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# Interrelationships of Staphyliniform groups inferred from 18S and 28S rDNA sequences, with special emphasis on Hydrophiloidea (Coleoptera, Staphyliniformia)

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## Abstract

The series Staphyliniformia is one of the mega-diverse groups of Coleoptera, but the relationships among the main families are still poorly understood. In this paper we address the interrelationships of staphyliniform groups, with special emphasis on Hydrophiloidea and Hydraenidae, based on partial sequences of the ribosomal genes 18S rDNA and 28S rDNA. Sequence data were analysed with parsimony and Bayesian posterior probabilities, in an attempt to overcome the likely effect of some branches longer than the 95% cumulative probability of the estimated normal distribution of the path lengths of the species. The inter-family relationships in the trees obtained with both methods were in general poorly supported, although most of the results based on the sequence data are in good agreement with morphological studies. In none of our analyses a close relationship between Hydraenidae and Hydrophiloidea was supported, contrary to the traditional view but in agreement with recent morphological investigations. Hydraenidae form a clade with Ptiliidae and Scydmaenidae in the tree obtained with Bayesian probabilities, but are placed as basal group of Staphyliniformia (with Silphidae as subordinate group) in the parsimony tree. Based on the analysed data with a limited set of outgroups Scarabaeoidea are nested within Staphyliniformia. However, this needs further support. Hydrophiloidea s.str., Sphaeridiinae, Histeroidea (Histeridae + Sphaeritidae), and all staphylinoid families included are confirmed as monophyletic, with the exception of Hydraenidae in the parsimony tree. Spercheidae are not a basal group within Hydrophiloidea, as has been previously suggested, but included in a polytomy with other Hydrophilidae in the Bayesian analyses, or its sistergroup (with the inclusion of Epimetopidae) in the parsimony tree. *Helophorus* is placed at the base of Hydrophiloidea in the parsimony tree. The monophyly of Hydrophiloidea s.l. (including the histeroid families) and Staphylinoidea could not be confirmed by the analysed data. Some results, such as a placement of Silphidae as subordinate group of Hydraenidae (parsimony tree), or a sistergroup relationship between Ptiliidae and Scydmaenidae, appear unlikely from a morphological point of view.

**Key words:** Staphyliniformia – phylogeny – Hydraenidae – Histeroidea – Hydrophiloidea – 18S rDNA – 28S rDNA

## Introduction

Staphyliniformia is a very large and diverse group of beetles with a worldwide distribution. Based on morphological features they appear to be one of the most basal groups of Polyphaga (Crowson 1960; Lawrence and Newton 1982). However, despite substantial contributions (e.g. Hansen 1991, 1997; Archangelsky 1998) the position and the phylogenetic interrelationships of the subgroups are not sufficiently clarified.

In the classification presented by Lawrence and Newton (1995) Staphyliniformia are divided into two superfamilies, Hydrophiloidea (including the histeroid families) and Staphylinoidea (including Hydraenidae). Following this scheme, two lineages can be recognized within Hydrophiloidea: a 'histerid' lineage composed by the families Histeridae, Sphaeritidae and Synteliidae and a 'hydrophilid' lineage, composed by the family Hydrophilidae (*sensu lato*). Other authors (Hansen 1991, 1997; Archangelsky 1998) favour a scheme that recognizes two distinct superfamilies, Hydrophiloidea (divided into six families) and Histeroidea, which implies a monophyletic origin of each of them. This would be in contrast to a placement of Histeroidea as a subordinate group of Hydrophiloidea in the strict sense, which at present cannot be ruled out (Beutel 1999).

Scarabaeoidea were placed as the sistergroup of both taxa by Hansen (1997), but were treated as a separate lineage (Scarabaeiformia) by Lawrence and Newton (1995). The placement of this highly diverse group, which is here addressed for the first time with molecular data, is one of the major issues in polyphagan systematics.

The position of Hydraenidae has been a matter of long controversy. Traditionally, Hydraenidae were considered as a family of Hydrophiloidea (excluding the histeroid families; e.g. d'Orchymont 1916, 1919; van Emden 1942; Crowson 1955), a view also adopted by Beutel (1994). However, in studies based on larval morphology, a placement within Staphylinoidea was already proposed by Böving and Craighead (1931) and Paulian (1941). The latter approach was strongly supported in more recent contributions made by Lawrence and Newton (1982) and Hansen (1991, 1997). These studies were exclusively based on morphological characters, and internal features were scarcely taken into consideration. Furthermore, Hydraenidae were only represented by *Ochthebius* in Hansen (1991), a genus which is characterized by many autapomorphies, and Hydraenidae and Hydrophiloidea (excluding the histeroid families) were treated as terminal taxa in Hansen (1997). In recent investigations, it has turned out that an insufficient taxon sampling may be problematic in this context, as character states entered in data matrices may not represent the groundplan of Hydraenidae (e.g. absence of five-segmented club and pubescence from the antennae of Orchymontinae) (Beutel et al. 2003).

Another strongly disputed question addressed in the present study is the position of Spercheidae. They display a remarkable number of seemingly plesiomorphic features of the larval head (Beutel 1994, 1999), which suggest a basal position within Hydrophiloidea. Other features, especially the presence of a complex larval stigmatic atrium, strongly suggest that spercheids form a clade with Hydrochidae and Hydrophilidae (Archangelsky 1998).

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Presently, only few molecular studies within subgroups of Staphyliniformia are available. They address the phylogeny of Silphidae (Staphylinoidea) and of the genus *Aleochara* (Staphylinidae) using mitochondrial cytochrome oxidase sequences, which produce good resolution at these systematic levels (Dobler and Müller 2000; Maus et al. 2001). Furthermore, Caterino and Vogler (2002) studied the phylogeny of Histeroidea based on 18S rDNA sequences and also morphological data, which yielded similar trees.

The purpose of this contribution is to provide more evidence for the investigation of the phylogeny of Staphyliniformia, with emphasis on the aquatic groups and Histeroidea, and to evaluate the usefulness of genes for this taxonomic level and the groups in question. For the analysis we chose complete 18S rDNA and the 5′ region of the 28S rDNA.

Within beetles sequences of 18S rDNA were used to reconstruct phylogeny of Adephaga (Shull et al. 2001), Hydradephaga (Ribera et al. 2002) and Carabidae (Maddison et al. 1998), and to clarify the interrelationships between the suborders of Coleoptera (Caterino et al. 2002). This gene also yielded a good resolution within Histeridae, as more than the 'normal' sequence variation was found within this taxon (Caterino and Vogler 2002). However, it should also be mentioned that in some studies the 18S rDNA could not resolve phylogenetic relationships sufficiently, e.g. basal relationships within carabids (Maddison et al. 1999) or subfamilial relationships in Curculionidae (Marvaldi et al. 2002).

The 5′ region of the 28S rDNA was used to resolve the phylogenetic relationships within subgroups of Carabidae (Kim et al. 2000; Cryan et al. 2001; Ober 2002) and Curculionoidea (Sequeira et al. 2000).

## Materials and methods

### Taxon sampling

All extant families and main subfamilies of Hydrophiloidea and two of three families of Histeroidea are included in the analyses. Sampling within Staphylinoidea is less dense, but the included taxa represent all major lineages. Agyrtidae is the only staphylinoid family not included. The complete list of analysed taxa is presented in Table 1. The outgroup comprises representatives of two families, Buprestidae (*Trachys troglodytes* Gyllenhal, 1817 and *Agrilus populneus* Schaefer, 1946) and Dascillidae (*Dascillus cervinus* Linnaeus, 1758). Specimens were collected directly in 95% ethanol and stored at −20°C for preservation of nucleic acids.

Table 1. Taxa sampled

| Taxon | |
|---|---|
| **Hydrophiloidea** | |
| Helophoridae | *Helophorus aquaticus* (Linnaeus, 1758) |
| | *Helophorus nivalis* (Giraud, 1851) |
| Georissidae | *Georissus* sp. |
| Hydrochidae | *Hydrochus elongatus* (Schaller, 1783) |
| Spercheidae | *Spercheus emarginatus* (Schaller, 1783) |
| Hydrophilinae | *Enochrus testaceus* (Fabricius, 1801) |
| | *Hydrobius fuscipes* (Linnaeus, 1758) |
| | *Berosus luridus* (Linnaeus, 1761) |
| Sphaeridiinae | *Sphaeridium bipustulatum* (Fabricius, 1781) |
| | *Coelostoma orbiculare* (Fabricius, 1775) |
| Epimetopidae | *Epimetopus* sp. |
| Histeridae | |
| Histerinae | *Hololepta plana* (Sulzer, 1776) |
| | *Margarinotus brunneus* (Fabricius, 1775) |
| Dendrophilinae | *Dendrophilus punctatus* (Herbst, 1792) |
| Sphaeritidae | *Sphaerites glabratus* (Fabricius, 1792) |
| **Staphylinoidea** | |
| Hydraenidae | |
| Hydraeninae | *Hydraena gracilis* (Germar, 1824) |
| | *Hydraena iberica* (d'Orchymont, 1936) |
| Ochthebiinae | *Ochthebius minimus* (Fabricius, 1792) |
| | *Ochthebius melanescens* (Dalla Torre, 1877) |
| Scydmaenidae | *Palaeostigus* sp. |
| Leiodidae | |
| Cholevinae | *Catops picepes* (Fabricius, 1787) |
| | *Nargus velox* (Spence, 1815) |
| Silphidae | |
| Silphinae | *Oiceoptoma thoracica* (Linnaeus, 1758) |
| | *Silpha obscura* (Linnaeus, 1758) |
| Staphylinidae | |
| Staphylininae | *Quedius mesomelinus* (Marsham, 1802) |
| | *Ocypus opthalmicus* (Scopoli, 1763) |
| Omaliinae | *Lesteva punctata* (Erichson, 1839) |
| Ptiliidae | |
| Acrotrichinae | *Acrotrichis* sp. (isolates a,b,c) |
| **Scarabaeoidea** | |
| Scarabaeidae | |
| Cetoniinae | *Cetonia aurata* (Linnaeus, 1758) |
| Aphodiinae | *Aphodius* sp. |
| **Buprestoidea** | |
| Buprestidae | |
| Agrilinae | *Agrilus populneus* (Schaefer, 1946) |
| | *Trachys troglodytes* (Gyllenhal, 1817) |
| **Dascilloidea** | |
| Dascillidae | |
| Dascillinae | *Dascillus cervinus* (Linnaeus, 1758) |

### DNA extraction, PCR amplification and sequencing

DNA was extracted from frozen specimens, which were pulverized in 1.5 ml microfuge tubes with a pestle following standard methods such as the DTAB-protocol (Gustincich et al. 1991).

Complete 18S rDNA sequences were amplified with the universal eukaryote-specific primer pair F01 (5′-AACCTGGTTGATCCTGCCAGT-3′) and R01 (5′-TGATCCTTCCGCAGGTTCACCTAC-3′) complementary to the 5′ and 3′ end of the gene (Medlin et al. 1988). A ca. 0.8 kb fragment of the nuclear 28S rRNA gene was amplified using the primers 28S-01 (5′-GACTACCCCCTGAATTTAAGCAT-3′) and 28SR-01 (5′-GACTCCTTGGTCCGTGTTTCAAG-3′) (Kim et al. 2000).

For the 18S rDNA the PCR conditions were as follows: 5 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1.5 min at 45°C, 2 min at 72°C, and a final single extension step 10 min at 72°C, using 50 µl reactions containing 0.1 µM of each primer, 200 µM dNTP, 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, template DNA and 1 unit of *Taq* DNA polymerase (Sigma, Munich, Germany). The amplification products were purified and concentrated with Microcon 30 (Millipore, Schwalbach, Germany) and cloned using the pGEM-T Vector System-Kit (Promega, Mannheim, Germany).

Amplification of the 28S rDNA was carried out using the same standard three-step PCR conditions with 52°C annealing temperature and the products were purified and sequenced directly with the PCR primers.

Sequences were determined on an automated LI-COR DNA Sequencer 4000L (MWG-Biotech, Ebersberg, Germany) using the Thermo Sequenase fluorescent-labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham, Freiburg, Germany). For sequencing of the complete 18S rDNA we used the universal primer pair M13 Universal CS and M13 Reverse CS and additional internal sequencing primers: 590F: 5′-CGGTAATTCCAGCTCCAATAGC-3′, 1280F: 5′-TGCATGGCCGTTCTTAGTTGGTG-3′, 600R: 5′-GCTATTGGAGCTGGAATTAC-3′, 1300R: 5′-CACCAACTAAGAACGGCCATGC-3′ (Medlin et al. 1988). The GenBank accession numbers are AJ810714–AJ810783.

### Alignment and phylogenetic analyses

The analyses was performed in a two-step procedure (Phillips et al. 2000), aligning sequences in Clustal W (Higgins et al. 1992) and tree searches on the aligned matrix using parsimony analysis in PAUP 4.0b6 (Swofford 2000). Due to the length variation of the sequences (see below), a sensitivity analysis (*sensu* Wheeler 1995) was undertaken. We used three different alignments: alignment 1, using the program Clustal W with default values (gap cost 15, extension cost 6.6); alignment 2, using Clustal W with gap cost 2 and extension cost 1; and alignment 3, manual. The preferred parameter combination was selected according to two criteria, maximum character congruence among genes and maximum phylogenetic signal. Character congruence was measured with the incongruence-length difference test (ILD) (Farris et al. 1994), and the modified ILD (WILD; Wheeler and Hayashi 1998), which presumably represents a normalized ILD allowing for more general comparisons among analyses. Phylogenetic signal was measured with the retention index (RI), a measure of the amount of similarity than can be measured as synapomorphies (Kitching et al. 1998).

In all the parsimony analyses gaps were coded as a fifth character (Giribet and Wheeler 1999), and trees were rooted with the two species of Buprestidae. PAUP searches consisted of TBR heuristic explorations of 10 000 replicates. The significance of the congruence among genes was tested with the partition homogeneity test (PHT) (Farris et al. 1994; Swofford 2000) as implemented in PAUP. Node support was measured with non-parametric bootstrap (Felsenstein 1985), with 1000 iterations of 100 TBR replicates, and with partitioned Bremer support (PBS) values (Baker and DeSalle 1997) obtained by searching on constraint trees generated with TreeRot (Sorenson 1996).

Comparisons of the optimal tree obtained with parsimony and previous phylogenetic hypothesis of the group were conducted with the non-parametric Templeton (1983) test. The hypothesis to be tested were used as a backbone constraint in a parsimony search in PAUP following the above procedure, and the resulting trees were compared with the optimal, unconstrained tree.

The sum of all parsimony branch lengths leading to the terminal taxa (species path lengths) were examined for the possible existence of exceedingly long branches. The species path lengths are the sum of multiple individual functions (the probability of character change in the time between two speciation events), and thus assumed to follow a normal distribution with parameters estimated from the data (Sokal and Rohlf 1995). Species path lengths longer than the 95% cumulative probability of this normal distribution were considered to be long.

To avoid the effect of possible long branches, the data were also analysed with Bayesian posterior probabilities, as implemented in MrBayes V3.04 (Huelsenbeck and Ronquist 2001). We used a general time reversible model (Rodríguez et al. 1990), with a proportion of invariant sites and unequal rates (GTR + I + G), as this was the model considered to be optimal by Modeltest (Posada and Crandall 1998) using the Akaike (1973) information criteria for both genes separately. The parameters of the different partitions (18S and 28S rDNA) were unlinked, and thus allowed to be estimated independently. We used defaults priors (i.e. uniform probabilities) and random trees as a starting point, running the analyses for 1 000 000 generations. The three heated and one cold Markov chains were sampled at intervals of 100 generations, rendering a total of 10 000 data points. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time, and visually determined when the log-likelihood values reached a stable equilibrium. To avoid the risk of the analysis being trapped in local optima, we repeated the whole procedure twice, beginning with different (random) starting trees (Huelsenbeck et al. 2002). If the log-likelihood scores were similar, indicating convergence of the two analyses, the trees (once burn-in samples were discarded) were combined in a single majority consensus topology, and the percentage of the nodes was taken as posterior probability (Huelsenbeck and Ronquist 2001). The 95% confidence interval of the topology was used to check the inclusion of alternative phylogenetic hypothesis congruent with previous proposals.

## Results

### Sequence variability

All amplifications were successful, with fragments ranging from 1839 bp (*Sphaeridium*) to 1858 bp (*Ocypus*) for 18S rDNA, and from 535 bp (*Ochthebius minimus*) to 541 bp (the two species of Silphidae) for 28S rDNA. Genetic distances on the optimal alignment (see below) ranged from $p = 0.001$ (equivalent to two nucleotide changes) between the two species of *Helophorus* to $p = 0.11$ (204 changes) between *Acrotrichis* sp. and *Palaeostigus* for 18S rDNA; and from $p = 0.00$ for the two species of *Hydraena* (with identical sequences) to $p = 0.22$ (116 changes) between *Oiceoptoma* and *Palaeostigus* for 28S rDNA.

Of the three alignments tested, the optimal, both based on the ILD and WILD and the RI, was the manual (Table 2). All subsequent analyses were thus conducted on this alignment.

### Parsimony analysis

The parsimony analysis on the optimal alignment resulted in a single tree of 2212 steps (Table 2, Fig. 1). The two genes were marginally incongruent as measured with the PHT ($p = 0.054$), although the correlation between the PBS values was very high ($r^2 = 0.72$, $p < < 0.0001$), indicating a good agreement between the topologies supported by the two partitions. Nodes with considerable incongruence were the grouping of Ptiliidae with Scydmaenidae, Silphidae with *Ochthebius*, and Staphylinidae plus Scarabeidae with Hydrophiloidea (Fig. 1). In all of these, the negative value corresponds to 28S rDNA. To further check the effect of the alignment, an additional search was conducted deleting all characters with gaps from the optimal alignment (105 characters). The search resulted in three equally parsimoni-

Table 2. Tree statistics in the sensitivity analysis

| Alignment | 18S | 28S | Combined | No. cha | Inf. cha. | ILD | WILD | CI | RI |
|---|---|---|---|---|---|---|---|---|---|
| A | 1246 | 944 | 2212 | 2428 | 484 | 22 | 0.010 | 0.521 | 0.509 |
| B | 1238 | 953 | 2218 | 2426 | 478 | 27 | 0.012 | 0.516 | 0.491 |
| C | 1344 | 1123 | 2491 | 2605 | 494 | 24 | 0.010 | 0.543 | 0.472 |

Alignment, A, manual; B, Clustal W default values (gap opening 15, gap extension 6.6); C, Clustal W (gap opening 2, gap extension 1). 18S, length of the optimal tree for the 18S rRNA partition. 28S, length of the optimal tree for the 28S rRNA partition. Combined, length of the optimal tree for the combined data. No. cha., total number of characters in the combined matrix. Inf. cha., number of informative characters in the combined matrix. ILD, inconsistency length difference [ILD = combined−(18S + 28S)]. WILD, Wheeler ILD (WILD = ILD/combined). CI, consistency index. RI, retention index.

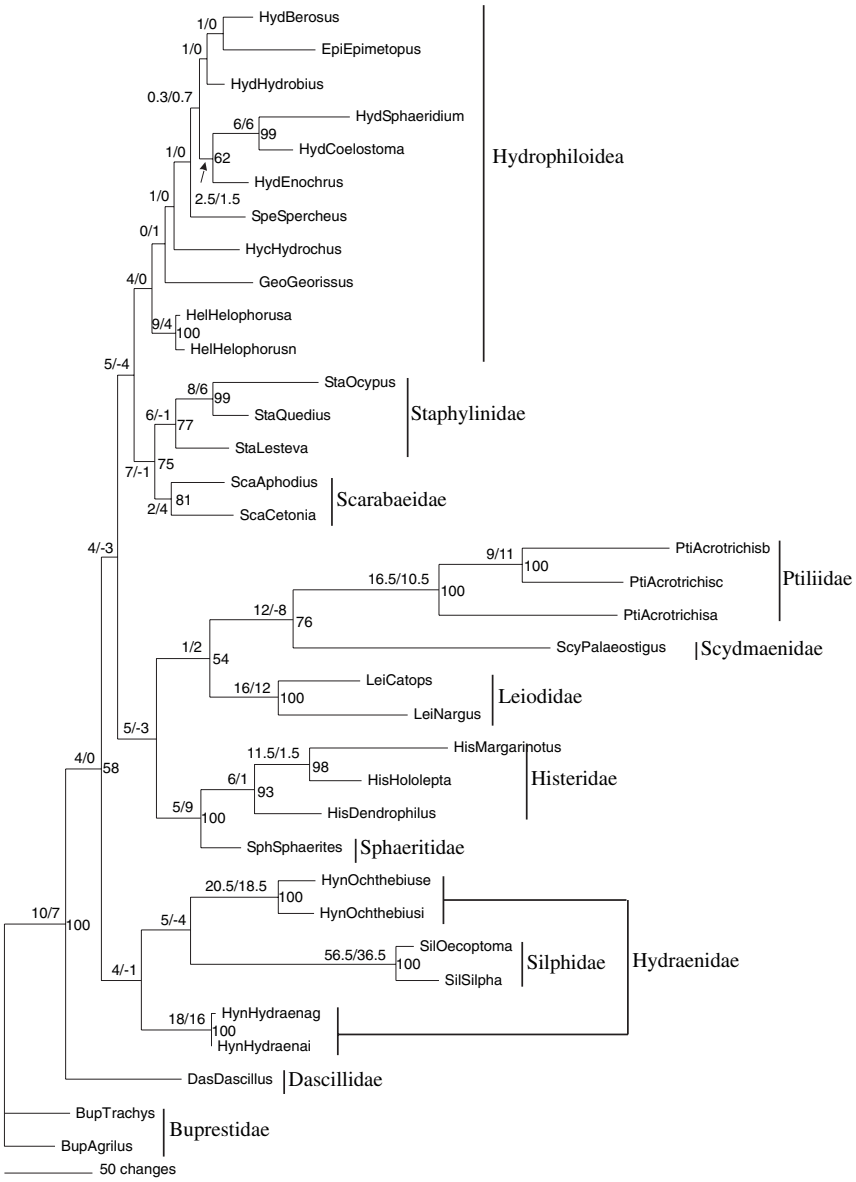


Fig. 1. Phylogram of the single most parsimonious tree found in the combined analysis of the 18S rRNA and 28S rRNA genes using the optimal (manual) alignment. Number inside nodes, bootstrap support (> 50%). Above branches, partitioned Bremer support values (18S/28S). See Table 1 for the names of the species

ous trees of 1766 steps (CI = 0.518, RI = 0.509). The topology of the consensus tree was very similar to that of the tree obtained with the full set of characters [the comparison among the two topologies using the Templeton (1983) non-parametric test is not significant, p = 0.64], although considerably less resolved. The only change of importance is the shift in the position of Georissidae, which is placed basal to Hydrophiloidea.

The estimated distribution of the species path lengths in the most parsimonious tree had an average of 143.6 steps and a variance of 74.5. The 95% cumulative probability interval included all taxa with the exception of the three species of Ptiliidae and the only Scydmaenidae, which were sister groups (Fig. 2). When these four species were excluded, the 95% confidence interval of the new estimated distribution (average 121.6 steps, variance 44.4) excluded *Nargus*, *Margarinotus* and the two included species of Silphidae (Fig. 2). The accumulation of long branches in the clades grouping Ptiliidae + Scydmaenidae + Leiodidae + Histeridae, and Hydraenidae + Silphidae (Fig. 1), strongly suggest the possibility of an artefact due to long branch attraction (Felsenstein 1978).

There is a general low support in the internal nodes of the tree, other than for intra-family relationships. Very few inter-family nodes have bootstrap values higher than 50%, and Bremer support values above 4, which are usually considered as thresholds for a minimum reliability (Swofford 2000; DeBry 2001).

### Posterior Bayesian probabilities

The two independent runs of MrBayes reached stability at around the 30 000 generation, although 50 000 generations (i.e. 500 data points) were discarded as burn-in as a precautionary measure. The arithmetic mean of the estimated marginal likelihood was very similar in the two runs (−13 699.59 and −13 697.57 respectively), and the estimated parameters were well within the 95% confidence interval of each other. The topologies of the majority consensus rule of the sampled trees were identical, with very similar posterior

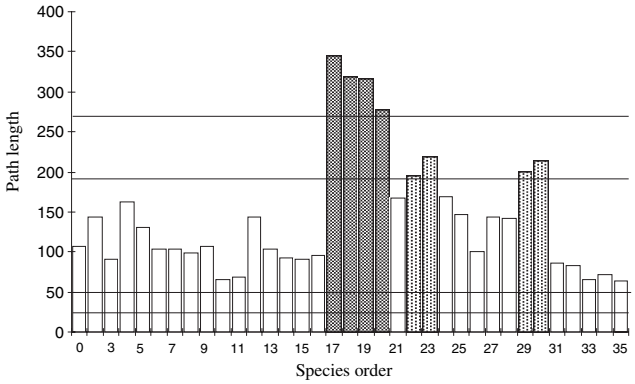


Fig. 2. Species path lengths in the single most parsimonious tree obtained with the combined analysis of the 18S rRNA and 28S rRNA genes using the optimal (manual) alignment (see Fig. 1). Solid lines, 95% cumulative probability of a normal distribution estimated from all 35 species. Dashed lines, 95% cumulative probability of a normal distribution estimated from all species with the exception of the Ptiliidae and Scydmaenidae (see text). Bars with dense pattern, Ptiliidae and Scydmaenidae; bars with clear pattern, *Nargus*, *Margarinotus*, *Oiceoptoma* and *Silpha* respectively (see Fig. 1 and Table 1)

probabilities in all the nodes. In consequence, the trees obtained in both runs were merged (once the burn-in trees were discarded), and the majority consensus rule is represented in Fig. 3.

Well-supported inter-family nodes in both trees (parsimony and Bayesian) are the sister relationship between Scydmaenidae and Ptiliidae (76% bootstrap, 94% posterior Bayesian probability, Figs 1 and 3), although the branches were among those identified as 'long' according to our criteria. On the contrary, the relationship between Scarabaeidae and Staphylinidae, also well supported with both methods (75% bootstrap, 99% posterior Bayesian probability), does not involve long branches.

Well-supported nodes only with one of the methods were Hydrophiloidea (90% posterior Bayesian probability, but less than 50% boostrap support, and a decay index of 4); the placement of *Spercheus* within the 'higher' Hydrophiloidea (97% posterior Bayesian probability, but less than 50% boostrap support, and a decay index of 1); the sister relationship of Hydraenidae and Scydmaenidae + Ptiliidae (94% posterior Bayesian probability, not present in the parsimony tree); and finally the sister relationship between Histeridae and Leiodidae (94% posterior Bayesian probability, including Scydmaenidae + Ptiliidae in the parsimony tree, although with very low support) (Figs 1 and 3).

### Comparison with previous phylogenetic hypotheses

Five hypotheses were tested: the tree of Archangelsky (1998), a composite tree reflecting the phylogenetic hypotheses of Hansen (1991, 1997), and three specific nodes: *Spercheus* as sister to the remaining Hydrophiloidea (Beutel 1999), Hydraenidae sister to Hydrophiloidea (Crowson 1960; Beutel 1994), and Hydraenidae sister to Ptiliidae (Lawrence and Newton 1982; Hansen 1991, 1997; Grebennikov and Beutel 2002).

The comparison of the single most parsimonious tree obtained with the manual alignment and the trees resulting from the searches with the backbone constraint of the hypotheses tested was significant only for the trees of Hansen

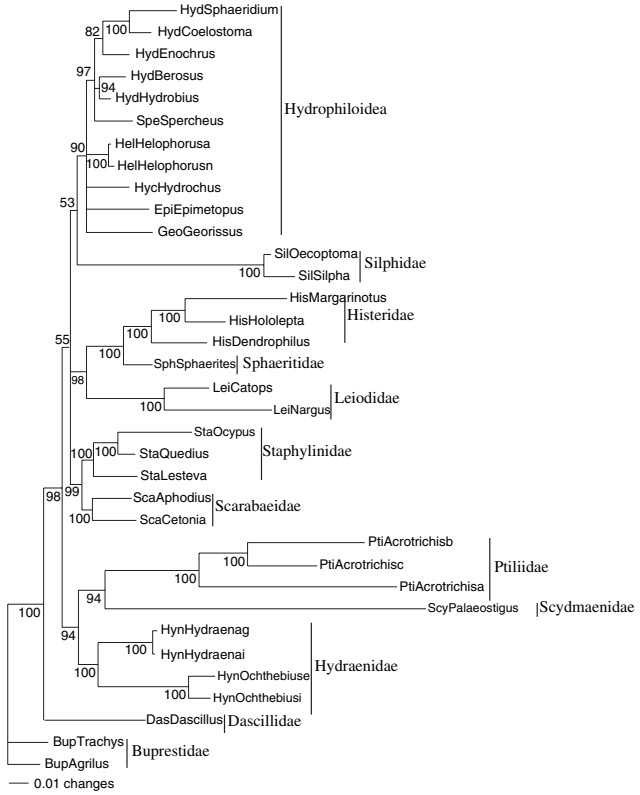


Fig. 3. Majority rule consensus phylogram of the combined independent runs of MrBayes with the combined data set (optimal alignment), after deleting the burn-in trees (500 of 10 000 trees in each of the two runs). Numbers refer to posterior probabilities (> 50%). See Table 1 for the names of the species

(1991 and 1997) and Archangelsky (1998), as measured with a non-parametric Wilcoxon sign test (Templeton 1983) (Table 3). The trees obtained after constraining individual nodes were not significantly different from the best tree (Table 3).

The 95% confidence interval of the topology obtained in the combined two independent runs of the Bayesian probabilities was extremely wide, with a total of 3838 trees of 4798 different trees sampled in the Monte Carlo Markov chains. The strict consensus of these 3838 trees (not shown) is almost fully

Table 3. Templeton (1983) non-parametric test for the comparison of topologies with the most parsimonious tree

| Constrain | No. trees | Length | Rank sums | p (two-tailed) |
|---|---|---|---|---|
| Unconstrained | 1 | 2212 | – | – |
| Archangelsky (1998) | 2 | 2235 | 1242/−588 | 0.0076 |
| Hansen (1991, 1997) | 1 | 2269 | 3533.5/−1122.5 | < 0.0001 |
| Hydraenidae + Hydrophiloidea | 4 | 2224 | 1124/−767 | n.s. |
| Hydraenidae + Ptiliidae | 2 | 2225 | 1659/−1191 | n.s. |
| Spercheus + Hydrophiloidea | 6 | 2218 | 790/−641 | n.s. |

No. trees, number of trees obtained in PAUP in a search with a backbone constraint. Length, length of the trees obtained. Rank sums, Wilcoxon-signed ranks for the comparison between the unconstrained (best) tree and the tree obtained with the backbone constraint. n.s., non significant.

unresolved, with the exception of closely related species of the same genus, and closely related genera of the same family (Leiodidae, Silphidae, *Sphaeridium* and *Coelostoma*, *Ocypus* and *Quedius*). Only one family group is recovered: Histeridae plus Sphaeritidae. Based on this confidence interval for the topologies, none of the alternative hypotheses could be rejected.

As an additional test of the topologies, we used the Shimodaira–Hasegawa test with the RELL approximation (Shimodaira and Hasegawa 1999), with 1000 bootstrap replicates, as implemented in PAUP. We used the GTR + I + G maximum likelihood model with parameter values as estimated by Modeltest for the combined data, as PAUP does not allow the use of different parameter estimations for the partitions in a combined analysis. The Shimodaira–Hasegawa test was considered to be appropriate as our topologies were selected *a posteriori* (Goldman et al. 2000). Confirming the results of the topological confidence interval in MrBayes, none of the comparisons was significant at the standard 0.05 level. Only the comparisons with the topologies of Hansen (1991, 1997) and Archangelsky (1998) could be marginally rejected ($p < 0.1$), as well as some of the trees obtained when the sister group relationship between Hydraenidae and Hydrophiloidea was constrained ($p < 0.1$).

## Discussion

Although the PHT is marginally significant, indicating some degree of incongruence among genes, there is a very good agreement among the topologies (as measured with the partition Bremer support), and most of the incongruent nodes involve long branches. The PHT (or its equivalent, the ILD) is a good measure of general congruence, but has been recently criticized as a criteria for combining data or doing separate analysis (Dolphin et al. 2000; Barker and Lutzoni 2002; Darlu and Lecointre 2002). We opted for a combined analysis (see e.g. Grant and Kluge 2003 and references therein), using the congruence as a criteria for choosing among alternative alignments. The main results were, however, not very sensitive to the alignment parameters, and even after the deletion of all characters with gaps, the main topological change on the trees obtained with parsimony was the position of Georissidae, which become basal to Hydrophiloidea.

The method used to detect and quantify the exceedingly long branches can only be taken as indicative. The assumption of a normal distribution of the path lengths could be violated in case a subclade of the tree has faster rates of molecular evolution than the rest without the presence of 'long branch attraction' (Felsenstein 1978). Similarly, it is still possible that nodes whose branches are within the 95% cumulative probability of the estimated normal distribution are the product of random synapomorphies ('long' branches). However, it is an easy way to select nodes with the potential of being the product of an analysis artefact, in particular when they do not have strong morphological support. The fact that the trees obtained with Bayesian methods (which in principle are less sensitive to long branch artefacts; Huelsenbeck et al. 2002) differ in the placement of most of the taxa involved in the branches identified as 'long' according to our criteria supports this interpretation.

The results of both analyses are characterized by the poor support of most deep branches. In addition, some of the well-supported nodes in the parsimony analysis may be the result of long branch attraction, as they involve unusually long branches (as detected by our criteria). Some of these nodes have negative partition Bremer support values for 28S rDNA, showing a certain degree of incongruent signal between the two genes used. The final topology is always dominated by the signal of the 18S rDNA gene, probably due to its much larger number of nucleotides. The use of Bayesian posterior probabilities seems to at least partially overcome the 'long branch attraction' effect, but the support (although higher, as could be expected with the use of Bayesian methods; Simmons et al. 2004) is still low.

Comparison of the most parsimonious trees with the trees obtained after constraining individual nodes including some of the branches considered to be 'long' (as e.g. the position of Hydraenidae) were not significant. This indicates that the nodes involving these taxa have, in general, low support, but that their placement in different nodes of the tree does not affect its general topology in a major way. On the contrary, when the constraint involves multiple nodes (Hansen 1991, 1997; Archangelsky 1998) differences were significant. On the Bayesian trees, despite the high posterior probabilities of some nodes, the 95% confidence interval for the topology is extremely wide, not allowing to discard almost any meaningful alternative, in agreement with recent papers suggesting that posterior probabilities are an overestimation of node support (e.g. Simmons et al. 2004). Results which are in good agreement with those of morphological studies (e.g. Hansen 1991, 1997; Beutel and Komarek 2004) are the monophyly of Hydrophiloidea (*sensu* Hansen 1991), Hydrophilidae (Bayesian tree), Sphaeridiinae, Histeroidea (Sphaeritidae + Histeridae), and Histeridae (Caterino et al. 2002). The families of Staphylinoidea included also turned out as monophyletic. A clade formed by Spercheidae and Hydrophilidae supports the hypotheses presented by Hansen (1991), Archangelsky (1998), and Beutel and Komarek (2004), but is in contrast to Beutel (1994, 1999), who proposed a basal position of this group within Hydrophiloidea. The former option is strongly supported by the presence of a large stigmatic atrium in the larvae, but implies that a considerable number of seemingly plesiomorphic features of the larval head (e.g. presence of a broad gula and a well-developed lacinia; Beutel 1999), are in fact due to reversal. In the parsimony trees, Hydrochidae – the third family with a larval stigmatic atrium – groups with Spercheidae and Hydrophilidae. However, *Epimetopus*, which lacks the stigmatic atrium and is likely closely related with Georissidae (e.g. Hansen 1991; Beutel 1999; Beutel and Komarek 2004), is nested within the latter family. A placement of Hydraenidae within Staphylinoidea is tentatively supported by the present results. They never group with Hydrophiloidea, but form a clade with Ptiliidae and with *Palaeostigus* (Scydmaenidae) in the Bayesian tree. It is likely that this placement of the scydmaenid genus does not reflect the true position of this family (e.g. Hansen 1997).

An interesting result is that Scarabaeidae are well nested within Staphyliniformia in the parsimony and Bayesian trees. This is in agreement with recent morphological analyses (Hansen 1997; Beutel and Komarek 2004), and suggests that their treatment as a separate series Scarabaeiformia is probably not justified. However, they do not group with Hydrophiloidea s.l. (including Histeroidea) as suggested by Hansen (1997), but in both trees as sistergroup of Staphylinidae, which appears quite unlikely considering the morphological evidence. The branching of Scarabaeoidea within Staphyliniformia has

to be verified (or falsified) in future analyses with more taxa including outgroup representatives of other polyphagan series as well as other scarabaeoid families.

Several results are problematic or unlikely from a morphological point of view. Histeroidea do never form a clade with Hydrophiloidea as suggested by Lawrence and Newton (1982), Hansen (1997); Beutel (1999), and Beutel and Komarek (2004), and Staphylinoidea are highly polyphyletic in both trees. Furthermore, the positions of two families, Silphidae and Leiodidae, change markedly between the trees (Figs 1 and 3). Based on morphology the positioning of Silphidae as the sistergroup of Hydrophiloidea as well as Leiodidae as sistergroup of Sphaeritidae + Histeridae is implausible (Fig. 3). In contrast, the parsimony tree (Fig. 1) shows Leiodidae as the sistergroup of Scydmaenidae + Ptiliidae, which is compatible with current hypotheses (e.g. Hansen 1997). However, the placement of Silphidae within Hydraenidae in the same tree is highly unlikely. For a better resolution of the phylogenetic relationships of these families, as well as for the whole Staphyliniformia, the inclusion of more taxa, especially of Agyrtidae and further staphylinid subfamilies, should be helpful. Rydin and Källersjö (2002) show that taxon sampling is a crucial issue in molecular phylogenetic studies. It is expected that more taxa could be included in further analyses to address the phylogenetic relationships within Staphyliniformia.

The data presented suggest some interesting relationships, in part also supported by morphology, but generally with low support. As in other recent molecular contributions (e.g. Maddison et al. 1998; Shull et al. 2001; Caterino et al. 2002; Marvaldi et al. 2002), they are not sufficient for a reliable clarification of deep relationships, but may be useful in combination with more molecular data and morphological characters in future analyses.

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## Zusammenfassung

*Untersuchungen zur Phylogenie der Staphyliniformia (Coleoptera), unter besonderer Berücksichtigung der Hydrophiloidea, basierend auf 18S und 28S rDNA Sequenzen*

Die Serie Staphyliniformia ist eines der artenreichsten Taxa der Coleoptera. Die bisher postulierten Verwandtschaftsbeziehungen zwischen den Familien sind nach wie vor nicht ausreichend abgesichert. Die vorliegende Arbeit ist ein Beitrag zur Aufklärung der Phylogenie mit Hilfe von Teilsequenzen der 18S rDNA und 28S rDNA, unter besonderer Berücksichtigung der Hydrophiloidea und Hydraenidae. Die Sequenzdaten wurden mit Parsimonie-Verfahren und um wahrscheinliche „long-branch' Effekte zu vermeiden auch mit dem Bayesschen Verfahren analysiert. Mit beiden Analysemethoden werden die Verwandtschaftsbeziehungen zwischen den meisten Familien nur schwach unterstützt. Allerdings stimmen die meisten Resultate gut mit morphologischen Arbeiten überein. Keine unserer Analysen ergab eine nahe Verwandtschaft zwischen den Hydraenidae und Hydrophiloidea. Dies steht in Widerspruch zur traditionellen Auffassung, stimmt aber mit aktuellen morphologischen Untersuchungen überein. Nach dem auf der Bayesschen Methode beruhenden Kladogramm bilden die Hydraenidae ein Monophylum mit den Ptiliidae und Scydmaenidae. Dagegen stehen sie nach den Ergebnissen der Parsimonie-Analyse an der Basis der Staphyliniformia (mit den Silphidae als untergeordneter Teilgruppe). Die Scarabaeoidea stehen innerhalb der Staphyliniformia. Die Hydrophiloidea s.str., Sphaeridiinae, Histeroidea (Histeridae + Sphaeritidae), und alle Familien der Staphylinoidea wurden als monophyletische Gruppen bestätigt. Nur die Hydraenidae bilden in dem auf der Parsimonie-Methode beruhenden Kladogramm kein Monophylum. Die Spercheidae stellen keine basale Gruppe der Hydrophiloidea dar, sondern bilden mit Teilgruppen der Hydrophilidae eine Multifurkation (Bayes'sches Kladogramm), oder sie sind die Schwestergruppe der Hydrophilidae inklusive Epimetopidae (Parsimonie-Analyse). Nach der Parsimonie-Methode bildet *Helophorus* die Schwestergruppe zu den übrigen Hydrophiloidea. Die Monophylie der Hydrophiloidea s.l. (inklusive Histeroidea) und der Staphylinoidea konnte durch die Analysen nicht bestätigt werden. Einige Resultate wie die Stellung der Silphidae als untergeordnete Teilgruppe der Hydraenidae (Parsimonie-KladogrammAnalyse), oder das Schwestergruppenverhältnis zwischen den Ptiliidae und Scydmaenidae erscheinen in Anbetracht der morphologischen Befunde unwahrscheinlich.

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