Monophyly of terrestrial adephagan beetles as indicated by three nuclear genes (Coleoptera: Carabidae and Trachypachidae)

David R. Maddison, Wendy Moore, Michael D. Baker, Tana M. Ellis, Karen A. Ober, Jamie J. Cannone & Robin R. Gutell

Submitted: 28 March 2008
Accepted: 9 July 2008
doi:10.1111/j.1463-6409.2008.00359.x

Maddison, D. R., Moore, W., Baker, M. D., Ellis, T. M., Ober, K. A., Cannone, J. J. & Gutell, R. R. (2009). Monophyly of terrestrial adephagan beetles as indicated by three nuclear genes (Coleoptera: Carabidae and Trachypachidae). — *Zoologica Scripta*, *38*, 43–62.

The beetle suborder Adephaga is traditionally divided into two sections on the basis of habitat, terrestrial Geadephaga and aquatic Hydradephaga. Monophyly of both groups is uncertain, and the relationship of the two groups has implications for inferring habitat transitions within Adephaga. Here we examine phylogenetic relationships of these groups using evidence provided by DNA sequences from all four suborders of beetles, including 60 species of Adephaga, 4 Archostemata, 3 Myxophaga, and 10 Polyphaga. We studied 18S ribosomal DNA and 28S ribosomal DNA, aligned with consideration of secondary structure, as well as the nuclear protein-coding gene *wingless*. Independent and combined Bayesian, likelihood, and parsimony analyses of all three genes supported placement of Trachypachidae in a monophyletic Geadephaga, although for analyses of 28S rDNA and some parsimony analyses only if Coleoptera is constrained to be monophyletic. Most analyses showed limited support for the monophyly of Hydradephaga. Outside of Adephaga, there is support from the ribosomal genes for a sister group relationship between Adephaga and Polyphaga. Within the small number of sampled Polyphaga, analyses of 18S rDNA, *wingless*, and the combined matrix supports monophyly of Polyphaga exclusive of Scirtoidea. Unconstrained analyses of the evolution of habitat suggest that Adephaga was ancestrally aquatic with one transition to terrestrial. However, in analyses constrained to disallow changes from aquatic to terrestrial habitat, the phylogenies imply two origins of aquatic habit within Adephaga.

Corresponding author: *David R. Maddison, Department of Entomology, University of Arizona, Tucson, AZ, 85721. E-mail: beetle@ag.arizona.edu*
Wendy Moore, Department of Entomology, University of Arizona, Tucson, AZ, 85721. Present address: Department of Entomology, California Academy of Sciences, Golden Gate Park, 55 Music Concourse Drive, San Francisco, CA 94118. E-mail: wmoore@calacademy.org
Michael D. Baker, Department of Entomology, University of Arizona, Tucson, AZ, 85721. Present address: DNA Sequencing and Synthesis Facility, Office of Biotechnology, Iowa State University, Ames, IA 50011. E-mail: mdbaker@iastate.edu
Tana M. Ellis, Department of Entomology, University of Arizona, Tucson, AZ, 85721. Present address: Department of Forest Science, Oregon State University, 321 Richardson Hall, Corvallis, OR 97331. E-mail: Tana.Ellis@oregonstate.edu
Karen A. Ober, Department of Entomology, University of Arizona, Tucson, AZ, 85721. Present address: Department of Biology, College of the Holy Cross, Worcester, MA 01610. E-mail: kober@holycross.edu
Jamie J. Cannone, Robin R. Gutell, Center for Computational Biology and Bioinformatics, Section of Integrative Biology, University of Texas, Austin, TX 78712. E-mails: cannone@mail.utexas.edu, robin.gutell@mail.utexas.edu

Note about contributions by the authors: WM, MDB, TME, DRM, and KAO gathered the new sequence data; DRM conducted the phylogenetic analyses and wrote the first draft; WM organized and helped process the data; JJC and RRG aligned the ribosomal DNA sequences based on secondary structure.

Introduction

Of the four suborders of beetles, Adephaga is the second largest, with over 36 000 known species (Nilsson 2001; Lorenz 2005). Most species of Adephaga are predatory, although there are a few lineages that feed on organisms other than animals; for example, haliplid larvae feed on algae (Seeger 1971), rhysodine carabids on slime molds (Bell 1994), and some members of the carabid tribe Harpalini on seeds (Thiele 1977). This diversity of species and habits has a Mesozoic origin. The earliest adephagan fossils, from the late Triassic, include both terrestrial (Trachypachidae) and apparently aquatic (Triaplidae) forms (Ponomarenko 1977). A recent dating analysis places the most recent common ancestor of living Adephaga at about 237 mya, in the early Triassic (Hunt *et al*. 2007). By the Jurassic, many modern lineages were present, and in some deposits they are the dominant beetles (Ponomarenko 1977). Adephagan monophyly is undisputed and is supported by several apomorphic features of both larvae and adults (Beutel 1995; Beutel & Haas 2000; Beutel *et al*. 2007). The relationships of major lineages within Adephaga, however, is not clear.

Adephagan beetles can be separated into two groups based on habitat: the terrestrial Geadephaga (Carabidae and Trachypachidae) and the aquatic Hydradephaga (Amphizoidae, Aspidytidae, Dytiscidae, Gyrinidae, Haliplidae, Hygrobiidae, Meruidae, and Noteridae). Crowson (1960) proposed the hydradephagan families to be monophyletic and sister to the terrestrial Geadephaga. Since then, family level relationships within Adephaga have been extensively examined based on various morphological character systems (e.g. see Beutel 1993, 1995; Beutel & Haas 1996; Beutel *et al*. 2007), including head structure (Beutel 1989), thoracic sclerites and musculature (Bell 1966, 1967; Kavanaugh 1986; Beutel 1988, 1990, 1992b), female abdominal structure (Bils 1976; Burmeister 1976, 1990b, 1990a; Deuve 1993), ventral nerve cord (Heath & Evans 1990), digestive system (Yahiro 1990), wing venation (R. D. Ward 1979), larvae (e.g. Bousquet 1986; Arndt 1989; Beutel 1992a,c, 1993; Beutel & Roughley 1993; Arndt & Beutel 1995; Alarie & Bilton 2005; Alarie & Michat 2007), defensive glands (Forsyth 1972; Kanehisa & Murase 1977), and defensive secretions (e.g. Kanehisa & Murase 1977; B. P. Moore 1979; Dettner 1985; Attygalle *et al*. 2004). These studies provide conflicting views of some aspects of family level relationships. In particular, it is unclear whether Geadephaga and Hydradephaga are each monophyletic. Central to this question is the phylogenetic placement of Trachypachidae.

Trachypachidae is a small, amphitropical family containing six extant species classified in two genera, *Trachypachus* Motschulsky (endemic to western North America and northern Europe) and *Systolosoma* Solier (endemic to southern South America) (Fig. 1). Discerning the phylogenetic placement of Trachypachidae is thought to be a key to understanding the evolution of Adephaga as a whole (Bell 1983). Most commonly trachypachids are considered as either sister to Carabidae (e.g. Erwin 1985; Kavanaugh 1986; Beutel *et al*. 2006) resulting in a monophyletic Geadephaga, or as sister to all or part of Hydradephaga (Crowson 1960; Bell 1966, 1967; Bils 1976; Burmeister 1976; Baehr 1979; Hammond 1979; Ward 1979; Roughley 1981; Evans 1982; Bell 1983; Nichols 1985; Beutel & Roughley 1988; Deuve 1993; Beutel 1994; Arndt & Beutel 1995; Beutel 1998). Although trachypachids are terrestrial and appear superficially similar to Carabidae,

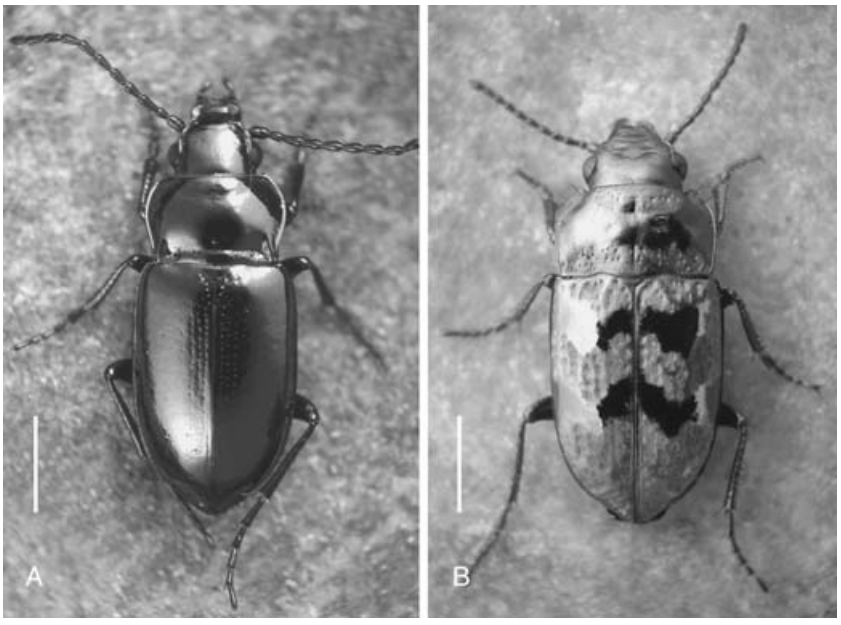

Fig. 1 A, B. Representatives of the two living genera of trachypachids. Scale bar: 1 mm. —A. *Trachypachus holmbergi*, USA: Oregon: Lincoln Co., Cape Perpetua Campground on route 101′S of Yachats, 44.2809′N 124.1014′W. —B. *Systolosoma breve*, CHILE: Reg. IX: P.N. Nahuelbuta, 37.8042′S 73.0281′W.

they share several derived traits with dytiscimorph hydradephagans including glabrous antennae, medial binding patches on their hindwings, and medial fusion and immobilization of the metacoxae, among others (*loc. cit.*).

The monophyly of Hydradephaga, as suggested by Crowson (1960), is also controversial. Some morphological studies suggest that Hydradephaga is not monophyletic for reasons other than the phylogenetic placement of trachypachids. For example, Kavanaugh (1986) suggested that the hydradephagan family Haliplidae is sister to a monophyletic Geadephaga. Beutel & Roughley (1988) proposed that Gyrinidae is the sister group of the rest of Adephaga, and thus that the other families of Hydradephaga are more closely related to geadephagans than to gyrinids.

Conflicting hypotheses of these basal relationships lead to different conclusions regarding the number of times adephagan beetles have colonized aquatic habitats. Crowson (1960) stated, 'No serious coleopterist has ever suggested that terrestrial caraboids are derived from the aquatic ones; it is universally assumed that the derivation has been in the reverse sense.' Following that assumption, phylogenies that suggest a non-monophyletic Hydradephaga imply multiple, independent colonisations and adaptations to an aquatic lifestyle.

Molecular studies designed to infer the deep phylogenetic structure within Adephaga have been based largely on the nuclear ribosomal small subunit gene (SSU or 18S rDNA) (D. R. Maddison *et al.* 1999; Shull *et al.* 2001; Caterino *et al.* 2002; Ribera *et al.* 2002; Hunt *et al.* 2007). Results of some of these analyses provide support for hydraphagean and/or geadephagan monophyly. Hunt *et al.* (2007) included sequence data of the mitochondrial gene 16S ribosomal DNA for hydradephagans, trachypachids, and other geadephagans. While they did not present results of a separate analysis of 16S data, their combined analysis, which also included the mitochondrial gene cytochrome oxidase I for some adephagans, but not trachypachids, recovered a monophyletic Geadephaga and a monophyletic Hydradephaga, a result consistent with previous analyses based solely on 18S rDNA.

Here, we revisit adephagan beetle phylogeny, focusing on the placement of trachypachids, with new sequence data of 18S rDNA, as well as two markers not yet used for this question, the nuclear ribosomal large subunit gene (LSU or 28S rDNA) and the protein-coding gene *wingless*. We analyse data from each gene in both separate and combined analyses. We also re-analyse 16S rDNA data available from Hunt *et al.* (2007) to explore the evidence it provides regarding trachypachid placement.

In addition, we briefly examine some of the suggested relationships in beetles outside of Adephaga, including relationship of the suborders. In light of recent evidence suggesting the monophyly of the majority of Polyphaga exclusive of Scirtoidea and Derodontoidea (Lawrence 1999, 2001; Caterino *et al.* 2002; Friedrich & Beutel 2006; Hunt *et al.* 2007), we also touch on the basal splits within Polyphaga.

Past studies of ribosomal DNA in Adephaga have not used detailed secondary structure information to aid in the alignment, instead using either standard multiple sequence alignment methods followed by tree inference (D. R. Maddison *et al.* 1999; Shull *et al.* 2001; Caterino *et al.* 2002) or simultaneous alignment and tree inference (Shull *et al.* 2001; Ribera *et al.* 2002), that is, 'tree alignment' (Sankoff 1975). In this paper, we align the ribosomal DNA sequences using the secondary structure models that have been developed and confirmed by crystal structure and covariation analysis (Woese *et al.* 1980; Noller *et al.* 1981; Gutell *et al.* 2002).

Methods

Taxon sampling

Tables 1–3 list focal taxa and genes that were sequenced for each. Voucher specimens are stored in the collection of DRM for eventual deposition into a public collection. Voucher ID numbers and localities are provided in the Supplementary Materials.

Five of the six living species of trachypachids are included (only the Palearctic *Trachypachus zetterstedti* (Gyllenhal) is absent), as well as representatives of all adephagan families other than Meruidae and Aspidytidae. Four species in two families of Archostemata, three families of Myxophaga, and a sample of polyphagan superfamilies serve as primary outgroups to the Adephaga.

DNA extraction and sequencing

Most nucleic acid extractions were performed as described in Maddison *et al.* (1999), with the remainder using the DNeasy Tissue Kit from Qiagen.

Fragments for each gene were amplified using the Polymerase Chain Reaction on either a Perkin Elmer DNA Thermal Cycler, MJ Research PTC-150 Minicycler or an Eppendorf Mastercycler Thermal Cycler, using either GibcoBRL Taq Polymerase or Eppendorf Hotmaster Taq and the basic protocols recommended by the manufacturers. Amplification and sequencing primers used are given in Table 4. About 30–40 cycles were used, with annealing temperatures of 50 °C (18S rDNA), 53–54 °C (28S rDNA), or 51–56 °C (*wingless*). For some species (most notably members of Hydradephaga, Archostemata, and Megaloptera), amplification of ribosomal genes required addition of 5 μL glycerol and 3 μL dimethyl sulfoxide (DMSO) to the 50 μL PCR reaction. The amplified products were cleaned using Microcon-100 Microconcentrators (Amicon) and sequenced at the University of Arizona's Genomic and Technology Core Facility using a 377 Applied Biosystems automatic sequencer, or cleaned, quantified, and sequenced at the University of Arizona's Genomic and Technology Core Facility using

Table 1 Taxon Sampling and GenBank numbers of sequences of species outside of Adephaga. Sequences with GenBank numbers beginning with 'EU' are novel.

		18S rDNA	28S rDNA	*wingless*
Megaloptera				
Sialidae	*Sialis* sp.	EU797399	EU797384	EU797281
Neuroptera				
Ithonidae	*Oliarces clara* Banks	AF012527	EU797371	EU797314
Mantispidae	*Mantispa* sp.	EU797400	EU797366	EU797311
Coleoptera: Archostemata				
Cupedidae	*Cupes capitatus* Fabricius	EU797406	EU797351	EU797298
	Priacma serrata (LeConte)	EU797411	EU797380	EU797322
	Tenomerga cinerea (Say)	EU797417	EU797392	EU797330
Micromalthidae	*Micromalthus debilis* LeConte	EU797409	EU797367	—
Coleoptera: Myxophaga				
Hydroscaphidae	*Hydroscapha natans* LeConte	AF012525	EU797359	—
Sphaeriusidae	*Sphaerius* sp.	EU797414	EU797386	—
Torridincolidae	*Torridincola rhodesiaca* Steffan	AF201420	EU797393	—
Coleoptera: Polyphaga				
Buprestidae	*Acmaeodera* sp.	—	EU797336	EU797282
	Acmaeodera sp. C36	AF423771	—	—
Byrrhidae	*Byrrhus* sp.	—	EU797344	EU797290
	Byrrhus pilula (Linnaeus)	AF427604	—	—
Clambidae	*Clambus arnetti* Endrödy-Younga	EU797403	EU797347	EU797292
	Clambus seminulum Horn	EU797404	EU797293	EU797346
Endomychidae	*Aphorista morosa* (LeConte)	EU797402	EU797342	EU797288
Hydrophilidae	*Tropisternus ellipticus* (LeConte)	EU797419	EU797397	EU797334
Scarabaeidae	*Dynastes granti* Horn	AF002809	EU658919	EU658921
Scirtidae	*Cyphon* sp.	—	EU797352	EU797299
	Cyphon hilaris Nyholm	AF201419	—	—
	Prionocyphon discoideus Say	EU797412	EU797381	EU797323
Staphylinidae	*Xanthopygus cacti* Horn	AF002810	EU797398	EU797335

either a 3730 or 3730 XL Applied Biosystems automatic sequencer. For 28S rDNA and *wingless* two bidirectional sequencing reactions were obtained for each sequence; for 18S rDNA, six reactions were obtained.

Results of individual sequencing reactions were assembled and base calls made using Sequencher (Gene Codes Corp.) or using Mesquite's Chromaseq package (D. R. Maddison & Maddison 2007; W. P. Maddison & Maddison 2008), in conjunction with Phred (Green & Ewing 2002) and Phrap (Green 1999). Multiple peaks at a single position in both reads were coded using IUPAC ambiguity codes.

A total of 21 novel 18S rDNA sequences were obtained, as well as 63 28S rDNA, and 55 *wingless* sequences.

Alignment

18S rDNA and 28S rDNA were aligned by JJC and RRG. An unaligned matrix of 18S rDNA sequences was initially provided to JJC and RRG with names of sequences removed, to eliminate the possibility of biasing the alignment due to presumptions about phylogenetic relationships; the 28S rDNA matrix was provided with names visible. JJC and RRG aligned these sequences, as well as made judgements about confidence in the homology of each position. A general description of the alignment methods is given in Alverson *et al*. (2006). In brief, patterns of sequence conservation and variation, including covariation analysis (Gutell *et al*. 1985) of a large and phylogenetic diverse set of sequences available at the CRW Site (http://www.rna.ccbb.utexas.edu/DAT/3C/Alignment/) have been used to determine secondary structure models of large and small subunit ribosomal RNAs. These models were then applied to the sequences in this paper. The few regions that could not be accommodated with high confidence in these models, in part because of extensive variation within Adephaga, were not included in the phylogenetic analysis.

The 18S rDNA sequences range from 1777 to 2633 long nucleotides (nt); with the regions of low confidence excluded, the range in sequence length is 1566–1693. 28S rDNA ranges from 837 to 1325 nt, of which 598 to 813 nt were used in the analyses.

The 5′ and 3′ ends of the *wingless* gene fragment show no insertions and deletions, and thus are easy to align; the central region, however, shows a great deal of length variation, and there is much less certainty in the alignment. This central region was treated in one of two ways. In the first treatment, called 'ModClustal' for modified Clustal alignment, the

Table 2 Taxon Sampling and GenBank numbers of sequences of Hydradephaga. Sequences with GenBank numbers beginning with 'EU' are novel.

		18S rDNA	28S rDNA	*wingless*
Amphizoidae	*Amphizoa lecontei* Matthews	AJ318678	EU797340	EU797286
	Amphizoa insolens LeConte	EU797401	EU797339	EU797285
Dytiscidae	*Agabus semivittatus* LeConte	—	EU797337	EU797283
	Agabus bipustulatus (Linnaeus)	AJ318687	—	—
	Copelatus chevrolatei renovatus Guignot	AF012524	EU658918	EU797296
	Hydroporus axillaris LeConte	—	EU797358	EU797305
	Hydroporus planus (Fabricius)	AJ318734	—	—
	Hydrotrupes palpalis Sharp	EU797408	EU797360	EU797306
	Hydrovatus pustulatus (Melsheimer)	—	EU797361	EU797307
	Hydrovatus nigrita Sharp	AJ318717	—	—
	Laccophilus pictus Castelnau	—	EU797363	EU797309
	Laccophilus poecilus Klug	AJ318714	—	—
	Liodessus affinus species complex	—	EU797364	EU797310
	Liodessus sp IR96	AJ318728	—	—
	Rhantus gutticollis (Say)	—	EU797382	EU797324
	Rhantus suturalis (MacLeay)	AJ318696	—	—
	Stictotarsus corvinus (Sharp)	EU797415	EU797387	EU797327
Gyrinidae	*Gyretes iricolor* Young	AJ318663	—	EU797301
	Gyretes torosus Babin	—	EU797354	—
	Gyrinus woodruffi (Fall)	—	EU797355	EU797302
	Gyrinus sp. VLS-1999	AF201412	—	—
	Spanglerogyrus albiventris Folkerts	EU797413	EU797385	EU797326
Haliplidae	*Haliplus eremicus* Wells	—	EU797356	—
	Haliplus sp.	—	—	EU797303
	Haliplus lineatocollis Marsham	AJ318666	—	—
	Peltodytes dispersus Roberts	EU797410	EU797379	EU797321
Hygrobiidae	*Hygrobia hermanni* Fabricius	AF201414	EU797362	EU797308
Noteridae	*Hydrocanthus oblongus* Sharp	AF201415	EU797357	—
	Hydrocanthus atripennis Say	—	—	EU797304
	Noterus clavicornis De Geer	AF201416	EU797368	—
	Notomicrus nanulus (LeConte)	—	EU797370	EU797313
	Notomicrus tenellus (Clark)	AJ318671	—	—
	Suphis inflatus LeConte	AF012523	EU797388	—
	Suphisellus gibbulus (Aubé)	—	EU797389	—
	Suphisellus sp. IR110	AJ318669	—	—

amino acid translation was aligned using Clustal W version 1.83 (Chenna *et al*. 2003) using gap opening cost of 5, gap extension cost 0.2, and a Gonnet series matrix. The Clustal amino acid alignment was subjected to a neighbor-joining analysis in PAUP* (Swofford 2002), and the subsequent tree was read into MacClade (D. R. Maddison & Maddison 2005). The taxa were reordered in MacClade so that the order in the matrix matched the order in the tree. The names of the taxa then were hidden in MacClade, and DRM realigned amino acids in the central region by eye to ensure that common motifs present in almost all sequences were aligned. In particular, an NSIH sequence (asparagine, serine, isoleucine, histidine) was not always judged homologous by Clustal; this was manually corrected in the ModClustal alignment. The nucleotides were then aligned to match this amino acid alignment.

The second treatment used Opal (Wheeler & Kececioglu 2007) to align the amino acids with default settings (gap open, gap extension, terminal gap open costs, and terminal gap extension values of 60, 38, 15, 36, respectively), which the advisor function (Wheeler & Kececioglu 2007) judged to be best. Unlike Clustal, Opal successfully aligned the NSIH motif in all sequences. Both the ModClustal and Opal alignments were then divided into well-aligned regions and indel-rich regions with uncertain alignment. Two matrices were examined for each alignment, one with the indel-rich regions removed, and the other with all data included.

With all nucleotides included, the *wingless* sequences varied from 381 to 546 nt; with the indel-rich region excluded, the sequences range from 324 to 402 nt.

Combined matrices were produced by concatenation of data from all three genes. For most genera with more than

Table 3 Taxon Sampling and GenBank numbers of sequences of Geadephaga. Sequences with GenBank numbers beginning with 'EU' are novel.

		18S rDNA	28S rDNA	*wingless*
Trachypachidae	*Trachypachus gibbsii* LeConte	AF002808	EU797394	EU797331
	Trachypachus slevini Van Dyke	EU797418	EU797396	EU797333
	Trachypachus holmbergi Mannerheim	AF002807	EU797395	EU797332
	Systolosoma lateritium Négre	AF012522	EU797391	EU797329
	Systolosoma breve Solier	EU797416	EU797390	EU797328
Carabidae	*Metrius contractus* Eschscholtz	AF012515	AF398687	AF398605
	Pachyteles striola species complex	AF012517	EU797377	EU797319
	Arthropterus sp.	AF012516	AF398644	AF398570
	Amblycheila baroni Rivers	AF423057	EU797338	EU797284
	Ctenostoma erwini Naviaux	EU797405	EU797350	EU797297
	Oxycheila nigroaenea Bates	AF201393	—	—
	Oxycheila cf *glabra* Waterhouse	—	EU797376	EU797318
	Cicindela sedecimpunctata Klug	AF012518	AF398660	AF398579
	Dhysores sp.	EU797407	EU797353	EU797300
	Omoglymmius hamatus (LeConte)	AF012520	EU797372	AF398609
	Clinidium calcaratum LeConte	AF012521	—	—
	Clinidium baldufi Bell	—	EU797348	EU797294
	Nebria hudsonica LeConte	AF002805	AF398676	AF398608
	Opisthius richardsoni Kirby	AF012511	EU797374	EU797316
	Notiophilus semiopacus Eschscholtz	AF002804	EU797369	EU797312
	Loricera foveata LeConte	AF012503	EU797365	—
	Elaphrus californicus Mannerheim	AF012514	AF398639	AF398563
	Omophron obliteratum G.H. Horn	AF012513	EU797373	EU797315
	Antarctonomus complanatus Blanchard	AF012504	EU797341	EU797287
	Carabus nemoralis O.F. Müller	AF012507	EU797345	EU797291
	Scaphinotus petersi catalinae Van Dyke	AF002801	EU658920	EU658922
	Pasimachus obsoletus atronitens Casey	AF002794	EU797378	EU797320
	Scarites subterraneus Fabricius	AF002795	AF398708	AF398625
	Clivina ferrea LeConte	AF002796	EU797349	EU797295
	Schizogenius falli Whitehead	AF002797	EU797383	EU797325
	Oregus aereus White	AF012500	EU797375	EU797317
	Broscosoma relictum Weissmandl	AF012502	EU797343	EU797289
	Bembidion levettei carrianum Casey	AF002791	AF398647	AF398571
	Diplochaetus planatus G.H. Horn	AF002789	AF438060	AF437938
	Diplous californicus (Motschulsky)	AF002785	AF398699	AF398587
	Patrobus longicornis (Say)	AF002786	AF398700	AF398613
	Amblytelus curtus (Fabricius)	AF012484	AF398683	AF398566
	Pterostichus melanarius (Illiger)	AF002779	AF398707	AF398623
	Galerita lecontei lecontei Dejean	AF002780	AF398686	AF398590
	Chlaenius ruficauda Chaudoir	AF002777	AF398680	AF398578

one species represented in our data (see Tables 1–3), the sequences from different species were merged into a chimera representing the genus; the species of *Clambus* and trachypachids were not so merged and were kept distinct. The 16S rDNA data were not combined with the three genes we sequenced because of paucity of taxa in common between the matrices.

Phylogenetic inference

Most parsimonious trees were sought using PAUP* (Swofford 2002). For each search, 4000 replicates were conducted, each beginning with a starting tree formed by the random addition sequence option, with subsequent TBR branch rearrangement, with each replicate saving no more than 25 trees. The number of most parsimonious trees found for each matrix ranged between 4 and 12 712.

For parsimony bootstrap analyses in PAUP*, 1000 bootstrap replicates were examined, each of which used a heuristic search with five replicates, each beginning with a starting tree formed by the random addition sequence option, with TBR branch rearrangement, with each replicate saving no more than 25 trees.

Models of nucleotide evolution were chosen with the aid of MODELTEST (Posada 2005). For 18S and 28S rDNA, the model chosen by the Akaike Information Criterion (AIC) was

Table 4 Primers used for DNA amplification and sequencing.

Gene	Primer	Syn	Dir	Kind	Sequence	References
28S	LS58F	D1	F	A	GGGAGGAAAAGAAACTAAC	Ober 2002
	NLF184/21		F	A	ACCCGCTGAAYTTAAGCATAT	Van der Auwera *et al.* 1994
	LS998R	D3	R	A	GCATAGTTCACCATCTTTC	Ober 2002
	LS1041R		R	A	TACGGACRTCCATCAGGGTTTCCCCTGACTTC	Maddison 2008
	LS30F	D1mod	F	A	ACCCCCTRAATTTAAGCATAT	Moore 2008
	LS264F	D2F	R	A	CGTGTTGCTTGATAGTGCAGC	This paper
	LS770R	D2R	R	A	TCAAGACGGGTCCTGAAAGT	This paper
18S	SS27F	518S	F	A	TATGCTTGTCTCAAAGATTAA	
	S1893R	18L	R	A	CACCYACGGAAACCTTGTTACGACTT	
	SS398F	18Sai	F	S	CCTGAGAAACGGCTACCACATC	Wray *et al.* 1993
	SS1054F	760F	F	S	ATCAAGAACGAAAGT	Wray *et al.* 1993
	SS1090R	18Sbi	R	S	GAGTCTCGTTCGTTATCGGA	Wray *et al.* 1993
	SS1554R	909R	R	S	GTCCTGTTCCATTATTCCAT	Maddison *et al.* 1999
wg	wg550F		F	A	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	Wild & Maddison, 2008
	wgAbRZ		R	A	CACTTNACYTCRCARCACCARTG	Wild & Maddison, 2008
	wg578F		F	A	TGCACNGTGAARACYTGCTGGATG	Ward & Downie 2005
	wgAbR		R	A	YTCGCAGCACCARTGGAA	Ward & Downie 2005
	B5wg1		F	A	GARTGYAAGTGTCAYGGYATGTCTGG	Maddison 2008
	5wg		F	A	GARTGYAARTCYCAYGGYATGTCTGG	This paper
	5wgB		F	A	ACBTGYTGGATGCGNCTKCC	Maddison 2008
	3wg2		R	A	CTCGCARCACCARTGGAATGTRCA	This paper
	B3wg2		R	A	ACTCGCARCACCAGTGGAATGTRCA	Maddison 2008
	3wg		R	A	ACTCGCARCACCARTGGAATGTRCA	This paper

Dir: direction of primer, either forward (F) or reverse (R). Syn: primer synonym. Kind: primer used for original PCR amplification and sequencing (A) or primer used only for sequencing (S). Ref: reference for original description of primer, if known.

a General Time Reversible rate matrix with a proportion of sites being invariant and the remainder following a gamma distribution (the GTR + I + Γ model). For the *wingless* gene, the GTR + I + Γ model was chosen for the region without extensive insertions and deletions, but for the indel-rich region an HKY + I + Γ model was preferred. When codon positions were allowed separate models, a GTR + I + Γ was preferred for each.

For the *wingless* amino acid matrix, ProtTest (Drummond & Strimmer 2001; Guindon & Gascuel 2003; Abascal *et al.* 2005) was used to choose the model of amino acid evolution. The model chosen for the region without extensive insertions and deletions was JTT + I + Γ for the ModClustal alignment, and JTT + Γ for the Opal alignment, and VT + Γ for the indel-rich region for both alignments.

Bayesian analyses were conducted using MrBayes (Huelsenbeck & Ronquist 2005). Two runs of four chains each were run for between 6 million and 300 million generations, with trees sampled every 1000 generations. Runs were terminated once the average standard deviation of split frequencies went below 0.01 (Huelsenbeck & Ronquist 2005). Two analyses did not reach this level: (i) the 28S matrix, which reached 0.0135 after 100 million generations, and (ii) the *wingless* nucleotide matrix based on the ModClustal alignment, partitioned by codon position, which reached 0.012 after 300 million generations. For each analysis, the trees in a burn-in period were excluded, and the majority-rule consensus tree of the remaining trees was calculated by PAUP* to determine Bayesian Posterior Probability (BPP) of each clade. The burn-in period was at least 25% of the total length of the run (as only the remaining 75% were used to calculate the average standard deviation of split frequencies used as a convergence diagnostic), and extended until the likelihood scores and all parameter values reached a stable plateau, as judged by visualization tools in Tracer (Rambaut & Drummond 2004). The burn-in period ranged from 2.6 million generations to 280 million generations. The number of trees sampled for each analysis varied from 6800 to 60 000.

Likelihood analyses of nucleotide data were conducted using Garli version 0.951 (Zwickl 2006); for *wingless* amino acid data RAxML version 2.2.3 (Stamatakis 2006) was used. For each matrix, 25 search replicates were conducted. Two hundred non-parametric bootstrap replicates and likelihood searches were used to calculate bootstrap values for groups of interest.

Some analyses yielded a non-monophyletic Coleoptera or non-monophyletic Adephaga. Equivalent analyses were also conducted constraining Coleoptera or Adephaga to be monophyletic, as appropriate. That these two groups are monophyletic is not in dispute, and is well-supported by

morphological evidence (Beutel 1997; Beutel & Haas 2000; Beutel *et al*. 2008). Constraining these groups to be monophyletic is one way to incorporate this previously acquired knowledge into the analysis, just as we include broader knowledge of the tree of life through our choice of outgroups.

A reanalysis of the mitochondrial large subunit ribosomal DNA (16S rDNA) data from Hunt *et al*. (2007) included a subset of sequences designed to match the taxon sampling in this paper: all non-polyphagan sequences, all Scirtoidea and Derodontoidea, and the closest relative of each of the six other polyphagan genera sampled for 18S rDNA, 28S rDNA, and *wingless* (*Endomychus* for *Aphorista*, *Agrilus* for *Acmaeodera*, *Byrrhus*, *Heteronychus* for *Dynastes*, *Enochrus* for *Tropisternus*, and *Pseudopsis* for *Xanthopygus*). Because of the differences in taxon sampling, the 16S rDNA data could not be combined with the other genes. These data were examined to see if they contribute to our understanding of geadephagan boundaries. The alignment of Hunt *et al*. (2007) was used, except for modification of two misaligned sequences (*Clambus* and *Chrysopa*). Parsimony, Bayesian, and likelihood analyses were conducted as described above. The Bayesian analysis ran for 68 million generations, and reached an average standard deviation of split frequencies of 0.017; the last 10 million generations were examined. The model chosen by AIC using ModelTest was HKY + I + Γ.

Habitat transitions

The evolution of habitat in Adephaga was investigated on trees inferred in one of the Bayesian analyses, which allowed uncertainty in the tree structure to be considered. The habitats of adults were tabulated with all hydradephagans, *Tropisternus*, *Hydroscapha*, and *Torridincola* recorded as aquatic, with the remaining species recorded as terrestrial. The habitats of larvae were similarly coded, but with *Sialis*, *Cyphon*, and *Prionocyphon* also recorded as aquatic. The posterior probabilities of different numbers of transitions between terrestrial and aquatic habitat were calculated using the 11 226 trees sampled by the Bayesian analysis of the combined data matrix (*wingless* amino acids, excluding the indel-rich region, plus 18S rDNA and 28S rDNA) using a method proposed by Huelsenbeck *et al*. (2000). Each of these trees was examined using Mesquite's (W. P. Maddison & Maddison 2008) Summarize Changes on Selected Branches feature. For each tree, all most parsimonious reconstructions (MPR) of habitat change were examined, and the number of each sort of change was calculated within Adephaga for each MPR; the fraction of MPRs with 0, 1, 2, or more changes of each sort was then recorded for that tree. For example, if there are six MPRs for a tree, and two of these showed one terrestrial to aquatic change, and four showed two terrestrial to aquatic changes, then the fraction of MPRs showing one terrestrial to aquatic change is one-third, and the fraction showing two changes is two-third. The posterior probability of a particular number of changes (e.g. two aquatic to terrestrial changes) was then estimated by calculating the average fraction of MPRs showing that number of changes across all trees. If each tree has only one MPR for habitat, then the average fraction of MPRs showing that number of changes is equivalent to the fraction of trees showing that number of changes; multiple MPRs are accommodated in that each contributes an equal fraction to the count for a given tree. Two analyses were conducted in this fashion for each of adult habitat and larval habitat, one unconstrained, and other only counting MPRs in which there were no changes from aquatic to terrestrial habitat.

The ancestral habitat of Adephaga was reconstructed over all 11 226 trees using Mesquite's Trace Character Over Trees procedure, using both parsimony and maximum likelihood methods; the latter employed Lewis's (2001) mk1 model. The maximum likelihood methods depend upon branch lengths, and the branch lengths are inferred by MrBayes using the divergences evident in the three genes. However, habitat is unlikely to evolve according to the same rate-variation patterns as the molecular data, and more appropriate branch lengths would be ones proportional to time of divergence. An additional analysis was therefore conducted on the Bayesian trees modified to be 'chronograms', that is, trees with branch length patterns consistent with relative time of divergence. These chronograms were calculated using the penalized likelihood algorithm described in Sanderson (2002), as implemented by the chronopl function in APE version 2.2 (Paradis *et al*. 2004), with lambda of 2.0.

Results

The maximum likelihood trees for each of the three genes are shown in Figs 2–4; each of these is constrained to have either Coleoptera or Adephaga monophyletic. Figures of trees from unconstrained Bayesian and parsimony analyses are presented in the Supplementary Information. The majority-rule consensus tree of Bayesian trees for a combined matrix is presented in Fig. 5. The maximum likelihood tree for the subset of Hunt *et al*.'s (2007) mitochondrial 16S rDNA data is shown in Fig. 6. A summary of the support for or against monophyly of Geadephaga and Hydradephaga is given in Fig. 7.

Wingless

Results for the *wingless* gene were somewhat sensitive to the inclusion of the indel-rich region, the alignment used, and whether data were analysed as nucleotides or amino acids. Trees inferred with the indel-rich region excluded, and with data analysed as nucleotides, differ more from trees supported by morphological data. For example, Coleoptera is strongly supported as monophyletic in all analyses except for parsimony analysis of nucleotides with the indel-rich region excluded.

Fig. 2 Maximum likelihood tree inferred from all *wingless* amino acids (Opal alignment) under the constraint that Adephaga is monophyletic (striped branch). Arrow indicates placement of Polyphaga if constraint is not enforced. Branches with dots have Bayesian Posterior Probability of 0.90 or more for the Opal alignment of all amino acids. Branches that are slightly thickened have an additionally property, in that they appear in the most parsimonious trees. The thickest branches have, additionally, a parsimony bootstrap value of ≥ 80 for all nucleotides based upon the Opal alignment, a likelihood bootstrap value ≥ 80 for all Opal alignment amino acids, and a Bayesian Posterior Probability of 0.90 or more for the ModClustal alignment of all nucleotides. Dots and thickened branches are based upon unconstrained analyses. The Geadephaga branch is indicated by an asterisk.

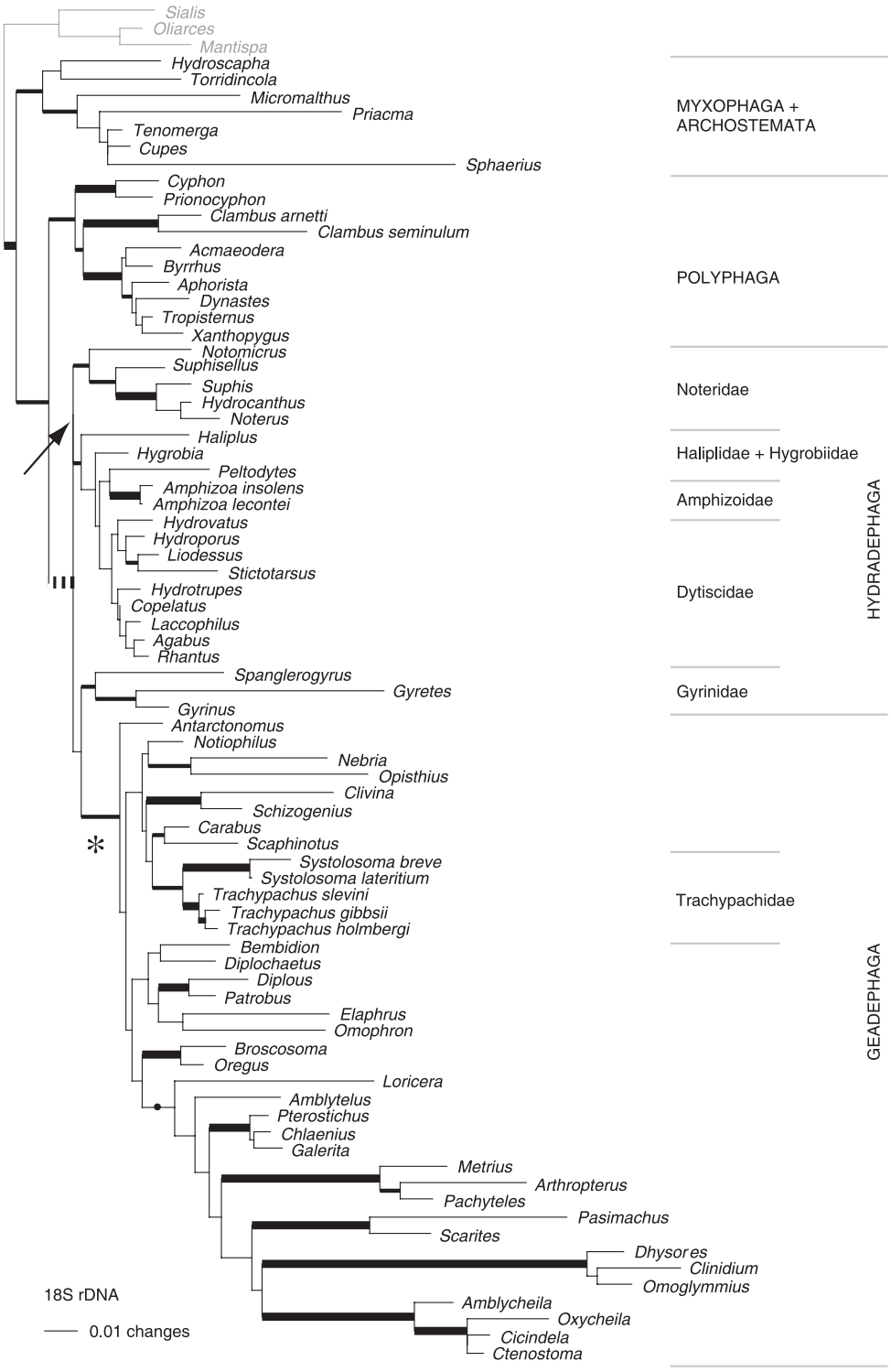

Fig. 3 Maximum likelihood tree inferred from 18S rDNA under the constraint that Adephaga is monophyletic (striped branch). Arrow indicates placement of Polyphaga (as sister to Hydradephaga excluding Gyrinidae) if the constraint is not enforced. Branches with dots have Bayesian Posterior Probability of 0.90 or more. Branches that are slightly thickened also appear in the most parsimonious trees. The thickest branches have, in addition, a parsimony bootstrap value of ≥ 80, a likelihood bootstrap value ≥ 80. Dots and thickened branches are based upon unconstrained analyses. The Geadephaga branch is indicated by an asterisk.

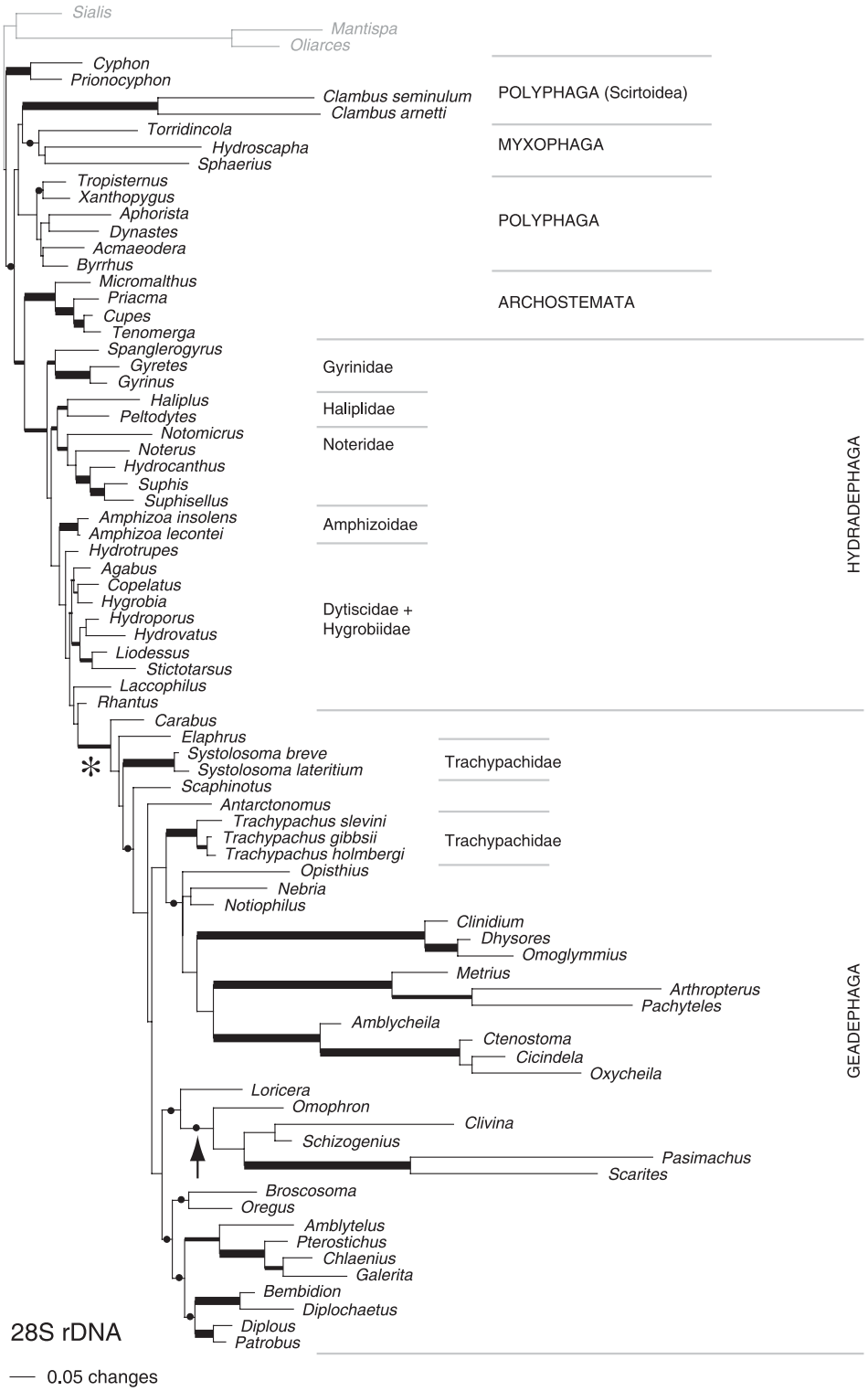

Fig. 4 Maximum likelihood tree inferred from 28S rDNA under the constraint that Coleoptera is monophyletic. Arrow indicates placement of *Oliarces* and *Mantispa* if constraint is not enforced. Branches with dots have Bayesian Posterior Probability of 0.90 or more. Branches that are slightly thickened also appear in the most parsimonious trees. The thickest branches have, in addition, a parsimony bootstrap value of ≥ 80, a likelihood bootstrap value ≥ 80. Dots and thickened branches are based upon analyses with Coleoptera constrained to be monophyletic. The Geadephaga branch is indicated by an asterisk.

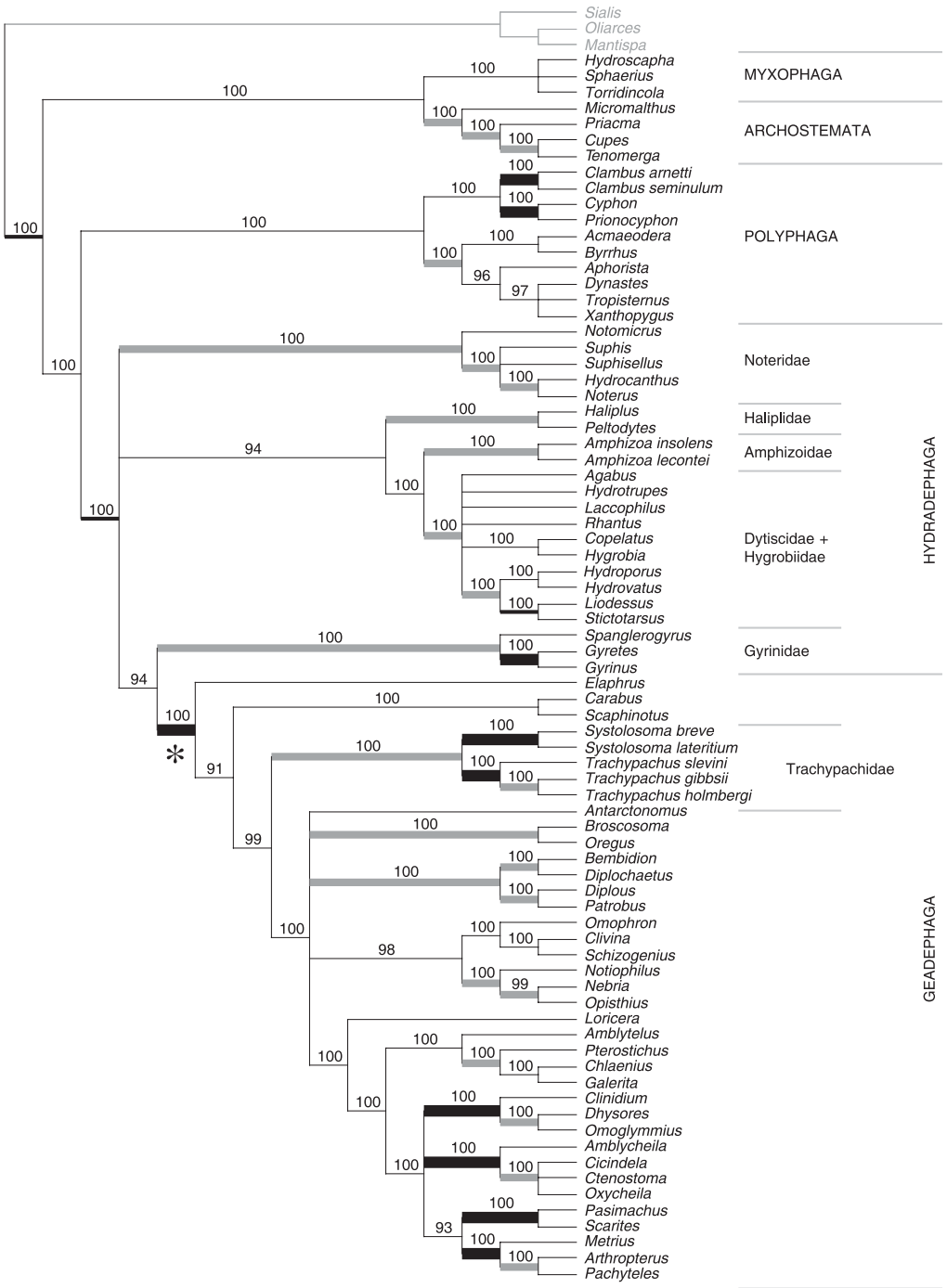

Fig. 5 A 90% majority-rule consensus tree of 11 226 postburn-in trees from the Bayesian analysis of the combined data matrix (*wingless* amino acids, excluding the indel-rich region, plus 18S rDNA and 28S rDNA), with Bayesian posterior probability percentage estimates on each branch. The four slightly thickened branches are those clades that are strongly supported in two additional analyses with the combined nucleotide matrix (all *wingless* nucleotides, ModClustal alignment, plus 18S rDNA and 28S rDNA): a Bayesian Posterior Probability of 0.80 or more, and a likelihood bootstrap value of 80 or more. The moderately thick, grey branches indicate clades that are strongly supported in those analyses in addition to having a parsimony bootstrap value ≥ 80 for the combined nucleotide matrix (with Coleoptera constrained to be monophyletic). The ten very thick black branches are strongly supported in the same way, and in addition show strong support from each of the three genes independently (for each gene BPP ≥ 90, and in MPTs; for 28S rDNA with Coleoptera constrained to be monophyletic and for *wingless* with Adephaga constrained to be monophyletic). The Geadephaga branch is indicated by an asterisk.

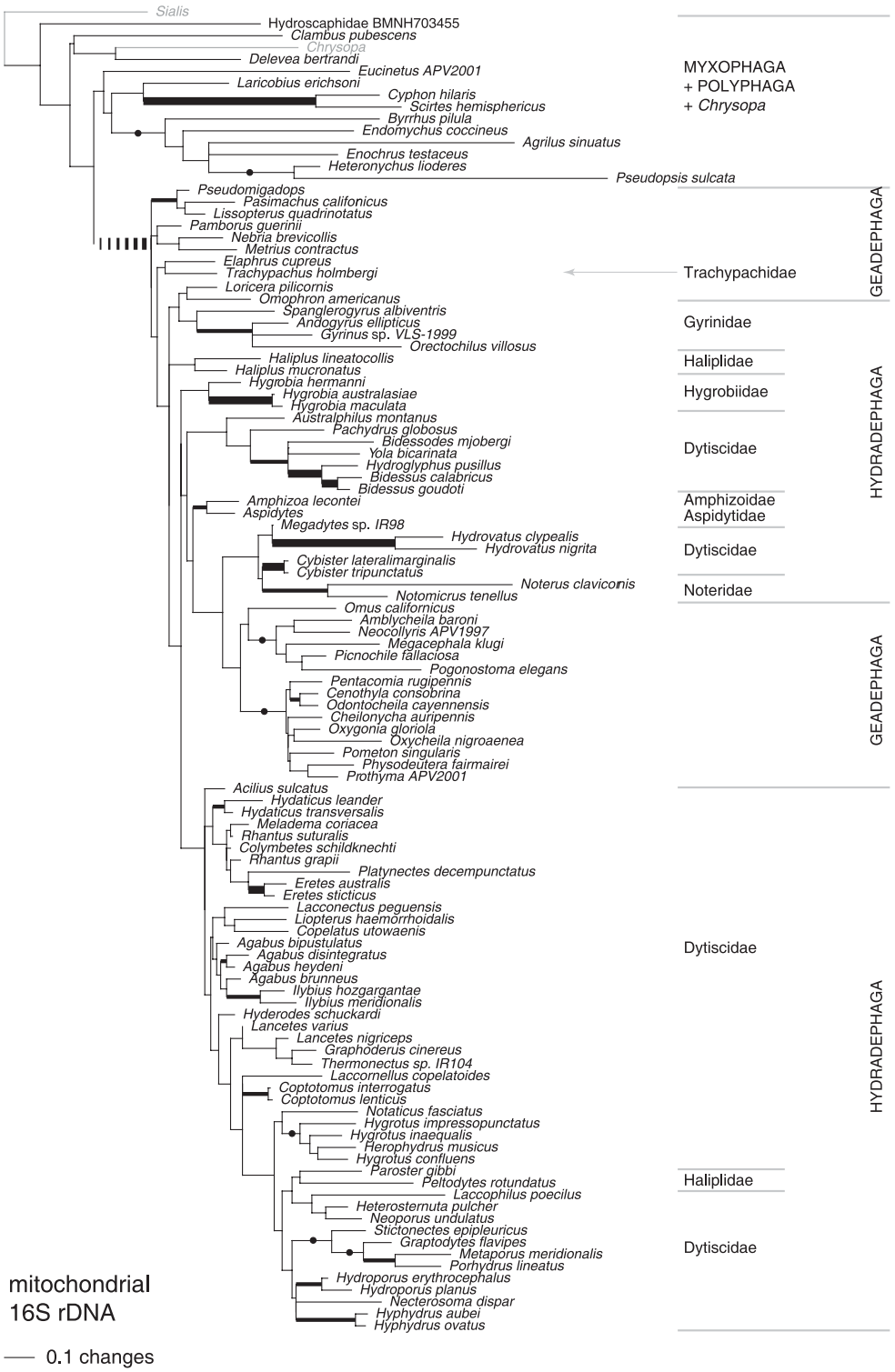

Fig. 6 Maximum likelihood tree inferred from mitochondrial 16S rDNA, under the constraint that Adephaga is monophyletic (striped branch). If this constraint is not enforced, then Polyphaga is the sister of the carabid genus *Elaphrus*. Branches with dots have Bayesian Posterior Probability of 0.90 or more. Branches that are slightly thickened in addition appear in the most parsimonious trees. The thickest branches have in addition a parsimony bootstrap value of ≥ 80, a likelihood bootstrap value ≥ 80. Dots and thickened branches are based upon unconstrained analyses.

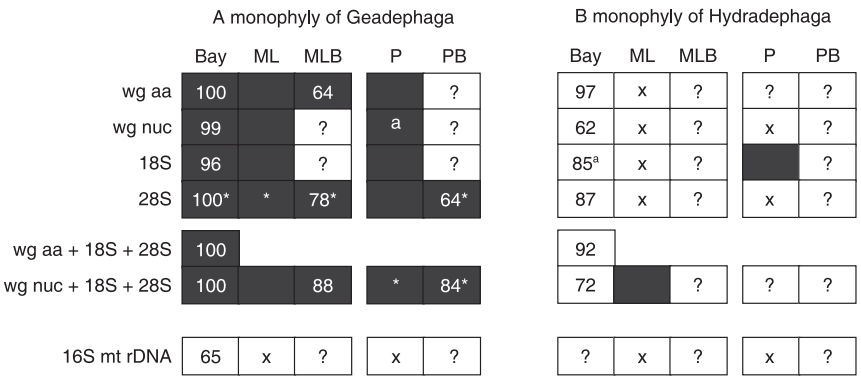

Fig. 7 A, B. Summary of support for monophyly of —A. Geadephaga and —B. Hydradephaga. Each row represents a different matrix, each column a different analysis. Dark squares indicate support for the clade for that matrix and analysis; white squares indicate lack of support or support for non-monophyly. Matrices: 'wg aa': all *wingless* amino acids, Opal alignment; 'wg nuc': all *wingless* nucleotides, ModClustal alignment; '18S': 18S rDNA; '28S': 28S rDNA; 'wg aa + 18S + 28S': combined matrix of *wingless* amino acids (Opal alignment, excluding indel-rich region), 18S rDNA, and 28S rDNA; 'wg nuc + 18S + 28S': combined matrix of all *wingless* nucleotides (ModClustal alignment), 18S rDNA, and 28S rDNA. 16S mt rDNA: mitochondrial 16S rDNA data from Hunt *et al*. (2007). Analyses: 'Bay': Bayesian Posterior Probability percentage of clade (if cell is dark) or of contradictory clade (if cell is white); 'ML': Maximum Likelihood Tree; 'MLB': Maximum Likelihood Bootstrap value; 'P': Most Parsimonious Trees; 'PB': Parsimony Bootstrap value. ?: No support for or against the clade; x: contradictory clade present; *: analysis done enforcing monophyly of Coleoptera; a: analysis done enforcing monophyly of Adephaga.

While monophyly of Adephaga has high BPP in some analyses (e.g. 0.99 for all amino acids in the Opal alignment), in others there is high BPP against monophyly (e.g. the BPP for inclusion of some polyphagans within Hydradephaga is 0.96 for analysis of the ModClustal nucleotide alignment with the indel-rich region excluded).

The *wingless* gene strongly supports a monophyletic Geadephaga in all Bayesian analyses of nucleotide or amino acid data, except for the analysis of nucleotides in which the indel-rich region was excluded. For the Opal alignment, Geadephaga is monophyletic in the most parsimonious trees and maximum likelihood trees if all sites are included or if the data are analysed as amino acids, but bootstrap values are less than 50%. In some analyses of the ModClustal alignment, Geadephaga is not evidently monophyletic, but there is no evidence for paraphyly or polyphyly. Support for monophyly of Geadephaga is not affected by enforcing monophyly of Adephaga. The sister group to Geadephaga in most *wingless* analyses is the Gyrinidae in whole or in part; this is supported by a BPP of 0.64–0.97, depending upon the analysis.

Trachypachids are also supported as monophyletic except for some parsimony analyses. Their sister group varies from analysis to analysis; when evident it is either tiger beetles (Cicindelinae) or carabites (Carabini + Cychrini) plus Migadopini.

Hydradephaga is not monophyletic in any analysis, and with strong evidence against in some Bayesian analyses (which places gyrinids with Geadephaga with BPP of 0.54–1.00 depending upon the analysis).

The monophyly of Polyphaga exclusive of Scirtoidea is recovered in all Bayesian and likelihood analyses with the indel-rich region included, with strong support (BPP 0.90–0.98), but not if the indel-rich region is excluded, and not in parsimony analyses.

18S rDNA

Coleoptera is strongly supported in all analyses of 18S rDNA, with BPP of 1.00, likelihood bootstrap value of 98, and parsimony bootstrap value of 97. Adephaga + Polyphaga are monophyletic in most analyses (with a BPP of 1.00, a likelihood bootstrap of 60, but a parsimony bootstrap value < 50). Adephaga is monophyletic in the most parsimonious trees, but not in most other analyses, in which Polyphaga is found within Hydradephaga.

Geadephaga is strongly supported in the Bayesian analysis (BPP = 0.96); it is monophyletic in the most parsimonious and maximum likelihood trees, but for parsimony and likelihood the bootstrap values are < 50. The sister group of Geadephaga is Hydradephaga or Hydradephaga + Polyphaga in most analyses in which it is evident, although this is not strongly supported. The single exception is the Bayesian analysis with Adephaga constrained to be monophyletic, which has Gyrinidae as the sister to Geadephaga (with BPP of 0.85).

Trachypachids are recovered as monophyletic in most analyses, with a BPP of 0.99, but with bootstrap values < 60. The sister group of trachypachids is only evident in the most parsimonious trees (for which the sister group is Carabitae) and in the maximum likelihood tree (for which it is the carabid tribe Broscini).

Hydradephaga is monophyletic in the most parsimonious trees, but not in any other analyses. For example, if Adephaga

is constrained to be monophyletic, the BPP of Geadephaga + Gyrinidae is 0.85, rendering Hydradephaga paraphyletic.

Polyphaga exclusive of Scirtoidea is recovered in the Bayesian analysis (BPP = 1.00), parsimony analysis (bootstrap value of 91), and likelihood analysis (bootstrap value of 95).

28S rDNA

All standard analyses of 28S rDNA show Coleoptera as paraphyletic because of inclusion of the two Neuroptera sequences (*Oliarces* and *Mantispa*) within Carabidae, near some relatively long branches (arrow in Fig. 4).

Adephaga is monophyletic in the Bayesian analysis (BPP = 1.00), most parsimonious trees, and maximum likelihood tree if Coleoptera is constrained to be monophyletic, but the bootstrap values are less than 50.

Geadephaga is recovered as monophyletic in all 28S rDNA analyses if Coleoptera is constrained to be monophyletic, with a BPP of 1.0, a parsimony boostrap value of 64, and a likelihood bootstrap value of 78. The sister of Geadephaga is either *Rhantus* or *Laccophilus* in these analyses.

Trachypachids are not recovered as monophyletic in any analysis, and thus their sister group is undetermined.

Hydradephaga is also not monophyletic in any 28S rDNA analysis, with moderate support against monophyly (BPP against of 0.73 if Coleoptera is not constrained to be monophyletic, 0.87 if it is).

The monophyly of Polyphaga exclusive of Scirtoidea is supported in all analyses, with BPP values between 0.72 and 0.78, and bootstrap values between 65 and 76.

Combined matrix

Bayesian analysis of the combined matrix shows strong support for monophyly of Coleoptera, each of the four suborders, Geadephaga, Trachypachidae, and the non-scirtoid Polyphaga, with BPP between 0.99 and 1.0 (Fig. 5).

Geadephaga is not supported as monophyletic in the parsimony analyses because of inclusion of *Oliarces*, *Mantispa*, and the two *Clambus* species within Carabidae, again associated with long branches within Carabidae. Enforcing the constraint of Coleoptera monophyly yields a monophyletic Geadephaga in parsimony analyses, with bootstrap values between 82 and 84. Geadephaga is monophyletic in all likelihood analyses of the combined data, with bootstrap values from 80 to 88. The sister group of Geadephaga in all analyses was Gyrinidae, unless all nucleotides (as opposed to amino acids) for *wingless* were analysed, in which case the sister was Hydradephaga.

The sister group of Trachypachidae in combined analyses is a large clade comprising almost all carabids; only *Elaphrus*, the carabites, and (in some analyses) migadopines are excluded.

Hydradephaga is monophyletic only in most parsimonious trees and maximum likelihood trees of the combined nucleotide matrix with all *wingless* positions included. Hydradephaga is not monophyletic with *wingless* treated as amino acids and all Bayesian analyses; in the latter analyses, the BPP against monophyly, that is, in support of a paraphyletic Hydradephaga, is between 0.72 and 0.95.

Non-scirtoid Polyphaga is strongly supported as monophyletic with the combined data, with a BPP of 1.00, parsimony bootstrap values of 77–95, and likelihood bootstrap values of 98–100.

The combined matrix also indicates a sister group relationship between Myxophaga plus Archostemata and Polyphaga plus Adephaga. The BPP of Polyphaga plus Adephaga is 0.99, the parsimony bootstrap value is 51, and the likelihood bootstrap value is 50 in one analysis, < 50 in the other.

Mitochondrial 16S rDNA

Mitochondrial large subunit ribosomal DNA (16S rDNA) yields trees that are less compatible with morphological data than the other genes. For example, dytiscids, haliplids, and carabids each appear polyphyletic in the analyses (Fig. 6), suggesting that this gene is not as appropriate for studying family level divergences within Adephaga.

The single trachypachid included, *Trachypachus holmbergi*, groups with a carabid, *Elaphrus*, with BPP 0.73, and in the most parsimonious and maximum likelihood trees (but with bootstrap values < 50). Because of the lack of congruence of this gene with other data, the evidence provided about the placement of *Trachypachus* should be considered weaker. Nonetheless, 16S mitochondrial rDNA does not provide evidence that trachypachids are related to dytiscoids.

The monophyly of Polyphaga exclusive of scirtoids and derodontoids is supported with this gene as well, with BPP 1.0; this clade is also present in the most parsimonious and maximum likelihood trees, but with bootstrap values < 50.

Habitat transitions

For both adult and larval habitat, the unconstrained Bayesian analysis does not strongly favour two transitions to an aquatic lifestyle (BPP 0.432 for adults, BPP 0.307 for larvae) or one change from aquatic to terrestrial lifestyle (BPP 0.502 for adults, BPP 0.628 for larvae) within Adephaga, although the latter is slightly favoured (Table 5). However, if it is considered unlikely that an aquatic to terrestrial transition took place (Crowson 1960), and MPRs with such changes are excluded, then the Bayesian analysis strongly supports two changes from terrestrial to aquatic habit (Table 5).

The ancestral habitat of Adephaga was reconstructed overwhelmingly as aquatic using likelihood analysis on trees with unsmoothed branches (Table 6); in contrast, parsimony analysis and likelihood analyses with chronogram branch lengths do not as clearly choose between an aquatic or terrestrial ancestor.

Table 5 Posterior probabilities (BPP) of numbers of transitions between habitats within Adephaga, calculated from the Bayesian analysis of the combined data matrix (wingless amino acids, excluding the indel-rich region, plus 18S rDNA and 28S rDNA).

Change	Number	Unconstrained	No aquatic → terrestrial
Adult habitat			
terrestrial → aquatic	0	0.502	0.0
	1	0.066	0.071
	2	0.432	0.929
Aquatic → terrestrial	0	0.498	1.0
	1	0.502	0.0
	2	0.0	0.0
Larval habitat			
Terrestrial → aquatic	0	0.628	0.0
	1	0.065	0.075
	2	0.307	0.925
Aquatic → terrestrial	0	0.372	1.0
	1	0.628	0.0

The 'unconstrained' values are the BPP if all transitions are allowed; 'no aquatic → terrestrial' are the values only over reconstructions with no transitions from aquatic to terrestrial habits.

Table 6 Proportion of the 11 226 Bayesian trees from the combined data matrix (with *wingless* amino acids, excluding the indel-rich region, plus 18S rDNA and 28S rDNA) that support either a terrestrial or aquatic ancestral habitat for adults and larvae of Adephaga, as reconstructed by both likelihood and parsimony.

Reconstruction method	Ancestral state	Adult habitat	Larval habitat
Likelihood	Terrestrial	0.03	0.0
	Aquatic	0.97	1.0
	Number of trees	*10 421*	*10 486*
Likelihood (chronogram)	Terrestrial	0.389	0.199
	Aquatic	0.611	0.801
	Number of trees	*2098*	*2989*
Parsimony	Terrestrial	0.488	0.353
	Aquatic	0.512	0.647
	Number of trees	*1520*	*2018*

For likelihood analyses, trees were only counted if one ancestral state has a –ln-likelihood value at least 2.0 units higher than the other states. The first likelihood analysis used branch lengths as inferred from the molecular data; the second used chronogram branch lengths. For parsimony analyses, trees were counted if there is only a single most parsimonious ancestral state at the ancestral node of Adephaga. The number of trees that support one state over the other is also given; the remainder of the 11 226 trees do not support one state unequivocally.

Discussion

Geadephaga

The monophyly of Geadephaga is supported by individual analyses of 18S rDNA, 28S rDNA, *wingless*, and in the combined analyses (Fig. 7). This holds true for Bayesian, likelihood, and parsimony methods, although the support is only strong for Bayesian analyses. The congruence of these data sources, as well as the consistency of the result with some morphological analyses (Beutel & Haas 1996; Beutel *et al.* 2006), confirms the placement of Trachypachidae within Geadephaga.

The sister group of Trachypachidae within Geadephaga is not evident, however. It varies from analysis to analysis, and gene to gene, but in all cases trachypachids are placed among the 'basal grade' of Carabidae, for example, with Carabitae, migadopines, or elaphrines.

The composition of the family Carabidae has varied in the literature, with three groups in particular being variously included or excluded in classifications: Cicindelinae, Rhysodinae, and Trachypachidae. In most of our analyses Carabidae is not monophyletic, no matter which of these groups are included or excluded from the family, unless one considers Carabidae to include all of these groups, that is, that Carabidae is equivalent to Geadephaga. Additional analyses and data are required to determine appropriate boundaries for Carabidae, short of all Geadephaga, but these are beyond the scope of this paper.

The sister group of Geadephaga also varies among analyses. The *wingless* gene suggests gyrinids in whole or in part as the sister; 18S rDNA indicates Hydradephaga, Hydradephaga + Polyphaga, or gyrinids; 28S rDNA suggests part of Dytiscidae. The combined data suggest gyrinids or Hydradephaga as the sister group of Geadephaga, depending upon the analyses, and those appear to be the best hypotheses to examine with future research.

Hydradephaga

In contrast to previous studies (Shull *et al.* 2001; Ribera *et al.* 2002; Hunt *et al.* 2007) we found the monophyly of Hydradephaga to be poorly supported; it is only supported by parsimony analysis of 18S rDNA, and in two combined analyses with all nucleotide data. Bayesian analyses of each gene and the combined matrix provide evidence against monophyly (Fig. 7).

Coleoptera outside of Adephaga

The relationships of the suborders of beetles are controversial, with several conflicting hypotheses. Several authors (e.g. Crowson 1960; Beutel 1997; Beutel & Haas 2000; Friedrich & Beutel 2006) have proposed a sister group relationship between Polyphaga and Myxophaga. Others (Lawrence & Newton 1982; Kukalová-Peck & Lawrence 1993) consider Polyphaga to be the sister group of the remaining beetles. A sister group relationship between Adephaga and Polyphaga has been previously suggested by ribosomal DNA (Shull *et al.* 2001; Caterino *et al.* 2002; Hunt *et al.* 2007). This is supported by the current study (Fig. 5), which has a denser sampling of

ribosomal genes for Archostemata than has previously been available. Unfortunately, because of our lack of *wingless* data for Myxophaga, this result is based only upon the ribosomal genes, and we cannot now provide a new, independent source of information about the relationships of suborders. A clearer picture of subordinal relationships awaits sampling of more genes and addition of more insects other than beetles.

The placement of scirtoids as 'basal' polyphagans, that is, the bulk of Polyphaga, exclusive of scirtoids, is monophyletic, is strongly supported by 18S rDNA, *wingless*, and combined analyses. This placement has previously been suggested from 18S rDNA data alone (Caterino *et al*. 2002; Hunt *et al*. 2007). While scirtoids have previously been proposed to be basal polyphagans based on morphological data (Lawrence 1999, 2001; Friedrich & Beutel 2006), a recent analysis of morphological data favours the placement of Scirtoidea within Elateriformia rather than as basal (Friedrich & Beutel 2006). We suggest further morphological examination of Polyphaga exclusive of Scirtoidea and Derodontoidea might yield additional synapomorphies of that large group.

The scirtoids appear as monophyletic in only some of the analyses, and the molecular diversity within this group is notable. For example, the uncorrected ('P') distance between the two species of *Clambus* studied (*C. seminulum* and *C. arnetti*) is 0.203, whereas the greatest distance between any two non-scirtoid polyphagans is 0.151. The branch lengths of scirtoids (*Clambus*, *Cyphon*, *Prionocyphon*) in all three genes (Figs 2–4) are unusually long, suggesting either a high evolutionary rate or an ancient divergence.

Fig. 8 A, B. Habitats of two species of trachypachids. —A. *Trachypachus slevini*, USA: Oregon: Lincoln Co., Moolack Beach, 44.7093°N 124.0605°W. —B. *Systolosoma breve*, CHILE: Reg. IX: Rio Pedregoso, E of El Pastal, 646 m, 39.1676°S 72.0001°W. Trachypachids were found in areas indicated by arrows, on the slope of material eroded from the neighbouring bank.

Morphology and the evolution of habitat

An unconstrained likelihood analysis of habitat on the Bayesian trees suggests that the ancestral adephagan was aquatic, and parsimony analysis of changes between states shows the highest posterior probability for the hypothesis of one change to terrestrial larval habitat within Adephaga. However, if reversal from aquatic to terrestrial habitat is considered unlikely on other grounds (as suggested by Crowson 1960), and disallowed, then the data favour two origins of aquatic larvae from a terrestrial ancestor in Adephaga.

Whatever the ancestral state of Adephaga and the number of habitat transitions, our data indicate that trachypachids are members of Geadephaga, with terrestrial ancestors, and have likely gained the derived morphological states they share with Dytiscoidea independently. As noted in Shull *et al.* (2001), the three North American species of *Trachypachus* are most frequently found on loose soil (Fig. 8A) in which they quickly burrow, as if swimming. More recent observations by DRM of *Systolosoma breve* in Chile indicate that they occur in similar habitats: in dry, very loose soil of *Nothofagus-Araucaria* forests, or on loose soil eroded along cutbanks of roads (Fig. 8B). K.W. Will (pers. commun., 2006) reports a similar habitat for *S. lateritium*. While it is speculative that morphological traits for 'swimming' in loose soil might converge upon those found in aquatic beetles, this hypothesis could be tested by examining the traits of other beetles that have independently entered similar habits.

Acknowledgements

Authors thank those who provided specimens: Daniel Gustafson *(Priacma)*, Clarke Scholtz *(Dhysores)*, David Kavanaugh *(Loricera)*, S. Endrödy-Younga *(Torridincola)*, Peter Johns *(Oregus)*, Kip Will *(Broscosoma*, *Systolosoma breve)*, Jeffrey Burne *(Cupes)*, Rolf Beutel *(Agabus*, *Liodessus*, *Copelatus*, *Noterus)*, Al Newton and Margaret Thayer *(Systolosoma lateritium*, *Antarctonomus)*, V. Shull *(Hygrobia)*, Wayne Maddison *(Amblycheila*, *Pachyteles)*, Harold Greeney *(Oxycheila)*, A.E. Arnold *(Ctenostoma*, *Acmaeodera)*, Dan Young *(Micromalthus)*, Lyle Browning *(Oliarces)*, Carl Olson *(Xanthopygus)*. We also thank Betsy Arnold, Julia Amerongen Maddison, Helen Amerongen, and David Swofford for their help with field work.

Authors are grateful to those who helped identify specimens for us: Rob Roughley (dytiscids), Jennifer Babin (gyrinids), Rex Kenner and Sharon Jasper (haliplids), P.M. Johns *(Oregus)*, S. Endrödy-Younga *(Torridincola)*, and Ricardo Sciaky *(Brosocoma)*.

Authors thank Betsy Arnold and Alex Wild for helpful comments that improved the manuscript, and Wayne Maddison for use of computers. Travis Wheeler conducted the Opal alignment for us. Thanks to Emmanuel Paradis for help with the use of APE.

This project was funded primarily by NSF grant DEB-9420219 to DRM, with EF-0531754 funding primer development. RRG and JJC's alignment work was funded through NIH grant GM067317.

References

Abascal, F., Zardoya, R. & Posada, D. (2005). ProtTest: Selection of best-fit models of protein evolution. *Bioinformatics*, *21*, 2104–2105.

Alarie, Y. & Bilton, D. T. (2005). Larval morphology of Aspidytidae (Coleoptera: Adephaga) and its phylogenetic implications. *Annals of the Entomological Society of America*, *98*, 417–430.

Alarie, Y. & Michat, M. C. (2007). Primary setae and pores on the maxilla of larvae of the subfamily Hydroporinae (Coleoptera: Adephaga: Dytiscidae): Ground plan pattern reconsidered. *Coleopterists Bulletin*, *61*, 310–317.

Alverson, A. J., Cannone, J. J., Gutell, R. R. & Theriot, E. C. (2006). The evolution of elongate shape in diatoms. *Journal of Phycology*, *42*, 655–668.

Arndt, E. (1989). Carabid larvae (Coleoptera, Carabidae) as subject of phylogenetical research. *Entomologische Nachrichten und Berichte*, *33*, 255–260.

Arndt, E. & Beutel, R. G. (1995). Larval morphology of *Systolosoma* Solier and *Trachypachus* Motschulsky (Coleoptera: Trachypachidae) with phylogenetic considerations. *Entomologica Scandinavica*, *26*, 439–446.

Attygalle, A. B., Wu, X. G., Ruzicka, J., Rao, S., Garcia, S., Herath, K. *et al*. (2004). Defensive chemicals of two species of *Trachypachus* Motschulski. *Journal of Chemical Ecology*, *30*, 577–588.

Baehr, M. (1979). Vergleichende Untersuchungen am Skelett und an der Coxalmuskulatur des Prothorax der Coleoptera. Ein Beitrag zur Klarung der phylogenetischen Beziehungen der Adephaga (Coleoptera, Insecta). *Zoologica (Stuttgart)*, *44*, 1–76. No. 130.

Bell, R. T. (1966). *Trachypachus* and the origin of the Hydradephaga (Coleoptera). *Coleopterists Bulletin*, *20*, 107–112.

Bell, R. T. (1967). Coxal cavities and the classification of the Adephaga (Coleoptera). *Annals of the Entomological Society of America*, *60*, 101–107.

Bell, R. T. (1983). What is *Trachypachus*? (Coleoptera: Trachypachidae). *Coleopterists Bulletin*, *36*, 590–596.

Bell, R. T. (1994). Beetles that cannot bite: Functional morphology of the head of adult rhysodines (Coleoptera: Carabidae or Rhysodidae). *Canadian Entomologist*, *126*, 667–672.

Beutel, R. G. (1988). Studies of the metathorax of the trout-stream beetle, *Amphizoa lecontei* Matthews (Coleoptera, Amphizoidae) — contribution towards clarification of the systematic position of Amphizoidae. *International Journal of Insect Morphology and Embryology*, *17*, 63–81.

Beutel, R. G. (1989). The head of *Spanglerogyrus albiventris* Folkerts (Coleoptera: Gyrinidae) contribution towards clarification of the phylogeny of Gyrinidae and Adephaga. *Zoologische Jahrbuecher Abteilung Fuer Anatomie und Ontogenie der Tiere*, *118*, 431–461.

Beutel, R. G. (1990). Metathoracic features of *Omoglymmius hamatus* and their significance for classification of Rhysodini (Coleoptera, Adephaga). *Entomologia Generalis*, *15*, 185–201.

Beutel, R. G. (1992a). Larval head structures of *Omoglymmius hamatus* and their implications for the relationships of Rhysodidae (Coleoptera, Adephaga). *Entomologica Scandinavica*, *23*, 169–184.

Beutel, R. G. (1992b). Phylogenetic analysis of thoracic structures of Carabidae (Coleoptera, Adephaga). *Zeitschrift Fur Zoologische Systematik und Evolutionsforschung*, *30*, 53–74.

Beutel, R. G. (1992c). Study on the systematic position of Metriini based on characters of the larval head (Coleoptera, Carabidae). *Systematic Entomology*, *17*, 207–218.

Beutel, R. G. (1993). Phylogenetic analysis of Adephaga (Coleoptera) based on characters of the larval head. *Systematic Entomology*, *18*, 127–147.

Beutel, R. G. (1994). Study on the systematic position of *Systolosoma breve* Solier (Adephaga, Trachypachidae) based on characters of the thorax. *Studies on Neotropical Fauna and Environment*, *29*, 161–167.

Beutel, R. G. (1995). The Adephaga (Coleoptera): phylogeny and evolutionary history. In J. Pakaluk & S. A. Slipinski (Eds) *Biology, Phylogeny, and Classification of Coleoptera* (pp. 173–218). Warzawa: Museum Zoologii PAN.

Beutel, R. G. (1997). Über Phylogenese und Evolution der Coleoptera (Insecta), insbesondere der Adephaga. *Abhandlungen des Naturwissenshaftlichen Vereins in Hamburg*, *31*, 1–164.

Beutel, R. G. (1998). Trachypachidae and the phylogeny of Adephaga. In: G. E. Ball, A. Casale & A. V. Taglianti (Eds) *Phylogeny and Classification of Carabidea (Coleoptera: Adephaga). Proceedings of XX International Congress of Entomology Symposium, 28 August 1996, Florence, Italy* (pp. 81–106). Torino, Italy: Museo Regional Science Naturali.

Beutel, R. G. & Haas, A. (1996). Phylogenetic analysis of larval and adult characters of Adephaga (Coleoptera) using cladistic computer programs. *Entomologica Scandinavica*, *27*, 197–205.

Beutel, R. G. & Haas, F. (2000). Phylogenetic relationships of the suborders of Coleoptera (Insecta). *Cladistics — the International Journal of the Willi Hennig Society*, *16*, 103–141.

Beutel, R. G. & Roughley, R. E. (1988). On the systematic position of the family Gyrinidae (Coleoptera, Adephaga). *Zeitschrift Fur Zoologische Systematik und Evolutionsforschung*, *26*, 380–400.

Beutel, R. G. & Roughley, R. E. (1993). Phylogenetic analysis of Gyrinidae based on characters of the larval head (Coleoptera, Adephaga). *Entomologica Scandinavica*, *24*, 459–468.

Beutel, R. G., Balke, M. & Steiner, W. E. (2006). The systematic position of Meruidae (Coleoptera, Adephaga) and the phylogeny of the smaller aquatic adephagan beetle families. *Cladistics*, *22*, 102–131.

Beutel, R. G., Ribera, I. & Bininda-Emonds, O. R. P. (2007). A genus-level supertree of Adephaga (Coleoptera). *Organisms Diversity and Evolution*, 7, 255–269.

Beutel, R. G., Ge, S.-Q. & Hörnschemeyer, T. (2008). On the head morphology of *Tetraphalerus*, the phylogeny of Archostemata and the basal branching events in Coleoptera. *Cladistics*, *24*, 270–298.

Bils, W. (1976). Das Abdomende weiblicher, terrestrisch lebender Adephaga (Coleoptera) und seine Bedeutung für die Phylogenie. *Zoomorphologie*, *84*, 113–193.

Bousquet, Y. (1986). Description of 1st-instar larva of *Metrius contractus* Eschscholtz (Coleoptera, Carabidae) with remarks about phylogenetic-

relationships and ranking of the genus *Metrius* Eschscholtz. *Canadian Entomologist*, *118*, 373–388.

Burmeister, E. G. (1976). Ovipositor of Hydradephaga (Coleoptera) and its phylogenetic significance, with special considerations of Dytiscidae. *Zoomorphologie*, *85*, 165–257.

Burmeister, E. G. (1990a). On the systematic position of Amphizoidae, emphasizing features of the female genital organs (Insecta: Coleoptera: Adephaga). *Quaestiones Entomologicae*, *26*, 245–272.

Burmeister, E. G. (1990b). The female genital structures of *Spanglerogyrus albiventris* Folkerts, 1979: a contribution to the systematic position of the Gyrinidae. *Spixiana*, *13*, 253–265.

Caterino, M. S., Shull, V. L., Hammond, P. M. & Vogler, A. P. (2002). Basal relationships of Coleoptera inferred from 18S rDNA sequences. *Zoologica Scripta*, *31*, 41–49.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G. *et al*. (2003). Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research*, *31*, 3497–3500.

Crowson, R. A. (1960). The phylogeny of Coleoptera. *Annual Review of Entomology*, *5*, 111–134.

Dettner, K. (1985). Ecological and phylogenetic significance of defensive compounds from pygidial glands of Hydradephaga (Coleoptera). *Proceedings of the Academy of Natural Sciences of Philadelphia*, *137*, 156–171.

Deuve, T. (1993). L'abdomen et les genitalia des femelles de Coléoptères Adephaga. *Mémoires du Muséum National D'Histoire Naturelle*, *155*, 1–184.

Drummond, A. & Strimmer, K. (2001). PAL: an object-oriented programming library for molecular evolution and phylogenetics. *Bioinformatics*, *17*, 662–663.

Erwin, T. L. (1985). The taxon pulse: a general pattern of lineage radiation and extinction among carabid beetles. *Series Entomologica (Dordrecht)*, 437–472.

Evans, M. E. G. (1982). Early evolution of the Adephaga — some locomotor speculations. *Coleopterists Bulletin*, *36*, 597–607.

Forsyth, D. J. (1972). The structure of the pygidial defence glands of Carabidae (Coleoptera). *Transactions of the Zoological Society of London*, *32*, 249–309.

Friedrich, F. & Beutel, R. G. (2006). The pterothoracic skeletomuscular system of Scirtoidea (Coleoptera: Polyphaga) and its implications for the high-level phylogeny of beetles. *Journal of Zoological Systematics and Evolutionary Research*, *44*, 290–315.

Green, P. (1999). PHRAP. *Version 0.990329*. Available at URL: http://phrap.org.

Green, P. & Ewing, B. (2002). Phred. *Ver. 0.020425c*. Available via http://phrap.org.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*, 696–704.

Gutell, R. R., Lee, J. C. & Cannone, J. J. (2002). The accuracy of ribosomal RNA comparative structure models. *Current Opinion in Structural Biology*, *12*, 301–310.

Gutell, R. R., Weiser, B., Woese, C. R. & Noller, H. F. (1985). Comparative anatomy of 16S-like ribosomal RNA. *Progress in Nucleic Acid Research and Molecular Biology*, *32*, 155–216.

Hammond, P. M. (1979). Wing-folding mechanisms of beetles, with special reference to investigations of Adephagan phylogeny (Coleoptera). In T. L. Erwin, G. E. Ball, D. R. Whitehead & A. L. Halpern (Eds) *Carabid Beetles: Their Evolution, Natural History, and Classification* (pp. 113–180). The Hague: Dr W. Junk bv.

Heath, R. V. & Evans, M. E. G. (1990). The relationship between the ventral nerve cord, body size and phylogeny in ground beetles (Coleoptera: Carabidae). *Zoological Journal of Linnean Society*, *98*, 259–294.

Huelsenbeck, J. P., Rannala, B. & Masly, J. P. (2000). Accommodating phylogenetic uncertainty in evolutionary studies. *Science*, *288*, 2349–2350.

Huelsenbeck, J. & Ronquist, F. (2005). MRBAYES. *Version 3.1.2*. Available at URL: http://www.mrbayes.net.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O. S., Wild, R. *et al*. (2007). A Comprehensive Phylogeny of Beetles Reveals the Evolutionary Origins of a Superradiation. *Science*, *318*, 1913–1916.

Kanehisa, K. & Murase, M. (1977). Comparative study of the pygidial defensive systems of carabid beetles. *Applied Entomology and Zoology*, *12*, 225–235.

Kavanaugh, D. H. (1986). A systematic review of Amphizoid beetles (Amphizoidae: Coleoptera) and their phylogenetic relationships to other Adephaga. *Proceedings of the California Academy of Sciences*, *44*, 67–109.

Kukalová-Peck, J. & Lawrence, J. F. (1993). Evolution of the hind wing in Coleoptera. *Canadian Entomologist*, *125*, 181–258.

Lawrence, J. F. (1999). The Australian Ommatidae (Coleoptera: Archostemata): new species, larva and discussion of relationships. *Invertebrate Taxonomy*, *13*, 369–390.

Lawrence, J. F. (2001). A new genus of valdivian Scirtidae (Coleoptera) with comments on Scirtoidea and the beetle suborders. *Special Publication of the Japanese Coleoptera Society, Osaka*, *1*, 351–361.

Lawrence, J. F. & Newton, A. F. Jr (1982). Evolution and classification of beetles. *Annual Review of Ecology Evolution and Systematics*, *13*, 261–290.

Lewis, P. O. (2001). A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, *50*, 913–925.

Lorenz, W. (2005). *A Systematic List of Extant Ground Beetles of the World (Coleoptera 'Geadephaga': Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae)*. Tutzing: Lorenz, W.

Maddison, D. R. (2008). Systematics of the North American beetle subgenus *Pseudoperyphus* (Coleoptera: Carabidae: *Bembidion*) based upon morphological, chromosomal, and molecular data. *Annals of Carnegie Museum*, 77, 147–193.

Maddison, D. R. & Maddison, W. P. (2005). MACCLADE *4: Analysis of Phylogeny and Character Evolution*, *Version 4.08*. Sunderland, Massachusetts: Sinauer Associates.

Maddison, D. R. & Maddison, W. P. (2007). CHROMASEQ: a Mesquite module for analyzing sequence chromatograms. *Version 0.92*. Available at URL: http://mesquiteproject.org/packages/chromaseq.

Maddison, D. R., Baker, M. D. & Ober, K. A. (1999). Phylogeny of carabid beetles as inferred from 18S ribosomal DNA (Coleoptera: Carabidae). *Systematic Entomology*, *24*, 103–138.

Maddison, W. P. & Maddison, D. R. (2008). MESQUITE: a modular system for evolutionary analysis. *Version 2.5*. Available at URL: http://mesquiteproject.org.

Moore, B. P. (1979). Chemical defense in carabids and its bearing on phylogeny. In T. L. Erwin, G. E. Ball, D. R. Whitehead & A. L. Halpern (Eds) *Carabid Beetles: Their Evolution, Natural History, and Classification* (pp. 193–203). The Hague: Dr. W. Junk bv.

Moore, W. (2008). Phylogeny of the Western Hemisphere Ozaenini (Coleoptera: Carabidae: Paussinae) based on DNA sequence data. *Annals of Carnegie Museum*, 77, 79–92.

Nichols, S. W. (1985). *Omophron* and the origin of Hydradephaga (Insecta: Coleoptera: Adephaga). *Proceedings of the Academy of Natural Sciences of Philadelphia*, *137*, 182–201.

Nilsson, A. N. (2001). Dytiscidae (Coleoptera). *World Catalogue of Insects*, *3*, 1–395.

Noller, H. F., Kop, J., Wheaton, V., Brosius, J., Gutell, R. R., Kopylov, A. M. *et al*. (1981). Secondary structure model for 23S ribosomal RNA. *Nucleic Acids Research*, *9*, 6167–6189.

Ober, K. A. (2002). Phylogenetic relationships of the carabid subfamily Harpalinae (Coleoptera) based on molecular sequence data. *Molecular Phylogenetics and Evolution*, *24*, 228–248.

Paradis, E., Claude, J. & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 289–290.

Ponomarenko, A. G. (1977). Suborder Adephaga. In L. V. Arnoldi, V. V. Zherikin, L. M. Nikritin & A. G. Ponomarenko (Eds) (pp. 1–104). Moscow: Trudi Paleontologiceskogo Instituta, Academija Nauk SSSR.

Posada, D. (2005). MODELTEST: A tool to select the best-fit model of nucleotide substitution. *Version 3.7*. Available at URL: http://darwin.uvigo.es.

Rambaut, A. & Drummond, A. (2004). Tracer. *Version 1.3*. Available at URL: http://evolve.zoo.ox.ac.uk/software.html?id=tracer.

Ribera, I., Hogan, J. E. & Vogler, A. P. (2002). Phylogeny of hydradephagan water beetles inferred from 18S rRNA sequences. *Molecular Phylogenetics and Evolution*, *23*, 43–62.

Roughley, R. E. (1981). Trachypachidae and Hydradephaga (Coleoptera) — a monophyletic unit? *Pan-Pacific Entomologist*, *57*, 273–285.

Sanderson, M. J. (2002). Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution*, *19*, 101–109.

Sankoff, D. D. (1975). Minimal mutation trees of sequences. *SIAM Journal of Applied Mathematics*, *28*, 35–42.

Seeger, W. (1971). Morphologie, Bionomie und Ethologie von Halipliden, unter besonderer Berücksichtigung funktionsmorphologischer Gesichtspunkte (Haliplidae; Coleoptera). *Archiv für Hydrobiologie*, *68*, 400–435.

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. *Systematic Biology*, *50*, 945–969.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, btl446.

Swofford, D. L. (2002). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version. 4.0b10*. Sunderland: Sinauer Associates.

Thiele, H. (1977). *Carabid Beetles in Their Environments: a Study on Habitat Selection by Adaptations in Physiology and Behaviour*. Berlin: Springer-Verlag.

Van der Auwera, G., Chapelle, S. & De Wachter, R. (1994). Structure of the large ribosomal subunit RNA of *Phytophthora megasperma*, and phylogeny of the oomycetes. *FEBS Letters*, *338*, 133–136.

Ward, R. D. (1979). Metathoracic wing structures as phylogenetic indicators in the Adephaga (Coleoptera). In T. L. Erwin, G. E. Ball, D. R. Whitehead & A. L. Halpern (Eds) *Carabid Beetles: Their Evolution, Natural History, and Classification* (pp. 181–191). The Hague: Dr W. Junk bv.

Ward, P. S. & Downie, D. A. (2005). The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. *Systematic Entomology*, *30*, 310–335.

Wheeler, T. J. & Kececioglu, J. D. (2007). Multiple alignments by aligning alignments. *Bioinformatics*, *23*, i559–i568.

Wild, A. L. & Maddison, D. R. (2008). Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution*, *48*, 877–891.

Woese, C. R., Magrum, L. J., Gupta, R., Siegel, R. B., Stahl, D. A., Kop, J. *et al*. (1980). Secondary structure model for bacterial 16S ribosomal RNA: phylogenetic, enzymatic and chemical evidence. *Nucleic Acids Research*, *8*, 2275–2293.

Wray, C. G., Lee, J. J. & Desalle, R. (1993). Extraction and Enzymatic Characterization of Foraminiferal DNA. *Micropaleontology*, *39*, 69–73.

Yahiro, K. (1990). A comparative morphology of the alimentary canal in the adults of ground-beetles (Coleoptera). I. Classification into the types. *Esakia*, *60*, 35–44.

Zwickl, D. J. (2006). Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Thesis. The University of Texas at Austin.