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

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Abstract

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, four Archostemata, three Myxophaga, and ten 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.

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

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.x

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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 as 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, 1992c, 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, amphotropical 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; R. D. 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, 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 and Roughley (1988) proposed that Gyrinidae is the sister group of remaining Adephaga, with the remainder comprised of two lineages: Haliplidae, and Dytiscoidea plus Geadephaga.

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 colonizations 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 analyze data from each gene in both separate and combined analyses. We also re-analyze 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 through 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. 30 to 40 cycles were used, with annealing temperatures of 50°C (18S rDNA), 53°C to 54°C (28S rDNA), or 51°C to 56°C (*wingless*). For some species (most notably members of Hydradephaga, Archostemata, and Megaloptera), amplification of ribosomal genes required addition of 5µl glycerol and 3µl dimethyl sulfoxide (DMSO) to the 50µl 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 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 judgments 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 in length from 1777 to 2633 nucleotides (nt); with the regions of low confidence excluded, the range in sequence length is 1566 to 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 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 one species represented in our data (see Tables 1 through 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 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 1,000 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: (1) the 28S matrix, which reached 0.0135 after 100 million generations, and (2) 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 burnin 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 6,800 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 1/3, and the fraction showing two changes is 2/3. 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 another 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", i.e., 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 are 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 analyzed as nucleotides or amino acids. Trees inferred with the indel-rich region excluded, and with data analyzed 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.

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 analyzed as amino acids, but bootstrap values are less than 50 percent. 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 to 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 to 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 Carabidae) 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 bootstrap 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.

Hydradeephaga 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 analyzed, in which case the sister was Hydradeephaga.

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.

Hydradeephaga is monophyletic only in most parsimonious trees and maximum likelihood trees of the combined nucleotide matrix with all *wingless* positions included. Hydradeephaga 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 Hydradeephaga, 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 to 95, and likelihood bootstrap values of 98 to 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, less than 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 favor 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 favored (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.

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 Carabidae, 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 is 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; Kukulová-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, i.e., that 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 favors 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.

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 favor 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. 7A) 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. 7B). K.W. Will (pers. comm., 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.

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Fig. 1.

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.

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 *Oligarces* 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 unconstrained analyses. The Geadephaga branch is indicated by an asterisk.

Fig. 5.

90% majority-rule consensus tree of 11,226 post-burn-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 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, gray 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 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.

A monophyly of Geadeephaga						B monophyly of Hydradeephaga					
	Bay	ML	MLB	P	PB		Bay	ML	MLB	P	PB
wg aa	100		64		?		97	x	?	?	?
wg nuc	99		?	a	?		62	x	?	x	?
18S	96		?		?		85 ^a	x	?		?
28S	100*	*	78*		64*		87	x	?	x	?
wg aa+18S+28S	100						92				
wg nuc+18S+28S	100		88	*	84*		72		?	?	?
16S mt rDNA	65	x	?	x	?		?	x	?	x	?

Fig. 7.

Summary of support for monophyly of (A) Geadeephaga and (B) Hydradeephaga. 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 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.

Fig. 8.

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, 646m, 39.1676°S 72.0001°W. Trachypachids were found in areas indicated by arrows, on the slope of material eroded from the neighboring bank.

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

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

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	<i>Trachypachus gibbsii</i> LeConte	AF002808	EU797394	EU797331
	<i>Trachypachus slevini</i> Van Dyke	EU797418	EU797396	EU797333
	<i>Trachypachus holmbergi</i> Mannerheim	AF002807	EU797395	EU797332
	<i>Systolosoma lateritium</i> Négre	AF012522	EU797391	EU797329
	<i>Systolosoma breve</i> Solier	EU797416	EU797390	EU797328
Carabidae	<i>Metrius contractus</i> Eschscholtz	AF012515	AF398687	AF398605
	<i>Pachyteles striola</i> species complex	AF012517	EU797377	EU797319
	<i>Arthropterus</i> sp.	AF012516	AF398644	AF398570
	<i>Amblycheila baroni</i> Rivers	AF423057	EU797338	EU797284
	<i>Ctenostoma erwini</i> Naviaux	EU797405	EU797350	EU797297
	<i>Oxycheila nigroaenea</i> Bates	AF201393	-	-
	<i>Oxycheila cf. glabra</i> Waterhouse	-	EU797376	EU797318
	<i>Cicindela sedecimpunctata</i> Klug	AF012518	AF398660	AF398579
	<i>Dhysores</i> sp.	EU797407	EU797353	EU797300
	<i>Omoglymmius hamatus</i> (LeConte)	AF012520	EU797372	AF398609
	<i>Clinidium calcaratum</i> LeConte	AF012521	-	-
	<i>Clinidium balduffi</i> Bell	-	EU797348	EU797294
	<i>Nebria hudsonica</i> LeConte	AF002805	AF398676	AF398608
	<i>Opisthius richardsoni</i> Kirby	AF012511	EU797374	EU797316
	<i>Notiophilus semiopacus</i> Eschscholtz	AF002804	EU797369	EU797312
	<i>Loricerafoveata</i> LeConte	AF012503	EU797365	-
	<i>Elaphrus californicus</i> Mannerheim	AF012514	AF398639	AF398563
	<i>Omophron oblitteratum</i> G.H. Horn	AF012513	EU797373	EU797315
	<i>Antarctonomus complanatus</i> Blanchard	AF012504	EU797341	EU797287
	<i>Carabus nemoralis</i> O.F. Müller	AF012507	EU797345	EU797291
	<i>Scaphinotus peter si catalinae</i> Van Dyke	AF002801	EU658920	EU658922
	<i>Pasimachus obsoletus atronitens</i> Casey	AF002794	EU797378	EU797320
	<i>Scarites subterraneus</i> Fabricius	AF002795	AF398708	AF398625
	<i>Clivinaferrea</i> LeConte	AF002796	EU797349	EU797295
	<i>Schizogenius falli</i> Whitehead	AF002797	EU797383	EU797325
	<i>Oregus aereus</i> White	AF012500	EU797375	EU797317
	<i>Brososoma relictum</i> Weissmandl	AF012502	EU797343	EU797289
	<i>Bembidion levettei carrianum</i> Casey	AF002791	AF398647	AF398571
	<i>Diplochaetus planatus</i> G.H. Horn	AF002789	AF438060	AF437938
	<i>Diplous californicus</i> (Motschulsky)	AF002785	AF398699	AF398587
	<i>Patrobus longicornis</i> (Say)	AF002786	AF398700	AF398613

	18S rDNA	28S rDNA	wingless
<i>Amblytelus curtus</i> (Fabricius)	AF012484	AF398683	AF398566
<i>Pterostichus melanarius</i> (Illiger)	AF002779	AF398707	AF398623
<i>Galerita lecontei lecontei</i> Dejean	AF002780	AF398686	AF398590
<i>Chlaenius ruficauda</i> Chaudoir	AF002777	AF398680	AF398578

Primers used for DNA amplification and sequencing. 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).

Table 4

Gene	Primer	Syn	Dir	Kind	Sequence	Ref
28S	LS58F	D1	F	A	GGGAGAAAAGAACTAAC	5
	NLF184/21		F	A	ACCCGCTGAAYTTAAGCATAT	3
	LS998R	D3	R	A	GCATAGTTCACCATCTTTC	5
	LS1041R		R	A	TACGGACRTCCATCAGGGTTTCCCTGACTTC	7
	LS30F	D1mod	F	A	ACCCCTRAATTAAAGCATAT	9
	LS264F	D2F	R	A	CGTGTGCTTGATAGTGCAGC	1
	LS770R	D2R	R	A	TCAAGACGGGTCCTGAAAAGT	1
18S	SS27F	518S	F	A	TATGCTTGCTCAAAAGATTAA	
	S1893R	18L	R	A	CACCYACGGAAACCTTGTACGACTT	
	SS398F	18Sai	F	S	CCTGAGAAACGGCTACCAACATC	2
	SS1054F	760F	F	S	ATCAAGAACGAAAGT	2
	SS1090R	18Sbi	R	S	GAGTCTCGTTCTGTTATCGGA	2
	SS1554R	909R	R	S	GTCCTGTTCCATTATTCCAT	4
wg	wg550F		F	A	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	8
	wgAbRZ		R	A	CACTTNACYTCRCARCACCCARTG	8
	wg578F		F	A	TGCACNGTGAARACYTGCTGGATG	6
	wgAbR		R	A	YTCGACGCACCARTGGAA	6
	B5wg1		F	A	GARTGYAAAGTGTCA YGGYATGTCTGG	7
	5wg		F	A	GARTGYAARTCYCAYGGYATGTCTGG	1
	5wgB		F	A	ACBTGYTGGATGCGNCTKCC	7
	3wg2		R	A	CTCGCARCACCACTGGAAATGTRCA	1
	B3wg2		R	A	ACTCGCARCACCACTGGAAATGTRCA	7
	3wg		R	A	ACTCGCARCACCACTGGAAATGTRCA	1

Ref: reference for original description of primer, if known.

1 : this paper

2 : Wray et al., 1993

- ³ : Van der Auwera et al., 1994
- ⁴ : Maddison et al., 1999
- ⁵ : Ober, 2002
- ⁶ : Ward and Downie, 2005
- ⁷ : Maddison, 2008
- ⁸ : Wild and Maddison, in press
- ⁹ : Moore, 2008

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). 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.

Adult habitat			
change	number	unconstrained	no aquatic→terrestrial
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			
change	number	unconstrained	no aquatic→terrestrial
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
	2	0.0	0.0

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. 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 state. 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.

reconstruction method	ancestral state	Adult Habitat	Larval Habitat
likelihood	terrestrial	0.03	0.0
	aquatic	0.97	1.0
	<i>number of trees</i>	<i>10421</i>	<i>10486</i>
likelihood (chronogram)	terrestrial	0.389	0.199
	aquatic	0.611	0.801
	<i>number of trees</i>	<i>2098</i>	<i>2989</i>
parsimony	terrestrial	0.488	0.353
	aquatic	0.512	0.647
	<i>number of trees</i>	<i>1520</i>	<i>2018</i>