Zootaxa 3173: 37–53 (2012)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

A new interstitial species of the *Hydroporus ferrugineus* group from north-western Turkey, with a molecular phylogeny of the *H. memnonius* and related groups (Coleoptera: Dytiscidae: Hydroporinae)

CARLES HERNANDO[1], PEDRO AGUILERA†[2], AGUSTÍN CASTRO[3] & IGNACIO RIBERA[4, 5]
[1]*Apartado de Correos 118, E-08911 Badalona, Barcelona, Spain. E-mail: leptotyphlus@gmail.com*
[2] *Deceased 15.2.2009*
[3]*IES Clara Campoamor, E-14900 Lucena, Córdoba, Spain. E-mail: hydraena@telefonica.net*
[4]*Institut de Biologia Evolutiva (IBE, CSIC-UPF), Passeig Maritim de la Barceloneta 37, E-08003 Barcelona, Spain.*
E-mail: ignacio.ribera@ibe.upf-csic.es
[5]*Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, E-28006 Madrid, Spain*

Abstract

We describe *Hydroporus bithynicus* **sp. n.** (Coleoptera, Dytiscidae, Hydroporinae) from the Bolu province in north-western Turkey. The species belongs to the newly defined *H. ferrugineus* group, and can be separated from the other two members (*H. ferrugineus* Stephens, 1829 and *H. sanfilippoi* Ghidini, 1958) by its more flattened shape, less developed eyes and shape of male genitalia. Its external morphology and the habitat in which all specimens were found (a small pool with upwelling spring water next to a stream) suggest an interstitial habitat, similar to that reported for other species of the group. We present a molecular phylogeny of the species of the *H. memnonius* and *H. longulus* groups, including some representatives of the main lineages within the genus, based on ca. 2 kb of four mitochondrial genes. We redefine the *H. memnonius* group and recognise the *H. ferrugineus*, *H. obsoletus* and *H. morio* groups of species as separate entities. *Hydroporus neglectus* Schaum, 1845 was found to be related to the species of the *H. angustatus,* but not the *H. memnonius* group.

Key words: Coleoptera. Dytiscidae, Hydroporinae, phylogeny, taxonomy, new species, interstitial, Turkey

Introduction

The genus *Hydroporus* Clairville, 1806, with 181 described species including the one described here (35 of them known from Turkey) (Nilsson 2001; Fery & Hendrich 2011), is one of the largest among diving beetles (Dytiscidae). The traditional subgenera are no longer considered valid (Nilsson 2001), and it is currently divided in "species groups" based on characters of external morphology. Recent molecular phylogenies of the genus have shown that some of these groups largely correspond with monophyletic lineages (Ribera *et al*. 2003), although the incomplete sampling of this study did not allow to obtain general conclusions.

The finding of a new species of *Hydroporus* apparently related to *H. ferrugineus* Stephens, 1829 in north-western Turkey in 2006 prompted us to re-evaluate the composition and the phylogenetic relationships of the species of the *H. memnonius* group, to which *H. ferrugineus* was assumed to belong (Fery 1999; Nilsson 2001), as well as that of closer species groups, such as the *H. longulus* group (former *Sternoporus* Falkenström, 1930), found to be sister to *H. memnonius* Nicolai, 1822 plus *H. melanarius* Sturm, 1835 in Ribera *et al*. (2003).

Material and methods

Taxon sampling. We use a wide representation of species of *Hydroporus*, including *Suphrodytes dorsalis* (Fabricius, 1787), shown to be nested within *Hydroporus* in Ribera *et al*. (2003) (Table 1). As outgroup we use three spe-

Accepted by H. Fery: 31 Oct. 2011; published: 25 Jan. 2012

cies of *Hydrocolus* Roughley & Larson, 2000 confirmed to be sister to *Hydroporus* in Ribera *et al*. (2008), as hypothesised by Larson *et al.* (2000).

Specimens were preserved in absolute ethanol in the field. Extractions of single specimens were non-destructive, using a standard phenol-chloroform method or the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). Vouchers and DNA samples are kept in the collections of the MNCN and IBE (see abbreviations below).

We amplified fragments of four mitochondrial genes: 3' end of *cox1*; 3' end of *rrnL*; full *trnL*; 5' end of *nad1* (see Ribera *et al*. 2002 and 2010 for primers and general PCR conditions). Sequences were assembled and edited using Sequencher TM 4.1.4 (Gene Codes, Inc., Ann Arbor, MI). New sequences have been deposited in GenBank (EMBL) (see Table 1 for the Accession Numbers).

Abbreviation of collections

The specimens included in this study are deposited in the following institutional and private collections:

BMNH	Natural History Museum, London, UK
CAC	Collection of Agustin Castro, Córdoba, Spain
CCH	Collection of Carles Hernando, Barcelona, Spain
CHF	Collection of Hans Fery, Berlin Germany (property of NMW)
IBE	Institut de Biologia Evolutiva (CSIC-UPF), Barcelona, Spain
MNCN	Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain
NMW	Naturhistorisches Museum, Wien, Austria

Phylogenetic analyses. We aligned the sequences using MAFFT online v.6 and the Q-INS-i algorithm (Katoh & Toh 2008), a progressive pair-wise method with secondary refinement. Bayesian analyses were conducted on a combined data matrix with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001), which runs two independent, simultaneous analyses, using four partitions corresponding to the four genes and with a GTR+I+Γ evolutionary model, the most complex evolutionary model available. MrBayes ran using default values, saving trees at each 500th generation. Convergence and "burn-in" values were established after visual examination of a plot of the standard deviation of the split frequencies between two simultaneous runs.

We also conducted maximum likelihood searches in Garli v.0.951 (www.bio.utexas.edu/faculty/garli/Garli.html), which uses genetic algorithms (Zwickl 2006), with an estimated GTR+I+Γ model for the combined sequence and the default settings. Support was measured with 1,000 bootstrap replicates, reducing the number of generations without improving the topology necessary to complete each replicate to 5,000.

Estimation of the ages of diversification. To calibrate the tree we use molecular clock-methods, with a combined rate for the mitochondrial genes of 2.3% per MY (i.e. the standard arthropod clock, Brower 1994), shown to be accurate for a mixture of protein coding and ribosomal mitochondrial genes in Coleoptera (Papadopoulou *et al*. 2010; Ribera *et al*. 2010). To obtain an ultrametric tree we used Bayesian estimations as implemented in Beast 1.4.7 (Drummond & Rambaut 2007). Well supported nodes according to the results were constrained to be monophyletic, and a GTR+I+Γ model was enforced with an uncorrelated lognormal relaxed clock and a Yule process speciation model. Priors and other parameters were left with default values, with the exception of the prior of the evolutionary rate, which was set to a normal distribution with mean of 0.0115 substitutions/site/MY and a standard deviation of 0.001. The results of two independent runs were merged with Tracer v1.4 and TreeAnnotator v1.4.7 (Drummond & Rambaut 2007).

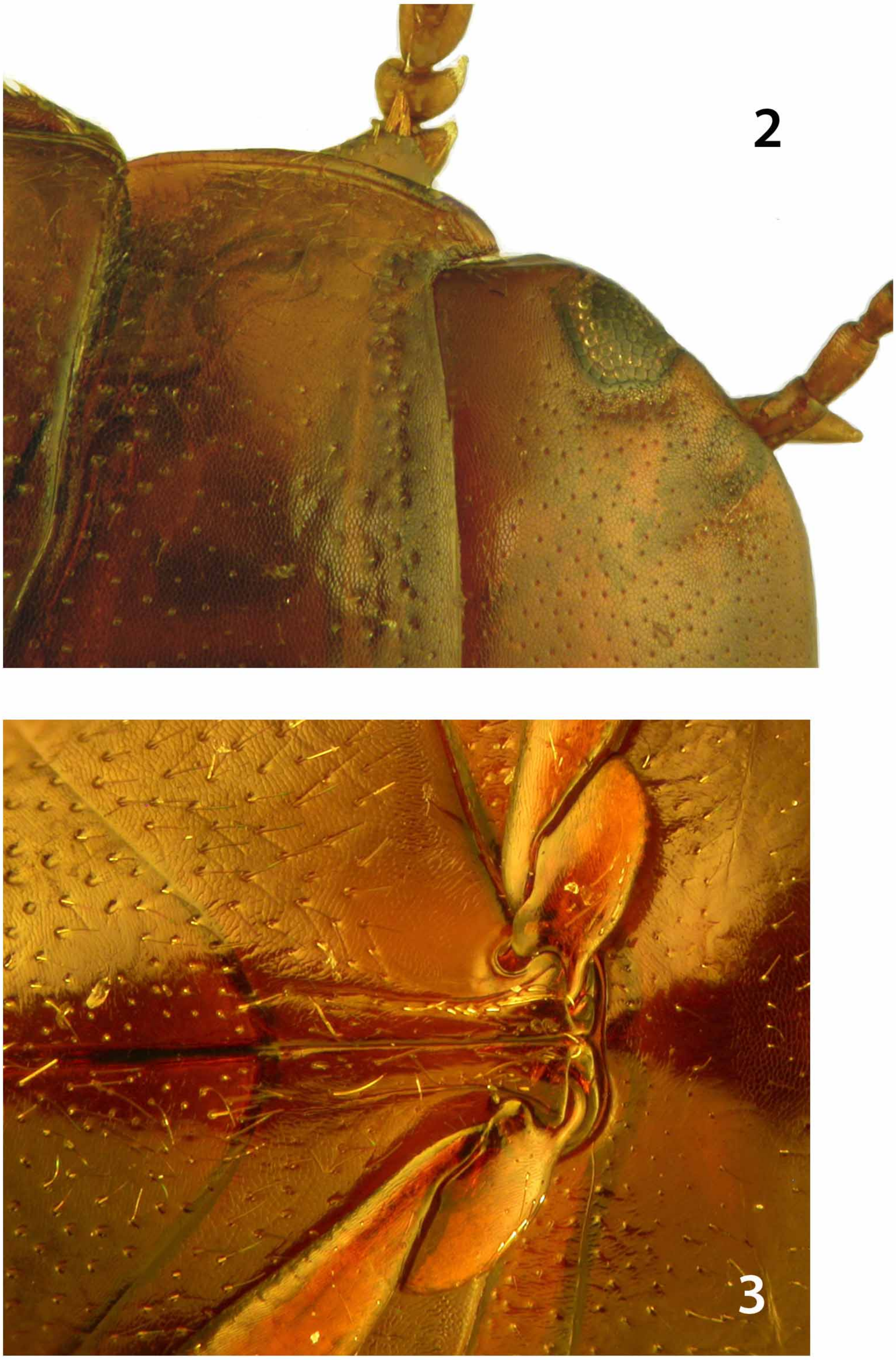
Taxonomy

Hydroporus bithynicus sp. n.

(Figs 1–6)

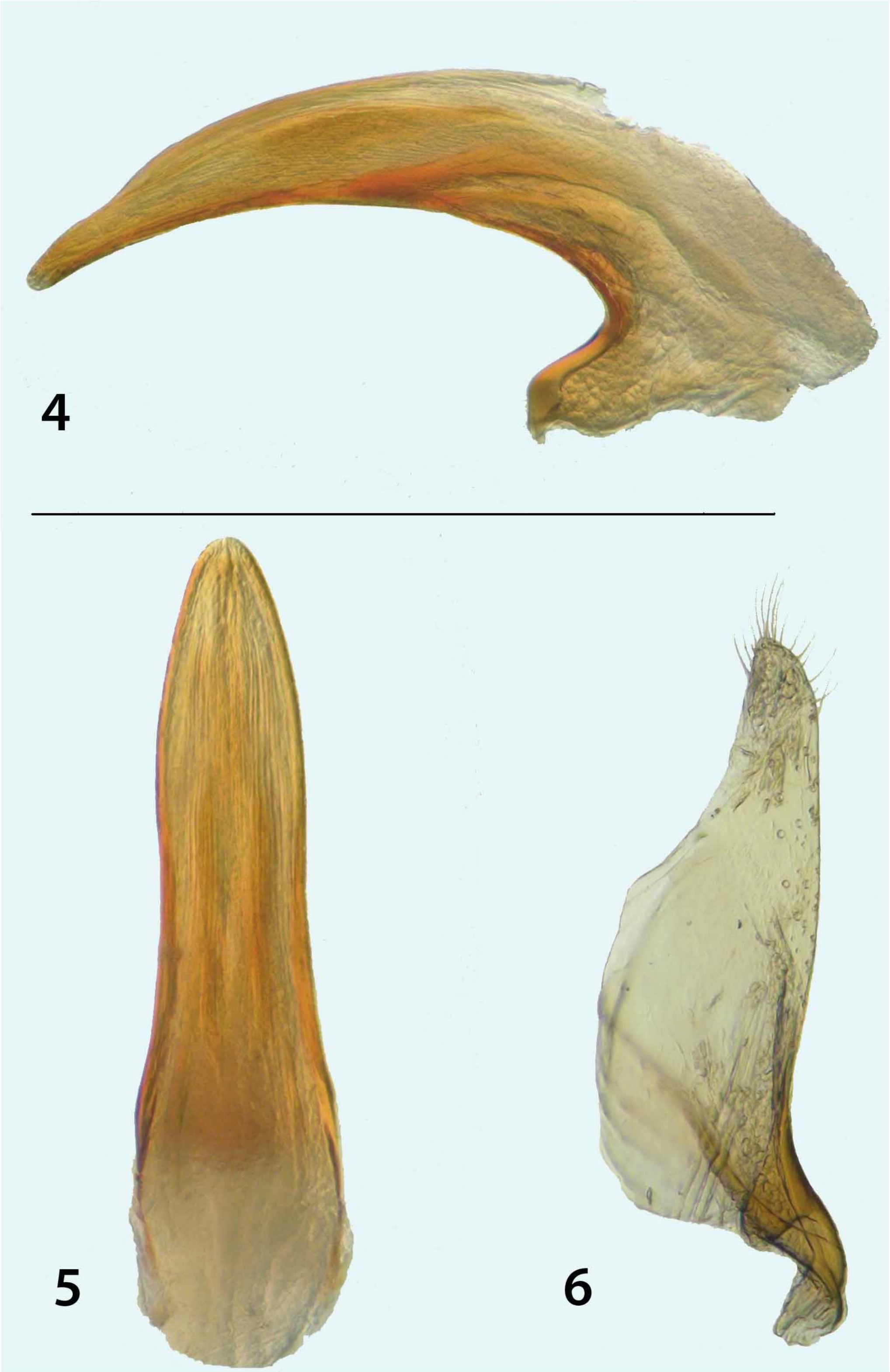
Type locality. Turkey, Bolu province, stream between Yeniçaga and Mengen.

FIGURE 1. Habitus of *Hydroporus bithynicus* **sp. n.** (paratype male, NMW, photo: M. Brojer).



FIGURES 2–3. *Hydroporus bithynicus* **sp. n.** (paratype male, DNA voucher specimen MNCN-AI782); 2) head and pronotum; 3) detail of the metacoxa (photos: A. Castro).

Type material. HOLOTYPE (MNCN): male, labelled "TURKEY 05 BOLU 24.4.2006 / Rd. 750 btw Yeniçaga & Mengen / fast stream in mixed forest / 844 m N40º50'49" E32º03'47.5" / Hernando,Aguilera,Castro&Ribera leg.", plus printed red Holotype label. Aedeagus extracted and mounted in DMHF on a transparent label pinned with the specimen. PARATYPES (IBE, MNCN, NMW, BMNH, CAC, CCH, CHF): 5 males, 3 females, same data as holotype with paratype labels. One male paratype was used for DNA extraction, voucher No. MNCN-AI782 (ref MNCN-ADN collection 24128).



FIGURES 4–6. *Hydroporus bithynicus* **sp. n.** (paratype male, DNA voucher specimen MNCN-AI782); 4) median lobe of the aedeagus, lateral view; 5) median lobe of the aedeagus, ventral view; 6) right paramere, lateral view. Scale bar: 1 mm (photos: A. Castro).

Diagnosis. *Hydroporus bithynicus* **sp. n.** has to be included among the species of the *H. ferrugineus* subgroup sensu Fery (1999) as it has uniform pale colour, lateral depressions at the base of the pronotum with a deeper punctation, a dorsally symmetric male aedeagus and the female gonocoxa do not have an angularity on the inner side

(Fery 1999). Of the known species of the *H. ferrugineus* subgroup, *H. bithynicus* **sp. n.** is the flattest, narrowest and more parallel sided. The eyes are also smaller than in the other two species, which is particularly evident in ventral view. The aedeagus has also a characteristic shape, unlike *H. ferrugineus* and *H. sanfilippoi* Ghidini, 1958 (see Fery 1999: figs 68, 70).

Description. Total length 3.7–4.0 mm, maximum width 1.7–1.8 mm. Body elongate, narrow, sides parallel; lateral outline almost continuous; pronotum slightly wider than base of elytra, as wide as maximum width of elytra (Fig. 1). Colour uniformly testaceous, ventral side darker.

FIGURES 7–9. Habitat of *Hydroporus bithynicus* **sp. n.**; 7) detail of the spring in which all specimens were found; 8) general view of the stream between Yeniçaga and Mengen; 9) two of the authors (P. Aguilera and C. Hernando) next to the spring (photos: I. Ribera).

Head (Figs 1, 2): wide; surface finely microreticulated, with polygonal, isodiametric cells; covered with very fine, dense punctation; space between punctures 2–3 times wider than their diameter. Two small clypeal fossae; periocular area slightly depressed. Eyes small, not prominent. Maximum diameter of eye (seen from above) ca. 11–12 ommatidia (15–16 in *H. sanfilippoi*); surface of ommatidia transparent, pigmented area below cuticle smaller than area covered by ommatidia (Fig. 2).

Pronotum (Figs 1, 2): transverse; sides strongly bordered, slightly arched. Surface microreticulate, stronger and with larger cells than on head; punctation on disk sparser and stronger than on head; near posterior angles punctation denser, with a more rugose appearance. Anterior margin with irregular row of coarse punctures. Anterior angles with a group of very long setae. Hind wings apparently well developed.

Elytra (Fig. 1): parallel sided, maximum width behind middle; surface covered with dense and strong punctation; microreticulation strong, cells wider than on pronotum and head, isodiametric. Without regular rows of punctures; with some isolated coarser punctures with setae less erect that those of regular punctures, forming loose series on the elytra. Margins with a series of very long setae, from shoulder to apex.

Ventral darker than dorsal side; metaventrum and first two or three abdominal ventrites dark brown; surface of metaventrite covered with regular, coarse and sparse punctation, space between punctures ca. 3 times wider than their diameter; covered by strong reticulation, cells slightly transverse (Fig. 3). Abdominal ventrites with weaker punctation, more disperse; surface microreticulated, with smaller cells than on metaventrite (Fig. 3). Posterior margin of metacoxal process slightly sinuate, medially protruded backwards (Fig. 3).

Male: pro- and mesotarsi slightly dilated; claws unmodified. Aedeagus as in Figs 4-6.

Etymology. Named after Bithynia, ancient kingdom of the NW coast of Anatolia and Roman province. The specific epithet is an adjective in the nominative singular.

Distribution and habitat. So far only known from the type locality, a fast-flowing stream in a well preserved mixed forest in north-west Turkey (Figs 7–9). All specimens were found in a small pool with upwelling spring water (ca. 1 m diameter, few centimetres deep) on the side of the river. The pool had a stony substratum without vegetation, with some algal and bacterial grow, and was fed by underground water upwelling through the bottom (Figs 7, 9).

The habitat and the external morphology, in particular the reduced eye size and pigmentation and the presence of long sensory setae on pronotum and elytra, suggest that the species is an inhabitant of the interstitial water, only accessible in particular circumstances – in this case, an upwelling spring. The other two species of the group, although with an apparently less modified morphology (they are less flat, with larger eyes) have been reported from similar habitats, or, in the case of *H. ferrugineus*, from deep inside caves (Franciscolo 1979; Foster & Friday 2011). The specimen of *H. sanfilippoi* sequenced here (Table 1) was collected in the small pools of a minuscule stream formed by upwelling water while there was heavy rain: short after the specimen was collected the rain stopped and the stream dried out completely. Only after some additional rain during the next days it was flowing again, and more specimens could be found. The preference for very small running water bodies or interstitial habitats seems to be shared between the species of the *H. ferrugineus*, *memnonius* and *longulus* groups (see below), and has been suggested as a possible reason for the abundance of the local endemics in the group (Fery 2009; Hájek & Fikáček 2010).

Phylogeny of the *Hydroporus memnonius* and related groups

The concatenated sequence had a final length of 1615 nucleotides, 826 corresponding to the *cox1* gen (without any indel) and 729 to the *rrnL+trnL+nad1* fragment (with 4–5 indels as aligned with MAFFT). MrBayes run for $5 \cdot 10^6$ generations, after which the standard deviation of the split frequencies between the two simultaneous runs reached values of ca. 0.01, indicating a good convergence. The two methods used for the phylogenetic reconstruction (Bayesian probabilities and Maximum Likelihood with a heuristic genetic algorithm) resulted in very similar topologies, with only the position of some species differing between them (*H. submuticus* Thomson, 1874, *H. tessellatus* (Drapiez, 1819), Fig. 10). The alternative topologies for these nodes did not change the composition of the species groups, and their support was, in any case, always low, with all well supported nodes (Bayesian posterior probability, Bpp > 0.9, ML bootstrap, MLb > 70%) congruent between methods.

The monophyly of *Hydroporus* plus *Suphrodytes* was well supported (Bpp = 1, MLb = 100%; Fig. 10), but the phylogenetic position of *Suphrodytes* not: it appears nested within *Hydroporus* with low support in the Bayesian analysis, but unresolved at the base of *Hydroporus* with ML (Fig. 10). With our data we, thus, cannot exclude the status of *Suphrodytes* and *Hydroporus* as separate genera (Angus 1985).

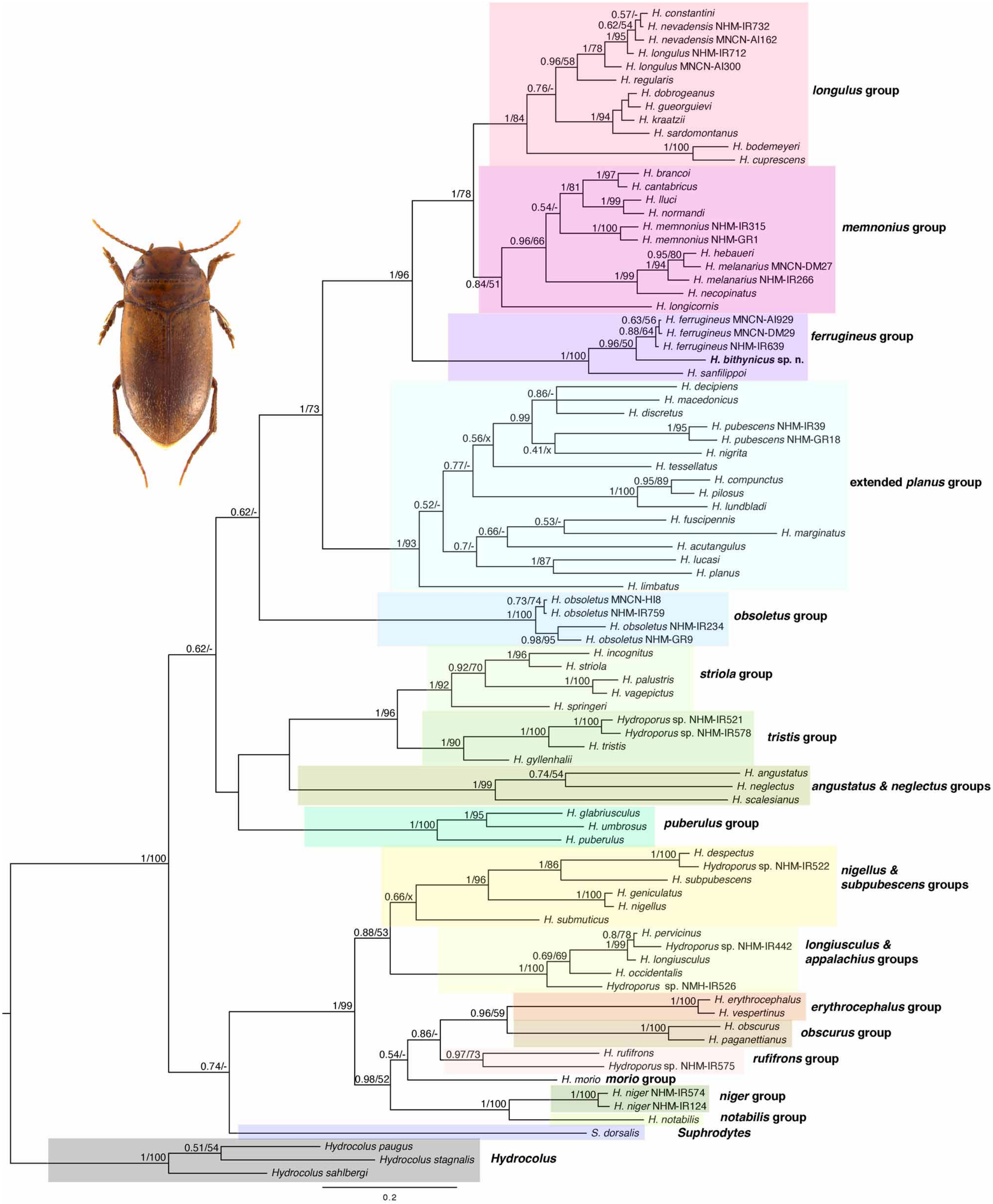


FIGURE 10. Phylogram obtained with MrBayes. Above nodes, Bayesian posterior probabilities (when > 0.5) / bootstrap support values in Garli (when > 50%). "-", node not resolved; "x", node not present. See Table 1 for the localities and voucher reference of the specimens.

TABLE 1. Material used in the molecular study, with GenBank accession numbers (new sequences in bold). See text for detailed locality data of type material. Species groups follow Nilsson (2001), except for the modifications introduced here (see text).

No	sp. group	species	voucher	country	locality	year	legit	*cox1*	*rrnL* + *trnL* + *nad1*
1	*angustatus*	*H. angustatus* Sturm, 1835	NHM-GR16	UK	England, Eping Forest	1999	I. Ribera	AY365300	AY365266
2	*angustatus*	*H. scalesianus* Stephens, 1828	NHM-IR38	UK	Dorset, Wareham, Morden bog	1998	I. Ribera	AF518309	AF518278
3	*appalachius*	*Hydroporus* sp.	NHM-IR526	Canada	British Columbia, 40 km N Hope	2000	I. Ribera & A. Cieslak	AY365295	AY365261
4	*appalachius*	*H. occidentalis* Sharp, 1882	NHM-IR514	Canada	British Columbia, Yoho National Park	2000	I. Ribera & A. Cieslak	AY365314	AY365280
5	*erythrocephalus*	*H. erythrocephalus* (Linné, 1758)	NHM-GR24	UK	England, Sommerset Levels	1998	I. Ribera	AF518291	AF518261
6	*erythrocephalus*	*H. vespertinus* Fery & Hendrich, 1988	NHM-IR144	Portugal	Guarda, Serra da Estrela	1999	D.T. Bilton	AY365325	AY365291
7	*ferrugineus*	*H. bithynicus* sp. n.	MNCN-AI782	Turkey	Bolu, between Yeniçaga and Mengen	2006	I. Ribera	**HE599650**	**HE599681**
8	*ferrugineus*	*H. ferrugineus* Stephens, 1829	MNCN-AI929	Germany	Bayern, Rehau	2003	A. Skale	**HE599657**	**HE599683**
9	*ferrugineus*	*H. ferrugineus*	MNCN-DM29	UK	England, North Hampshire, Wick Hanger	2000	J. Denton	**HE599658**	-
10	*ferrugineus*	*H. ferrugineus*	NHM-IR639	UK	England, North Hampshire, Wick Hanger	2000	J. Denton	**HE599659**	**HE599698**
11	*ferrugineus*	*H. sanfilippoi* Ghidini, 1958	MNCN-AH189	Italy	Emilia Romagna, Berceto	2008	I. Ribera	**HE599672**	**HE599674**
12	*longiusculus*	*Hydroporus* sp.	NHM-IR442	Canada	British Columbia, Champion L. Provincial Park	2000	I. Ribera & A. Cieslak	AY365292	AY365258
13	*longiusculus*	*H. longiusculus* Gemminger & Harold, 1868	NHM-MA410	US	California, Mendocino co., Manchester	2000	I. Ribera & A. Cieslak	AY365305	AY365271
14	*longiusculus*	*H. pervicinus* Fall, 1923	NHM-MA359	US	California, Shasta co., Lake McCumber	2000	I. Ribera & A. Cieslak	AY365316	**HE599695**
15	*longulus*	*H. b. bodemeyeri* Ganglbauer, 1900	MNCN-AI110	Greece	Chios, Kapouries	2004	G.N. Foster	**HE599651**	**HE599675**
16	*longulus*	*H. constantini* Hernando & Fresneda, 1996	MNCN-AI285	Spain	Burgos, Cruz de la Demanda	2004	I. Ribera	**HE599654**	**HE599679**

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TABLE 1. (continued)

No	sp. group	species	voucher	country	locality	year	legit	*cox1*	*rrnL* + *trnL* + *nad1*
17	*longulus*	*H. cuprescens* K.W. Miller & Fery, 1995	NHM-IR647	Cyprus	Ayia, Paphos forest	2001	K.W. Miller	**HE599655**	**HE599699**
18	*longulus*	*H. dobrogeanus* Ieniştea, 1962	MNCN-DM85	Serbia	Canyon of River Pschinja	2006	V. Pešić	**HE599656**	**HE599689**
19	*longulus*	*H. gueorguievi* Wewalka, 1975	MNCN-DM20	Bulgaria	Pirin Mountains, cottongrass bog above Dobriniste	2005	D.T. Bilton	**HE599660**	**HE599685**
20	*longulus*	*H. kraatzii* Schaum, 1868	MNCN-DM1	France	Isère, Lac Punay	2005	G.N. Foster	**HE599662**	**HE599684**
21	*longulus*	*H. longulus* Mulsant & Rey, 1861	NHM-IR712	Scotland	Inverness, Loch Einich	2001	G.N. Foster	AY365326	-
22	*longulus*	*H. longulus*	MNCN-AI300	Italy	Brescia, Val Trompia	2002	I. Ribera & A. Cieslak	**HE599664**	**HE599680**
23	*longulus*	*H. nevadensis* Sharp, 1882	MNCN-AI162	Spain	Granada, Sierra Nevada	2004	A. Castro	**HE599668**	**HE599677**
24	*longulus*	*H. nevadensis*	NHM-IR732	Spain	Santander, Reinosa	2001	H. Fery	AF518295	AF518265
25	*longulus*	*H. regularis* Sharp, 1882	NHM-IR166	France	Corsica, Vizzavona, Cascades des Anglais	1999	I. Ribera & A. Cieslak	AY365318	AY365284
26	*longulus*	*H. sardomontanus* Pederzani, Rocchi & Schizzerotto, 2004	MNCN-DM62	Italy	Sardinia, Nuoro, Gennargentu	2006	P. & V. Ponel	**HE599673**	**HE599688**
27	*memnonius*	*H. brancoi* Rocchi, 1981	MNCN-AI228	Portugal	Minho, Serra de Arga	2005	D.T. Bilton	**HE599652**	**HE599678**
28	*memnonius*	*H. cantabricus* Sharp, 1882	MNCN-AI122	Spain	Burgos, Sierra de Valnera	2004	H. Fery	**HE599653**	**HE599676**
29	*memnonius*	*H. hebaueri* Hendrich, 1990	MNCN-AI789	Turkey	Bartin, between Topallar and Kakraz	2006	I. Ribera	**HE599661**	**HE599682**
30	*memnonius*	*H. lluci* Fery, 1999	NHM-IR498	Spain	Mallorca, Lluc	2000	I. Ribera & A. Cieslak	AY365307	**HE599697**
31	*memnonius*	*H. longicornis* Sharp, 1871	MNCN-DM22	Sweden	Vasterbotten, Vindeln	2000	A.N. Nilsson	**HE599663**	**HE599686**
32	*memnonius*	*H. melanarius* Sturm, 1835	MNCN-DM27	Sweden	Vasterbotten, Vindeln	2000	A.N. Nilsson	**HE599665**	**HE599687**
33	*memnonius*	*H. melanarius*	NHM-MA266	Sweden	Vasterbotten, Vindeln	2000	A.N. Nilsson	**HE599666**	AF518268
34	*memnonius*	*H. memnonius* Nicolai, 1822	NHM-GR1	UK	England, New Forest	1999	I. Ribera	AF518300	AF518269

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TABLE 1. (continued)

No	sp. group	species	voucher	country	locality	year	legit	*cox1*	*rrnL* + *trnL* + *nad1*
35	*memnonius*	*H. memnonius*	NHM-IR315	Switzerland	St. Gottardo pass	2000	I. Ribera & A. Cieslak	**HE599667**	**HE599694**
36	*memnonius*	*H. necopinatus* Fery, 1999	NHM-IR146	Portugal	Guarda, Serra da Estrela	1999	D.T. Bilton	AY365309	**HE599692**
37	*memnonius*	*H. normandi* Régimbart, 1903	NHM-IR629	Spain	Zaragoza, Santed	2000	P. Aguilera	AY365312	AY365278
38	*morio*	*H. morio* Aubé, 1838	NHM-ER22	Sweden	Vasterbotten, Ansele	1999	A.N. Nilsson	AY365308	AY365274
39	*neglectus*	*H. neglectus* Schaum, 1845	NHM-GR15	UK	England, Norfolk, East Walton Common	1999	I. Ribera	AY365310	AY365276
40	*nigellus*	*Hydroporus* sp.	NHM-IR522	Canada	British Columbia, Stump Lake	2000	I. Ribera & A. Cieslak	AY365294	AY365260
41	*nigellus*	*H. despectus* Sharp, 1882	NHM-IR483	Canada	Alberta, Waterton lakes National Park	2000	I. Ribera & A. Cieslak	AY365302	**HE599696**
42	*nigellus*	*H. geniculatus* C.G. Thomson, 1856	NHM-ER3	Sweden	Vasterbotten, Vindeln, Skatan	2001	A.N. Nilsson	AY365303	AY365269
43	*nigellus*	*H. nigellus* Mannerheim, 1853	NHM-IR506	Sweden	S. Ha. Onsala, Kustgol	2000	B. Andren	AY365311	AY365277
44	*nigellus*	*H. submuticus* C.G. Thomson, 1874	NHM-ER23	Sweden	Vasterbotten, Strycksele	1999	A.N. Nilsson	AY365321	AY365287
45	*nigellus*	*H. subpubescens* LeConte, 1852	NHM-MA411	US	California, Mendocino co., Manchester	2000	I. Ribera & A. Cieslak	AY365322	AY365288
46	*niger*	*H. niger* Say, 1823	NHM-IR574	US	Vermont, Bennington	2000	C. Hernando	AY365296	AY365262
47	*niger*	*H. niger*	NHM-IR124	US	New York, Tompkins co., Ithaca	1998	K.B. Miller	AF518301	AF518270
48	*notabilis*	*H. notabilis* LeConte, 1850	NHM-ER20	Sweden		1999	A.N. Nilsson	AY365313	AY365279
49	*obscurus*	*H. obscurus* Sturm, 1835	NHM-ER4	Sweden	Vasterbotten, Vindeln, Skatan	2002	A.N. Nilsson	AF518303	AF518272
50	*obscurus*	*H. paganettianus* Scholz, 1923	NHM-IR145	Portugal	Guarda, Serra da Estrela	1999	D.T. Bilton	AY365324	AY365290
51	*obsoletus*	*H. obsoletus* Aubé, 1838	NHM-GR9	Portugal	San Lourenco de Montaria, Serra Do Minho	1998	I. Ribera	AF518304	AF518273
52	*obsoletus*	*H. obsoletus*	NHM-DW234	Morocco	Ait-Iftene, river Ait-Baha	2000	I. Ribera	**HE599670**	**HE599690**
53	*obsoletus*	*H. obsoletus*	NHM-IR759	Tunisia	Between Tabarka and Aïn-Draham	2001	I. Ribera & A. Cieslak	**HE599671**	**HE599701**

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TABLE 1. (continued)

No	sp. group	species	voucher	country	locality	year	legit	*cox1*	*rrnL* + *trnL* + *nad1*
54	*obsoletus*	*H. obsoletus*	MNCN-HI8	Algeria	Aïn-El-Barraka	2006	S. Bouzid	**HE599669**	-
55	*planus*	*H. acutangulus* C.G. Thomson, 1856	NHM-ER1	Sweden	Vasterbotten, Vindeln, Skatan	2004	A.N. Nilsson	AF518286	AF518256
56	*planus*	*H. compunctus* Wollaston, 1865	NHM-IR181	Spain	La Gomera, Garajonay	2000	D.T. Bilton	AF518289	**HE599693**
57	*planus*	*H. decipiens* Sharp, 1878	NHM-ER31	Spain	Avila, Sierra de Gredos, S Hoyos del Espino	1999	H. Fery	AF518287	AF518257
58	*planus*	*H. discretus* Fairmairei & Brisout de Barneville, 1859	NHM-GR6	Spain	Albacete, Paterna del Madera, Arroyo de la Fuenfria	1998	I. Ribera	AF518290	AF518260
59	*planus*	*H. fuscipennis* Schaum, 1868	NHM-MA263	Sweden	Vasterbotten, Vindeln	2000	A.N. Nilsson	-	AF518262
60	*planus*	*H. limbatus* Aubé, 1838	NHM-GR4	Morocco	Delta du Moulouya	1999	I. Ribera	AF518294	AF518264
61	*planus*	*H. lucasi* Reiche, 1866	NHM-GR7	Spain	Granada, Güejar Sierra, Barranco de las Viboras	1998	I. Ribera	AF518296	AF518266
62	*planus*	*H. lundbladi* (Falkenström, 1938)	NHM-IR664	Portugal	Madeira, Paul do Serra	2001	D.T. Bilton	AF518297	-
63	*planus*	*H. macedonicus* Fery & Pešić, 2006	MNCN-AI251	Macedonia	Kozuf Mountain, Dve Usi	2004	V. Pešić	DQ195528	DQ195529
64	*planus*	*H. marginatus* (Duftschmid, 1805)	NHM-GR10	Spain	Burgos, Pineda de la Sierra, river Arlanzon	2005	I. Ribera	AF518298	AF518267
65	*planus*	*H. nigrita* (Fabricius, 1792)	NHM-GR3	Spain	Burgos, Pineda de la Sierra, river Arlanzon	1998	I. Ribera	AF518302	AF518271
66	*planus*	*H. pilosus* (Guignot, 1949)	NHM-IR37	Spain	Tenerife, Anaga, Roque Chinobre	1997	D.T. Bilton	AF518305	AF518274
67	*planus*	*H. planus* (Fabricius, 1781)	NHM-IR690	Russia	Astrakhan oblast, Nishniy Baskuntyak	2001	A.N. Nilsson	AF518306	AF518275
68	*planus*	*H. pubescens* (Gyllenhal, 1808)	NHM-GR18	UK	England, New Forest	1999	I. Ribera	AF518307	AF518276
69	*planus*	*H. pubescens*	NHM-IR39	Spain	Burgos, Pineda de la Sierra, river Arlanzon	1998	I. Ribera	AF309300	EF419327
70	*planus*	*H. tessellatus* (Drapiez, 1819)	NHM-GR8	Portugal	Alto Alentejo, Serra de Sao Mamede	1998	I. Ribera	AF518310	AF518279
71	*puberulus*	*H. glabriusculus* Aubé, 1838	NHM-GR33	UK	England, Norfolk, East Walton Common	1999	I. Ribera	AY365304	AY365270

continued next page

TABLE 1. (continued)

No	sp. group	species	voucher	country	locality	year	legit	*cox1*	*rrnL + trnL + nad1*
72	*puberulus*	*H. puberulus* LeConte, 1850	NHM-ER2	Sweden	Vasterbotten, Vindeln, Skatan	1999	A.N. Nilsson	AY365317	AY365283
73	*puberulus*	*H. umbrosus* (Gyllenhal, 1808)	NHM-IR152	Finland		1999	T. Berendonk	AF518311	AF518280
74	*rufifrons*	*Hydroporus* sp.	NHM-IR575	US	Vermont, Bennington	2000	C. Hernando	AY365297	AY365263
75	*rufifrons*	*H. rufifrons* (O.F. Müller, 1776)	NHM-IR694	UK	Scotland, Argyll, Strath Orchy, Dalmally	2001	G.N. Foster	AF518308	AF518277
76	*striola*	*H. incognitus* Sharp, 1869	NHM-GR35	Czech Republic	Jizerske Hory Mts., Nova Louka	1999	J. Šťastný	AY365306	AY365272
77	*striola*	*H. palustris* (Linné, 1761)	NHM-IR561	Italy	Veneto, Le Bine	2000	M. Toledo	AY365315	AY365281
78	*striola*	*H. springeri* J. Müller, 1924	NHM-IR632	Italy	Lombardia, Mantova	2001	M. Toledo	AY365319	AY365285
79	*striola*	*H. striola* (Gyllenhal, 1826)	NHM-GR25	UK	England, Sommerset Levels	1998	I. Ribera	AY365320	AY365286
80	*striola*	*H. vagepictus* Fairmaire & Laboulbène, 1855	NHM-IR28	Portugal	Guarda, Serra da Estrela	1998	I. Ribera	AF518312	AF518281
81	*tristis*	*Hydroporus* sp.	NHM-IR578	US	New Jersey, High Point State Park	2000	C. Hernando	AY365298	AY365264
82	*tristis*	*Hydroporus* sp.	NHM-IR521	Canada	British Columbia, Stump Lake	2000	I. Ribera & A. Cieslak	AY365293	AY365259
83	*tristis*	*H. gyllenhalii* Schiödte, 1841	NHM-GR2	UK	England, New Forest	1999	I. Ribera	AF518293	AF518263
84	*tristis*	*H. tristis* (Paykull, 1798)	NHM-ER5	Sweden	Vasterbotten, Vindeln, Skatan	2006	A.N. Nilsson	AY365323	AY365289
85	outgroup	*Hydrocolus paugus* (Fall, 1923)	MNCN-AC43	Canada	Nova Scotia, Cumberland, Mapleton	2007	I. Ribera & A. Cieslak	**HE599648**	-
86	outgroup	*Hydrocolus sahlbergi* Nilsson, 2001	NHM-ER21	Sweden	Vasterbotten, Ansele	1999	A.N. Nilsson	AJ850629	**HE599691**
87	outgroup	*Hydrocolus stagnalis* (Gemminger & Harold, 1868)	NHM-IR579	US	New Jersey, High Point State Park	2000	C. Hernando	**HE599649**	-
88	outgroup	*Suphrodytes dorsalis* (Fabricius, 1787)	NHM-IR67	UK	England, Norfolk	1998	D.T. Bilton	AF309299	**HE599700**

Within the wider *Hydroporus*+*Suphrodytes* clade the species groups defined by Nilsson & Holmen (1995) and Nilsson (2001) are in general recovered as monophyletic, and with good support. This agreement between morphological and genetic discontinuities among the species, allowing the recognition of phenotypic species groups and providing good support for the same clades based on genetic data, is most remarkable giving the independence of both sets of characters. Thus, when plotting the average support of the nodes according to their topological level in the tree in Fig. 10, the level at which the support is maximum (nodes at the fifth level, average support Bpp = 0.91, Fig. 11) is also the one at which there is the highest number of nodes defining species groups (seven). One possible cause of this agreement is that both set of characters are neutral and evolve at a rate proportional to time (Ahrens & Ribera 2009).

In some cases our results do not agree with the established species groups. *Hydroporus morio* Aubé, 1838 was included in the *H. nigellus* group in Nilsson & Holmen (1995), but listed under the *H. puberulus* group in Nilsson (2001). We found it in a rather isolated position, although more related to the *H. nigellus* than to the *H. puberulus* group (Fig. 10). We found *H. neglectus* Schaum, 1845 closer to *H. angustatus* Sturm, 1835 than to *H. scalesianus* Stephens, 1828, suggesting the need to merge both species groups, but in any case not related to the species of the *H. memnonius* or *H. longulus* groups, as traditionally suggested (see Fery 2009 for an overview). Similarly, *H. pervicinus* Fall, 1923, placed in the *H. nigellus* group in Nilsson (2001), was found to be more closely related to the species of the *H. longiusculus* group (Fig. 10). *Hydroporus subpubescens* LeConte, 1852 appears nested within the species of the *H. nigellus* group, suggesting that it should be included here rather than in a separate species group as previously considered (Nilsson, 2001).

Hydroporus obsoletus Aubé, 1838 has been traditionally included in the *H. memnonius* group, close to the species of the *H. ferrugineus* subgroup (Fery 1999, 2009). We found it in an isolated position as sister to a large part of the Palaearctic species, although with low support, which in any case warrants its consideration as a separate species group. *Hydroporus obsoletus* is widely distributed through Europe and north Africa, and despite the homogeneity of the male genitalia through his range (Fery 1999) there are deep genetic divergences among the specimens from Algeria and Tunisia and those of the western Mediterranean (SW Morocco and Portugal) (Fig. 10, Table 1).

We found strong support for an extended *Hydroporus planus* group, including the *H. marginatus*, *H. tessellatus* and *H. nigrita* groups, in agreement with Ribera *et al*. (2003) (their "*H. fuscipennis sensu lato*"). The internal phylogeny of this extended group was not well resolved, but it seems that the elytral reticulation (traditionally used to separate these species groups) is a very labile character with very weak phylogenetic information. The extended *H. planus* group is sister to a large clade including the species of the *H. memnonius* and *H. longulus* groups sensu Fery (1999). Within this large clade the species phylogenetically close to *H. ferrugineus*, including *H. bithynicus* **sp. n.**, are sister to the rest, thus rendering the *H. memnonius* group as traditionally defined paraphyletic. *Hydroporus bithynicus* **sp. n.** is sister to the central and north European *H. ferrugineus*, and both sister to *H. sanfilippoi* from Italy. These three species should be considered a group within the genus *Hydroporus*, the *H. ferrugineus* group.

Within the *H. memnonius* group (in its restricted sense defined here, i.e. excluding the species of the *H. ferrugineus* and *H. obsoletus* groups), the species with the angularity in the female gonocoxa form a well supported clade, corresponding to the *H. memnonius* subgroup sensu Fery (1999). However, *Hydroporus longicornis* Sharp, 1871 was found to be sister to the rest of the species of the group, and not included in the *H. melanarius* complex, as suggested by Fery (1999).

The *Hydroporus longulus* group (the former subgenus *Sternoporus*, see Fery 2009 for a review) was found monophyletic with strong support, but highly derived within the genus *Hydroporus*, so that its reinstatement as valid subgenus would render *Hydroporus* s. str. highly paraphyletic. Among the sampled species of the *H. longulus* group we found three linages: the eastern Mediterranean *H. bodemeyeri bodemeyeri* Ganglbauer, 1900 plus *H. cuprescens* K.W. Miller & Fery, 1995 from Cyprus (as hypothesised in Hájek & Fikáček 2010); the *H. kraatzii* complex, including *H. sardomontanus* Pederzani, Rocchi & Schizzerotto, 2004 from Sardinia; and the *H. longulus* complex plus the Corsican *H. regularis* Sharp, 1882. It is interesting to note that each of these lineages has an inland endemic, and that the species from Corsica and Sardinia (*H. regularis* and *H. sardomontanus*) are not sister species.

Within the *H. longulus* complex, and despite the clear morphological differences, the mitochondrial markers used here do not recover the species as monophyletic, probably as a result of their recent divergence (see below). The same happens with the *H. melanarius* complex.

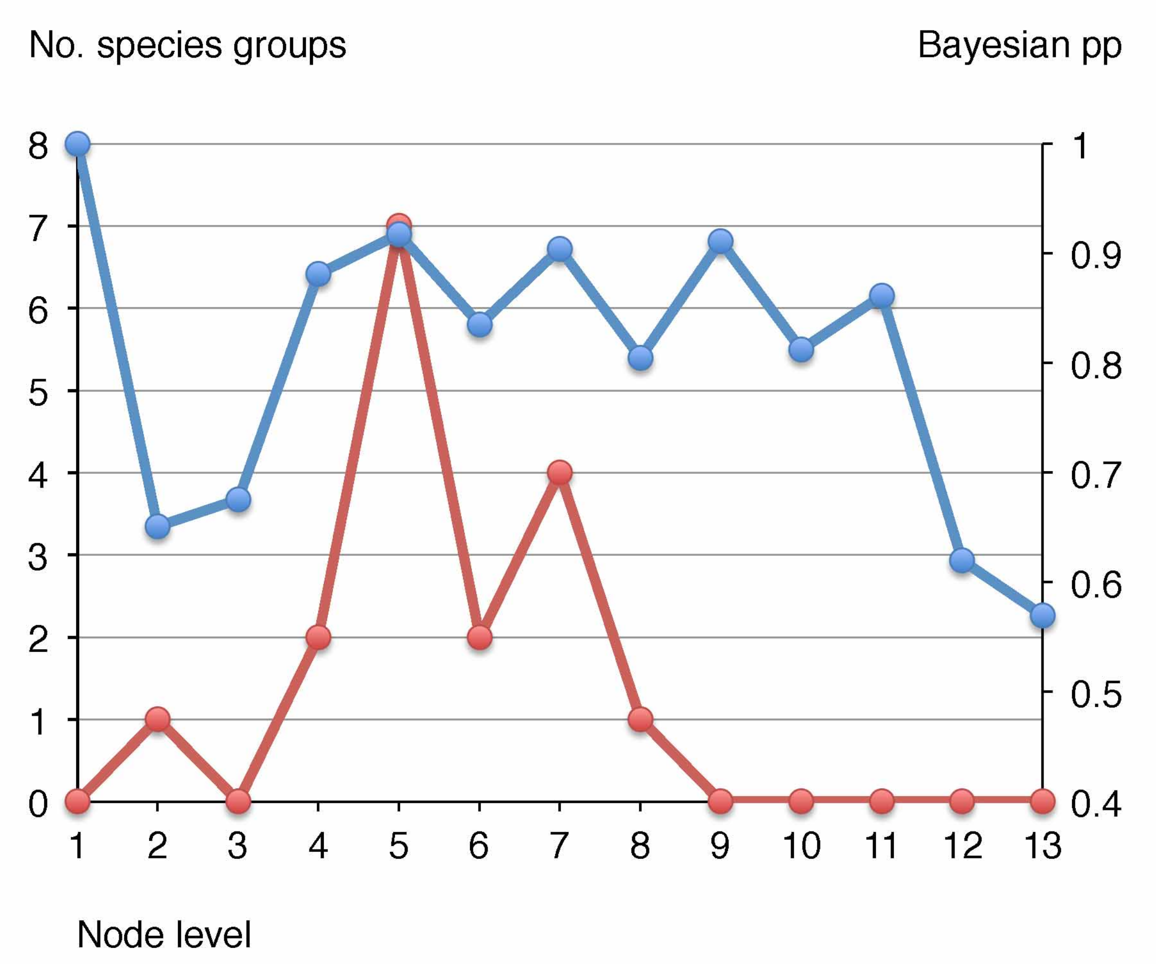

FIGURE 11. Average node support (Bayesian posterior probability) (blue line) and number of species groups defined (red line) in each node level, counting from the root of the genus *Hydroporus* in the tree of Fig. 10.

Estimation of divergence times

We restricted our analyses of divergence times to the clade of the *H. memnonius*, *H. longulus* and *H. ferrugineus* groups, for which the sampling was most complete. Using an evolutionary rate of 0.0115 substitutions/site/MY, the origin of the whole clade was estimated to be at late Miocene (Fig. 12), and that of the three species groups at Pliocene. As usual when using a single constraint for the estimation (in this case, the average evolutionary rate), the intervals of 95% confidence for the estimated ages are very large, so they have to be taken with caution. Even considering that our sampling is incomplete, with some species missing from the tree, all the speciation events were estimated to have occurred during the Pleistocene with the only possible exception of *H. longicornis*, which origin was estimated to be at early Pliocene (Fig. 12). The separation between *H. ferrugineus* and *H. bithynicus* **sp. n.** was estimated to be at middle Pleistocene.

Acknowledgements

We thank all the people listed in Table 1 for allowing us to study their material. Ana Izquierdo, David Molina and Rocío Alonso are thanked for their lab work. We also thank the comments of Hans Fery and Garth Foster on the taxonomy of *Hydroporus*, Michaela Brojer (NMW) for Fig. 1, and the comments of Jiři Hájek and two anonymous referees. Funding was through projects CGL2007-61665 and CGL2010-15755 to IR.

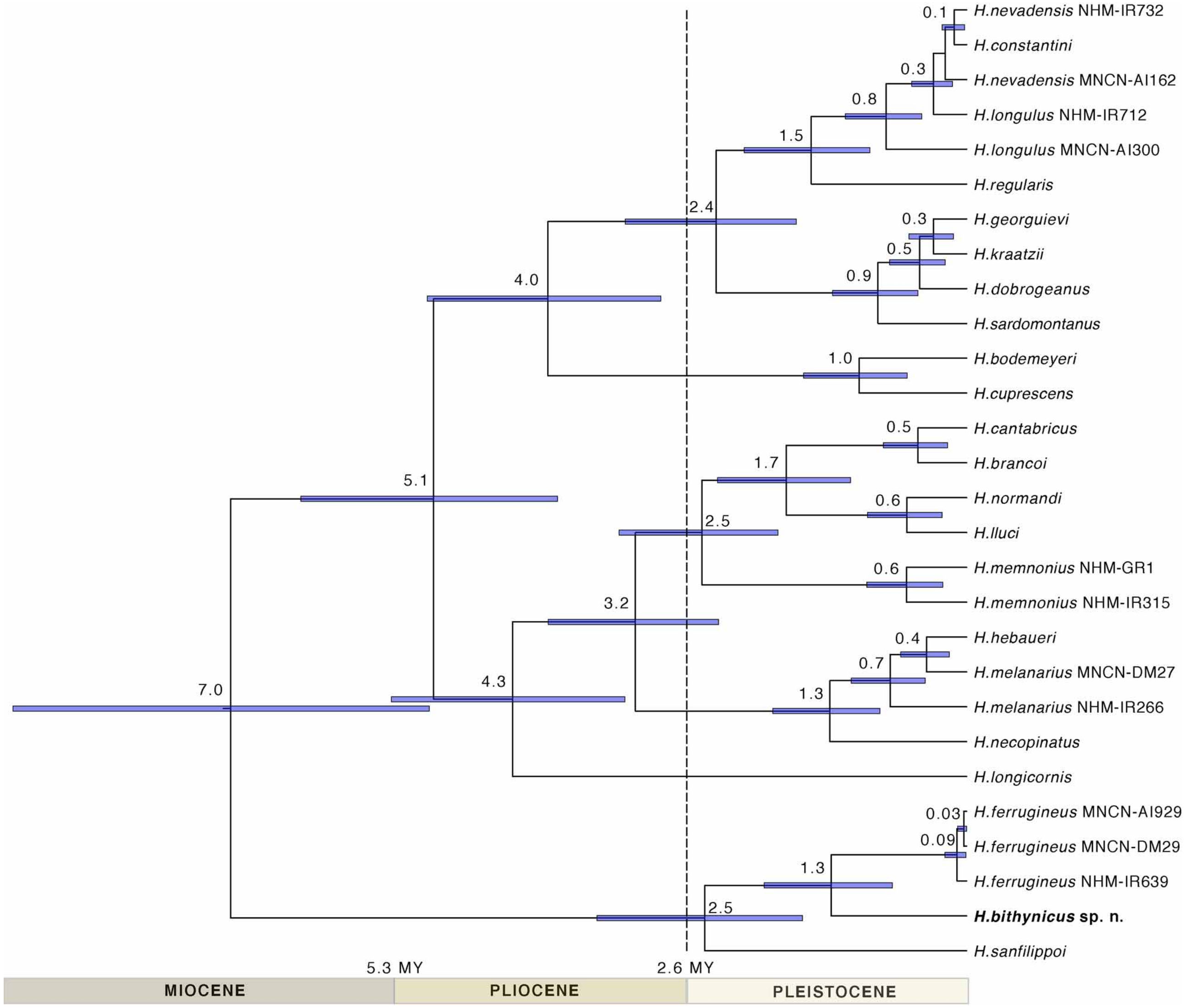

FIGURE 12. Ultrametric tree obtained with Beast and an evolutionary rate of 0.0115 substitutions/site/MY for the *H. memnonius*, *H. longulus* and *H. ferrugineus* groups. Numbers in nodes, estimated ages; blue bars, 95% confidence intervals.

References

Ahrens, D. & Ribera, I. (2009) Inferring speciation modes in a clade of Iberian chafers from rates of morphological evolution in different character systems. *BMC Evolutionary Biology,* 9, 234.

Angus, R.B. (1985) *Suphrodytes* des Gozis a valid genus, not a subgenus of *Hydroporus* Clairville (Coleoptera: Dytiscidae). *Entomologica Scandinavica,* 16, 269–275.

Brower, A.V.Z. (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial-DNA evolution. *Proceedings of the National Academy of Sciences of the United States of America,* 91, 6491–6495.

Drummond, A. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology, 7,* 214.

Fery, H. & Hendrich, L. (2011) *Hydroporus esseri* sp. n., a new diving beetle from southern Turkey (Coleoptera, Dytiscidae, Hydroporinae). *Zootaxa,* 2909, 38–46.

Fery, H. (1999) Revision of a part of the *memnonius*-group of *Hydroporus* Clairville, 1806 (Insecta: Coleoptera: Dytiscidae) with the description of nine new taxa, and notes on other species of the genus. *Annalen des Naturhistorischen Museums in Wien Serie B Botanik und Zoologie,* 101B, 217–269.

Fery, H. (2009) New species of the *Hydroporus longulus*-group from Iran, Armenia and Turkey with a synopsis of the group

(Coleoptera: Dytiscidae). *Acta Entomologica Musei Nationalis Pragae*, 49, 529–558.

Foster, G.N. & Friday, L. (2011) *Keys to adults of the water beetles of Britain and Ireland (Part 1).* Handbooks for the identification of British insects, Vol. 4 Part 5, 2nd ed. Shrewsbury, UK, 144 pp.

Franciscolo, M.E. (1979) *Coleoptera. Haliplidae, Hygrobiidae, Gyrinidae, Dytiscidae*. Fauna d'Italia, 14. Edicioni Calderini, Bologna, 804 pp.

Hájek, J. & Fikáček, M. (2010) Taxonomic revision of the *Hydroporus bodemeyeri* species complex (Coleoptera: Dytiscidae) with a geometric morphometric analysis of body shape within the group. *Journal of Natural History*, 44, 1631–1657.

Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics,* 17, 754–755.

Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics*, 9, 286–298.

Larson, D.J., Alarie, Y. & Roughley, R.E. (2000) *Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic Region, with emphasis on the fauna of Canada and Alaska*. NRC Research Press, Ottawa, 982 pp.

Nilsson, A.N. & Holmen, M. (1995) *Fauna Entomologica Scandinavica, vol. 32. The aquatic Adephaga (Coleoptera) of Fennoscandia and Denmark. II. Dytiscidae.* E. J. Brill, Leiden, 192 pp.

Nilsson, A.N. (2001) *World catalogue of insects, Vol. 3. Dytiscidae*. Apollo Books, Stenstrup, 395 pp.

Papadopoulou, A., Anastasiou, I. & Vogler, A.P. (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution,* 27, 1659–1672.

Ribera, I., Vogler, A.P. & Balke, M. (2008) Molecular phylogeny and diversification of diving beetles (Coleoptera, Dytiscidae). *Cladistics,* 24, 563–590.

Ribera, I., Beutel, R.G., Balke, M. & Vogler, A.P. (2002) Discovery of Aspidytidae, a new family of aquatic Coleoptera. *Proceedings of the Royal Society of London, Series B*, 269, 2351–2356.

Ribera, I., Bilton, D.T., Balke, M. & Hendrich, L. (2003) Evolution, mitochondrial DNA phylogeny and systematic position of the Macaronesian endemic *Hydrotarsus* Falkenström (Coleoptera: Dytiscidae). *Systematic Entomology*, 28, 493–508.

Ribera, I., Fresneda, J., Bucur, R., Izquierdo, A., Vogler, A.P., Salgado, J.M. & Cieslak, A. (2010) Ancient origin of a Western Mediterranean radiation of subterranean beetles. *BMC Evolutionary Biology,* 10, 29.

Zwickl, D. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. thesis, The University of Texas at Austin, USA.