From terrestrial to aquatic habitats and back again — molecular insights into the evolution and phylogeny of Hydrophiloidea (Coleoptera) using multigene analyses

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The phylogenetic relationships within Hydrophiloidea have been a matter of controversial discussion for many years and the supposedly repeated changes between aquatic and terrestrial lifestyles are not well understood. In order to address these issues we used an extensive molecular data set comprising sequences from six nuclear and mitochondrial genes. The analyses accomplished with the entire data set resulted in largely congruent tree topologies concerning the main branches, independent from the analytical procedures. However, only Bayesian analyses yielded sufficient high posterior probabilities, whereas bootstrap support values for most nodes were generally low. Our results are only partially congruent with hypotheses based on morphological analyses. Spercheidae were placed as the sister group of the remaining hydrophiloid subgroups. Hydrophiloidea excluding Spercheidae split into two clades: the 'helophorid lineage' comprising the small groups Epimetopidae, Hydrochidae, Georissidae and Helophoridae, and the largest family, Hydrophilidae. Within Hydrophilidae, Hydrophilinae do not form a monophylum. The predominantly terrestrial Sphaeridiinae were placed as a subordinate clade within this subfamily. Furthermore, our data suggest a single origin of the aquatic lifestyle in Hydrophiloidea, with numerous secondary changes to terrestrial habits and tertiary changes to aquatic habitats within Sphaeridiinae.

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Introduction

Hydrophiloidea (*sensu stricto* [= *sensu* Hansen 1991, 1997a]) includes *c.* 2800 species and has a worldwide distribution. Its monophyly is well supported by morphological characters (Hansen 1991, 1997a; Beutel & Komarek 2004). Hansen (1991) and Archangelsky (1998, 2004) divided this superfamily into six families: Helophoridae, Epimetopidae, Georissidae, Hydrochidae, Spercheidae and Hydrophilidae. This concept is followed here. An alternative concept was proposed by Lawrence & Newton (1982, 1995) and also used by Archangelsky *et al*. (2005). In their classifications these families are treated as subfamilies and together form the Hydrophilidae (*sensu lato*). The Hydrophiloidea (*s.l.*) as defined by Lawrence & Newton (1995) includes the histeroid families. Together with Staphylinoidea (and probably Scarabaeoidea) it forms the staphyliniform lineage (see e.g. Beutel & Leschen 2005).

The diversity of five of the six families of Hydrophiloidea is low and they are not subdivided into subfamilies. In contrast, Hydrophilidae comprises more than 2300 species (Archangelsky 2004) and is currently divided into four subfamilies: Horelophinae, Horelophopsinae, Hydrophilinae and Sphaeridiinae

(Archangelsky 2004). However, Archangelsky *et al*. (2005) did not integrate them as subgroups of the Hydrophilidae, but gave them the same rank as all other six groups mentioned above.

Adults and larvae of Hydrophiloidea (*s.s.*) can be found in a variety of habitats. Most species (*c*. 75%) are aquatic, others semiaquatic or riparian, while others are fully terrestrial (e.g. Hansen 1997b; Archangelsky *et al*. 2005). Notably, most species of Sphaeridiinae live in terrestrial habitats, albeit usually in substrates with a high water content such as dung or decaying plant material. Furthermore, species of Georissidae are found in terrestrial and riparian habitats like wet sand or soil.

Adult hydrophiloids usually feed on plant material and decaying organic matter. Adults of Spercheidae are highly specialized and are the only known filter feeders among beetles (Rothmeier & Jäch 1986). In contrast, most known hydrophiloid larvae are predacious (Hansen 1997b). Spercheid larvae differ again by feeding on detritus with a specific filter mechanism or on carrion (Archangelsky 2001), while larvae of some species of Helophoridae feed on plants and may even cause economic damage (Petherbridge 1928).

The phylogenetic relationships within Hydrophiloidea have been controversial for many years (e.g. Crowson 1955; Hansen 1991, 1997a; Beutel 1994, 1999; Lawrence & Newton 1995; Archangelsky 1998; Archangelsky *et al*. 2005). Traditionally, Hydraenidae were included within Hydrophiloidea (e.g. Crowson 1955; Beutel 1994). However, morphological (e.g. Beutel *et al*. 2003; Beutel & Komarek 2004; Beutel & Leschen 2005) as well as molecular studies (Korte *et al*. 2004; Caterino *et al*. 2005) have clearly showed that this group does not belong to Hydrophiloidea, but to Staphylinoidea. Unusual morphological features of hydraenid larvae indicate a sister-group relationship with Ptiliidae (see e.g. Hansen 1997a; Beutel & Leschen 2005).

Within the remaining Hydrophiloidea, Hansen (1991, 1997a) proposed two major lineages mainly based on characters of adults: the 'helophorid lineage' containing the Helophoridae, Epimetopidae, Georissidae, Hydrochidae, and the 'hydrophilid lineage' including the Spercheidae and Hydrophilidae (Fig. 1). Based on adult and larval characters Archangelsky (1998) favoured a slightly different branching pattern, shifting the Hydrochidae to the 'hydrophilid lineage'.

In contrast to these concepts based on structural features of larvae, Beutel (1994, 1999) suggested a basal position of the Spercheidae within Hydrophiloidea; the grouping of the other families also differs clearly. In a cladogram presented by Beutel & Komarek (2004) based on thoracic features of adults, Helophoridae branch off first, followed by Hydrochidae, then by a clade comprising Epimetopidae and Georissidae, and then by Spercheidae as the sister group of Hydrophilidae.

Within Hydrophilidae most authors assume a sister-group relationship between the aquatic Hydrophilinae and the terrestrial Sphaeridiinae (Hansen 1997a; Archangelsky 2004; Archangelsky *et al*. 2005). However, some morphological analyses (Anton & Beutel 2004; Beutel & Komarek 2004) have not confirmed this hypothesis, proposing that Sphaeridiinae branches off within Hydrophilinae. Consequently, the paraphyly of Hydrophilinae should be considered as a possible option. Interestingly, a similar branching pattern was also found in phylogenetic analyses using SSU and LSU rDNA sequences (Korte *et al*. 2004) or SSU rDNA sequences alone (Caterino *et al*. 2005). However, both studies were focused on the phylogenetic relationships within the entire Staphyliniformia using a broad range of taxa and offer only a limited resolution within Hydrophiloidea.

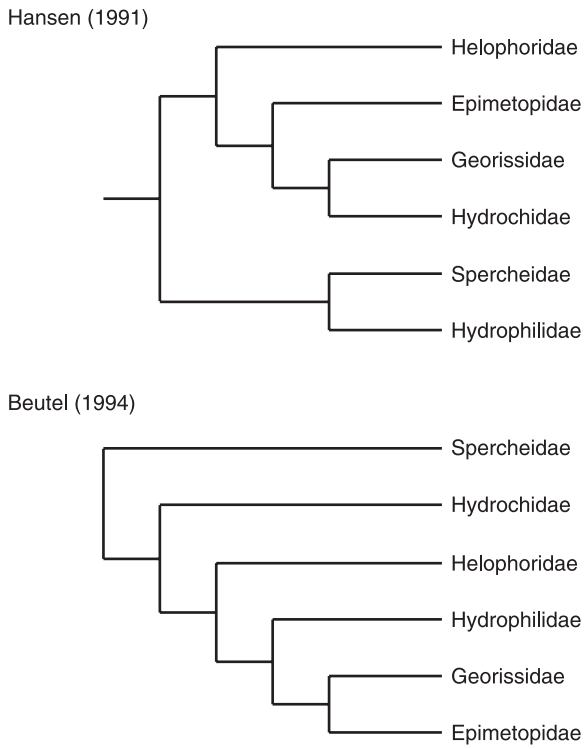

Fig. 1 Two alternative proposals of the phylogeny of Hydrophiloidea proposed by Hansen (1991) and Beutel (1994).

The different hypotheses concerning hydrophiloid phylogeny also raise questions about the transition between terrestrial and aquatic habitats and vice versa. The terrestrial lifestyle of Sphaeridiinae is generally thought to be secondary (Hansen 1997b). A secondary change to terrestrial habitats can also be assumed for some genera or species within groups of mainly aquatic species (e.g. some species of Helophoridae or *Anacaena* belonging to Hydrophilinae). However, it is not clear if the switch to aquatic habitats evolved in the common ancestor of Hydrophiloidea or in different lineages independently.

In order to address some of these questions concerning phylogeny and shifts between habitats, we carried out an

analysis using representatives of all families and subfamilies (*sensu* Hansen 1991, 1997a) of Hydrophiloidea with only two exceptions. Horelophinae and Horelophopsinae, each represented by one rare species occurring in New Zealand and Iryan Jaya, respectively, were not included as no suitable material was available. To obtain a better resolution within Hydrophiloidea (compared to Korte *et al*. 2004) we expanded the data set to six genes (nuclear SSU rDNA, LSU rDNA and mitochondrial 12S rDNA, 16S rDNA, COI, COII). We set out to address the following questions. (1) Are there two separate 'helophorid' and 'hydrophilid' lineages of families within the Hydrophiloidea (*s.s.*)? (2) If so, does the branching pattern match the proposal of Hansen (1991, 1997a) or that of Archangelsky (1998) and Archangelsky *et al*. (2005)? (3) Alternatively, are the Spercheidae the most basal group within the Hydrophiloidea? (4) Are the morphologically well-defined Hydrophilinae monophyletic or paraphyletic with Spaeridiinae as a subordinate group? (5) What can be concluded from the phylogenetic results about transitions between terrestrial and aquatic habitats and vice versa?

Materials and methods

Taxon sampling

Representatives of all families of Hydrophiloidea and of two of the four subfamilies of Hydrophilidae were included in the analyses. In total, 22 species were examined. Eighteen belonged to Hydrophiloidea and four species of Histeroidea (Histeridae and Sphaeritidae) were used as outgroup. All taxa are listed in Table 1 (classification following the concept of Hansen 1991, 1997a).

DNA extraction, PCR amplification and sequencing

The 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 (Gustinich *et al*. 1991).

Complete nuclear SSU 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). An approx. 0.7 kb fragment of the nuclear LSU rDNA was amplified using the primers 28S-01 (5′-GACTACCCCCTGAATTTAAGCAT-3′) and 28SR-01 (5′-GACTCCTTGGTCCGTGTTTCAAG-3′) (Kim *et al*. 2000).

Four regions of the mitochondrial genome were amplified using the primers from Simon *et al*. (1994): (1) a region of approx. 350 bp of the 12S rDNA with SR-J-14233 and SR-N-14588; (2) a fragment of approx. 500 bp of the 16S rDNA with LR-N-13398 and LR-J-12887; (3) a fragment of approx. 1200 bp of the COI gene with C1-J-1751 and TL2-N-3014 from which only about 700 bp could be sequenced; (4) a fragment of approx. 700 bp including the complete COII gene with slightly modified versions of the primers TL2-J-3037 (5′-TAATATGGCAGATTAGTGCA-3′) and TK-N-3785 (5′-GTTTAAGAGACCAGTACTT-3′).

For the SSU rDNA and LSU rDNA the PCR conditions were as follows: 5 min at 95 °C, followed by 40 cycles of 1 min at 95 °C, 1 min at 45 °C (SSU rDNA) or 48 °C (LSU rDNA), 2 min at 72 °C, and a final single extension step, 10 min at 72 °C. Mitochondrial genes were amplified under the same conditions with some modifications in the annealing and elongation steps: 1 min at 45 °C (12S rDNA), 1.5 min at 45 °C (16S rDNA), 1.5 min at 50 °C (COI), 1 min at 50 °C (COII) and 1.5 min at 72 °C (12S rDNA, COI).

Sequencing reactions were performed for both DNA strands using the PCR primers, with additional internal sequencing primers for SSU rDNA (see Korte *et al*. 2004) and for COI: CoxI460F (5′-CATATTATTAGACAAGAAAGAGG-3′) and CoxI470R (5′-GTTTCCTTTTTTCCTCTTTCTTG-3′) on an ABI PRISM 3100 Genetic Analyser.

Phylogenetic analyses

Sequences of each gene were aligned separately using the ClustalW algorithm implemented in MEGA v. 3.1 (Kumar *et al*. 1993) with default parameters. Protein coding sequences were aligned at the amino-acid level and translated back into nucleotides. The alignments were concatenated to three different data sets: combined mitochondrial sequences (12S rDNA, 16S rDNA, COI, COII), combined nuclear sequences (SSU rDNA, LSU rDNA), and a data set comprising all six gene sequences with a total 4968 aligned characters. MEGA v. 3.1 was used to calculate sequence statistics.

Phylogenies were estimated using different procedures. MODELTEST v. 3.07 (Posada & Crandall 1998) was used to find the most appropriate model of DNA substitution. For the whole data set, both the hierarchical likelihood ratio test and the Akaike information criterion selected the same best-fit model GTR, with a proportion of invariant sites (0.5208) and unequal rates (0.7950) (GTR + I + Γ); this was then used for maximum likelihood (ML), Neighbour-joining (NJ) and Bayesian analyses.

ML and NJ analyses were calculated with PAUP* 4.0b10 (Swofford 2002). Bayesian analyses were performed with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001), which was used to run 1 000 000 generations, with a sampling frequency of 10 generations. From the 100 000 trees found, the first 5000 were discarded after MrBayes reached stability.

Maximum parsimony (MP) analyses were performed with PAUP* using the heuristic search method with 10 random stepwise additions and the TBR branch swapping option.

Bootstrap analyses (Felsenstein 1985) were used to examine the robustness of the resulting bifurcations within the trees. MP and NJ trees were tested with 2000 and 10 000 replicates,

Table 1 Taxa used in this analysis classification of taxa follows the concept of Hansen (1991, 1997a), and GenBank accession number.

		Accession No.					
Taxon		SSU rDNA	LSU rDNA	16S rDNA	12S rDNA	COI	COII
Hydrophiloidea							
Helophoridae	*Helophorus aquaticus* (Linnaeus, 1758)	AJ810714	AJ810749	AM287056	AM287034	AM287078	AM287100
	Helophorus arvernicus Mulsant, 1846	AM287122	AM287130	AM287057	AM287035	AM287079	AM287101
	Helophorus guttulus Motschulsky, 1860	AM287123	AM287131	AM287058	AM287036	AM287080	AM287102
	Helophorus nivalis (Giraud, 1851)	AJ810715	AJ810750	AM287059	AM287037	AM287081	AM287103
Epimetopidae	*Epimetopus* sp.	AJ810724	AJ810759	AM287060	AM287038	AM287082	AM287104
Georissidae	*Georissus* sp.	AJ810716	AJ810751	AM287061	AM287039	AM287083	AM287105
Hydrochidae	*Hydrochus carinatus* Germar, 1824	AM287124	AM287132	AM287062	AM287040	AM287084	AM287106
Spercheidae	*Spercheus emarginatus* (Schaller, 1783)	AJ810718	AJ810753	AM287063	AM287041	AM287085	AM287107
Hydrophilidae							
Hydrophilinae	*Anacaena globulus* (Paykull, 1798)	AM287125	AM287133	AM287064	AM287042	AM287086	AM287108
	Berosus luridus (Linnaeus, 1761)	AJ810721	AJ810756	AM287065	AM287043	AM287087	AM287109
	Cymbiodyta marginella (Fabricius, 1792)	AM287126	AM287134	AM287066	AM287044	AM287088	AM287110
	Enochrus testaceus (Fabricius, 1801)	AJ810719	AJ810754	AM287067	AM287045	AM287089	AM287111
	Enochrus quadripunctatus (Herbst, 1797)	AM287127	AM287135	AM287068	AM287046	AM287090	AM287112
	Helochares obscurus (O. F. Müller, 1776)	AM287128	AM287136	AM287069	AM287047	AM287091	AM287113
	Hydrobius fuscipes (Linnaeus, 1758)	AJ810720	AJ810755	AM287070	AM287048	AM287092	AM287114
Sphaeridiinae	*Cercyon ustulatus* (Preyssler, 1790)	AM287129	AM287137	AM287071	AM287049	AM287093	AM287115
	Coelostoma orbiculare (Fabricius, 1775)	AJ810723	AJ810758	AM287072	AM287050	AM287094	AM287116
	Sphaeridium bipustulatum Fabricius, 1781	AJ810722	AJ810757	AM287073	AM287051	AM287095	AM287117
Histeroidea							
Histeridae							
Histerinae	*Margarinotus brunneus* (Fabricius, 1775)	AJ810726	AJ810761	AM287074	AM287052	AM287096	AM287118
	Hololepta plana (Sulzer, 1776)	AJ810725	AJ810760	AM287075	AM287053	AM287097	AM287119
Dendrophilinae	*Dendrophilus punctatus* (Herbst, 1792)	AJ810727	AJ810762	AM287076	AM287054	AM287098	AM287120
Sphaeritidae	*Sphaerites glabratus* (Fabricius, 1792)	AJ810728	AJ810763	AM287077	AM287055	AM287099	AM287121

respectively. Only 100 bootstrap resamplings were carried out in the ML analyses in order to avoid excessive computational time.

Four species of the families Histeridae and Spharitidae (Histeroidea) were used to root the trees.

For the evaluation of life history changes we mapped the habitats of adults and larvae on the trees resulting from the analyses.

Results

The results are based on analyses of six genes isolated from 22 species (Table 1). Sequence lengths, AT content and number of informative characters are listed in Table 2 for all genes separately and for the combined data sets. All four mitochondrial genes show the typical high A + T contents (average 71.9%), similar to the values in other mitochondrial genes of

Table 2 Length of alignments, AT content and number of informative characters for all genes separately and for the combined data sets.

	Alignment size	No. of parsimony informative characters	AT content (%)
SSU rDNA	1981	125	48.3
LSU rDNA	696	201	40.0
12S rDNA	362	180	76.8
16S rDNA	522	218	74.2
COI	711	303	68.4
COII	696	321	71.2
Nuclear genes	2677	326	46.2
Mitochondrial genes	2291	1022	71.9
Whole data set	4968	1348	58.3

insects (e.g. Clary & Wolstenholme 1985; Simon *et al*. 1994). The nucleotide composition in the nuclear genes was nearly unbiased, with a slight overabundance of G + C in the LSU rDNA fragment (60%).

Phylogenetic reconstruction was performed as follows: (1) with all genes separately; (2) with the combined data sets of mitochondrial and nuclear genes, respectively, and (3) with the whole data set consisting of all six genes. Analyses based on single genes generally resulted in a weak phylogenetic resolution, as differing tree topologies with low support values were obtained (not shown). The two combined data sets consisting of mitochondrial and nuclear genes, respectively, resulted in clearly better resolved branching patterns in each case. However, there were differences between the topologies created by the various analytical methods (results not shown).

In contrast, phylogenetic reconstructions using the whole data set with all six genes combined resulted in nearly identical tree topologies concerning the main branches, independent of the method used (Figs 2, 3). Slight variations were found in the branching pattern within Hydrophilidae and in the placement of Hydrochidae (Figs 2, 3). The support values of the nodes differ considerably. The posterior Bayesian

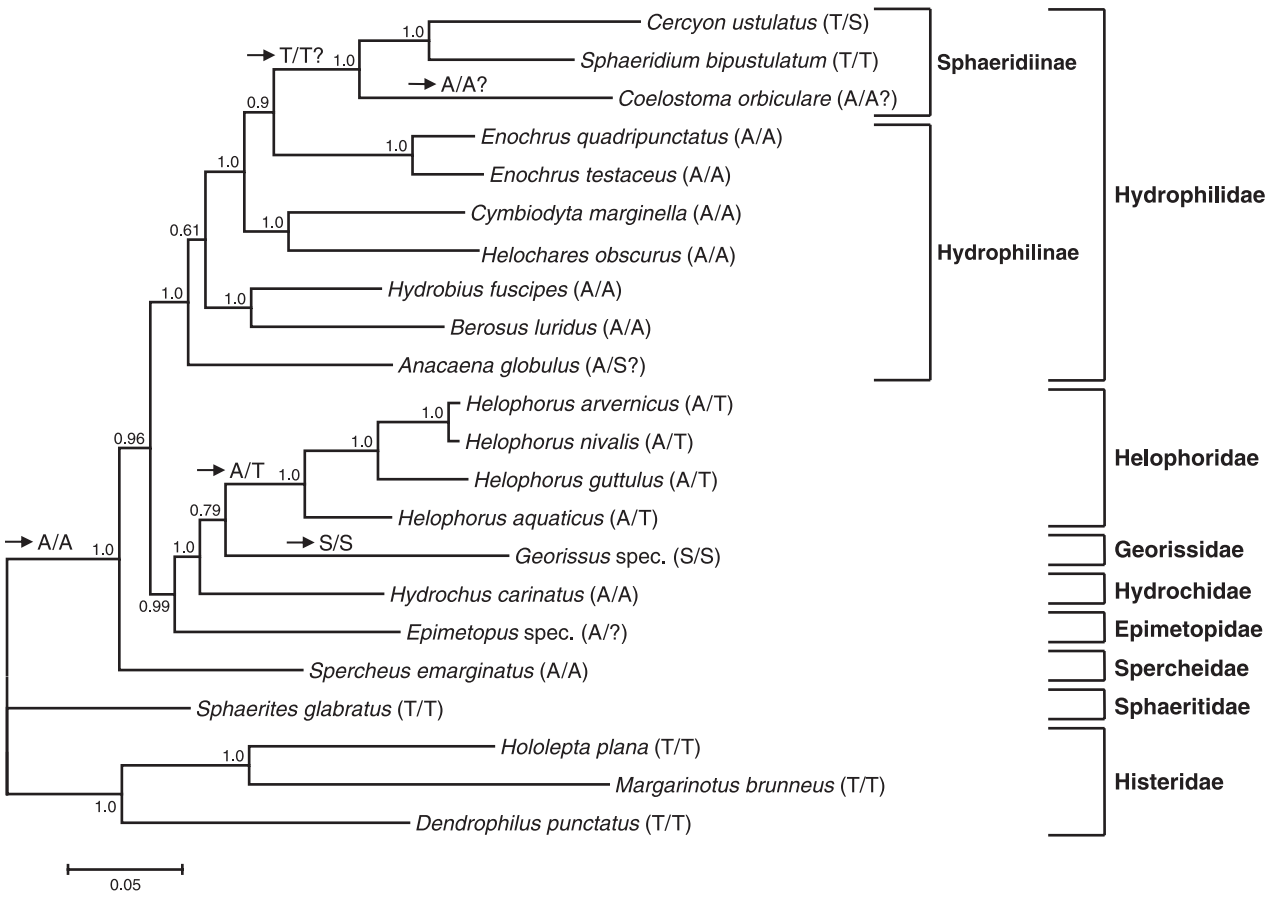

Fig. 2 Bayesian tree of the whole data set (SSU rDNA, LSU rDNA, 16S rDNA, COI, COII). Four species of the Histeroidea (Histeridae and Sphaeritidae) were chosen as outgroups. The number at each node refers to posterior probabilities. Habitats of adults (first letter) and larvae (second letter) are given in brackets. The assumed transitions in lifestyle are marked with arrows. A = aquatic, S = semiaquatic, T = terrestrial.

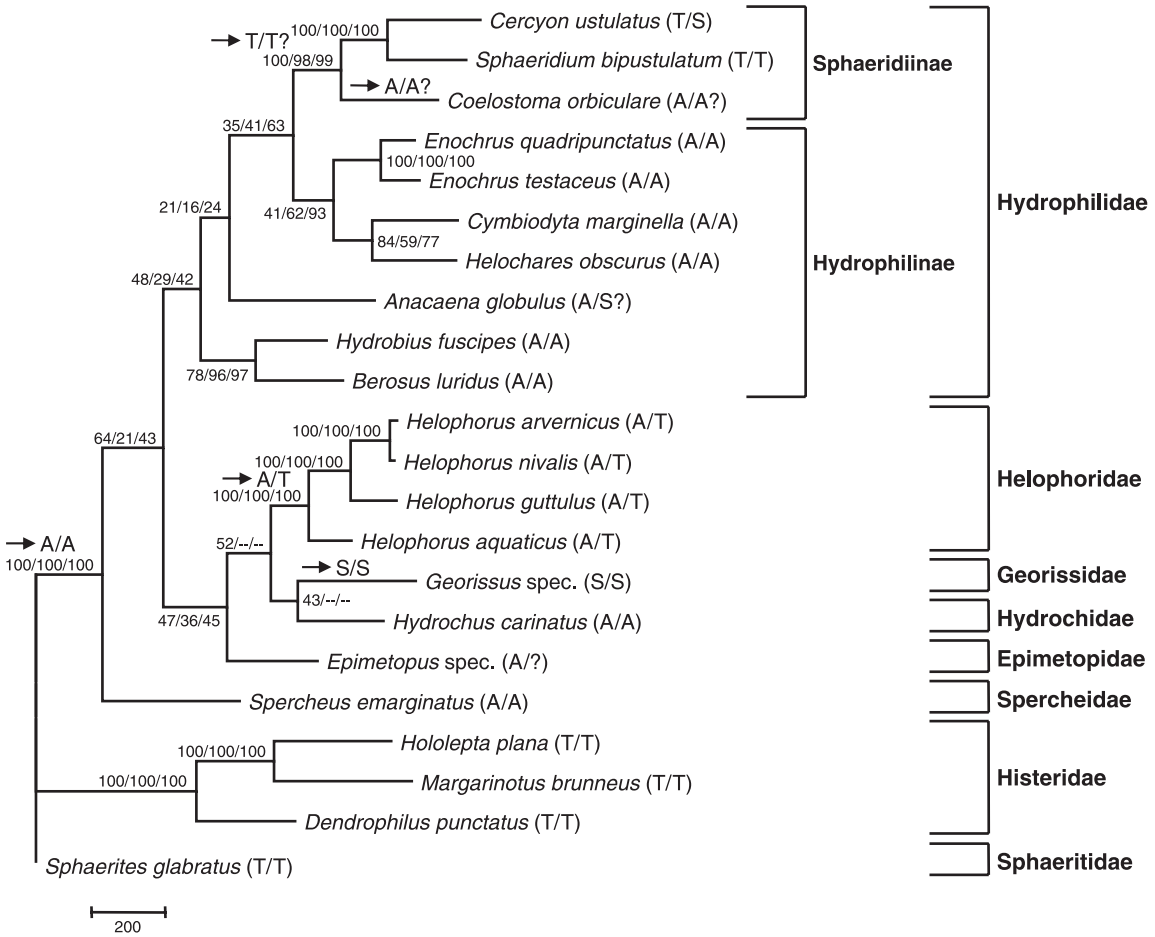

Fig. 3 Maximum parsimony tree of the whole data set (SSU rDNA, LSU rDNA, 16S rDNA, COI, COII). Four species of the Histeroidea (Histeridae and Sphaeritidae) were chosen as outgroups. The first number at each node refers to bootstrap values for 2000 bootstrap resamplings using the maximum parsimony method. The second number gives bootstrap values (100 bootstrap resamplings) using the maximum likelihood (ML) method (GTR + I + Γ). The third number represents bootstrap values out of 10 000 trees in the NJ analysis. Habitats of adults (first letter) and larvae (second letter) are given in brackets. The assumed transitions in lifestyle are marked with arrows. A = aquatic, S = semiaquatic, T = terrestrial.

probabilities are generally very high (mostly reaching 100%) while the bootstrap support values are often low.

The phylogenetic analyses with the complete data set yielded a well-supported monophyletic group comprising all members of Hydrophiloidea. The branching pattern within the outgroup is also well supported and corresponds with traditional views of histeroid phylogeny. The histerid species form a well founded clade; also, the two species belonging to the subfamily Histerinae branch together, separate from *Dendrophilus punctatus* (Dendrophilinae).

Within Hydrophiloidea, *Spercheus emarginatus* (Spercheidae) always branches off first followed by a split into two lineages. The first group comprises Epimetopidae, Hydrochidae, Georissidae, and Helophoridae, with Epimetopidae branching off first followed by Hydrochidae and Georissidae (Fig. 2). The same branching pattern was found in the ML analysis, whereas the position of Hydrochidae differs in the MP and NJ analyses. This family groups either with Georissidae (MP, Fig. 3) or with Epimetopidae (NJ), but the bootstrap values of both nodes are low. The sequence data also yielded a good resolution within the genus *Helophorus*. All included species of the subgenus *Atractelophorus* (*H. arvernicis*, *H. nivalis*, *H. guttulus*) form a clade, with *H. aquaticus* (*Helophorus* s.s.) branching at the base of the family. The second large lineage comprises all included species of Hydrophilidae (s.s.) belonging either to the subfamily Hydrophilinae or to the Sphaeridiinae. Hydrophilidae forms a clade with all analytical approaches, but is only well supported in the Bayesian analysis. The branching pattern within the family differs between the Bayesian analysis (Fig. 2) and the other methods (Fig. 3).

However, in all trees Hydrophilinae is paraphyletic as Sphaeridiinae is placed as a subordinate group within the subfamily. Slight differences occur in the Bayesian analysis (Fig. 2). The two *Enochrus* species form the sister group of Sphaeridiinae (Fig. 2); *Cymbiodyta marginella* and *Helochares obscurus* branch off first, whereas in the other analyses all four species together emerge as their sister group (Fig. 3). Paraphyly of Hydrophilinae was also found in our analyses using the combined data sets of mitochondrial or nuclear genes.

Discussion

Based on an evaluation of a comprehensive molecular data set we present an alternative view of the phylogenetic relationships and the evolution of aquatic and/or terrestrial lifestyles within Hydrophiloidea. The taxon sampling in our analyses was comparatively limited, although we included representatives of all families and subfamilies, with the exception of the monospecific Horelophinae and Horelophopsinae. From some other subgroups only single species could be analysed. However, in contrast to Hydrophilidae, the other families are small and monogeneric, with the exception of Epimetopidae, which comprises three genera and 35 species (Archangelsky *et al*. 2005).

Recent studies have demonstrated that the number of taxa is of less importance than the quantity of character data for the accuracy of phylogenetic results (e.g. Poe 1998; Rosenberg & Kumar 2001, 2003; Rokas & Carroll 2005). Moreover, increasing numbers of taxa can even correlate with a slight decrease in phylogenetic accuracy, while increasing numbers of genes have a significant positive effect (Rokas & Carroll 2005). Accordingly, previous molecular analyses using the SSU and LSU rDNA of many staphyliniform species (Korte *et al*. 2004; Caterino *et al*. 2005) have yielded only unstable and weakly supported branches with a very limited resolution within Hydrophiloidea.

Therefore, we focused our analyses on 22 species, using six different genes or gene fragments with a total of 4968 positions. Our analyses showed that the whole data set with all six genes resulted in stable and more consistent trees; it also produced higher support values than the nuclear or mitochondrial data sets or the single genes alone.

The results of our study clearly confirm the monophyly of Hydrophiloidea s.s. Within this clade, Beutel (1994, 1999) suggested a basal placement of Spercheidae based on structural features of larvae (see Beutel 1999 for a detailed discussion). This placement is in contrast to Hansen (1991), Archangelsky (1998), and Beutel & Komarek (2004), who proposed a sister-group relationship between Spercheidae and Hydrophilidae. The molecular data support the hypothesis of Beutel (1994). In all trees based on the entire molecular data set, *Spercheus emarginatus* is placed at the first branch (Figs 2, 3).

The same result was obtained using the nuclear data set (SSU and LSU rDNA), although the combined mitochondrial genes did not support this branching pattern unambiguously. Morphological characters of larvae in support of Hydrophiloidea excl. Spercheidae are the absence of intramaxillary mobility, the subdivision of the cardo, the mesally closed, tube-shaped stipes, the reduction of the lacinia, and the absence of a typical M. craniolacinialis. Further potentially plesiomorphic features of spercheid larvae suggesting a basal position are the subprognathous head, the presence of a broad gula, the posterior position of the posterior tentorial arms, the complete absence of the nasale and the adnasalia, and the retracted position of the mentum (Beutel 1994, 1999). However, this placement of Spercheidae implies that the complex stigmatic atrium of larvae, which is also present in Hydrochidae and Hydrophilidae (partim), has either evolved several times independently, or was secondarily reduced in some groups.

The relationships of the remaining families suggested by the results of our analyses are partly in agreement with the proposals of Hansen (1991, 1997a). As in Hansen's preferred cladogram (Hansen 1991: fig. 5) we obtained a monophyletic 'helophorid lineage' (Hansen 1991) comprising Helophoridae, Epimetopidae, Georissidae and Hydrochidae. The monophyly of Hydrophilidae was also confirmed. However, the branching pattern within the 'helophorid lineage' clearly differs from that proposed by Hansen (1991). Furthermore, our data do not support a modified version of the hypothesis of Hansen (1991) presented by Archangelsky (1998) and Archangelsky *et al*. (2005), i.e. a sister group between Hydrochidae and a clade comprising Spercheidae and Hydrophilidae (= 'hydrophilid lineage'). This group is strongly supported by the presence of the stigmatic atrium (see above; Archangelsky 1998). The molecular results are also in contrast to other current proposals of the phylogenetic relationships within Hydrophiloidea based on morphological data (Beutel & Komarek 2004; Beutel & Leschen 2005). A sister-group relationship between Epimetopidae and Georissidae appears well supported by morphological data (e.g. preocular clypeal excision in adults, mesally directed, serrate adnasal spines in larvae; e.g. Archangelsky 1998; Beutel 1999). However, our results suggest a closer relationship between the latter family and Helophoridae, and a sister-group relationship between Epimetopidae and the remaining 'helophorid lineage'.

Our results also disagree with traditional views of the phylogenetic relationships within the Hydrophilidae (see e.g. Hansen 1991; Archangelsky *et al*. 2005). Sphaeridiinae are placed as a subordinate group within Hydrophilinae. Therefore, Hydrophilinae appear as a paraphyletic assemblage. The *Enochrus* species (Fig. 2), *Cymbiodyta marginella*, and *Helochares obscurus* are more closely related to Sphaeridiinae than to the rest of Hydrophilinae. This is in contrast to Hansen

(1991, 1997a), Archangelsky (2004), and Archangelsky *et al*. (2005), but paraphyly of Hydrophilinae with Sphaeridiinae as a subordinate group was also suggested by Anton & Beutel (2004) and Beutel & Komarek (2004) based on analyses of characters of the adult head and thorax, respectively (see above). Similar topologies were found in molecular analyses focusing on the phylogeny within Staphyliniformia using SSU and LSU rDNA sequences (Korte *et al*. 2004; Caterino *et al*. 2005). Moreover, paraphyly of Hydrophilinae with Sphaeridiinae as a subordinate group was confirmed in all of our trees independent of data sets or analytical procedures.

Our results suggest some novel evolutionary interpretations concerning the transitions between aquatic and terrestrial lifestyles. Within Polyphaga multiple transitions to aquatic habitats occurred independently in Hydrophiloidea, Staphylinoidea (Hydraenidae), Byrrhoidea (e.g. Elmidae, Dryopidae), Chrysomelidae (Donaciinae) and Curculionidae (e.g. *Eubrychiopsis*, *Bagous*), sometimes only in the adult (Dryopidae, Helophoridae) or larval stages (Ptilodactylidae), but usually in both. Within the Adephaga, two or three invasions were suggested by Beutel (e.g. 1995, 1997) and Beutel *et al*. (2006) based on morphological investigations, whereas a single colonization of the aquatic medium by the Hydradephaga was supported by SSU rDNA sequence data (Ribera *et al*. 2002). A secondarily terrestrial lifestyle has evolved in Hydradephaga (e.g., *Geodessus*) and Hydrophiloidea several times, although more often in the latter group. The adaptations to an aquatic lifestyle are very different in Hydrophiloidea and Hydradephaga (Beutel & Komarek 2004), and in general most aquatic species of Hydrophiloidea are poor swimmers compared to species of most hydradephagan groups (Gyrinidae, Hygrobiidae, Dytiscidae). Only some representatives of the Hydrophilidae are good swimmers (e.g. Berosini). Therefore, Beutel & Komarek (2004) assumed that rapid swimming is not as important for herbivorous or saprophagous species as it is for predacious beetles. Therefore, adults of Hydrophiloidea are primarily adapted to moving slowly among water plants, a habit which is preserved by most members of the group.

Archangelsky *et al*. (2005) assumed a change to aquatic habits by the common ancestor of Spercheidae, Hydrochidae and Hydrophilidae (stigmatic atrium of larvae) and at least one further transition within the 'helophorid' lineage (adults of Helophoridae) based on the phylogenetic hypothesis of Archangelsky (1998). Hansen (1997b) stated that the ancestor of Hydrophiloidea was terrestrial and both larvae and adults occurred as saprophages among litter or in the upper soil layer, a habitat that is still maintained by some representatives of Hydrophiloidea (e.g. Horelophopsis; Archangelsky *et al*. 2005). In contrast, Beutel (1997) suggested a primarily terrestrial lifestyle for the adults and a primary preference for riparian habitats for the larvae. The molecular results as shown in Figs 2 and 3 indicate a single origin of the aquatic lifestyle by the common ancestor of Hydrophiloidea, at least in the adult stage. This interpretation is more parsimonious as it involves only one transition to a terrestrial lifestyle by the Sphaeridiinae and a further transition to semiaquatic habitats by the Georissidae (Figs 2, 3). In contrast, a terrestrial ancestor would require at least three independent invasions of the aquatic environment, and also secondarily terrestrial habits in Sphaeridiinae (or multiple invasions of aquatic habitats). Spercheidae and Hydrochidae are fully aquatic as larvae and adults, and Helophoridae as adults (with few exceptions). This interpretation implies that the riparian habits have secondarily evolved in Georissidae, which burrow in sand, and possibly in some representatives of Epimetopidae. The biology of the latter group is very poorly known (Archangelsky *et al*. 2005), but aquatic habits are at least reported for adults (Hansen 1991; see also Jäch 1998). Within the 'helophorid lineage' all aquatic species are poor swimmers, and are mainly adapted to climbing on water plants (Helophoridae, Hydrochidae). Adults of Spercheidae move along the underside of the surface film. This, and their unique filter feeding apparatus, are without doubt autapomorphies of this family. Within Helophoridae some species are terrestrial, but most adult representatives of the family are aquatic or at least semiaquatic.

Hydrophilidae comprises aquatic and terrestrial subgroups. An aquatic lifestyle is very likely a groundplan feature of this clade (larvae and adults) and secondarily a terrestrial lifestyle has likely evolved several or many times. Within the traditional subfamily Hydrophilinae most species are aquatic. The swimming ability of the majority is poor, but some are good swimmers (Berosini). Transitions to terrestrial habits occurred within the Chaetarthriini and Anacaenini (Hydrophilinae), and a terrestrial lifestyle is presumably ancestral and autapomorphic for Sphaeridiinae, traditionally treated as a separate subfamily. However, within this lineage tertiary shifts from terrestrial to aquatic habits also occurred, as is known for species of the genera *Cercyon* and *Coelostoma*. Despite of the aquatic habitats of *Coelostoma*, which branches off first within the Sphaeridiinae in our trees (Figs 2, 3), we assume that terrestrial habits are ancestral for Sphaeridiinae, even though this is less parsimonious with regard to the branching pattern of the taxa we included in the analyses. This is suggested by the terrestrial lifestyle of most members of Rgymodini, Tormissini and Andotypini (Hansen 1991). Furthermore, adults of several genera of Coelostomatini are also terrestrial.

Conclusions

Previous analyses based on morphological characters (e.g. Hansen 1991; Beutel 1994, 1999; Archangelsky 1998) and also DNA sequence data resulted in different reconstructions of the phylogeny of Hydrophiloidea (e.g. Caterino *et al*. 2005; Korte *et al*. 2005). Our results, based on analyses of a

comprehensive molecular data set, are partly in agreement with earlier hypotheses, but differ in some aspects. The monophyly of Hydrophiloidea, Helophoridae, Hydrophilidae and Sphaeridiinae is confirmed. We suggest a basal position of Spercheidae, as already proposed by Beutel (1994, 1999), a 'helophorid lineage' comprising Epimetopidae (as basal group), Hydrochidae and Georissidae as a sister group of Helophoridae.

Sphaeridiinae are placed as a subordinate group within Hydrophilinae, thus rendering the latter subfamily paraphyletic. More taxa need to be examined for a detailed reconstruction of the phylogeny of Hydrophilidae. Based on the obtained branching pattern we suggest that the ancestor of Hydrophiloidea was aquatic and that several changes to a terrestrial lifestyle took place within the group. A detailed examination of morphological features of Horelophinae and Horelophopsinae and extraction of DNA from suitably fixed material should have high priority. A combined analysis of molecular data and morphological characters or members of all subfamilies may finally yield a very solid phylogenetic reconstruction.

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