were weighted equal; co 1: va 1).

^a *Torrudincola* was included in Adephaga + Polyphaga.

only four nodes in the marginal consensus trees contradicted nodes present in other marginal consensus trees. The parameter values that resulted in the maximum number of key nodes in the marginal consensus trees were gap cost = 1 and V4 and V6 regions excluded for Analysis 1, and gap cost = 1 and relative weight of conserved and variable regions of 5:1 for Analyses 2 and 3 (Table 6).

The parameter values considered optimal were the only ones for which the marginal consensus trees recovered some widely accepted groups (e.g., Beutel,

1997), including a monophyletic Haliplidae (Analyses 1, 2, and 3), Noteridae (Analysis 1 only), Adephaga and Geadephaga (Analysis 2), and Dytiscoidea (= Dytiscidae + Hygrobiidae + Amphizoidea; Analyses 2 and 3) (Table 5). In the only marginal consensus tree that recovered Adephaga as monophyletic (Analysis 2, gap cost 1) Hydradephaga was also monophyletic, and Trachypachidae was included within Geadephaga (Table 5). A few key nodes were recovered in all of the marginal consensus trees, including the *Graptodytes* and *Hygrotus* groups of genera (key nodes 26 and 27; Table

TABLE 6

Presence and Absence of Key Nodes under the Preferred Parameter Combinations

Key node	No.	Analysis 1	Analysis 2		Analysis 3	
		POY	POY	PAUP	POY	PAUP
Adephaga + Polyphaga	1	+	+	108	n.a.	n.a.
Polyphaga	2	+	+	6	n.a.	n.a.
Adephaga	3	×	+	107	n.a.	n.a.
Geadephaga + Trachypachidae	4	×	+	18	n.a.	n.a.
Hydradephaga	5	+	+	106	n.a.	n.a.
Hydradephaga excluded Gyrinidae	6	×	+	105	+	83
Gyrinidae	7	+	+	24	+	83
Gyrininae	8	+	+	23	+	84
Noteridae	9	+	+	104	+	9
Noteridae excluded <i>Notomicrus</i>	10	+	+	103	+	8
Haliplidae	11	+	+	27	+	4
Dytiscidae + Hygrobiidae + Amphizoidae	12	+	+	98	+	81
Dytiscidae + Hygrobiidae	13	+	+	96	+	79
Dytis. + Hygrobiidae excl. Hydroporinae	14	×	×	×	+	49
Cybistrini	15	+	+	94	+	15
Colymbetini	16	×	+	40	+ ^a	21 ^a
Aciliini + Eretini	17	+	+	52	+	32
Aciliini	18	×	+	50	+	30
Dytiscinae + <i>Notaticus</i> (excl. Cybistrini)	19	+	+	53	+	33
<i>Hydaticus</i> (<i>Guignotites</i>) + <i>Notaticus</i>	20	+	+	46	+	26
Hydaticini (inc. <i>Notaticus</i>)	21	×	×	×	×	X
Hydaticini (inc. <i>Notaticus</i>) + <i>Hyderodes</i>	22	×	+	48	×	28
Laccophilinae	23	+	+	59	+	37
Bidessini	24	+	+	88	+	55
Methlini + Bidessini	25	+	+	89	+	56
<i>Graptodytes</i> gr	26	+	+	79	+	69
<i>Hygrotus</i> gr	27	+	+	69	+	73
<i>Hydroporus</i> gr	28	+	+	74	+	60
Total supported		20	26	26	21	22
Total contradicted		8	2	2	2	1

Note. +, Node supported; −, node unresolved but compatible with tree; ×, node contradicted; n.a., not applicable. See Material and Methods for weighting and alignment parameters used in Analyses 1, 2, and 3. Preferred alignment parameters were: Analysis 1, gap cost = 1 and excluding variable regions V4 and V6; Analyses 2 and 3, gap cost = 1, weight conserved to variable regions = 5:1. POY, best trees obtained with POY; PAUP, most parsimonious trees obtained by search on the implied alignment output of POY. Numbers in the PAUP columns refer to nodes in Figs. 2 and 3, respectively.

^a Including *Platynectes*.

5). The tribe Bidessini (key node 24) was always recovered except once. Other key nodes recovered in more than 50% of the marginal consensus trees grouped *Notaticus* (subfamily Aubehydrinae) plus *Hydaticus* (*Guignotites*) (key node 20), all species of the subfamily Laccophilinae (key node 23), and the tribes Aciliini plus Eretini (key node 17) (Table 5).

Trees Obtained under Preferred Parameter Combinations

Detailed phylogenetic analyses were performed on trees resulting from the best parameter combinations of Analyses 2 and 3. None of the parameter combinations applied in Analysis 1 performed equally well (Tables 5 and 6) and therefore the resulting tree topologies were not explored further. In Analysis 2, POY returned a single tree of cost 12,625 and CI = 0.49 under the

preferred alignment parameters of gap cost = 1 and weighting of conserved to variable regions 5:1. The PAUP search on the implied alignment resulted in 40 trees of cost 12,622 and CI = 0.49, with a topology almost identical to that of the original POY tree (Fig. 2). Adephaga + Polyphaga, Adephaga, and Geadephaga + Trachypachidae are each recovered as monophyletic. Trachypachidae is included within portions of Geadephaga but is sister to Geadephaga under gap cost = 2 and excluding the variable regions V4 and V6. Hydradephaga is thus monophyletic, with Gyrinidae basal. Amphizoidae is sister to Dytiscidae + Hygrobiidae, with Hygrobiidae in a derived position within the Dytiscidae. The large subfamily Hydroporinae is paraphyletic and placed at the base of Dytiscidae. Within Hydroporinae, Methlini is placed sister to Bidessini, and Hydroporini is paraphyletic. Bootstrap

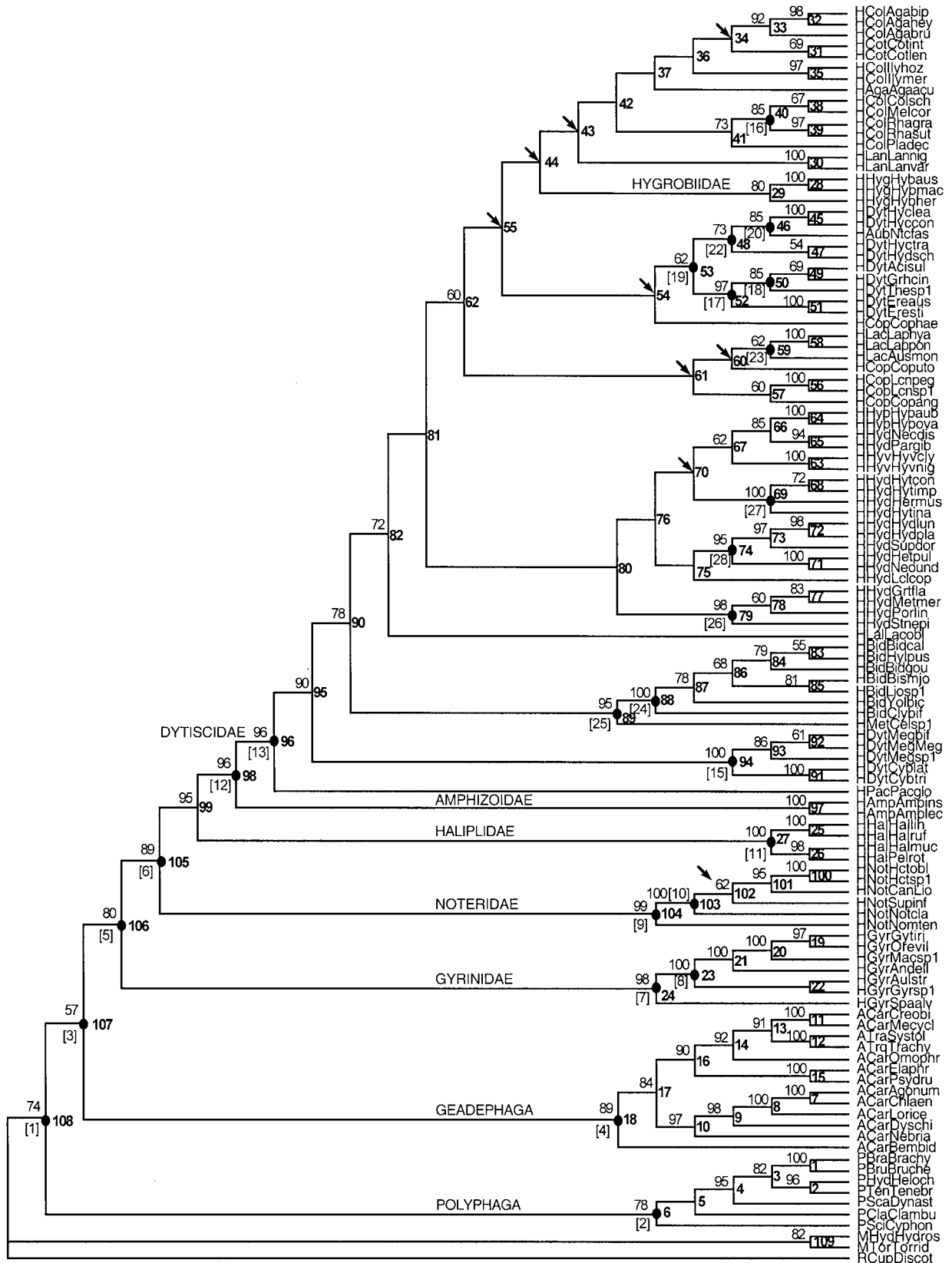


FIG. 2. One of 40 shortest trees obtained with PAUP, based on the implied alignment obtained in POY (see text) for Analysis 2 (all species included, variable regions V2, V4, and V6 included for Adephaga only, gap cost = 1, weight of conserved vs variable regions = 5:1). Bootstrap values (>50%) on top left of nodes; node number on right of the node (in boldface); arrows, unresolved nodes in the strict consensus tree; black dots, key nodes; number of key nodes in brackets refer to key nodes as listed in Tables 5 and 6. See Table 7 for the Bremer support values of the nodes, and Table 2 for species codes.

values were higher than 50% for most of the nodes, except for the relationships among the main groups within Hydroporinae and among the basal groups of the non-Hydroporinae clade (Fig. 2).

Analysis 3 (only Hydradephaga included) produced a single best tree of cost 7119 and CI = 0.56. The PAUP search based on the implied alignment resulted in two trees of cost 7116 and CI = 0.57 (Fig. 3). Relationships of family-level taxa were identical to those in Analysis 2 except for the change of position of Noteridae, which was sister to Amphizoidae + Hygrobiidae + Dytiscidae. Again, Hygrobiidae was placed within Dytiscinae. Hydroporinae was monophyletic except for two species of *Hyphdrus* (based on incomplete sequences) which were placed basal to Dytiscidae. As is apparent from the phylogram (Fig. 4) the parsimony branch lengths of Dytiscidae and Amphizoidae are remarkably homogeneous, and in general much shorter than those in Gyrinidae, Haliplidae, and Noteridae. Among Dytiscidae, only the terminal branches leading to Hygrobiidae, the Australian Hydroporini (*Necterosoma* and *Paroster*), and *Pachydrus* were apparently longer.

We also tested the relative contribution of variable and conserved regions to the phylogenetic signal and potential conflict between different parts of the molecule. The Partition Homogeneity Test indicated significant incongruence ($P < 0.01$ in Analysis 2; $P < 0.02$ in Analysis 3) in the character distribution among gene regions. PBS values for each region reflect this fact, with an abundance of negative values indicating conflict with the topology of the simultaneous analysis (Table 7; presented for Analysis 2 only). Some nodes with high negative values for the conserved regions linked species known to be closely related, e.g., the two species of *Coptotomus* (node 31 in Analysis 2) or the two species of *Lacconectus* (node 56, Table 7). In both analyses some key nodes defining well-established monophyletic groups were supported by a combination of conserved and variable regions, whereas others had low or negative values for some conserved regions (e.g., in Analysis 2, subfamily Laccophilinae, key node 23; tribe Acilini, key node 18; tribe Colymbetini, key node 16; Noteridae, key node 9). At deeper levels nodes were mostly supported by the conserved regions (Table 7), although some nodes such as Hydradephaga excluding Gyrinidae (key node 6) had a relatively high support from variable regions also.

The mixed contribution of the conserved and variable regions to the simultaneous analysis tree was more clearly reflected by the correlation among the PBS values (Table 8). Although the overall Bremer Support was significantly and positively correlated with each of the regions, many of the pairwise correlations between regions were not significant. There were no significant differences in the number of negative PBS values between conserved and variable regions in any of the two analyses (ANOVA, $P > 0.05$), but the

number of positive values in the variable regions was significantly higher than that in the conserved regions in Analysis 3 (average of 65 and 36, respectively; ANOVA, $P < 0.01$), suggesting a larger influence of the variable regions in the final topology of the tree.

To further explore the different roles of the conserved vs variable regions, PAUP searches were performed on the preferred alignment excluding one or the other. When only the conserved regions were included a loss of resolution at the intermediate node levels was observed. Most nodes with bootstrap values of less than 50% in the combined analysis (Figs 2 and 3) were unresolved, and several nodes with negative PBS values for the conserved regions were not maintained (e.g., subfamily Laccophilinae). When only the variable regions were included, the tree was remarkably similar to the one obtained with the whole dataset, indicating the strong contribution of the variable regions to the phylogenetic signal. In comparison with the simultaneous analysis, the main differences were that Gyrinidae was paraphyletic at the base of Hydradephaga and that Colymbetini was placed as sister to Dytiscinae.

DISCUSSION

DNA Sequence Alignment

The phylogenetic conclusions from this data set are highly sensitive to alignment parameters. Our exploration of the parameter space focused on two aspects, the relative costs of nucleotide changes to gaps and the weight of the highly variable regions relative to the conserved regions. The latter is an important issue, as the regions of the 18S rRNA constitute essentially two classes of characters with very different dynamics of variation. In an unweighted analysis the variable regions receive high weight because of their greater length differences, in particular when the cost of gaps is high. This may easily overwhelm the signal in the conserved regions, which exhibit little or no length variation, although they may provide data of generally higher quality, given their unambiguous homologies and potentially higher consistency. Downweighting the variable regions would ameliorate this effect, while still applying high costs for gaps relative to nucleotide changes.

To select among alignment parameters, accepted procedures based on topological congruence (Wheeler, 1995) or character congruence (Vogler and Pearson, 1996; Giribet and Wheeler, 1999) are not easily applied in Hydradephaga because there are no external data sets or strongly founded prior hypotheses for most groups. Without the possibility of congruence testing, we made use of the information contained in the 18S data themselves, by establishing "key nodes" whose recovery was not sensitive to changes in alignments over parts of the parameter space. The assumption is

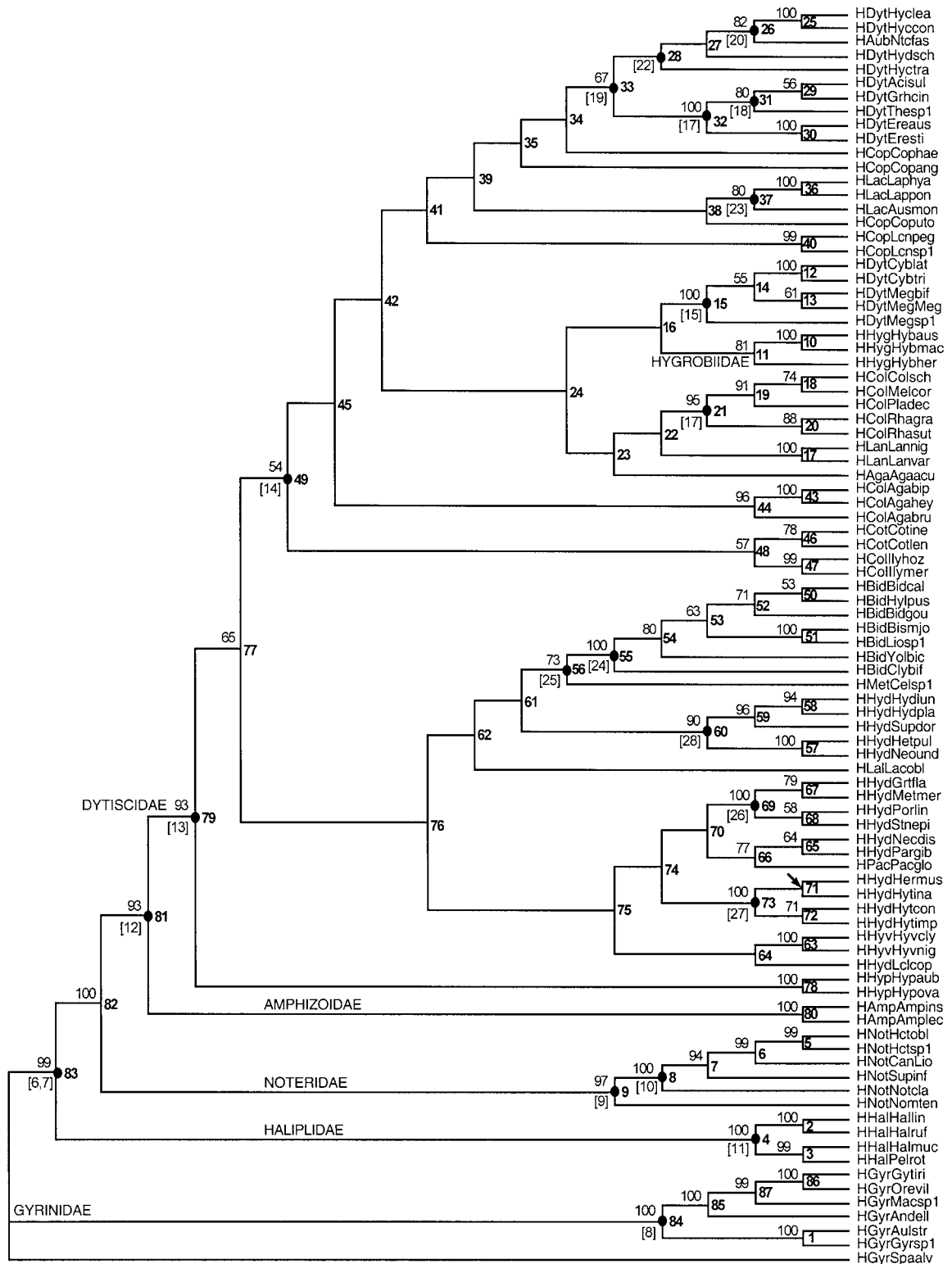


FIG. 3. One of the two trees obtained with a search in PAUP using the implied alignment given by POY for Analysis 3 (only Hydradephaga included, gap cost = 1, weight of conserved vs variable regions = 5:1). Bootstrap values (>50%) on top left of nodes; node number on right of the node (in boldface); arrows, unresolved node in the strict consensus tree; black dots, key nodes; number of key nodes in brackets refer to Tables 5 and 6. See Table 2 for the codes of the species.

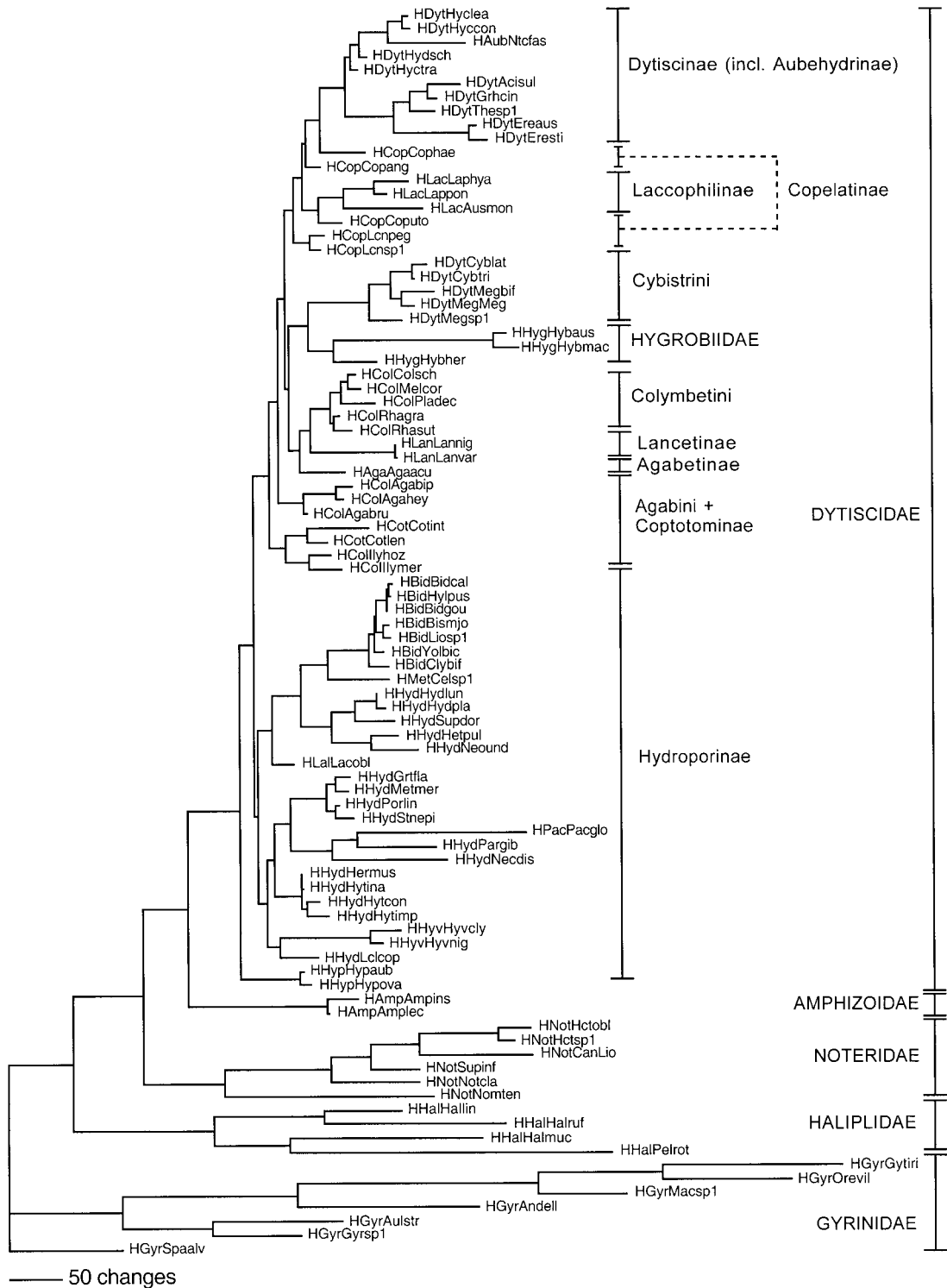


FIG. 4. Phylogram of the tree shown in Fig. 3. Families and subfamilies are indicated by bars. See Table 2 for species codes.

that the phylogenetic signal manifests itself by consistent recovery of certain nodes under a wide range of alignment parameters. Tree alignments considered optimal are those obtained with the parameter combinations that recover the maximum number of key nodes,

as they presumably capture the greatest amount of phylogenetic signal contained in the data.

Our method therefore assumes that the stability to variation of alignment parameters is due to the underlying phylogenetic signal (and that random sequences

TABLE 7
Partitioned Bremer Support for the Preferred Tree of Analysis 2

Node	Region								Total		
	C1	V2	C3	V4	C5	V6	C7	all	+	-	0
Cost	1205	407	2064	1804	3025	1428	2689	12622	7	0	0
1	5	0	5	0.1	15.6	0.5	28.9	55.1	6	0	1
2	15	-0.5	-4.4	-0.3	5	1.2	18.9	34.9	4	3	0
3	0	-0.7	0.6	-0.6	4.3	0.9	10.4	14.9	4	2	1
4	0	0.5	12.3	1.3	5.8	-0.9	3.6	9.9	5	2	0
5	-1.7	0.5	2.3	0.3	5.8	-0.9	3.6	9.9	5	2	0
6 (2)	10	0.2	5.6	2.3	0	0.8	1.1	20	6	0	1
7	15	0.2	15.6	0.3	0	0.8	26.1	58	6	0	1
8	10	5.2	20.6	10.1	18.8	7.8	23.6	96.1	7	0	0
9	0	4	5.6	4.3	14	9	-5.9	31	5	1	1
10	0	-0.4	-0.4	6.7	1	10	-1.9	15	3	3	1
11	15	5.8	10.6	11.2	8.1	8.8	5.5	65	7	0	0
12	0	0.4	15.6	11.1	-1.2	6.8	27.3	60	5	1	1
13	-5	2.4	15	4.9	0.6	2.4	-2.2	18.1	5	2	0
14	0	1.7	-9.4	47.7	-5.6	20.4	4.2	59	4	2	1
15	0	0.7	0.1	14.4	6	2.7	-0.9	23	5	1	1
16	0	3.6	0.6	5.9	-0.6	5.1	3.3	17.9	5	1	1
17	0	0	19.6	-0.9	0	1.2	5.1	25	3	1	3
18 (4)	0	-0.7	25.6	-0.8	-5.5	1.3	-4.9	15	2	4	1
19	0	1.5	-0.1	0.2	5.7	14.1	14.7	36.1	5	1	1
20	-25	-0.1	10.2	28.5	45	-5.5	-6	47.1	3	4	0
21	0	6.2	14.9	1.7	1.4	10.2	12.5	46.9	6	0	1
22	15	2.1	20.6	-1.4	9.3	6.2	3.2	55	6	1	0
23 (8)	10	0.2	10.6	13.3	32.5	8.3	11.1	86	7	0	0
24 (7)	0	3.6	14.2	-0.1	2.1	7.5	-0.3	27	4	2	1
25	5	3.1	5.6	19	29.5	10.2	18.6	91	7	0	0
26	0	1	-0.2	-0.9	10	7.8	15.3	33	4	2	1
27 (11)	0	-0.3	13.1	1.8	20	2.8	13.6	51	5	1	1
28	30	3.8	35.6	2.7	50	1.8	11.1	135	7	0	0
29	0	0.8	5.6	9.6	5	3.8	-12	13	5	1	1
30	25	3.2	0.6	12.3	25	1.8	1.1	69	7	0	0
31	-5	0	-5.2	1.3	-3.3	14.3	1.9	4	3	3	1
32	15	-0.2	5.6	-0.1	-0.6	1.9	2.3	23.9	4	3	0
33	0	0.2	0.6	1.8	0	3.3	6.1	12	5	0	2
34	0	0.7	0.6	-0.2	0	1.6	-2.7	0	3	2	2
35	0	1.2	4.9	0.3	10.7	1.2	4.7	23	6	0	1
36	0	-1.8	0.6	5.3	0	-1.2	1.1	4	3	2	2
37	5	-0.8	-1.1	-0.4	1.7	-1.5	1.1	4	3	4	0
38	0	-0.5	0.2	-1.2	0	0.6	4.9	4	3	2	2
39	0	4.2	0.6	6.3	0	1.8	-3.9	9	4	1	2
40 (16)	0	0.5	0.6	1.5	0	3.3	-3.9	2	4	1	2
41	0	-0.8	0.6	3.3	0	0.8	6.1	10	4	1	2
42	0	-0.4	0.6	1.1	0	1.6	0.1	3	4	1	2
43	0	-1.3	0.6	-0.2	-5	-0.2	6.1	0	2	4	1
44	0	-0.2	-0.4	-0.3	0	-0.2	1.1	0	1	4	2
45	0	0.7	-0.2	7.5	1.7	2.3	-3.9	8.1	4	2	1
46 (20)	-5	2.8	-0.4	5.9	12	2.6	-3.9	14	4	3	0
47	0	1	-0.4	2.3	2	0	-3.9	1	3	2	2
48 (22)	0	2.6	-0.1	1.4	1.4	0.5	-3.9	1.9	4	2	1
49	0	0.2	0.6	-0.5	0	3.3	2.3	5.9	4	1	2
50 (18)	0	4.8	0.6	1.3	0	1.2	-3.9	4	4	1	2
51	0	2.1	10.6	18.7	25	7.6	6.1	70.1	6	0	1
52 (17)	0	3.3	0.6	10.6	-1.4	3.8	1.1	18	5	1	1
53 (19)	-3	4.4	1.6	1.3	-0.5	3.1	2.1	9	5	2	0
54	0	-1.2	0.6	-1.7	-0.7	1.2	1.8	0	3	3	1
55	0	-1.2	0.6	-1.5	-1	1	2.1	0	3	3	1
56	5	2.5	-4.4	9.3	-1.7	4.1	-3.9	10.9	4	3	0
57	2.5	1.4	-1.9	4.9	-0.6	1.6	-3.9	4	4	3	0
58	-5	8.9	0.6	7.3	10	7.1	-3.9	25	5	2	0
59 (23)	-5	6.2	-4.4	3.3	15	-1.2	-3.9	10	3	4	0

TABLE 7—Continued

Cost	Region								Total		
	C1 1205	V2 407	C3 2064	V4 1804	C5 3025	V6 1428	C7 2689	all 12622	+7	- 0	0 0
60	0	-1.6	0.6	-1.1	-1	1	2.1	0	3	3	1
61	0	-0.8	0.6	-0.7	-5	-0.2	6.1	0	2	4	1
62	8.3	0.2	-0.2	2.8	-5	3	1.9	11	5	2	0
63	5	3.5	5.6	3.8	15	11.1	31.9	75.9	7	0	0
64	5	-0.2	15.6	14.1	8.8	0.9	-0.1	44.1	5	2	0
65	10	3.5	-0.2	6.8	0	2.6	0.3	23	5	1	1
66	0	3.6	-1.6	4.2	3.9	-0.9	2.8	12	4	2	1
67	2.8	1.8	-2.2	9.7	-9.4	2.9	-0.6	5	4	3	0
68	0	-0.2	0.6	0.3	-1	1.2	4.1	5	4	2	1
69 (27)	5	0.4	0.6	0.3	4	2.6	9.1	22	7	0	0
70	0	-0.3	0	0.3	0	0.2	-0.1	0.1	2	2	3
71	15	0.1	-4.4	0.7	7.9	5.7	1.1	26.1	6	1	0
72	5	0.4	5.6	1.8	-1.7	5.8	1.1	18	6	1	0
73	5	1.2	5.6	1.1	-1.2	3.8	3.6	19.1	6	1	0
74 (28)	5	0.5	-4.4	2.9	-0.6	7.1	9.4	19.9	5	2	0
75	5	1.3	-4.9	1	-3.2	3.4	-0.7	1.9	4	3	0
76	5	1.2	-6.1	-0.4	3.3	1.1	-2.2	1.9	4	3	0
77	-5	2.8	-1.3	-2.5	0	2	9.9	5.9	3	3	1
78	0	0	-0.5	-1.1	-1.7	1.4	5	3.1	2	3	2
79 (26)	-3.3	3.9	2.3	3.2	3.8	5.2	4	19.1	6	1	0
80	1.5	-0.1	-0.6	1.5	-4.6	4.8	-1.6	0.9	3	4	0
81	0	0.3	0.6	2.8	-5.5	5.3	-2.5	1	4	2	1
82	0	0.6	-1.4	5.3	0	5.4	-1.9	8	3	2	2
83	0	1.5	-0.1	0.6	0.7	0.1	-1.8	1	4	2	1
84	-5	1.2	0.6	3.3	5	7.8	-3.9	9	5	2	0
85	0	0.8	4.9	-0.1	0.7	0.1	-0.3	6.1	4	2	1
86	0	-1	0.6	-0.1	-1	2.9	0.6	2	3	3	1
87	0	2.2	0.6	3.3	-5	7.8	-3.9	5	4	2	1
88 (24)	11.1	0.1	8.7	0.9	13.9	6.6	2.8	44.1	7	0	0
89 (25)	5.7	1.3	4.9	1.5	-2.1	3.6	-2.8	12.1	5	2	0
90	5	0.2	-4.9	2	5	0.6	4.1	12	6	1	0
91	5	1.9	0.6	3.3	5	1.5	-2.2	15.1	6	1	0
92	0	-0.3	-4.4	5.9	-0.6	1.2	-0.8	1	2	4	1
93	0	0.4	0.6	1.9	5	0	-2.9	5	4	1	2
94 (15)	10	-0.1	0.6	7.4	-1	18	4.1	39	5	2	0
95	0	1.1	-0.5	9.6	11.7	3.8	-1.7	24	4	2	1
96 (13)	0	1.5	2.3	1.3	11.7	6.5	7.8	31.1	6	0	1
97	25	7.1	10.6	0.4	3.6	0.7	24.7	72.1	7	0	0
98 (13)	-5	-0.1	5.6	1	15	1.8	12.8	31.1	5	2	0
99	-5	0.2	5.6	2.3	15	1.8	11.1	31	6	1	0
100	0	2.9	0.6	13	4.4	1.2	-1.1	21	5	1	1
101	0	0.8	-4.4	1.8	15	13.2	-11	15	4	2	1
102	0	1	-4.4	0.5	9	-5.2	-0.9	0	3	3	1
103 (10)	5	-0.5	15.6	15.8	0	28.1	6.9	70.9	5	1	1
104 (9)	0	1.9	10.6	9.6	14.4	7.1	-6.7	36.9	5	1	1
105 (6)	0	0.2	3.6	2.9	3	4.2	6.1	20	6	0	1
106 (5)	0	0.2	5.6	0.8	-6.2	1	3.6	5	5	1	1
107 (3)	0	-0.8	0.6	-0.4	3.3	1.1	1.1	4.9	4	2	1
108 (1)	5	-0.4	0	-0.3	0.6	0.3	4.9	10.1	4	2	1
109	-11	0.2	0.6	0.3	15.8	1.1	17.8	25	6	1	0
Average	2.2	1.2	3.3	4.0	4.9	3.7	3.5	22.9			
std. dev.	6.8	2.0	7.1	6.7	9.7	4.8	8.1	25.1			
+	36	76	74	84	59	97	68	101			
-	14	29	33	25	31	10	41	0			
0	59	4	2	0	19	2	0	8			

Note. Analysis 2 includes all taxa but variable regions V4 and V6 of Adephaga only. The preferred alignment parameters in Analysis 2 were gap cost = 1 and weight of conserved variable regions = 5:1 (see text). Tree as shown in Fig. 2. Numbers refer to the relative Partitioned Bremer Support (i.e., the difference with the value obtained with no constraint). Node numbers in first column refer to Fig. 2. Numbers in parentheses are the key nodes used in the sensitivity analysis (see Tables 5 and 6).

TABLE 8

Spearman's Nonparametric Rank Correlation of PBS Values for Conserved and Variable Regions of 18S rRNA

	C1	V2	C3	V4	C5	V6	C7
Analysis 2							
C1	1						
V2	n.s.	1					
C3	n.s.	n.s.	1				
V4	n.s.	0.42	n.s.	1			
C5	n.s.	0.28	n.s.	n.s.	1		
V6	n.s.	0.35	n.s.	0.40	n.s.	1	
C7	0.30	n.s.	0.29	n.s.	n.s.	n.s.	1
all	0.38	0.33	0.56	0.41	0.57	0.48	0.46
Analysis 3							
C1	1						
V2	0.41	1					
C3	0.29	n.s.	1				
V4	n.s.	n.s.	0.37	1			
C5	0.24	0.50	n.s.	n.s.	1		
V6	n.s.	n.s.	0.52	0.49	n.s.	1	
C7	0.51	0.48	0.29	n.s.	n.s.	n.s.	1
all	0.34	0.44	0.66	0.45	0.55	0.50	0.43

Note. Rank correlations were calculated in pairwise analysis of PBS values obtained for each of the conserved (C1, C3, C5, C7) and variable (V2, V4, V6) regions. See Material and Methods for description of data used in Analyses 2 and 3. Alignment parameters in both analyses are gap cost = 1, weight conserved to variable regions = 5:1. n.s., not significant ($P < 0.05$).

would not produce the same trees under different alignment conditions). This assumption is supported by the recovery of uncontroversial nodes with this method, such as the monophyly of the smaller families Gyrinidae, Haliplidae, and Noteridae, demonstrating that the key node analysis produces phylogenetically meaningful results. This confirms that the stability of nodes to variation in alignment parameters indeed reflects phylogenetic signal and supports the assumption that the preferred alignment parameters will also correctly infer those (less widely supported) nodes that had not been recovered as key nodes in the marginal consensus trees.

The use of "marginal consensus trees" for the recovery of key nodes proved a useful tool. Very few nodes were universally found under all parameters tested, and in fact the consensus of topologies from all alignments in the current analysis (the consensus of all trees obtained under all parameter values) was almost entirely unresolved except for some "trivial" nodes which grouped very closely related sequences. However, only a small proportion of key nodes obtained from one marginal consensus tree is incompatible with those in another, and hence different alignment parameters appear to reveal different components of a universal topology, rather than to produce conflicting trees.

The determination of key nodes depends critically on the initial selection of alignment parameters. As the parameter space is vast and only a small proportion can be surveyed, the identification of key nodes may be simply a result of subjective selection of alignment parameters. However, while there is a strong possibility that particular nodes appear as key nodes (i.e., are recovered under a set of alignment parameters) due to the limited search of the parameter space, our procedure for identifying key nodes is conservative. By using strict consensus trees to assess the similarities of topologies, any differences between trees with regard to the position of a single taxon may result in the collapse of many nodes in the marginal consensus trees. The conservative character of the sensitivity analysis is reflected in the low number of "nontrivial" key nodes recovered (a total of 28, of 88 maximum nodes on the tree, of which 16 were considered "trivial"). Interestingly, these nodes encompassed the full range of hierarchical levels, from suborder to genus.

These alignments also revealed the complexity of the contribution of conserved and variable regions to the final tree. The Partition Homogeneity Test revealed significant incongruence in pairwise tests of the seven 18S rRNA gene regions, although the analysis of PBS values for the conserved regions shows that in general they were positively correlated, in particular in Analysis 3 (Table 8). Also, the PBS values for each of the seven gene regions were positively correlated with the global Bremer Support value (Table 8). This suggests that all regions contribute to the phylogenetic signal, but the lack of correlation in some of the pairwise comparisons may be due to the limited number of character changes in each region which are insufficient to recover fully an otherwise consistent signal.

It is interesting to note that different rates of evolution in conserved and variable regions do not correspond to different hierarchical levels of their respective phylogenetic signals, as the highly variable regions provide phylogenetic information even at deep levels, and the conserved regions contribute to the resolution at the shallow levels. Measures of divergence are thus not a good predictor of the information content.

Phylogenetic Conclusions

Our results support the monophyly of the aquatic families of Adephaga (Hydradephaga), in agreement with Shull *et al.* (2001) and with the traditional view of morphological studies (e.g., Crowson, 1960) but contrary to recent work proposing the polyphyly of aquatic lineages and the repeated colonization of aquatic habitat (Beutel, 1997 and references therein). In no case did our alignments produce trees consistent with this scenario. Alternative topologies that we obtained under nonoptimal parameter combinations placed Polyphaga amid different groups of Adephaga (sometimes derived within Dytiscidae), a most unlikely sit-

uation. But wherever the well-established Adephaga was recovered as monophyletic, Hydradephaga was also monophyletic.

Within Hydradephaga, in all analyses we recovered (a) Gyrinidae, Haliplidae, Noteridae, Amphizoidae, and Dytiscidae + Hygrobiidae as monophyletic, (b) Gyrinidae as the most basal node of Hydradephaga, and (c) a monophyletic Amphizoidae + Hygrobiidae + Dytiscidae. In Analysis 3 the Noteridae were also recovered within this monophyletic group, which has been widely accepted as Dytiscoidea (Lawrence and Newton, 1982; Beutel and Roughley, 1988; Belkaceme, 1991; Beutel and Haas, 1996; Beutel, 1997, 1998; Miller, 2001; Ruhnau, 1986; Burmeister, 1990b) and which is strongly supported by morphological characters. In Analysis 2, however, Noteridae and Haliplidae switch positions, and Dytiscoidea was not recovered (Figs. 2 and 3). The intrafamilial relationships of the smaller families Gyrinidae and Noteridae are better known (see Introduction), and our results are in almost perfect agreement with previous morphological analyses (Belkaceme, 1991; Beutel and Roughley, 1994), except that *Macrogyrus* is placed within Orectochilini and not sister to *Andogyrus* (Enhydrini). However, our incomplete sequence of *Macrogyrus* and the general difficulty of sequencing some parts of the 18S rRNA gene in gyrinids may limit the support for this conclusion.

Beyond the family-level relationships in Hydradephaga our analysis focused on the relationships within the largest family, Dytiscidae. Within this family, a major division can be established between the large subfamily Hydroporinae and all other subfamilies. These two groups are recovered as sister in Analysis 3 (with the exception of the incompletely sequenced *Hyphydrus*). In Analysis 2 the non-Hydroporinae clade also appears as monophyletic (with the exception of the divergent Cybistrini), but the Hydroporinae appear as paraphyletic and at the base of Dytiscidae. This basic split of the family is interesting in the light of species numbers, as the number of species of Hydroporinae is roughly similar to that of the remaining dytiscid subfamilies combined.

Within the Hydroporinae lineage, two main clades are found in Analysis 2. The most basal clades of both groups belong to tribes Hyphydrini, Hydrovatini, and Laccornini, the latter two also considered basal within Hydroporinae by Burmeister (1976), Wolfe (1985, 1988), Alarie and Harper (1988), Wolfe and Roughley (1990), and Alarie (1991). However, the largest tribe, Hydroporini, is not found to be monophyletic in any of the trees, but groups into four main clades: the *Hydroporus* group, the *Graptodytes* group (*sensu* Seidlitz, 1887), the *Hygrotus* group, and the two Australian genera (*Paroster* and *Necterosoma*). The austral-American *Laccornellus* was most frequently placed at the

base of the *Hydroporus* group. The first two groups are recovered as sisters in Analysis 3, in agreement with Alarie and Nilsson (1997), Alarie and Delgado (1999), and Alarie *et al.* (1999). Methlini is consistently placed sister to the tribe Bidessini in a derived position in all trees, and not related to Hydrovatini, as suggested by Wolfe (1985, 1988).

Within the non-Hydroporinae clade, several unexpected findings were obtained. In contrast to morphological evidence (Burmeister, 1976; Ruhnau and Brancucci, 1984; Ruhnau, 1986; Beutel, 1994, 1998), which considered the Copelatinae to be basal within Dytiscidae, they are placed in a more derived position, near Laccophilinae and Dytiscinae. Similarly, there is no evidence for the relationship between Agabetinae and Laccophilinae, as suggested by Burmeister (1976, 1990a), Nilsson (1989), and Miller (2001). The position of Laccophilinae, however, remains uncertain based on 18S rRNA, but it is generally placed as derived within the non-Hydroporinae clade and never sister to Hydroporinae, as suggested by Wolfe (1985) and Ruhnau and Brancucci (1984). We also found subfamily Colymbetinae paraphyletic with respect to Lancetinae and Copotominae, with tribe Colymbetini more closely related to Dytiscinae than to Agabini, in agreement with Miller (2001). The morphological evidence for the monophyly of Colymbetinae is weak, with a single synapomorphy in an analysis of the larval setation (Alarie, 1995, 1998), but their possible paraphyly needs corroboration, as Colymbetinae are recovered as monophyletic in our Analysis 2.

In all of our analyses the subfamily Dytiscinae includes the genus *Notaticus*, usually considered a separate subfamily (Aubehydrinae) but placed within Dytiscinae by Miller (2000), who erected a monotypic tribe for it. According to our data it should be clearly placed within the tribe Hydatcini, as originally suggested by Zimmermann (1928). Dytiscinae are recovered as monophyletic, with the exception of the tribe Cybistrini, which placement is highly unstable and most unlikely from a morphological point of view, which seems to indicate some data artifact.

In summary, our analyses show that some of the relationships among genera and tribes of Dytiscidae are well resolved with strong support, such as those within Dytiscinae (excluding Cybistrini) and several groups within Hydroporinae (*Hydroporus*, *Graptodytes*, and *Hygrotus* groups; tribe Bidessini). However, the basal relationships of Dytiscidae remain contentious, in particular the likely nonmonophyly of Colymbetinae and the less likely para- or polyphyly of Hydroporinae and Copelatinae.

CONCLUSIONS

Our study is the first to establish basal relationships in Hydradephaga from molecular characters. First, the

data lend support to the monophyly of Hydradephaga (excluding Trachypachidae), consistent with a single origin of the aquatic life style. Second, family relationships were resolved, with evidence for a basal Gyrinidae and a monophyletic Dytiscoidea. Third, within the large family Dytiscidae a preliminary arrangement of some subfamily relationships was established, but additional markers will be required for more strongly supported topologies.

The 18S rRNA gene proved a useful marker despite great rate heterogeneity between clades and between conserved and variable regions. While uninformative or misleading at higher hierarchical levels, within Hydradephaga the variable regions contributed significantly to establish deep and shallow relationships. Establishing marginal consensus trees (the consensus of trees produced under a range of alignment parameters) is a useful method for selecting preferred alignment parameters when no external data sets are available to assess the quality of alignments.

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