

Discovery of Aspidytidae, a new family of aquatic Coleoptera

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The six extant aquatic families of Hydradephaga (Coleoptera) known so far represent a diverse group of beetles morphologically highly modified for life in the water. We report the discovery of a new genus with two species from South Africa and China, which differ greatly from all extant families, but resemble the Jurassic–Cretaceous †Liadytidae (the dagger symbol indicates that the taxa are known only as fossils). Based on a combined phylogenetic analysis of molecular and morphological data we erect a new family, Aspidytidae, which is the sister group of Dytiscidae plus Hygrobiidae. We propose a new scenario for the evolution of swimming behaviour in adepghan beetles, in which the transition into the aquatic environment is followed by complex and repeated changes in lifestyles, including the secondary complete loss of swimming ability in Aspidytidae.

Keywords: Coleoptera: Adepghaga; Hydradephaga relationships; biodiversity; new species, genus and family; simultaneous phylogenetic analysis; evolution of swimming

1. INTRODUCTION

Over one-third of all described animal species are beetles, with estimates of the total number of species ranging from 1 to 30 million (Hammond 1994; Novotny *et al.* 2002). Although most of these remain undescribed, new high-level groups of Coleoptera are rarely discovered. In the suborder Adepghaga (ground beetles, diving beetles and allies), representatives of almost all the currently accepted families (Lawrence & Newton 1995) had been described by the end of the 1700s, with only the Amphizoidae unknown until 1853.

Most adepghan species occur in terrestrial habitats, but *ca.* 5500 species in six extant families, generally referred to as Hydradephaga, live in aquatic environments (Guignot 1933; Crowson 1981). Hydradephagan beetles are usually characterized by a hydrodynamically shaped body and fringes of swimming hairs on their middle and hind legs, which facilitate locomotion in the aquatic medium. However, these traits differ widely between groups, reflecting the diversity of habitats, life strategies and swimming behaviours. The diversity of hydradephagan beetles ranges from groups that lack swimming ability (Amphizoidae), through poor (Haliplidae) and moderate (Hygrobiidae) swimmers, to the advanced swimming in Dytiscidae (diving beetles), Gyrinidae (whirligig beetles) and some Noteridae. These groups typically exhibit a streamlined body, flattened hind legs with swimming hairs, strong hind-leg muscles and synchronous leg movements (Ribera & Nilsson 1995). The Gyrinidae exhibit several spectacular adaptations to swimming on the water surface. The great diversity of swimming strategies has also brought into question the monophyly of the aquatic families, where morphological

studies mostly support at least two, if not three (Beutel 1997), independent transitions to the aquatic medium. However, traditional taxonomy (Crowson 1981) and more recent molecular studies (Shull *et al.* 2001; Ribera *et al.* 2002) suggest the monophyly of all hydradephagan families and imply a single aquatic origin.

Whereas representatives of the larger families (such as Dytiscidae, with *ca.* 4000 species, or Gyrinidae, with *ca.* 1000 species) can be found in almost every running and standing water body worldwide, some families have very restricted distributions. There are only six species of Amphizoidae, three from China and three from western North America. Similarly, Hygrobiidae are species poor and widely scattered geographically, with four species in Australia, one in China and one in the western Palaearctic.

In addition, not all habitat types are equally abundant, nor have they been studied with equal intensity. In particular, hygropetric (rocks overflowing with water) habitats have only recently received more attention and this has led to the discovery of a set of uniquely adapted species from various aquatic families, among them Dytiscidae. They usually exhibit characteristic morphological features, including the loss of proper swimming abilities, reduction of swimming hairs on the legs, and shorter and more robust body appendages (Balke *et al.* 1997).

Here, we describe a new genus and species obtained from this kind of habitat in the Cape region of Southern Africa. We demonstrate, based on morphological and DNA characters, that this genus has no close extant relatives. It is therefore necessary to establish a new taxonomic group within the suborder Adepghaga at the level of a family, for the first time since the Amphizoidae was described 150 years ago. This discovery adds significantly to our knowledge of biological diversity, and, because of the phylogenetic position of the new taxon, it also helps our understanding of the evolution of swimming behaviour in Hydradephaga.

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2. MATERIAL AND METHODS

(a) Morphology

Scanning electron micrographs (SEMs) were taken with a LEO 1430VP and a FEI ESEM XL 30. We made drawings using a Leitz MZ12 dissecting microscope equipped with a camera lucida.

(b) Phylogenetic analysis

DNA was extracted non-destructively and used for PCR amplification. To determine phylogenetic relationships of the new taxon we obtained sequences of the complete 18S rRNA gene and partial 16S rRNA (*ca.* 510 bp) and cytochrome oxidase I (COI) (*ca.* 750 bp) genes for all families of aquatic Adephaga, with good representation of the basal lineages (Ribera *et al.* 2002). PCR amplification and DNA sequencing were as described in Shull *et al.* (2001) and Ribera *et al.* (2002). PCR primers for the 18S rRNA gene were from Shull *et al.* (2001), and primers for 16S rRNA and COI from Ribera *et al.* (2001). New sequences were deposited in GenBank under accession numbers AY071769–AY071818.

Phylogenetic analysis was performed using a one-step tree-alignment procedure (Sankoff 1975; Wheeler 1996), using POY software (Gladstein & Wheeler 1997). Owing to the high variability, the hypervariable regions V4 and V6 (Tautz *et al.* 1988) of the 18S rRNA gene were excluded from the analyses. We assessed sensitivity to four different gap costs (1, 2, 5 and 10) and six weighting schemes (relative weights of 18S, 16S and COI genes 1 : 1 : 1, 5 : 1 : 1 and 5 : 5 : 1, respectively, including and excluding third positions in COI) (Wheeler 1995; Phillips *et al.* 2000). Within these 24 parameter combinations, the preferred settings were selected according to two criteria (Wheeler 1995): maximum congruence among genes (as measured with the incongruence-length difference test; Farris *et al.* 1995), and congruence with accepted independent (morphological) evidence, specifically the monophyly, respectively, of Gyrinidae, Haliplidae, Noteridae, Amphizoidae, Hygrobiidae and Dytiscidae. Molecular and morphological data were combined in a simultaneous analysis in POY, using the preferred parameter combinations obtained in the above analysis. All searches were performed with POY v. 2.0 on a multiprocessor computer running in parallel. Bootstrap and partitioned Bremer support values (Bremer 1994) were obtained for the combined molecular and morphology dataset in PAUP v. 4.0b8 (Swofford 1998) under the preferred parameter conditions, using a matrix generated with the 'impliedalignment' option in POY.

3. RESULTS

(a) Taxonomy

(i) *Aspidytes gen. nov.*

Type species: *Aspidytes niobe* sp. nov. Western Cape Province, Republic of South Africa. An additional undescribed species is known from Shaanxi Province, China (Balke *et al.* 2002).

Diagnosis: mesal walls of metacoxae extensively fused; pedicellus very short and partly enclosed by scapus (figure 1c); legs without swimming hairs (figure 1a); transverse suture (very weakly impressed) and corresponding internal ridge of metaventricle present, median part of metacoxae elevated (figure 1b). *Aspidytes* can be readily separated from any genera of the other extant families of Dytiscoidea (Noteridae, Amphizoidae, Hygrobiidae, Dytiscidae

(Lawrence & Newton 1995); table 1; see Balke *et al.* (2002) for a key and discussion of morphology; full character matrix and references available at <http://www.bio.ic.ac.uk/research/apvogler/vogler.htm> and upon request). Similarities exist to fossil †Liadytidae, a dytiscoid family recorded from the Jurassic (Toarcian, 187 Myr ago) to the Cretaceous (Albian, 97 Myr ago) (Ponomarenko 1977; Labandeira 1994). †*Liadytes* and *Aspidytes* share a broad lateral part of the metathoracic praepisternum, a very similar metacoxal structure, a metathoracic anepisternum that reaches the midcoxal cavities, a transverse suture of the metaventricle (evident in *Aspidytes* mostly internally as the division of prae- and katepisternum III), and thin and longish metatibiae and tarsi. However, the inclusion of *Aspidytes* in the Liadytidae is not supported by any apomorphy shared only by these groups, and the shared characters are known to be present in other, unrelated, lineages. The character most clearly identifying *Aspidytes* (configuration of scapus and pedicellus) is not observable in the known specimens of †*Liadytes*.

Etymology: from the Greek *aspis* (shield) and *dytes* (diver).

(ii) *Aspidytes niobe* sp. nov.

Material studied: holotype male (in Natural History Museum, London (NHM)) from 7 km southwest of Ceres and paratype male (Naturhistorisches Museum, Wien) and female (NHM) from Limiet Berge, 19 km northeast of Wellington. Both sites are in the Western Cape Province, South Africa, I. Ribera & A. Cieslak leg. 24.3.2001. DNA extractions of both males are stored in the tissue collection of the NHM (IR600, IR627).

Diagnosis: prosternal process widest caudally, subtriangular in shape; metacoxal plates with round apex, margin regularly curved; median lobe of aedeagus asymmetrical, parameters flat and wide (see Balke *et al.* (2002) for a detailed account of morphology).

Etymology: Niobe, queen of Tebas, was still covered in tears after being transformed to stone.

(b) Phylogenetic relationships of *Aspidytes*

The parameters for sequence alignment and character weighting for which the incongruence-length difference was minimized were: weight of 18S rRNA = 5, 16S rRNA and COI = 1; third codon positions excluded from COI; gap cost = 2. The final length of the aligned sequences was 2894 bp, of which 507 bp were parsimony informative. Under these conditions, two equally parsimonious trees of cost = 5824 were found (CI = 0.66, RI = 0.65, partition homogeneity test not significant, $p = 0.94$). *Aspidytes niobe* was placed within superfamily Dytiscoidea, as sister of Dytiscidae plus Hygrobiidae (figure 2). Trees obtained under the five next-best parameter combinations consistently placed *Aspidytes* sister to Hygrobiidae and Dytiscidae. An alternative position of *Aspidytes* was as sister to *Amphizoa*, which was obtained under the character combination that ranked ninth out of the 24 tested, whereas under the seventh and eighth highest scoring parameter settings Dytiscidae were paraphyletic with respect to two or more other families.

A morphological matrix including 49 adult and larval characters was compiled from Beutel (1990), Belkaceme

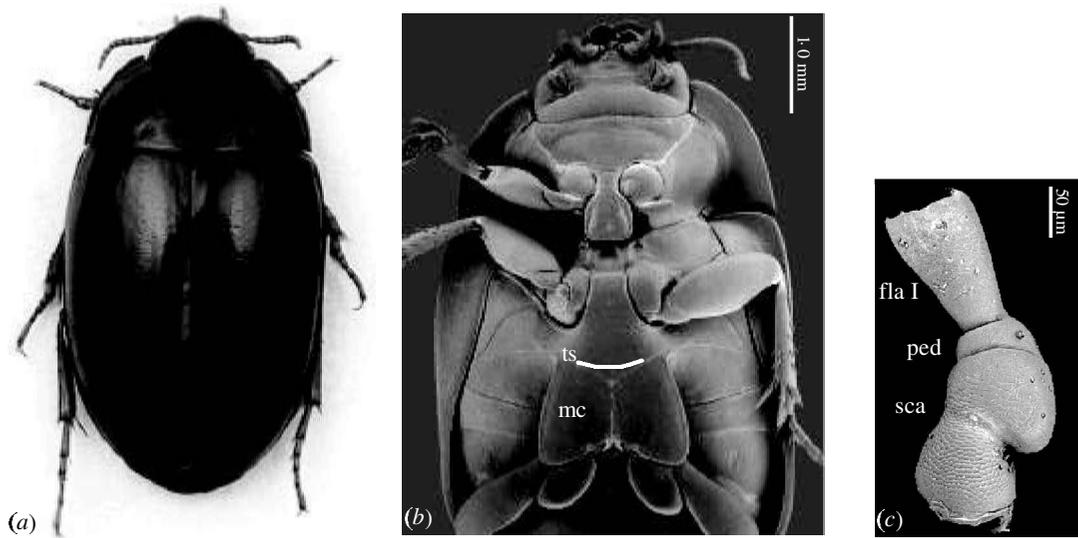


Figure 1. (a) *Aspidytes niobe*, new species, habitus of holotype. (b) *Aspidytes niobe*, new species, SEM of paratype male from Limiet Berge, South Africa, ventral view: mc, metacoxal plates; ts, position of the internal transverse suture of metaventricle, highlighted by white line. (c) *Aspidytes* sp. (undescribed Chinese species). SEM of female paratype: sca, configuration of scapus; ped, pedicellus; fla I, flagellomere I.

Table 1. Synoptic distribution of main morphological characters in hydradephagan families.

(Characters (C): 1, elongated head with protruding eyes (0), shortened and rounded laterally (1); 2, pedicellus not partly enclosed by scapus (0), partly enclosed by scapus (1); 3, prosternal process narrow and short (0), well developed (1); 4, pro- and metasternal process not in contact (0), in contact (1); 5, prothoracic defence glands absent (0), present (1); 6, mesoventrite large and flat (0), narrow with hexagonal groove (1); 7, mesocoxal cavity disjunct (0), complex (1); 8, mesocoxa triangular (0), globular or conical and sunk in deep cavities (1); 9, mesal metacoxal walls not fused or with small area of fusion (0), extensively fused (1); 10, metafurcal origin in metaventricle (0), intercoxal septum (1); 11, metasternal transverse ridge complete (0), partly reduced (1), absent (2); 12, cranial expansion of metacoxae absent (0), moderately expanded (1), strongly expanded (2); 13, metacoxal plates absent (0), moderately large (1), strongly enlarged (2), largely reduced (3); 14, swimming hairs absent (0), sparse fringes (1), dense fringes (2), feather-like (3), lamellae (4); 15, hind legs slender (0), broadened and flattened (1), with oar-like extension (2), short paddles (3). In parentheses, characters of *Spanglerogyrus* (Gyrinidae) and *Phreatodytes* (Noteridae). Some highly derived subterranean or terrestrial genera of Dytiscidae not scored.)

C	Gyrinidae	Haliplidae	Noteridae	Amphizoidae	Aspidytidae	†Liadytidae	Hygrobiidae	Dytiscidae
1	1	0	1	0	1	1	0	1
2	0	0	0	0	1	?	0	0
3	0	1	1	1	1	1	1	1
4	0	1	1(0)	0	0	0	1	1
5	0	0	0	0	0	?	1	1
6	0	1	1	1	1	1	1	1
7	0	0	0	1	1	1	0	0/1
8	0	1	1	1	1	1	1	1
9	0	0	1	1	1	1	1	1
10	0	0	1	1	1	1	1	1
11	2(0)	0	2	1	1	1	1	2
12	1/2(0)	0	2	1	1	1	1	2
13	0	2	1	1	1	1	3	3
14	4(3)	2	1/2(0)	1	0	2	2	2
15	3(2)	0	0/1	0	0	0	1	1

(1991), Beutel & Haas (1996) and original observations (full character matrix and references available at <http://www.bio.ic.ac.uk/research/apvogler/vogler.htm>). Parsimony analysis resulted in 226 trees of 89 steps (CI = 0.86, RI = 0.95), with the same topology as the preferred molecular tree, but with the node separating Amphizoidae, *Aspidytes* and Hygrobiidae plus Dytiscidae unresolved in the strict consensus. The internal topology of the families was either unresolved (Dytiscidae) or ident-

ical to that obtained with the molecular dataset (all other families).

Terminal taxa in the morphological study correspond to those used in the molecular analyses at the genus level. A simultaneous analysis of both datasets resulted in a single tree with the same topology as that obtained from molecular data. The tree from the simultaneous analysis was only one step longer than the sum of the separate analyses (partition homogeneity test not significant, $p = 0.98$), indi-

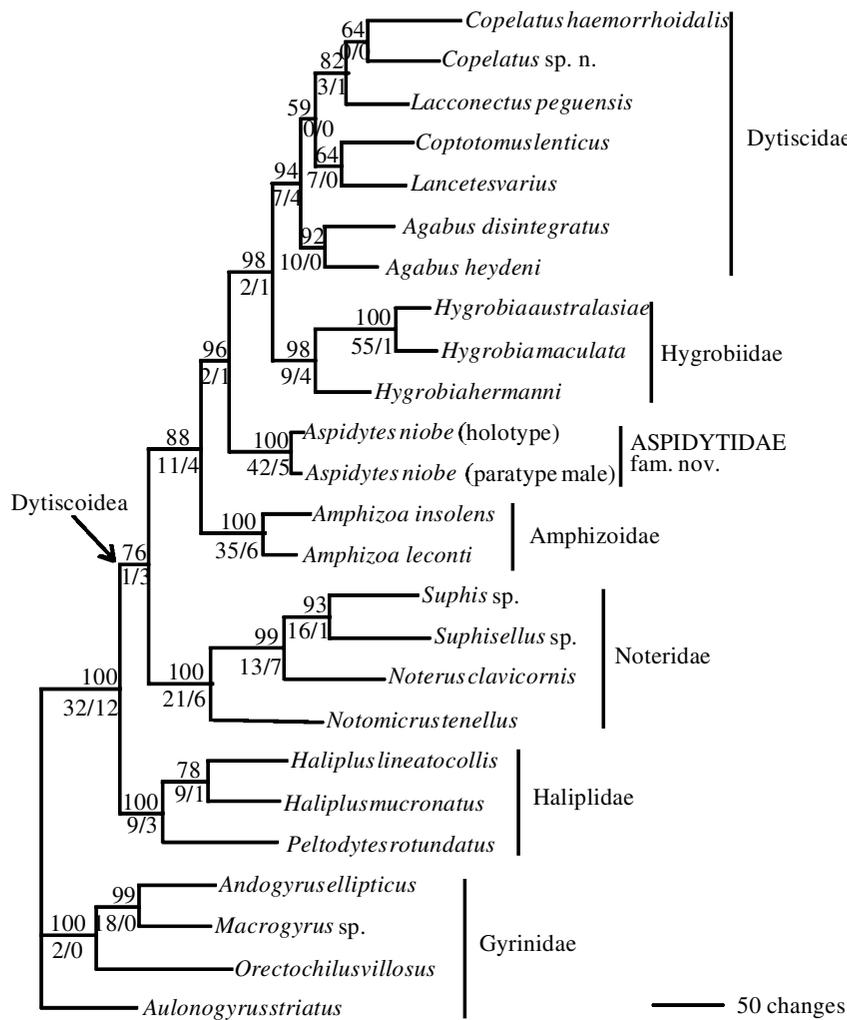


Figure 2. Single tree obtained in the simultaneous analysis of the molecular and morphological dataset, with the optimal parameter combination obtained in the molecular analysis (weight of 18S rRNA = 5, 16S rRNA, COI and morphology = 1; third codon positions excluded from COI; gap cost = 2). The tree is rooted on Gyrinidae, which are clearly basal to Dytiscoidea (Beutel 1997; Ribera *et al.* 2002). Branch lengths refer to molecular data only. Numbers above branches, bootstrap support values; below branches, partitioned Bremer support values (combined molecular/morphology).

cating minimal conflict between the two datasets. Most nodes had high Bremer and bootstrap support, including a 96% support value for the node determining the position of *Aspidytes* (figure 2).

(i) *Aspidytidae* fam. nov.

According to the morphological and phylogenetic evidence provided above, we classified our discovery as follows: Insecta; Coleoptera; Suborder Adephaga; Superfamily Dytiscoidea; *Aspidytidae* fam. nov. ('cliff water beetles'). This is a monogeneric family, erected for the South African *Aspidytes niobe* and an undescribed Chinese species (Balke *et al.* 2002). The diagnosis of the new family is the same as that of the genus *Aspidytes*.

4. DISCUSSION

(a) *Systematics*

Based on its morphology, the placement of *Aspidytes* within Dytiscoidea is strongly supported by the extensively fused mesal walls of the metacoxae. The inclusion in any of the extant families of Dytiscoidea is, however, not possible. The presence of a metasternal transverse ridge

excludes it from Dytiscidae and Noteridae. The absence of prothoracic glands also excludes it from Dytiscidae and Hygrobiidae, and the absence of spermathecal glands excludes it from Noteridae. The configuration of the pedicellus and scapus is unique, despite the superficial resemblance to the shortened scapus of Noteridae. Other characters of the meso- and metacoxae also exclude *Aspidytes* from any of the known Dytiscoidea families (table 1; Kavanaugh 1986; Balke *et al.* 2002).

The phylogenetic position of *Aspidytes* as sister to Hygrobiidae plus Dytiscidae has strong support from both the combined molecular and the morphological datasets, with very little conflict between the two. Accepting the convention of taxonomic classification on the basis of monophyly (Hennig 1966), the only alternative to establishing a new family would be the inclusion of Dytiscidae, Hygrobiidae and *Aspidytidae* in a single family. We consider this possibility to be less desirable, both because of the deep morphological and genetic differences between the three groups, and for reasons of stability, as Hygrobiidae and Dytiscidae have been considered as separate families for more than a century (Franciscolo 1979; Kavanaugh 1986).

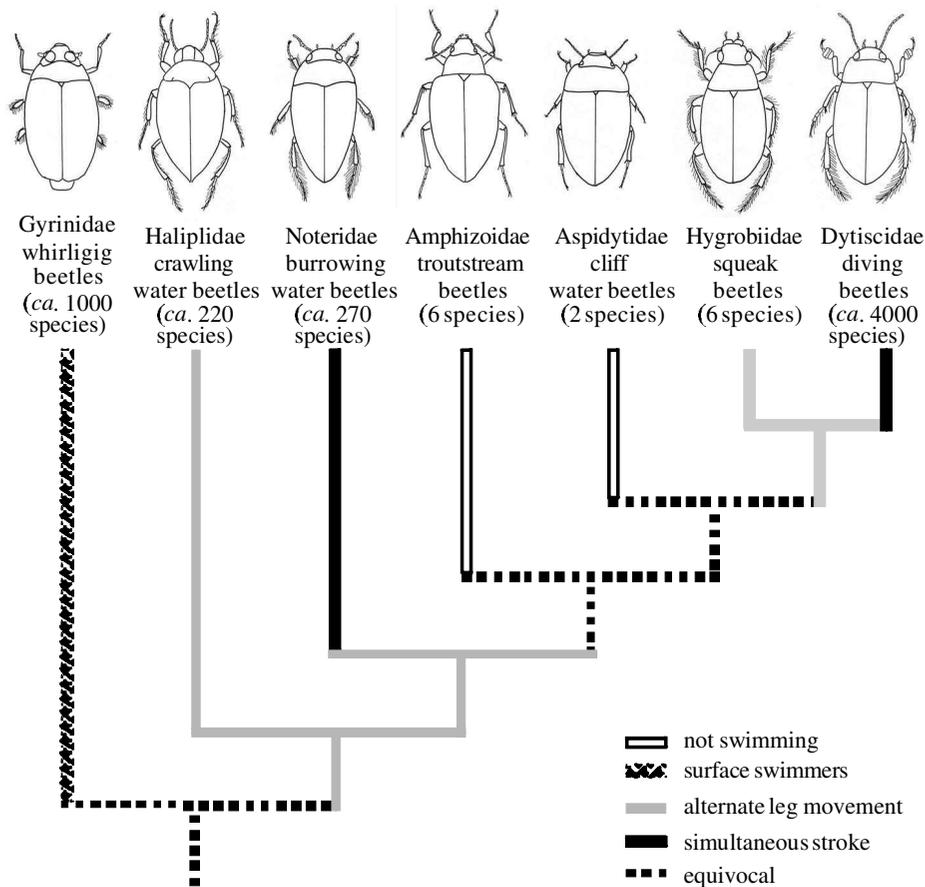


Figure 3. Evolution of swimming behaviour in the aquatic families of Hydradephaga, according to the phylogenetic hypothesis of figure 2. All families have well-developed swimming hairs except Amphizoidae (reduced) and Aspidytidae (absent). Modifications of the legs are different in Haliplidae (very slender, well-developed metacoxal plates, middle legs providing the main thrust) and Hygrobiidae (more robust, metacoxal plates reduced, hind legs providing the main thrust). Higher Noteridae have front legs modified for burrowing.

(b) Evolution of the swimming behaviour in adephagan beetles

The cladogram was used to evaluate scenarios for the evolution of swimming behaviour and associated morphological traits in Hydradephaga (figure 3). Once the transition to the aquatic medium had occurred in the stem species of Hydradephaga (Shull *et al.* 2001; Ribera *et al.* 2002), possibly excluding Gyrinidae (Beutel 1990; Beutel & Haas 1996), traditional views support a linear progression within Dytiscoidea from non-swimming forms (Amphizoidae) to imperfect swimming in Hygrobiidae and full swimming in Noteridae and Dytiscidae (Guignot 1933; Franciscolo 1979; also compatible with the phylogeny in Miller (2001); although see Beutel (1997) for an alternative view). We reveal a more complex situation, where idiosyncratic swimming morphologies and behaviours arose independently in various groups, but were constrained by functional requirements leading to convergence.

Basal to all other aquatic families, and possibly unrelated (Beutel 1997), is Gyrinidae, with unique and highly derived specializations for locomotion in the water surface film. Species of Haliplidae retain primitive swimming abilities, with slender legs that are moved alternately, similar to basal groups of Noteridae. Derived genera of Noteridae have advanced swimming abilities, with simultaneous movements of the middle and hind legs (Belkaceme

1991), and front legs adapted for burrowing. An independent origin of simultaneous leg movements is inferred in the Dytiscidae, which, together with gyrinids, have the most advanced swimming abilities (Nachtigall 1961; Balke 2003).

Hygrobiidae, as sister of Dytiscidae, retain alternate leg movements and exhibit dense fringes of long swimming hairs on all legs, with the forelegs developed for burrowing, similarly to 'higher' Noteridae. Finally, Amphizoidae and Aspidytidae live in fast-flowing rivers and on hydropetric surfaces, respectively, but are entirely unable to swim. Amphizoids exhibit very sparse fringes of swimming hairs, which are entirely absent in Aspidytidae. The phylogenetic placement of Amphizoidae and Aspidytidae amidst a clade of moderate ('basal' Noteridae, Hygrobiidae) and good swimmers indicates that swimming abilities and some associated morphological traits have been lost secondarily, possibly independently in each of the two groups.

Transitions into novel life zones produce opportunities for diversification (Simpson 1953). The spectacular ecological and morphological diversity of hydradephagan beetles was enhanced by independent and convergent diversification of aquatic life forms, rather than progressive refinement in a single lineage. However, the adaptive radiation of Hydradephaga, with 5500 species compared with over 24 000 species in their terrestrial counterpart

(Geadephaga), can hardly be considered an evolutionary success with regard to their taxonomic diversity. Aspidytidae remains a small apparently relictual group, but the finding of two species of *Aspidytes* in different parts of the globe highlights our ignorance of biological diversity in extreme or rare habitats and these habitats' importance for understanding the evolution and diversification process of extant lineages.

Alexandra Cieslak found the male paratype of *A. niobe*. Manfred A. Jäch trusted us with the Chinese species for study and helped in many ways. We thank Peter Forey, Peter Hammond and John Lawrence for comments on the manuscript. Konrad Dettner helped with the dissection of the prothorax. Kosmas Theodorides helped to compose the genus name. This work was supported by the Leverhulme Trust, FAZIT Foundation, Marie Curie fellowships of the EU, NERC and British Airways.

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Supplementary information

Morphological characters used in the analyses.

a) List of taxa:

Dytiscidae: *Agabus* spp., *Coptotomus* sp., *Lancetes* sp., *Copelatus haemorrhoidalis*, *Lacconectus* sp.

(species used for molecular analysis: *Agabus disintegratus*, *Agabus heydeni*, *Coptotomus lenticus*, *Lancetes varius*, *Copelatus haemorrhoidalis*, *Copelatus utowaensis*, *Lacconectus pegensis*)

Hygrobiiidae: *Hygrobia hermanni*

(species used for molecular analysis: *Hygrobia hermanni*, *H. australasiae*, *H. maculata*)

Amphizoidae: *A. insolens*, *A. lecontei* (same species for molecular analysis)

Noteridae: *Suphis* sp., *Suphisellus* sp., *Noterus clavicornis*, *Notomicrus* sp.

c) List of morphological characters used for phylogenetic analysis:

Adults

1. **Body shape:** (0) distinct pronoto-elytral angle; (1) laterally evenly rounded, streamlined. The body of adults is stream-lined and evenly rounded laterally in *Aspidytes* and in adults of Noterinae (Belkaceme 1991), Hygrobiidae and Dytiscidae. A distinct pronoto-elytral angle is present in all known species of Phreatodytinae (Uéno 1957, 1996), and in Amphizoidae.
2. **Head shape:** (0) not shortened and laterally rounded, eyes protruding (1) shortened, laterally rounded and streamlined, eyes not protruding.
3. **Compound eyes:** (0) undivided; (1) divided. The compound eyes are completely divided into an upper and a lower portion by a chitinous bridge in adults of Gyrinidae (and †Coptoclavidae; Ponomarenko 1977).
4. **Shape of scapus:** (0) parallel-sided, longer than wide; (1) large globular basal part; (2) basal and distal part globular; (3) strongly shortened but without enlarged globular part; (4) strongly enlarged, cup-shaped. A shortened scapus with a large globular basal part and a moderately elongate distal part is found in adults of Noteridae (Beutel & Roughley 1987; Belkaceme 1991). The scapus of Haliplidae is also strongly shortened, but without enlarged globular part. The distal part is also globular and very short in *Aspidytes*. A large cup-shaped scapus is present in Gyrinidae.
5. **Pedicellus:** (0) not enlarged, not strongly shortened and partly enclosed by scapus; (1) strongly shortened and enclosed by distal part of scapus; (2) ear-shaped, with fringe of sensory hairs. A strongly shortened pedicellus partly enclosed by the globular distal part of the scapus is characteristic for adults of *Aspidytes*.
6. **Flagellomeres of males:** (0) not broadened; (1) antennomeres 3-9 slightly broadened; (2) antennomeres 5 and more than one of the following segments distinctly broadened; (3) flagellomeres form a short and compact club-shaped structure.

Expansion of antennomeres in males (e.g. 5, 7, 9; see Belkaceme 1991) is a characteristic feature of Noteridae. Antennomeres 3-9 are slightly dilated in *Aspidytes*. The flagellum is compact and club-shaped in Gyrinidae.

7. **Galea:** (0) 2-segmented; (1) 1-segmented; (2) absent. The galea is one-segmented in Amphizoidae (Beutel 1988) and Gyrinini, and absent in Orectochilini and Enhydrini (Beutel & Roughley 1988).
8. **Elongate sensorial field of distal labial palpomere:** (0) absent; (1) present. A transverse, elongate sensorial field is present on the apex of the distal labial palpomere of adults of most noterid genera (not in *Notomicrus*; see Belkaceme 1991). A small sensorial field is present on each of the subapices in adults of *Copelatus* (coded as 0).
9. **Sensorial field on a protuberance of the dorsal side of the distal labial palpomere:** (0) absent; (1) present. A second sensorial field is present on a protuberance of the dorsal side of the distal labial palpomere of adults of all genera of Noterinae except for *Hydrocoptus* (Belkaceme 1991). It is elongate like the apical field in adults of *Renotus* (Belkaceme 1991).
10. **Shape of prosternal process:** (0) short and narrow; (1) well developed, converging towards apex; (2) apically rounded; (3) strongly broadened and apically truncate. The prosternal process is rounded posteriorly in adults of *Aspidytes*, *Phreatodytes*, *Amphizoa* and Trachypachidae. It is strongly broadened and apically truncate in adults of Haliplidae and some genera of Noteridae (*Siolius*, *Renotus*, *Suphis*, *Hydrocanthus*, *Canthydrus*, *Suphisellus*; Belkaceme 1991). The apical part is acuminate or at least converging towards the tip in adults of Dytiscidae and *Hygrobia*, and some noterids (e.g. *Notomicrus*; Belkaceme 1991: figs. 63-66).
11. **Ventral procoxal joint:** (0) with distinct coxal condyle; (1) coxal condyle obsolete. The ventral procoxal joint is absent in Gyrininae (well developed in *Spanglerogyrus*) and largely reduced in adults of Dytiscidae (Baehr 1979).

12. **Profemoral cleaning device:** (0) absent; (1) present. A profemoral cleaning device is present in adults of all noterid genera (Belkaceme 1991) and some representatives of Dytiscidae (e.g. *Copelatus*, *Laccophilus*).
13. **Protibial burrowing spur:** (0) absent; (1) present; (2) 2 large burrowing spurs. Presence of a strong, curved protibial burrowing spur is a characteristic feature of Noterinae excluding *Notomicrus*, *Hydrocoptus* and *Pronoterus* (Beutel & Roughley 1987). Two large, flattened burrowing spurs are present in adults of Hygrobiidae (Beutel 1986).
14. **Protibial row of flattened thorns on apical part of protibia:** (0) absent; (1) present, short; (2) present, extending to proximal part of tibia. A regular row of flattened, ribbed spines is present at the outer apical edge of Noterinae excluding *Notomicrus*, *Hydrocoptus* and *Pronoterus* (Belkaceme 1991). It is elongated towards the proximal part of the tibia in adults of *Hydrocanthus*, *Canthydrus* and *Suphisellus* (Belkaceme 1991).
15. **Outer edge of protibia;** (0) not rounded; (1) rounded. The outer edge of the protibial apex is rounded in adults of Noterinae excluding *Notomicrus*, *Hydrocoptus* and *Pronoterus* (Belkaceme 1991).
16. **Tibial groove or concavity for reception of burrowing spur:** (0) absent; (1) present. A tibial concavity for reception of the burrowing spur is present in adults of Noterinae excluding *Notomicrus*, *Hydrocoptus*, *Pronoterus* and *Noterus* (Belkaceme 1991). It is a furrow in adults of *Synchortus* and *Mesonoterus*, but a deep pit in adults of the remaining genera (Belkaceme 1991).
17. **Curved spurs on ventral side of protarsomeres 1-3:** (0) absent; (1) present. Curved spurs are present on the protarsomeres 1-3 of adults of Noterinae excl. *Notomicrus* (Belkaceme 1991).
18. **Prothoracic defense gland:** (0) absent; (1) present. These glands are present in *Hygrobia* and Dytiscidae.

19. **Mesoventrite:** (0) short, with hexagonal groove and anterolateral grooves for reception of the procoxae; (1) flat and extensive, without hexagonal groove and anterolateral grooves. A flat and extensive mesoventrite is characteristic for Gyrinidae. It is short in Trachypachidae, Dytiscoidea and Carabidae part. (e.g. *Nebria*) and the prosternal process articulates with a hexagonal groove.
20. **Mesocoxae:** (0) triangular; (1) globular or conical. Globular or conical mesocoxae inserted in deep coxal cavities and largely restricted to rotations are present in adult of all groups of Adephaga excluding Gyrinidae (Beutel & Roughley 1987).
21. **Mesocoxal cavity:** (0) laterally bordered by mesepimeron; (1) laterally bordered by mesepimeron and metathoracic anepisternum. In amphizoids and most dytiscids (e.g. *Copelatus*, *Laccophilus*), the mesocoxal cavities are laterally closed by the mesepimeron and the metathoracic anepisternum (complex-type *sensu* Bell 1967).
22. **Proximal pro- and mesotarsomeres:** (0) not elongated and broadened; (1) elongated and broadened. Elongated and broadened proximal pro- and mesotarsomeres are characteristic for adults of Noterinae excluding *Notomicrus* and *Hydrocoptus* (Belkaceme 1991).
23. **Middle- and hindlegs:** (0) elongate; (1) short and broad, paddle-like like. The transformation of the middle- and hindlegs into short paddle-like structures is a unique apomorphy of Gyrininae.
24. **Swimming hairs on meso- and metathoracic legs:** (0) absent; (1) sparse fringe of very thin and fine hairs; (2) dense row of longer hairs; (3) swimming lamellae. Swimming hairs are absent in adults of *Aspidytes*. Dense rows of hairs are present in adults of *Hygrobia*, Dytiscidae, and adults all noterine genera. Only a sparse fringe of very fine hairs is present in adults of *Amphizoa*. Characteristic swimming lamellae are present in adults of Gyrininae (not in *Spanglerogyrus*).

25. **Noterid platform of metaventricle:** (0) absent; (1) present. A flattened, platform-like median part of the metaventricle is characteristic for adults of Noterinae excl. *Notomicrus* (Beutel & Roughley 1987).
26. **Transverse ridge of metaventricle:** (0) complete; (1) partly reduced; (2) absent. The transverse ridge of the metaventricle is partly reduced in adults of *Aspidytes*, Hygrobiidae and Amphizoidae. It is absent in adults of Noteridae and Dytiscidae.
27. **Contact between pro- and metasternal process:** (0) absent; (1) present. The prosternal process articulates with the metasternal process in adults of Haliplidae, Noterinae, Hygrobiidae and Dytiscidae.
28. **Metafurca:** (0) originates from katepisternum; (1) originates from intercoxal septum. The metafurca originates from the intercoxal septum and not from the katepisternum in all adults of Dytiscoidea examined.
29. **Size of metafurca:** (0) large, with well developed lateral arms; (1) strongly reduced in size, lateral arms present; (2) narrow, without lateral arms. The metafurca of Amphizoidae is strongly reduced in size (Beutel 1988). It is strongly simplified and devoid of lateral arms in adults of Gyrininae (not in *Spanglerogyrus*).
30. **Mesal walls of metacoxa:** (0) free; (1) connected, contact area small; (2) connected along ventral edge; (3) connected, contact area extensive, intercoxal septum. A broad mesal attachment area and an extensive intercoxal septum is characteristic for all adults of Dytiscoidea (Beutel 1995). The attachment area is small in *Trachypachus*. Only the ventral edges are connected in Gyrininae (not in *Spanglerogyrus*).
31. **Anterior margin of the metacoxa:** (0) almost straight or very slightly extended anterolaterally; (1) with distinct angle; (2) rounded, strongly extended anteriorly; (3) oblique, strongly extended anteriorly, lateral part of metaventricle obsolete. A characteristic anteromesal angle of the metacoxa is present in all adults of Noterinae (Beutel & Roughley 1987). The metacoxae are strongly extended anteriorly in adults

of Gyrinini, Orectochilini, Dytiscidae and Phreatodytinae (Uéno 1957). The lateral parts of the metaventricle are obsolete in adults of the latter taxon (Uéno 1957).

32. **Metacoxal plates:** (0) absent; (1) well developed; (2) strongly enlarged; (3) largely reduced. Metacoxal plates are absent in adults of Gyrinidae (incl. †*Triadogyrus*; Beutel & Roughley 1988; Ponomarenko 1977) but present at least as vestiges in all other groups of Adephaga. They are largely reduced in adults of Dytiscidae and Hygrobiidae (and †Charonoscapinae; Ponomarenko 1977) and strongly enlarged in Haliplidae and †Triaplidae (Ponomarenko 1977).
33. **Lateral margin of metacoxal plates:** (0) very strongly diverging, reaching anterior margin of coxa at lateral edge; (1) slightly diverging anteriorly, not reaching anterior coxal margin; (2) distinctly diverging anteriorly, attaining anterior coxal margin; (3) slightly converging anteriorly, attaining anterior coxal margin; (4) very indistinct anteriorly. The lateral margin of the metacoxal plates is strongly diverging and reaching the anterior coxal margin at the lateral edge in adults of Trachypachidae and Carabidae. It is slightly diverging in *Aspidytes*, *Amphizoa* and *Hygrobia*. A clearly delimited lamina interna of the metacoxal plates with anteriorly converging external margin is characteristic for Noterinae.
34. **Mm. furca-coxalis anterior:** (0) present; (1) absent. The muscle is absent in adults of Dytiscoidea (incl. *Aspidytes*) and Gyrinidae excl. *Spanglerogyrus*.
35. **Mm. furca-coxalis anterior:** (0) present; (1) absent. Absent in adults of Dytiscoidea and *Orectochilus*.
36. **Abdominal segments III and IV:** (0) clearly separated; (1) largely or completely fused. The line separating the abdominal sternites III and IV is extremely indistinct or absent in adults of Noteridae (Uéno 1957; Belkaceme 1991: figs. 63, 65-69). It is distinct in *Aspidytes* and also in other adults of Adephaga.

37. **Bulges on anterior abdominal sternites:** (0) absent; (1) present. Bulging anterior abdominal sternites are present in adults of *Haliphus* (incl. *Algophilus* and *Apteraliphus*).
38. **Gonocoxosterna VIII:** (0) not exposed; (1) exposed, sternite-like. Fused, exposed and sternite-like gonocoxosterna VIII are an autapomorphy of Gyrininae.
39. **Ramen (GH VIII):** (0) not fused, not forming trough-like structure; (1) fused, forming trough-like structure. Fused genital appendages VIII, which form a trough-like structure for reception of the vaginal papillae, were considered as a synapomorphy of Amphizoidae, Hygrobiidae and Dytiscidae (Burmeister 1976).
40. **Laterotergite:** (0) orientation not strictly caudal; (1) strictly caudal, articulates with the cranial portion of the gonocoxa. In *Aspidytes* the laterotergite articulates with the cranial portion of the gonocoxa; the laterotergite has a strict caudal orientation (in resting position). This state is also present in females of all other groups of Dytiscoidea except for Noteridae, where the laterotergite has either a short apodeme which is extended cranially while the rest has a more or less caudal orientation (*Notomicrus*), or the laterotergite is much longer than the gonocoxa and completely orientated cranially (see Burmeister 1976).

Larvae

41. **Frontal suture:** (0) straight; (1) sinuate. The frontal suture is sinuate in all carabids larvae (incl. Rhysodidae) and all known larvae of Noterinae (obsolete in *Phreatodytes*).
42. **Lateral horizontal keel of head capsule (L₁):** (0) absent; (1) present. The presence of a lateral horizontal keel on the larval head capsule was considered a synapomorphy of Amphizoidae, Hygrobia and Dytiscidae (Ruhnau 1986). It is absent from later

instars of hygrobia and may be indistinct or absent in some larvae of Dytiscidae (Ruhnau 1986).

43. **Caudal tentorial arm:** (0) absent; (1) present, short; (2) present elongate. A short caudal tentorial arm is present in larvae of *Trachypachus* and *Noterus*. It is strongly elongate and attached to the ventral wall of the head capsule in larvae of Amphizoidae, Hygrobiidae and Dytiscidae.
44. **Maxillary groove:** (0) distinct; (1) partly reduced; (2) absent. The maxillary groove is distinct in larvae of Gyrinidae, partly reduced in larvae of Haliplidae and absent in other adepagan larvae.
45. **Intramaxillary moveability:** (0) fully retained; (1) absent. The intramaxillary moveability is fully retained in larvae of Gyrinidae but lost in other adepagan larvae.
46. **Cardo:** (0) as broad as stipital base; (1) narrowed or absent. A distinctly or completely (*Hygrobia*) reduced larval cardo is characteristic for larvae of Amphizoidae, Hygrobiidae and Dytiscidae.
47. **Lacinia:** (0) well developed and mobile; (1) immobilised, reduced or absent. The lacinia is hook-shaped and moveable in larvae of Gyrinidae but immobilised and partly or completely reduced in the other groups.
48. **Terminal segments:** (0) IX and X well developed; (1) IX strongly reduced, X absent. The abdominal segment IX is largely reduced in larvae of Dytiscoidea and segment X is absent.
49. **Spiracle VIII:** (0) normally developed; (1) enlarged, terminal; (2) reduced or closed. An enlarged terminal spiracle VIII is present in larvae of Noteridae, Amphizoidae and Dytiscidae.

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