

# Evolution, mitochondrial DNA phylogeny and systematic position of the Macaronesian endemic *Hydrotarsus* Falkenström (Coleoptera: Dytiscidae)

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**Abstract.** *Hydrotarsus* Falkenström (Coleoptera, Dytiscidae) is the only genus of aquatic beetle considered to be endemic to the Atlantic islands of Macaronesia. Its three known species (*H. lundbladi* Falkenström from Madeira, and *H. compunctus* (Wollaston) and *H. pilosus* Guignot from the Canary Islands) are revised, and their phylogenetic position studied based on sequences of the 16S rRNA and Cytochrome Oxidase I genes of mitochondrial DNA. Our results clearly indicate that the species of *Hydrotarsus* fall deep within the genus *Hydroporus* Clairville, in a clade formed by (in addition to *Hydrotarsus*) species currently included in the *H. fuscipennis*, *marginatus*, *nigrita* and *tessellatus* groups, and in consequence a new synonymy is proposed: *Hydrotarsus* Falkenström = *Hydroporus* Clairville syn.n. The origin of the species of '*Hydrotarsus*', based on molecular clock estimations, is late Miocene, relatively recent in the history of the Atlantic islands. They are therefore not palaeoendemics but highly derived, recently evolved elements in the Macaronesian fauna. The estimated ages of divergence among species are much more recent than the emergence of any of the islands on which they are found, suggesting rare long-range dispersal as the mechanism driving the diversification of the lineage. Morphological modifications of the species of '*Hydrotarsus*' are discussed, as well as those of other dytiscids from hygropetric habitats.

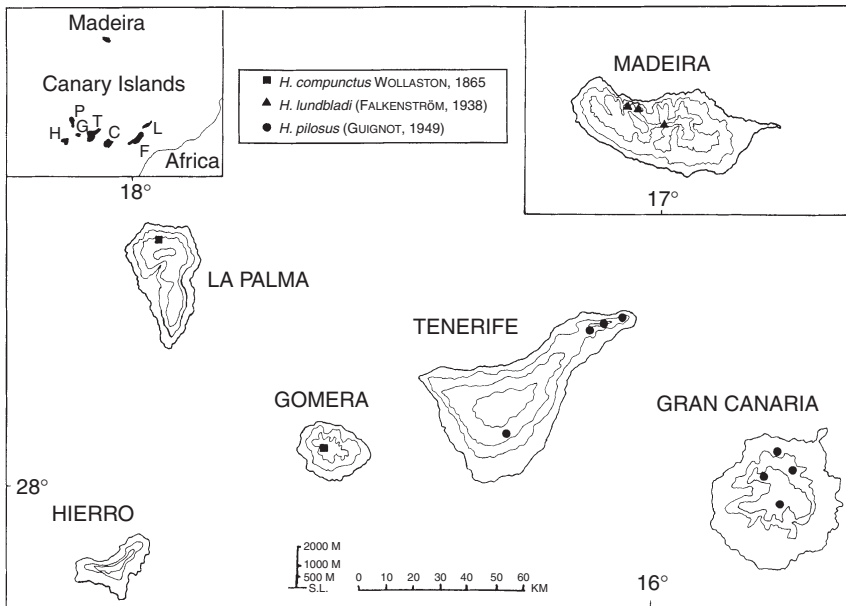
## Introduction

The islands of the central North Atlantic, particularly those belonging to the Macaronesian subregion (Madeira, Salvages, Canary Islands, Cape Verde), are well known for their endemic radiations of invertebrates (Kunkel, 1976; Juan *et al.*, 2000). Within the Coleoptera, intra-island and intra-archipelago radiations are characteristic of the fauna of Madeira and the Canary Islands (e.g. Wollaston, 1865; Machado, 1992; Machado & Oromí, 2000), many of these radiations being endemic to single islands or archipelagos at the generic level. However, amongst the aquatic Coleoptera

only a single genus of Dytiscidae is currently recognized as being endemic to Macaronesia: *Hydrotarsus* Falkenström, which currently includes three species, two of them endemic to the western Canary Islands (*H. compunctus* (Wollaston) and *H. pilosus* Guignot), and *H. lundbladi* Falkenström endemic to the main island of Madeira (Machado, 1987; Alarie & Bilton, 2001) (Fig. 1).

All three species of *Hydrotarsus* are specialist malicolous (see Balke *et al.*, 1997), being restricted as both larvae and adults to hygropetric habitats (running water films or small springs over exposed bedrock), which probably explains why they have been rarely collected by entomologists. In fact, the true habitat of the species was only discovered as recently as 1986 during a survey of Madeiran water beetles (Balke & Hendrich, 1989). The scarcity of *Hydrotarsus* species in collections has hampered adequate descriptions of the taxa, and made identification of specimens difficult in the past (Balke *et al.*, 1990). In addition, the phylogenetic

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**Fig. 1.** Distribution of the species of *Hydrotarsus* in the Macaronesian archipelago.

placement of this group of species has been highly problematic. The genus belongs to the tribe Hydroporini Sharp, which currently includes 36 genera worldwide (Nilsson, 2001). Along with *Necterosoma* MacLeay and *Sternopriscus* Sharp, species of *Hydrotarsus* are characterized by pentamerous pro- and mesotarsi (compared to pseudotetramerous in all other Hydroporinae, see Fig. 7). Although this character state has led to their basal placement within the Hydroporinae (Franciscolo, 1979; Machado, 1987) or even the erection of their own subfamily (Hyporinae, Falkenström, 1938), no other characters suggest a close relationship between these three genera.

Madicolous species of Dytiscidae are apparently subject to severe morphological constraint associated with the invasion of wet rock habitats (e.g. Balke *et al.*, 1997), and many features of *Hydrotarsus*, such as reduced swimming hairs, and indeed the tarsal structure may be associated with the evolution of madicoly. Recent work on the larva of *H. compunctus* (Alarie & Bilton, 2001) points to a close relationship between *Hydrotarsus* and the large Holarctic genus *Hydroporus* Clairville, rather than a basal placement within the subfamily, a finding backed up by preliminary results using ribosomal 18S rRNA sequence data (Ribera *et al.*, 2002b). Here we present a mitochondrial DNA (mtDNA)-based phylogeny of *Hydrotarsus* and other species of Hydroporinae, which demonstrates that, rather than representing an isolated basal lineage, species currently assigned to *Hydrotarsus* actually fall deep within the genus *Hydroporus*. We therefore synonymize *Hydrotarsus* with *Hydroporus* and provide a redescription of its species, as well as discussing the biogeographical history and ecology of the '*Hydrotarsus*' lineage.

## Materials and methods

### Morphological analysis

Morphometric measures were taken with an ocular micrometer attached to an Olympus (Hamburg, Germany) dissecting microscope at 40 $\times$ . Beetle structures were examined with a Zeiss (Jena, Germany) Stemi SV6, at 12–80 $\times$  (fluorescent bulb for diffuse light). Scanning electron micrographs were taken from gold–palladium-coated preparations with a Hitachi S2500 (Tokyo, Japan). Photographs of the habitus were taken with a Synoptics Automontage™ digital imaging system attached to a Zeiss Stemi SV6 at the Natural History Museum, London. Acronyms of the collections in which the material is deposited are as follows: BMNH, The Natural History Museum, London; MNHN, Muséum National d'Histoire Naturelle, Paris; NMW, Naturhistorisches Museum, Wien; CBH, coll. M. Balke & L. Hendrich (Berlin); CDB, coll. D. T. Bilton (Plymouth); CFP, coll. F. Pederzani (Ravenna); CGI, coll. G. Israelson (Uppsala); CGW, coll. G. Wewalka (Wien); CHF, coll. Hans Fery (Berlin); CNS, coll. N. Sanfilippo (Genoa).

### Taxon sampling and DNA sequencing

The three recognized species of *Hydrotarsus* were included in the analysis: *H. pilosus* from Tenerife, *H. compunctus* from La Gomera and *H. lundbladi* from Madeira (Machado, 1987; Balke & Hendrich, 1989; Balke *et al.*, 1990) (Table 1; Fig. 1). Preliminary analysis with a representation of different species groups of *Hydroporus*, together with other genera of Hydroporini, showed that *Hydrotarsus* belonged within the *H. fuscipennis* group (*sensu* Nilsson &

**Table 1.** Material studied, with species codes, species group (following Nilsson, 2001), geographical origin, collector, and GenBank accession numbers.

Species	Species gr	Country	Collector	16S	COI
<b>Ingroup</b>					
1 <i>Hydroporus scalesianus</i> Stephens	<i>angustatus</i>	U.K.	I. Ribera	AF518278	AF518309
2 <i>H. erythrocephalus</i> (L.)	<i>erythrocephalus</i>	U.K.	I. Ribera	AF518261	AF518291
3 <i>H. acutangulus</i> Thompson	<i>fuscipennis</i>	Sweden	A. N. Nilsson	AF518256	AF518286
4 <i>H. analis</i> Aubé	<i>fuscipennis</i>	Spain	H. Fery	AF518257	AF518287
5 <i>H. discretus</i> Fairm. & Brisout	<i>fuscipennis</i>	Spain	I. Ribera	AF518260	AF518290
6 <i>H. fuscipennis</i> Schaum	<i>fuscipennis</i>	Canada	I. Ribera	AF518262	AF518292
7 <i>H. limbatus</i> Aubé	<i>fuscipennis</i>	Morocco	I. Ribera	AF518264	AF518294
8 <i>H. lucasi</i> Reiche	<i>fuscipennis</i>	Spain	I. Ribera	AF518266	AF518296
9 <i>H. planus</i> (Fab.)	<i>fuscipennis</i>	Russia	A. N. Nilsson	AF518275	AF518306
10 <i>H. pubescens</i> (Gyllenhal)	<i>fuscipennis</i>	U.K.	I. Ribera	AF518276	AF518307
11 <i>H. basinotatus</i> Reiche	<i>marginatus</i>	Spain	I. Ribera	AF518258	AF518288
12 <i>H. marginatus</i> (Duftschmid)	<i>marginatus</i>	Spain	I. Ribera	AF518267	AF518298
13 <i>H. obsoletus</i> Aubé	<i>memnonius</i>	Portugal	I. Ribera	AF518273	AF518304
14 <i>H. melanarius</i> Sturm 1	<i>memnonius</i>	Bohemia	J. Statszny	–	AF518299
15 <i>H. melanarius</i> Sturm 2	<i>memnonius</i>	Sweden	A. N. Nilsson	AF518268	–
15' <i>H. memnonius</i> Nicolai	<i>memnonius</i>	U.K.	I. Ribera	AF518269	AF518300
16 <i>H. nigrita</i> (Fab.)	<i>nigrita</i>	Spain	I. Ribera	AF518271	AF518302
17 <i>H. obscurus</i> Sturm	<i>obscurus</i>	Sweden	A. N. Nilsson	AF518272	AF518303
18 <i>H. umbrosus</i> (Gyllenhal)	<i>puberulus</i>	Finland	T. Berendok	AF518280	AF518311
19 <i>H. rufifrons</i> (O.F.Müller)	<i>rufifrons</i>	U.K.	G. N. Foster	AF518277	AF518308
20 <i>H. longulus</i> Mulsant & Rey	<i>longulus</i>	Spain	H. Fery	AF518265	AF518295
21 <i>H. vagepictus</i> Fairm. & Labol.	<i>striola</i>	Portugal	I. Ribera	AF518281	AF518312
22 <i>H. tessellatus</i> (Drapiez)	<i>tessellatus</i>	Portugal	I. Ribera	AF518279	AF518310
23 <i>H. gyllenhalii</i> Schiödte	<i>tristis</i>	U.K.	I. Ribera	AF518263	AF518293
24 <i>H. niger</i> Say	<i>niger</i>	U.S.A.	K. B. Miller	AF518270	AF518301
25 <i>Hydrotarsus compunctus</i> (Wollaston)		Gomera	D. Bilton	AF518259	AF518289
26 <i>H. lundbladi</i> Falkenström		Madeira	D. Bilton	–	AF518297
27 <i>H. pilosus</i> Guignot		Tenerife	D. Bilton	AF518274	AF518305
<b>Outgroups</b>					
28 <i>Deronectes fosteri</i> Aguilera & Ribera		Spain	P. Aguilera	AF309260	AF309317
29 <i>Heterosternuta pulcher</i> (LeConte)		Canada	Y. Alarie	AF518252	AF518282
30 <i>Laccornis oblongus</i> (Stephens)		U.K.	D. Bilton	AF309241	AF309298
31 <i>Nebrioporus baeticus</i> (Schaum)		Spain	I. Ribera	AF309245	AF309302
32 <i>Neoporus dimidiatus</i> (Gemming & Harold)		U.S.A.	K. B. Miller	AF518253	AF518283
33 <i>Oreodytes davisii</i> (Curtis)		Spain	H. Fery	AF309244	AF309301
34 <i>Sanfilippodytes</i> sp.		USA	I. R. & A. Cieslak	AF518254	AF518284
35 <i>Scarodytes halensis</i> (Fab.)		Spain	H. Fery	AF309248	AF309305
36 <i>Stictonectes epipleuricus</i> (Seidlitz)		Spain	I. Ribera	AF518255	AF518285
37 <i>Stictotarsus duodecimpustulatus</i> (Fab.)		Spain	I. Ribera	AF309247	AF309304
38 <i>Suphrodytes dorsalis</i> (Fab.)		U.K.	D. Bilton	AF309242	AF309299

Holmen, 1995; Nilsson, 2001). A more comprehensive sampling of this group, and of morphologically similar groups, was thus conducted (Table 1). Most of the remaining Palaearctic species groups of *Hydroporus* are represented by single species.

Outgroups include all genera of the *Hydroporus* group as defined in Ribera *et al.* (2002b) (formerly included in the single genus *Hydroporus*, see, e.g. Alarie, 1991), with the exception only of the recently described *Hydrocolus* Roughley & Larson (Larson *et al.*, 2000): *Suphrodytes* Gozis, *Neoporus* Guignot, *Heterosternuta* Strand and *Sanfilippodytes* Franciscolo. Other included Hydroporini were the *Deronectes* group of genera (*sensu* Nilsson & Angus,

1992) (*Nebrioporus* Régimbart, *Deronectes* Sharp, *Stictotarsus* Zimmermann, *Scarodytes* Gozis and *Oreodytes* Seidlitz) and the *Stictonectes* group of genera *sensu* Ribera *et al.* (2002b) (*Stictonectes* Brinck). The tree was rooted in *Laccornis* Gozis (tribe Laccornini), which certainly lies outside Hydroporini and probably in a basal position within Hydroporinae (Wolfe, 1985; Miller, 2001; Ribera *et al.*, 2002b).

Soft tissue from single specimens collected in absolute ethanol was digested and DNA isolated using a phenol–chloroform extraction (as described in Vogler *et al.*, 1993) or using the DNeasy kit (Quiagen, Crawley, UK). Sequences of 16S rRNA were amplified in a single fragment of c. 500 bp, using primers 16Sa (5'-ATGTTTTTGTAAACAGGCG)

for the 5' end of the gene, and 16Sb (5'-CCGGTCTGAACT-CAGATCATGT) for the 3' end. A single fragment of c. 800 bp of Cytochrome Oxidase I (COI) (from the middle of the region E3 to the COOH end, Lunt *et al.*, 1996) was amplified using the primers 'Jerry' (5'-CAACATTTATTTT-GATTTTTTGG) for the 5' end of the gene, and 'Pat' (5'-TCCAATGCACTAATCTGCCATATTA) for the 3' end (Simon *et al.*, 1994). All sequences generated in this study were deposited in GenBank (Acc. Nos. AF518252–AF518312, Table 1).

The following cycling conditions were used: 1–2 min at 95°C; 30 s at 94°C, 30 s at 47–50°C (depending on the melting temperatures of the primer pair used), and 1–2 min at 72°C (repeated for 35–40 cycles); 10 min at 72°C. Amplification products were purified using a GeneClean II kit (Bio 101, Inc., Nottingham, U.K.). Automated DNA sequencing reagents were supplied by Perkin Elmer Applied Biosystems Ltd (Foster City, USA). (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit). Sequencing reactions were purified by ethanol precipitation and were electrophoresed on an ABI3700 sequencer. Sequencing errors/ambiguities were edited using the Sequencher 3.1 software package (Gene Codes Corporation, Ann Arbor, USA).

#### Phylogenetic analysis

Sequences for COI were not length variable, and 16S rRNA sequences differed in length only minimally, affecting mostly outgroup taxa (see Results). Alignment was therefore performed manually, by attempting to maximize sequence similarities. Phylogenetic analysis was performed with PAUP4.0b8 (Swofford, 1999), using parsimony procedures for tree reconstruction [a heuristic search with 1000 Tree-Bisection-Reconnection (TBR) replicas]. Constraint trees for determining Bremer Support values (Bremer, 1994) and partitioned Bremer Support values (Baker & DeSalle, 1997) were generated with TREEROT (Sorenson, 1996). The significance of the incongruence length difference (ILD) (Farris *et al.*, 1994) was assessed with the Partition Homogeneity Test as implemented in PAUP (using a heuristic search with 100 random-addition replicates). Non-parametric bootstrap support values (Felsenstein,

1985) were found in PAUP using 100 iterations of 100 TBR replicas each.

The COI nucleotide sequence was translated into an amino acid sequence using MACCLADE 4.0 (Maddison & Maddison, 2000), and analysed in PAUP. Owing to the low resolution attained, third codon positions were included in all analyses, and all characters were equally weighted.

#### Rate of diversification

The optimal evolutionary model was estimated using MODELTEST 3.04 (Posada & Crandall, 1998). To estimate node ages we fitted maximum likelihood (ML) branch lengths in the preferred tree assuming a molecular clock using the optimal model as estimated with MODELTEST, and compared the likelihood to that obtained assuming no clock (Felsenstein, 1981). When the two likelihood values are not significantly different (as measured with the likelihood ratio, which follows a chi-squared distribution, Felsenstein, 1981) it can be considered that the rate of evolution of the sequence is clock-like, and an ultrametric tree can be enforced.

To calibrate the branch lengths we used the standard 2% divergence per million years (Myr) for arthropod mitochondrial DNA (Brown *et al.*, 1979; Brower, 1994), corresponding to a base rate (per branch) of 0.01 substitutions per site Myr<sup>-1</sup>. In Ribera *et al.* (2001) it is shown that for species of Dytiscidae (genus *Agabus* Leach) the 2% Myr<sup>-1</sup> estimation for the combined COI plus 16S rRNA genes is equivalent to the much slower estimation of Gómez-Zurita *et al.* (2000) in a group of leaf beetles for the gene 16S rRNA alone (0.76% divergence Myr<sup>-1</sup>).

## Results

#### Phylogenetic analysis

Amplification of the COI gene was successful in all specimens, and a final interior continuous fragment of 769 bp was used for analyses. Uncorrected genetic distances among the taxa ranged from 1% to 17% (14% within the ingroup) (Table 2). The heuristic search resulted in two equally parsimonious trees (Table 3), with *Hydrotarsus* sister to

**Table 2.** Genetic distances. Min. D., minimum overall uncorrected *p* distance; Max. D., maximum overall *p* distance; Max. D. ingr., maximum *p* distance within the ingroup. See Table 1 for details of the species.

Gene	Min. D.	Max. D.	Max. D. ingr.
COI	<i>H. tessellatus</i> / <i>H. basinotatus</i> 0.01	<i>N. dimidiatus</i> / <i>L. oblongus</i> 0.17	<i>H. lundbladi</i> / <i>H. niger</i> 0.14
16S rRNA	<i>H. tessellatus</i> / <i>H. basinotatus</i> <i>H. pilosus</i> / <i>H. compunctus</i> 0.00	<i>H. melanarius</i> / <i>S. epipleuricus</i> 0.16	<i>H. umbrosus</i> / <i>H. tessellatus</i> 0.08
Combined	<i>H. tessellatus</i> / <i>H. basinotatus</i> 0.01	<i>H. lundbladi</i> / <i>S. halensis</i> 0.16	<i>H. lundbladi</i> / <i>H. niger</i> 0.14

**Table 3.** Tree statistics. CI, consistency index; RI, retention index; Inf. cha., number of parsimony informative characters.

Gene	No. trees	Length	CI	RI	No. cha	Inf. cha.
COI	2	1749	0.27	0.37	801	256
16S rRNA	80	567	0.40	0.60	515	132
Combined	1	2348	0.30	0.43	1316	388

*H. acutangulus* and *H. fuscipennis* (see Table 1 for the authors of the species included in the analyses), in a node including all the sampled species of the *H. fuscipennis* (excluding *H. limbatus*), *nigrita*, *tessellatus* and *marginatus* groups. This clade was sister to an unresolved group formed by the sampled species of the *H. longulus* and *memnonius* groups. *Suphrodytes* was included within *Hydroporus*. Outgroup relationships were similar to that of the combined tree (see Fig. 2).

Parsimony analysis of the protein sequence resulted in a largely unresolved tree. The *Hydroporus* group of genera (including *Hydrotarsus*) formed a monophyletic lineage with 69% bootstrap support. Within it, only three monophyletic groups were resolved: *Heterosternuta* + *Neoporus* (99% bootstrap support), *H. gyllenhalii* + *H. vagepictus* (bootstrap support lower than 50%) and *H. erythrocephalus* + *H. niger* + *H. obscurus* + *H. rufifrons* (58% bootstrap support) (all of them included in the combined tree, Fig. 2).

16S rRNA sequence varied in length between 506 and 512 bp, although within *Hydroporus*, *Sanfilippodytes* and *Suphrodytes* differences were restricted to a single base pair (a deletion either in position 14 or 45). Uncorrected genetic distances among the taxa ranged from 0% for some species pairs with identical sequence to 16% (8% within the ingroup) (Table 2). A parsimony search using gaps as a fifth character resulted in 80 equally parsimonious trees (Table 3). The two species of *Hydrotarsus* for which 16S rRNA could be obtained (*H. pilosus* and *H. compunctus*, Table 1) had an identical sequence, which was included in an unresolved monophyletic group including *H. basinotatus*, *H. tessellatus*, *H. analis*, *H. discretus*, *H. nigrita* and *H. pubescens*. This clade, together with the rest of the sampled species of the *H. fuscipennis*, *nigrita*, *tessellatus* and *marginatus* groups, formed an unresolved monophyletic group. *Suphrodytes* plus *Hydroporus* was also monophyletic although largely unresolved. The *Hydroporus* group of genera was monophyletic, and outgroup relationships were very similar to those of the combined analysis (see Fig. 2).

The combined analysis, using gap as a fifth character and both genes equally weighted, resulted in a single tree (hit 452 out of 1000 replicas) (Table 3 and Fig. 2). The incongruence among genes was not significant, as measured with the Partition Homogeneity Test ( $P=0.08$ ). Coding gaps as missing resulted also in a single tree of 2316 steps and  $CI=0.29$ , with identical topology to that shown in Fig. 2.

To assess the possible effect of alternative alignments of the outgroup sequence, a search was conducted using only the species of *Hydroporus* plus *Suphrodytes* (which were unambiguously aligned, see above). The single tree found (1362 steps,  $CI=0.37$ ) had identical topology to that including outgroups (when rooted in *Suphrodytes*).

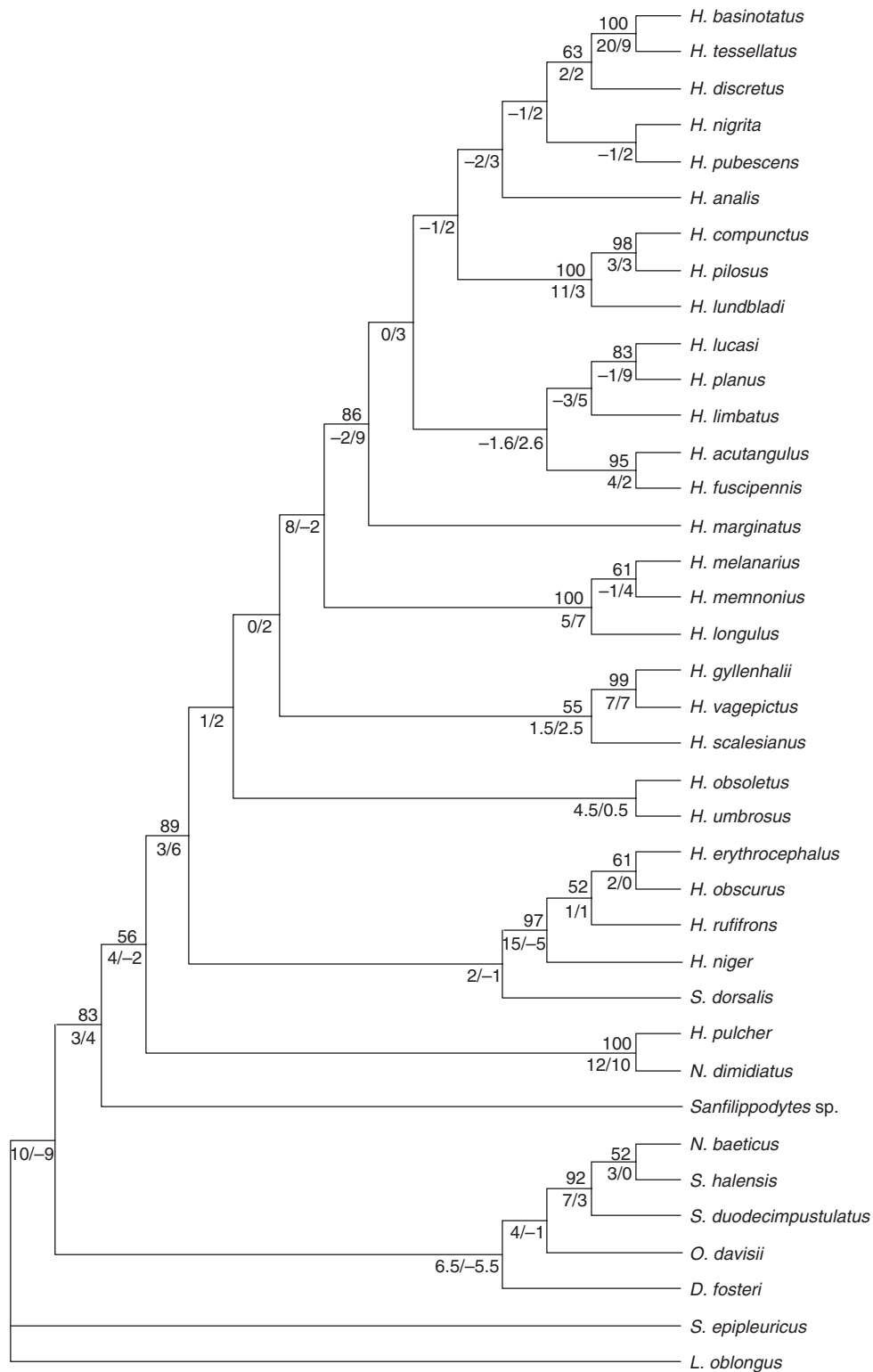
In the combined analysis, *Hydrotarsus* was included also in a monophyletic clade formed by the sampled species of the *H. fuscipennis*, *nigrita*, *tessellatus* and *marginatus* groups, hereafter referred to as the extended *H. fuscipennis* group (i.e. the less inclusive well-supported node including the species of *Hydrotarsus*), with high bootstrap support (86%) (Fig. 2). Within this group, relationships, although fully resolved, had low node support values.

The sister to the extended *H. fuscipennis* group was a clade formed by the sampled species of the *H. memnonius* and *longulus* groups (with the exception of *H. obsoletus*), with the remaining species of *Hydroporus* basal. Other clades within *Hydroporus* with bootstrap values above 50% are *H. gyllenhalii*, *H. vagepictus* and *H. scalesianus* (representing the *tristis*, *striola* and *angustatus* groups, respectively); and *H. erythrocephalus*, *H. rufifrons*, *H. niger* (representing the *erythrocephalus*, *rufifrons* and *niger* groups, respectively), plus *H. obsoletus* (Figs 2, 3, Table 1). *Suphrodytes* was included within *Hydroporus* as sister to the later clade, although with low node support. The clade formed by *Hydroporus* plus *Suphrodytes* was, however, highly supported (89% bootstrap), as well as the *Hydroporus* group of genera (83% bootstrap, Fig. 2).

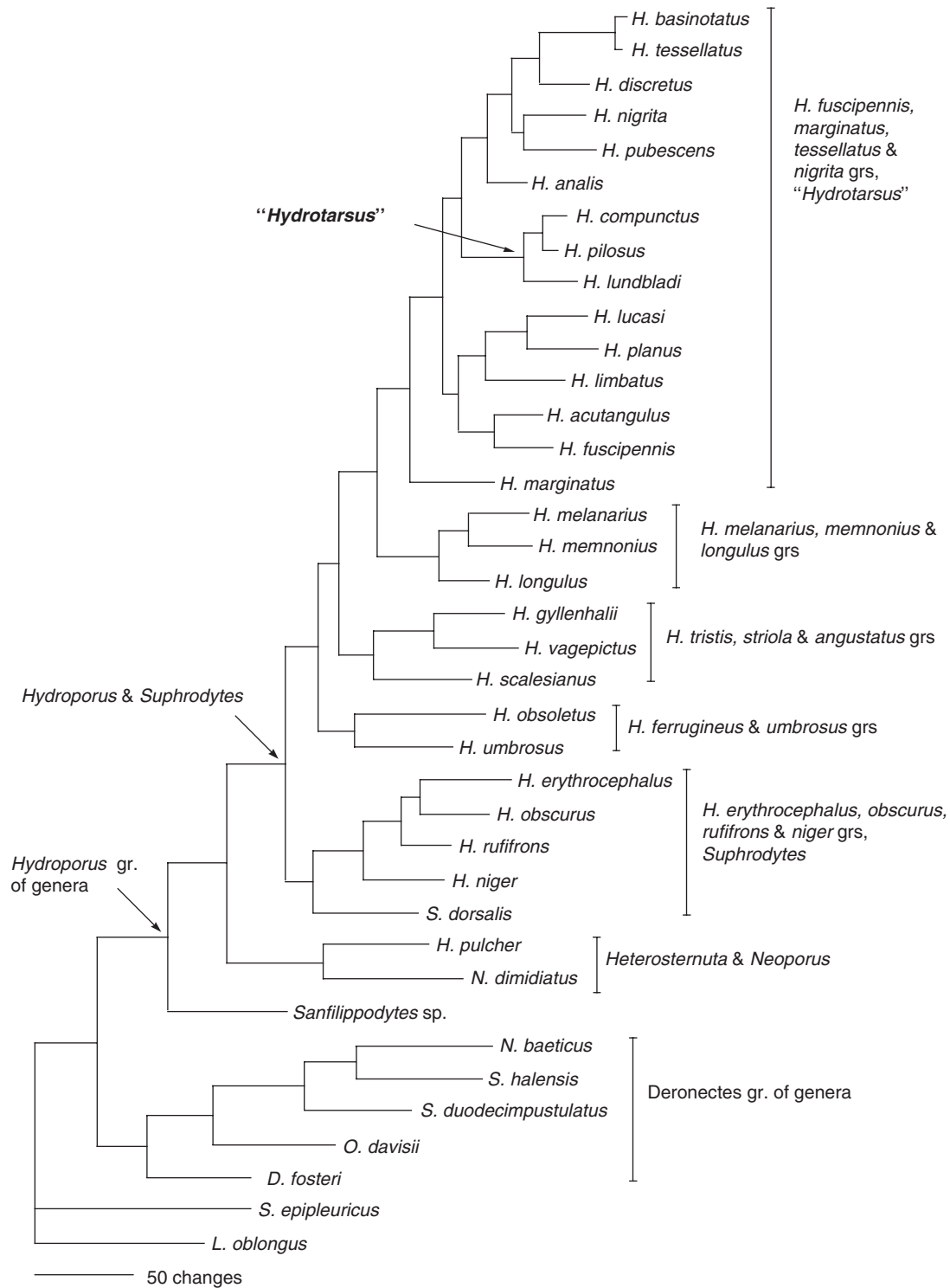
#### Rate variation and molecular clock estimates

Estimates of rate variation were restricted to the extended *H. fuscipennis* group (including *Hydrotarsus*). The best ML model (as estimated with MODELTEST, both using the Akaike Information Criterion or Hierarchical Likelihood Ratio Tests) was a complex GTR + I + G, with estimated base frequencies, among-site rate variation and a Gamma distribution shape parameter of 0.654. The likelihood ratio of the estimate enforcing and not enforcing a molecular clock was not significant ( $-\ln ML$  no clock 4413.1;  $-\ln ML$  clock 4421.9;  $-2 \times (\ln C - \ln NC) = 17.50$ , 13 d.f.,  $P=0.18$ ). The exclusion of *H. lundbladi* (with no 16S rRNA sequence) did not change the results ( $-\ln ML$  no clock 4282.9;  $-\ln ML$  clock 4292.2;  $-2 \times (\ln C - \ln NC) = 18.65$ , 12 d.f.,  $P=0.10$ ).

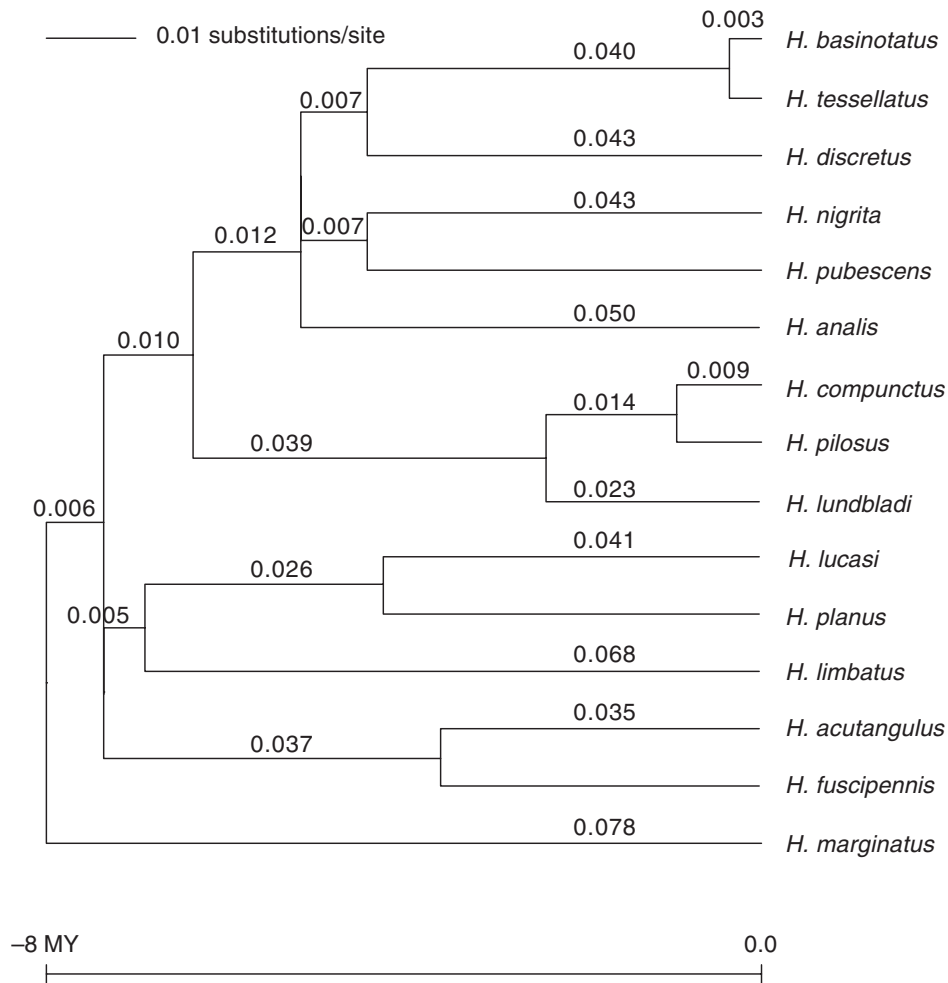
The estimated ultrametric tree using the optimal ML model and enforcing a molecular clock (Fig. 4) showed maximum divergences rates within the extended *H. fuscipennis* group of approximately 16% (0.08 substitutions/site/branch) (*H. marginatus* vs. remaining species). The species of *Hydrotarsus* differ by *c.* 12.5% from their sister clade, although within them maximum differences were 5% (*H. lundbladi*). *Hydrotarsus pilosus* and *H. compunctus* differ only by *c.* 2% (and only for the COI sequence, see above) (Fig. 4).



**Fig. 2.** Phylogenetic hypothesis for the relationships of ‘*Hydrotarsus*’ based on a combined parsimony analysis of the 16S rRNA and COI genes. Numbers below the branches are partitioned Bremer support values (COI/16S); numbers above the branches are bootstrap proportions (only shown if >50%). See Table 1 for details of species.



**Fig. 3.** Phylogram of the single most parsimonious tree obtained in the combined parsimony analysis of the 16S rRNA and COI genes, with the species names and the main clades. See Table 1 for details of species.



**Fig. 4.** Ultrametric tree for the extended *Hydroporus fuscipennis* group of species, obtained with maximum likelihood (optimal model) enforcing a molecular clock, with the topology of the parsimony tree including outgroups (see Figs 2, 3). Numbers on branches are their estimated length. The absolute timescale corresponds to an estimated rate of 0.01 substitutions per site per Myr per branch, equivalent to the standard divergence rate of 2% Myr<sup>-1</sup>. See Table 1 for details of species.

#### Taxonomy

##### *Hydroporus* Clairville, 1806

*Hydatoporus* Gistel, 1856

*Hydrocoptus* Motschulsky, 1853

*Hydroporidius* Guignot, 1949

*Hydroporinus* Guignot, 1945

*Schizoporus* Ádám, 1996

*Sternoporus* Falkenström, 1930

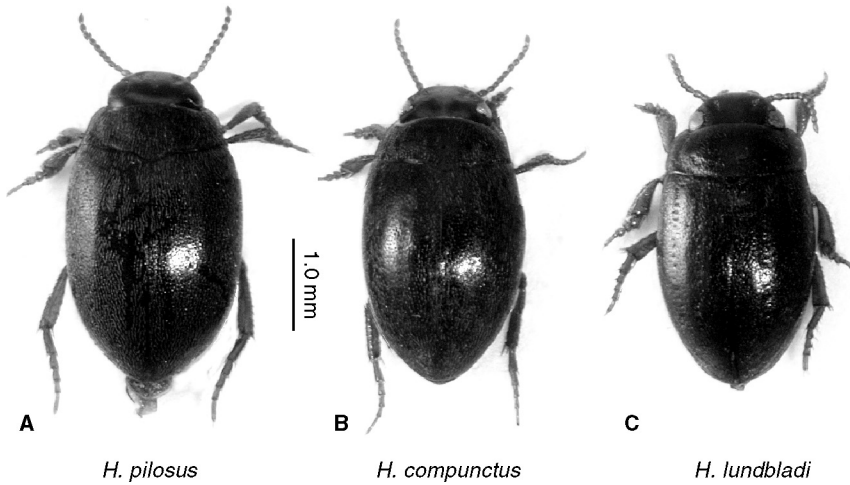
*Hydrotarsus* Falkenström, 1938, syn.n.

##### *Hydroporus compunctus* lineage

The three species of the former genus *Hydrotarsus* share a very similar external morphology: stout body (Fig. 5), antenna compact with roundish flagellomeres. Fore and especially middle tarsi clearly five segmented: 4th protarsomere

not mostly concealed by lateral lobes of 3rd tarsomere (pseudotetramerous condition, see Nilsson & Holmen, 1995: 56) (Fig. 7); 4th mesotarsomere fully exposed. Head dark brown, with contrasting orange areas in anterior, median and frontal parts; pronotum black with paler lateral and anterior margins; elytron dark brown; appendages orange; ventral side dark brown. Metacoxae slightly produced backwards medially, concave on each side (Fig. 7) (this character state is present also in *Heterosternuta*, Zimmermann, 1931; Larson *et al.*, 2000). Fringes of swimming hairs on legs rudimentary or absent, consisting of a dorsal row of few long hairs (Fig. 6E). Legs without swimming hairs on the ventral side (Fig. 6C,D,F,G) (only *H. compunctus* males possess a few stout hairs ventrally, Fig. 6A,B). Median lobe of the aedeagus very similar in all species (Fig. 7), and similar to other species of the group (see, for example, Wewalka, 1992; Nilsson & Holmen, 1995; Larson *et al.*, 2000).





**Fig. 5.** Habitus of the species of *H. compunctus* lineage. A, *H. pilosus*; B, *H. compunctus*; C, *H. lundbladi*.

*Key to the species of the Hydrotarus compunctus lineage*

1. Elytra and pronotum dull due to presence of strong microreticulation; angle in lateral outline between base of pronotum and elytron small (Fig. 5); males with first metatarsal segment strongly expanded, two first metatarsomeres with long, strong ventral setae (Fig. 6D,E); metacoxa densely covered with stout setae (Fig. 7). Madeira..... *H. lundbladi* (Falkenström)
- Elytra and pronotum shinier, with faint microreticulation; lateral outline of body continuous, without recognizable angle between base of pronotum and elytron (Fig. 5); first metatarsal segment in male not expanded, with fewer stout ventral setae (Fig. 6A–C,F,G); metacoxa less densely setose (Fig. 7). Canary Islands.....2
2. Body shape broadly oval (Fig. 5A); puncturation on pronotum coarser and denser; male two first metatarsal segments with several long, stout ventral setae,

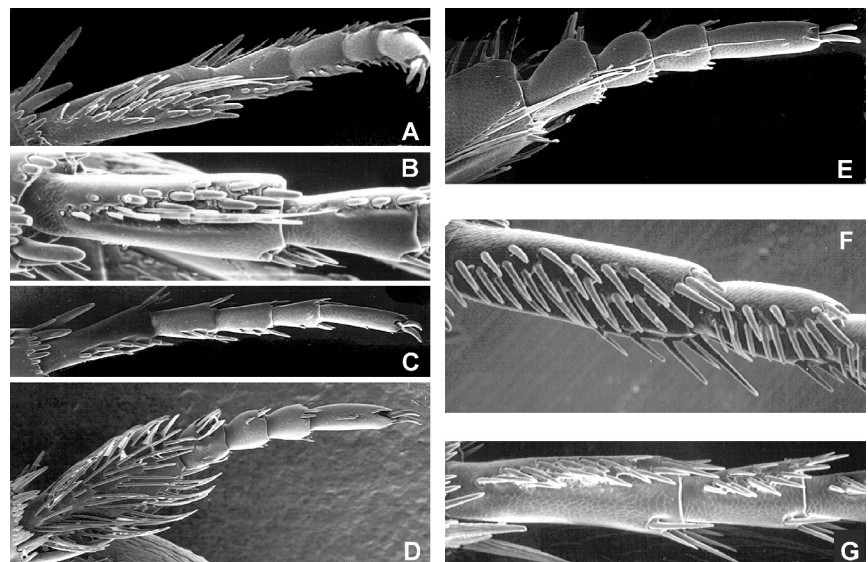
but without longer, stout hairs (Fig. 6F). Gran Canaria, Tenerife.....*H. pilosus* (Guignot)

– Body shape elongate oval (Fig. 5B); puncturation on pronotum slightly finer and sparser; male two first metatarsal segments with fewer stout setae and longer stout hairs (Fig. 6A,B). La Gomera, La Palma, Tenerife?..... *H. compunctus* Wollaston

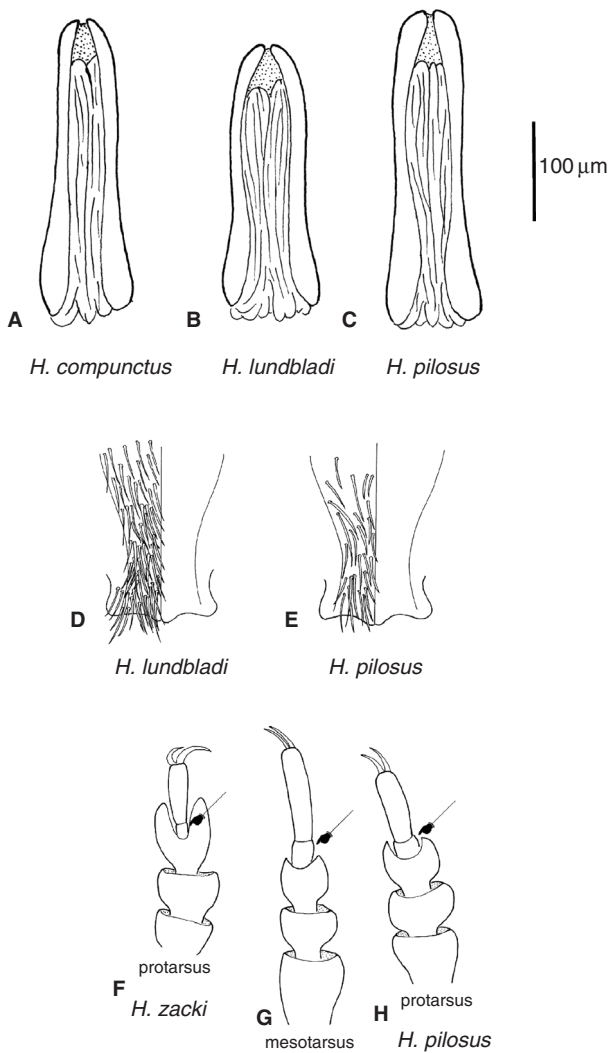
***Hydrotarus compunctus* Wollaston**

*Hydrotarus compunctus* Wollaston, 1865: 65; Sharp, 1882: 791; Régimbart, 1895: 28; Zimmermann, 1920: 82, 1931: 158; Winkler, 1924: 222.

? = *Hydrotarus pubescens* (Gyllenhal, 1808): Bedel 1925: 369.  
 ? = *Hydrotarus tessellatus* var. *pueli* (Barthe, 1916) [*Hydrotarus guernei* var. *pueli*: Nilsson 2001: 166]; Guignot 1959: 383 (note 284).



**Fig. 6.** Scanning electron micrographs of the hind tarsi of the species of the *H. compunctus* lineage. A & B, *H. compunctus*, male; C, *H. compunctus*, female; D & E, *H. lundbladi*, male; F, *H. pilosus*, male; G, *H. pilosus*, female.



**Fig. 7.** Medial lobe of the species of the *H. compunctus* lineage, dorsal view. A, *H. compunctus*; B, *H. lundbladi*; C, *H. pilosus*. Metacoxa of D, *H. lundbladi*; E, *H. pilosus*. F, protarsi of pseudotetramerous type (*Hydroporus zackii* Larson & Roughley); G & H, tarsi of pentamerous type (*H. pilosus*), G, mesotarsus; H, protarsus.

*Hydrotarsus compunctus* (Wollaston): Machado, 1987: 29; Balke *et al.* 1990: 361; Machado & Oromí, 2000: 31, 92; Alarie & Bilton, 2001: 341; Nilsson, 2001: 168.

**Table 4.** Morphometric measurements of the species of the *H. compunctus* lineage. TL-h, total length minus head (~96% of total length); TW, maximum width; Lp, length of pronotum medially; Wp, maximum width of pronotum. All measurements are in millimetres.

Species	Locality	N	TL-h (mean)	TW	Lp	Wp
<i>H. compunctus</i>	La Palma	10	2.60–3.00 (2.80)	1.60–1.80	0.60–0.65	1.30–1.52
<i>H. pilosus</i>	Gran Canaria	40	2.80–3.20 (3.00)	1.85–2.00	0.55–0.62	1.42–1.60
<i>H. pilosus</i>	Tenerife	15	2.80–3.10 (2.90)	1.80–1.85	0.60–0.62	1.45–1.60
<i>H. lundbladi</i>	Madeira	10	2.75–2.90 (2.80)	1.63–1.80	0.62–0.67	1.47–1.55

*Hydrotarsus pilosus* Guignot: Israelson *et al.* 1982: 113, 115 (partim).

*Type locality.* Tenerife, ‘? in the Barranco at Ycod el Alto’.

*Type material.* Holotype female in BMNH (Wollaston Canary Island Collection, ‘Supplementary Material’ drawer).

*Additional material examined.* 1 ex., La Palma, Garafia, Siete Fuentes, 15.vi.1966, G. Israelson leg. (CGI); 99 exx, La Palma, Garafia, env. Siete Fuentes, 18.vi.1989, 1250 m, Balke & Hendrich leg. (NMW, CBH, CHF, CGW). 20 exx, La Gomera, Garajonay, 1500 m, 1999, 15.i.2000, D. T. Bilton leg. (CDB, 1 ex., 15.i.2000 used for DNA extraction, BMNH ref. IR181).

*Diagnosis.* See Table 4 for measurements. Body form shortly oval; lateral outline not interrupted between pronotum and elytron (Fig. 5B). Pronotum widest at base, gently narrowing towards head. Head with distinct microreticulation, which is, however, only moderately deeply impressed. Pronotum and elytron both with faint microreticulation. First male metatarsal segment not enlarged, with only few stout ventral setae and with few longer, thick hairs (Fig. 6A,B); first female metatarsal segment only with few stout setae (Fig. 6C). Median lobe of aedeagus as in Fig. 7A.

*Distribution.* Canary Islands: La Gomera and La Palma. The type locality (Ycod el Alto, Tenerife) may be mislabelled.

*Ecology.* At Siete Fuentes, the beetles were found on a wet, almost vertical, 4–5-m-high rock surface in the main course of an otherwise dry streambed. There was a slight trickle of water, and the beetles were collected from underneath slices of stone, moss and washed out of small crevices in the rock. At Garajonay adults and larvae were collected from a vertical madicolous surface beside the road. Specimens were found crawling rapidly in the water film, and hiding in crevices and under dead leaves. Adults were observed feeding on moribund Oligochaeta.

*Remarks.* Wollaston (1865) noted: ‘The only specimen which I have seen of this *Hydroporus* was taken by the Messrs. Crotch, during the summer of 1864, in Tenerife- I believe, in the Barranco at Ycod el Alto’. There are thus some doubts on the true origin of the holotype.

***Hydroporus lundbladi* (Falkenström), comb.n.**

*Hydrotarsus lundbladi* Falkenström, 1938: 4; Gschwendtner, 1939: 39; Guignot, 1949: 3, 1959: 326; Balke & Hendrich, 1989: 60; Alarie & Bilton, 2001; Nilsson, 2001: 168.

*Type locality.* Madeira: Ribeira do Inferno.

*Type material.* Two syntypes (one male, one female) ‘Ribeira do Inferno [the female under moss near waterfall], 10.viii.1935’ in Naturhistoriska Riksmuseet Stockholm, not studied.

*Additional material examined.* 1 ex., Ribeira Joao Delgado, 19.vi.1935, 1450 m, d’Orchymont (MNHN); 94 exx, Pico de Ferreiro, 1500 m, 25–27.vii.1986, Balke & Hendrich leg. (NMW, CBH, CHF, CGW, CFP, CNS). 15 exx, Pico de Ferreiro, 1500 m, iii.1995; 8.iii.2001, D. T. Bilton leg. (CDB, 1 ex. 8.iii.2001 used for a non-destructive DNA extraction, BMNH ref. IR664).

*Diagnosis.* See Table 4 for measurements. Body form shortly oval, with a rather stout appearance, body appendages stout; angle between base of pronotum and base of elytron small but visible (Fig. 5C). Pronotum widest shortly anterior to base, gently narrowing towards head and slightly curved towards posterior angle. Head with distinct

microreticulation moderately to deeply impressed. Pronotum and elytron with distinct microreticulation. First male metatarsal segment strongly enlarged, with numerous long, stout ventral setae; without swimming hairs (Fig. 6D,E); second segment distinctly enlarged; third and fourth only slightly enlarged. This is the only known species of Dytiscidae in which males have expanded metatarsomeres. Median lobe of aedeagus as in Fig. 7B.

*Distribution.* Madeira.

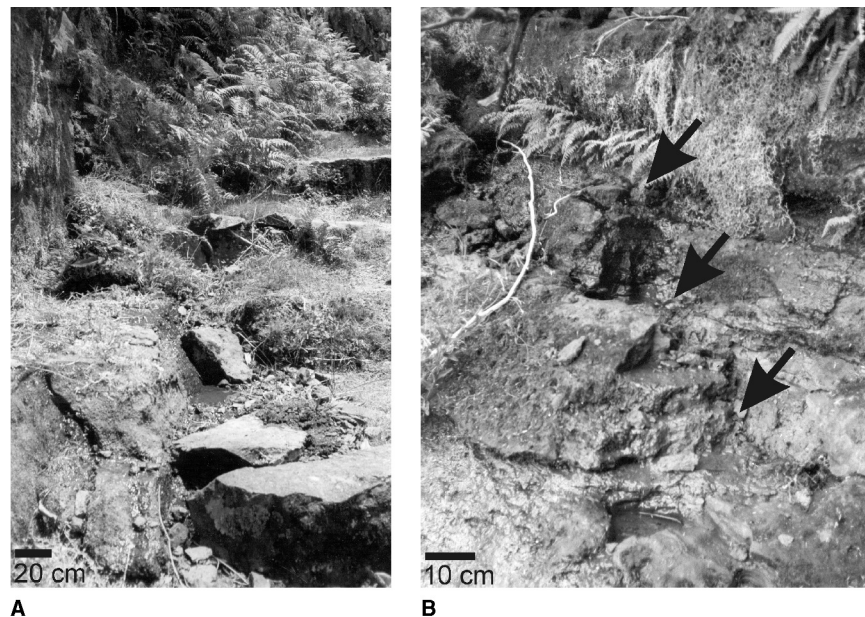
*Ecology.* At Pico de Ferreiro, M.B. and L.H. found specimens on a vertical rock surface, at the bottom of which a trickle of water had formed from a spring below the otherwise dry summit area. Specimens were found under hepatics, such as *Marchantia polymorpha* (L.), stones and slices of rock, which could be lifted with a chisel (Fig. 8). The same locality was visited by D.T.B. at wetter times of year, when specimens could be found crawling actively on vertical madicolous surfaces devoid of bryophyte cover. It is probable that the species retreats into crevices etc. in response to the seasonal drying of its wet rock habitat.

*Remark.* We do not consider the designation of a lectotype, as the description of this species is unambiguous.

***Hydroporus pilosus* (Guignot), comb.n.**

*Hydrotarsus pilosus* Guignot, 1949: 5, 1959: 328; Machado, 1987: 30; Balke *et al.*, 1990: 362; Malmquist *et al.*, 1995; Nilsson *et al.*, 1998: 418, 425; Machado & Oromí, 2000: 31, 92; Alarie & Bilton, 2001: 341; Nilsson, 2001: 168.

*Hydrotarsus* sp. Balke *et al.* 1990: 362 (partim).



**Fig. 8.** Habitat of *Hydroporus lundbladi* in Pico de Ferreiro, Madeira. A, general view of the rock wall and the streamlet; B, detail of the microhabitats.

*Type locality.* Gran Canaria: Teror.

*Type material.* 1 female syntype, Gran Canaria, Teror, 650 m, 11.v.1935, d'Orchymont leg. (MNHN, coll. F. Guignot). The remaining male syntype, which should be housed in the Institut Royal des Sciences Naturelles de Belgique, Bruxelles, is probably lost (cf. Machado, 1987: 31).

*Additional material examined.* Circa 650 exx, Gran Canaria, Pico de las Nieves area, 1200–1700 m, 5–7.vii.1989, Balke, Hendrich & Fery leg. (NMW, CBH, CHF, CGW, CFP, CNS); 12 exx, Tenerife, Anaga Massif, Roque Chinobre, xii.1997, D. T. Bilton leg. (CDB, 1 ex. used for DNA extraction, BMNH ref. IR37).

*Diagnosis.* See Table 4 for measurements. Body form shortly, broadly oval; lateral outline without noticeable angle between pronotum and elytron (Fig. 5A). Pronotum widest at base, gently narrowing towards head. Head with distinct microreticulation, only moderately deeply impressed. Pronotum and elytron with faint microreticulation; on elytron generally very hardly visible and often rudimentary. With well-developed metasternal wings (five specimens dissected). First male metatarsal segment not enlarged, with numerous stout ventral setae (Fig. 6F), female with fewer stout setae (Fig. 6G). Median lobe of aedeagus as in Fig. 7C.

*Distribution.* Canary Islands: Gran Canaria, Tenerife.

*Ecology.* On Gran Canaria specimens were found on wet, more or less vertical rock surfaces and overhanging rocks in an otherwise dry stream bed on a steep, isolated slope, hiding under stones, grass and moss. On Tenerife beetles were collected from water films on vertical rock surfaces beside roads and tracks in laurisylva.

*Remark.* We do not consider the designation of a lectotype, as the description of this species is unambiguous.

## Discussion

### *Phylogenetic position of 'Hydrotarsus' and the extended fuscipennis group*

Our results firmly support the monophyly of the three species of the former genus '*Hydrotarsus*' and its inclusion within *Hydroporus*. Several very robust nodes support this conclusion: the *Hydroporus* group of genera (*Hydroporus*, *Suphrodytes*, *Sanfilippodytes*, *Heterosternuta* and *Neoporus*, with 83% of bootstrap support), the genera *Hydroporus* plus *Suphrodytes* (89% of bootstrap support) and the clade we have designated as an extended *H. fuscipennis* group (see below) (86% bootstrap support). The genus *Hydrotarsus* was considered previously to be a basal offshoot of the subfamily Hydroporinae, mostly on the basis of its penta-

merous protarsi (Falkenström, 1938; Franciscolo, 1979). However, in Alarie & Bilton (2001) its close relationship with *Hydroporus* and *Sanfilippodytes* was noted, as well as the possibility that the divergent, and supposedly plesiomorphic features of the species of '*Hydrotarsus*' could in fact be derived characters related to their madicolous habits.

Our sequence data strongly support the monophyly of the clade we introduce as the extended *H. fuscipennis* group (we retain the name '*fuscipennis*' as this group is the most speciose of those combined here, Nilsson, 2001). We prefer this option to maintaining the individuality of former groups by re-distributing selected species because relationships within the extended *H. fuscipennis* group are not robust (see below), and are very likely to vary with the inclusion of new taxa.

The *Hydroporus fuscipennis* group (*planus* group *sensu* Zimmermann, 1931) so far contains 29 species, and has a Holarctic distribution (Nilsson, 2001). The group was revised partially by Wewalka (1992) (some Palaearctic species close to *H. planus*) and Larson *et al.* (2000) (Nearctic species). It was defined primarily on the basis of the absence of microreticulation on the elytral disc, which presents a smooth and shining surface between punctures (Nilsson & Holmen, 1995). Under our phylogenetic hypothesis, however, the ancestral condition of the extended *H. fuscipennis* group is with microreticulation (present in the most basal species, *H. marginatus*, as well as in their sister lineage, the *H. melanarius*, *longulus* and *memnonius* groups). As reconstructed with our data, the evolution of the elytral reticulation is ambiguous, with either four independent origins of a smooth surface (in *H. pubescens*, *H. discretus*, *H. analis* and the five species of the *H. planus* clade) using the 'delay changes' (DELTRAN) option in MACCLADE 4.0; or a single origin (at the base of the group, excluding *H. marginatus*) with three reversals to the plesiomorphic, reticulate condition (the '*Hydrotarsus*' lineage, *H. nigrita*, and *H. tessellatus* plus *H. basinotatus*), using the 'accelerate changes' option (ACTRAN). Regardless, this character is shown to be highly labile and can no longer be considered a reliable synapomorphy of the group.

The species included in our extended *H. fuscipennis* group all have a broad lateral beading of the pronotum, elytron with the lateral margin weakly ascending to the humeral angle, and the metacoxal processes with its posterior margin conjointly truncate (although the species of '*Hydrotarsus*' have a produced metacoxal process, see Zimmermann, 1931). On the basis of these characters they would, however, be part of a larger clade, including the species of the *memnonius* and *longulus* groups (the latter also with a produced metacoxal process) (Nilsson & Holmen, 1995). At present we cannot recognize any defining morphological synapomorphy for the species of an extended *H. fuscipennis* group. The utility of defining species groups based solely on molecular characters could of course be questioned, although in our opinion the information content of a monophyletic group, even when not well defined morphologically, is always higher than that of species assemblages based on homoplastic characters. Species groups within *Hydroporus*

were introduced originally by Zimmermann (1931), who dealt with the Palaearctic fauna. Since their inception, these groups have been modified and redefined, largely to incorporate a Holarctic perspective (Nilsson & Holmen, 1995; Larson *et al.*, 2000; Nilsson, 2001), and many remain rather ill-defined. As demonstrated here, whilst they may serve as convenient ways to link morphologically similar species, some of these species groups may not reflect phylogeny, which is a strong argument for refraining from affording them formal taxonomic status (Nilsson & Holmen, 1995).

Within the extended *H. fuscipennis* group the position of '*Hydrotarsus*' is less well established. In the combined analysis it is sister to a clade of species mostly with a western Mediterranean distribution, although with low bootstrap and Bremer support values. This uncertainty is not likely to result from incomplete sampling, as all the species of the extended *H. fuscipennis* group known to occur in Morocco (the continental area closest to Madeira and the Canary Islands, and the likely area of origin of the *H. compunctus* lineage) were included in the analysis (Wewalka, 1992; Nilsson, 2001).

In terms of the remaining species of the genus *Hydroporus*, our results support the conclusions of Nilsson (1989) in considering as synonyms the former subgenera *Sternoporus* and *Hydroporidius* (represented in our study by the species *H. longulus* and *H. melanarius*, respectively). These two groups are found to be closely related to the sampled species of the *H. memnonius* group (with 100% of bootstrap support), as suggested by different authors (see, for example, Nilsson, 1989; Fery, 1999). The species *H. obsoletus* was included formerly in the group *ferrugineus*, which was subsequently merged with the *H. memnonius* group by Nilsson (2001). Our results do not support the relationship between this species and those of the *H. memnonius* group.

We found the genus *Suphrodytes* nested within *Hydroporus*, although with low node support. The genus was included formerly in *Hydroporus*, but excluded from it by Angus (1985) based on a series of internal and external morphological characters. A more comprehensive sampling of the basal lineages of *Hydroporus* is necessary to clarify its systematic position.

The monophyly of the *Hydroporus* group of genera *sensu* Ribera *et al.* (2002b) (with the inclusion of *Sanfilippodytes*) is strongly supported. *Suphrodytes*, *Heterosternuta* and *Neoporus* formerly were considered subgenera within *Hydroporus* (e.g. Matta & Wolfe, 1981; Wolfe, 1984; Angus, 1985), as well as most of the species later transferred to *Sanfilippodytes*, which were known previously as the '*Hydroporus vilis* group' (Rochette, 1983). In a phylogenetic analysis of the larvae, Alarie (1991) provides several synapomorphies for the group, although subsequent morphological phylogenetic work found them paraphyletic with respect to the *Deronectes* group of genera (Alarie & Delgado, 1999; Alarie *et al.*, 1999). We found the *Hydroporus* and *Deronectes* groups of genera, respectively, monophyletic, in agreement with Alarie (1991) and traditional taxonomy.

### *Biogeography and ecology*

Species of the *Hydroporus compunctus* lineage are restricted to humid areas of the Canary Islands and Madeira, typically occurring in more or less permanent seepages on vertical rock faces in areas above 800 m (Balke & Hendrich, 1989; Balke *et al.*, 1990; Alarie & Bilton, 2001), primarily, although not exclusively, in areas of monteverde (laurisylva and fayal-breza). Such regions are subject to frequent precipitation in the form of rain and cloud banks, delivered by the north-easterly trade winds (Kunkel, 1976). *Hydroporus lundbladi* is restricted to the main island of Madeira, where it occupies areas on the central ridge. Within the Canary Islands, both *H. pilosus* and *H. compunctus* occur on two islands each. Such a lack of single-island endemism is typical of the Canarian Dytiscidae and other groups of aquatic insects (Bilton *et al.*, 2001; Kelly *et al.*, 2001), and contrasts considerably with most terrestrial coleopteran radiations. *Hydroporus pilosus* was described from Gran Canaria, and subsequently found on Tenerife. The precise distributional limits of *H. compunctus* are more problematic. The species was described from specimens apparently collected on Tenerife (Wollaston, 1865), but, following the discovery of this species on La Palma, Balke *et al.* (1990) considered this record to have resulted from mislabelling, known to have occurred with other species in Wollaston's material. The discovery of this species on La Gomera (Alarie & Bilton, 2001), however, demonstrates that this species is not endemic to the island of La Palma, and increases the possibility that the species does, or did, occur on Tenerife. As one of the youngest islands in the archipelago, La Palma has few unique endemic invertebrate taxa (e.g. Machado, 1992).

According to the standard rate of *c.* 2% divergence per Myr of insect mitochondrial DNA (Brower, 1994), the species of the *H. compunctus* lineage originated *c.* 6 Myr ago (late Miocene). *Hydroporus lundbladi* has been separated from the rest for *c.* 2.3 Myr, and the separation between *H. compunctus* and *H. pilosus* dates to *c.* 1 Myr. Based on these divergence time estimates, rather than being relictual, ancestral *Hydroporinae* (Falkenström, 1938; Franciscolo, 1979), this group is highly derived within the wider genus *Hydroporus*, and represents a relatively recent invasion of Macaronesia. Such a finding is in keeping with other molecular studies of Canarian Coleoptera, almost all of which suggest a recent origin of extant endemic species, rather than relictual status (e.g. Juan *et al.*, 1995; Emerson *et al.*, 1999, 2000a, b; Rees *et al.*, 2001; Ribera *et al.*, 2003; see Juan *et al.*, 2000, for a review). The unique character states of '*Hydrotarsus*' are likely to be associated with the switch to obligate madicolity (see below).

The radiation of the species of the *H. compunctus* lineage within Macaronesia cannot be clearly related to the emergence times of islands, based on mtDNA sequence divergence. Speciation within the clade clearly post-dates the appearance of individual islands, which range from 20 Myr (Madeira) to 2 Myr (La Palma) (Juan *et al.*, 2000). Interpretation of the colonization history of the group within and

between Macaronesian archipelagos must await further population-level sequence data, but radiation of these species seems likely to have involved rare, long-distance colonization events and subsequent divergence in allopatry.

#### *The hygropetric syndrome in Dytiscidae*

'Hygropetric' (Thienemann, 1905) or 'petrimadiculous' (Vaillant, 1956) habitats are those in which a thin layer of water (usually of only a few millimetres) runs over bare rock surfaces. These habitats are frequent in mountain rock outcrops and on coastal cliffs, and usually are fringed by dense layers of mosses, ferns and a diverse vascular vegetation. The aquatic beetle faunas of hygropetric habitats in most parts of the world have been largely overlooked, and some of their most characteristic species were considered until recently extremely scarce oddities of mysterious provenance (e.g. *Agabus aubei* Perris in Corsica, Balke *et al.*, 1997; or the species of '*Hydrotarsus*', Machado, 1987).

Most hygropetric species of Coleoptera show deviant morphological features, meaning that many were described originally as separate genera (e.g. *Metronectes aubei*, now *Agabus aubei*, Balke *et al.*, 1997; or '*Hydrotarsus*'), or even higher taxa (e.g. *Hydrotrupes palpalis* Sharp, considered to represent a monogeneric subfamily by Larson *et al.*, 2000, based on the phylogenetic analysis of Beutel, 1994; or the recently described hygropetric family Aspidytidae, Ribera *et al.*, 2002a). In some of the cases in which it has been possible to obtain molecular data, the phylogenetic analyses have demonstrated that characters considered to be plesiomorphic were instead secondarily derived, and the species usually more recent than expected (*A. aubei*, Ribera *et al.*, 2001; the former *Hydrotarsus*, this paper). Even in the case of *Aspidytes* Ribera *et al.*, in which combined analysis of three genes and a morphological matrix demonstrate an ancient origin, the lack of swimming abilities, and the primitive appearance of the legs (without any apparent modifications for aquatic life) are considered to be secondarily derived (Ribera *et al.*, 2002a). The study of the phylogenetic position of other hygropetric genera with a deviating morphology is still incomplete, in particular that of *Hydrotrupes* Sharp and of the genus *Africophilus* (considered to be basal within the Laccophilinae, Alarie *et al.*, 2000).

Despite having diverse phylogenetic origins, hygropetric species share a number of common features that give them a characteristic resemblance. Both larvae and adults have a stout body shape, with a compact appearance, a broad head with short and robust cephalic appendages, including antennae, short and robust legs, and reduction of the natatory setae (see Balke *et al.*, 1997, for adults and larvae of *A. aubei*; Larson *et al.*, 2000, and Alarie *et al.*, 1998, respectively, for adults and larvae of *H. palpalis*; Guignot, 1959, and Alarie *et al.*, 2000, respectively, for adults and larvae of *Africophilus* Guignot; and Ribera *et al.* 2002a, and Y. Alarie & D. T. Bilton *et al.* in prep., respectively, for adults and larvae of *Aspidytes*).

Another character set present only in some of the taxa is the structural reinforcement of the ventral side of the body, such as the sclerotized abdominal venters of the larvae of *Africophilus* (Alarie *et al.*, 2000) and the expanded metacoxa of *Aspidytes* (Ribera *et al.*, 2002a). Some of the larvae also show reduced urogomphi (e.g. *A. aubei*, *Africophilus* and *Hydrotrupes*, Alarie *et al.*, 1998, 2000), although not always (e.g. *Aspidytes*, Y. Alarie & D. T. Bilton, in prep.). Adults tend to be convex dorsally and flat ventrally, with strong spines on the tibia (particularly in *Hydrotrupes* and *Aspidytes*, Larson *et al.*, 2000; Ribera *et al.*, 2002a).

All these morphological modifications have been compared with that of other species known to be burrowers (such as Noteridae), which has led to the hypothesis that the morphological specializations of hygropetric species are in fact adaptations to burrowing among the layers of vegetation (algae, moss or angiosperm mats) usually present in or near the rock surfaces in which the beetles are found (Balke *et al.*, 1997; Larson *et al.*, 2000). In the case of '*Hydrotarsus*', owing to its estimated recent origin, these morphological modifications seem to have evolved over a relatively short timescale, despite their apparently complex nature (e.g. loss of the pseudotretamerous condition of the tarsi; loss of swimming adaptations of the legs). The fact that the derived morphology of the hygropetric species resembles in many cases the supposed plesiomorphic condition of the family Dytiscidae (or the group of aquatic families of Adephaga) suggest the involvement of relatively simple changes in regulatory pathways that could, however, result in major morphological modifications. This could also be the case for the aberrant morphology of the metatarsi of *H. lundbladi* (which, unique among dytiscids, have the same structure as the pro- and mesotarsi). It is clear that the detailed study of hygropetric habitats and their associated fauna is of maximum interest both for the understanding of the phylogeny of Dytiscidae and the evolution of their morphological adaptations to the aquatic life.

#### Acknowledgements

We thank Elisa Ribera for obtaining some of the sequences in the BMNH, Simon Rundle, Marcos Baez, Samantha Hughes and Lucy Kelly for assistance with fieldwork, and the persons listed in Table 1 for providing specimens for study. We also thank Alfried Vogler and Anders Nilsson for useful comments. Financial support was through the Leverhulme Trust to I.R., NERC (to Alfried Vogler) and the University of Plymouth to D.T.B. Michael Balke is a Marie Curie Research Fellow.

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Accepted 8 April 2003