



## Molecular and morphological analyses confirm two new species of the *Hydraena emarginata*–*saga* clade (Coleoptera, Hydraenidae) from Spain and France

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### Abstract

Using morphological and molecular analyses, the existence of two undescribed species, *H. diazi* from north–eastern Spain and French Pyrenees, and *H. fosterorum* from north–central Spain is confirmed. These species are members of a European endemic complex of hydraenid beetles, the *Hydraena emarginata*–*saga* clade, belonging to the "Haenydra" lineage. The two new species are described and the geographic range of the widespread *H. saga* is revised.

**Key words:** *Hydraena*, "Haenydra", new species, Western Europe, mtDNA

### Introduction

*Hydraena* Kugelann, with more than 850 species worldwide, is the most speciose genus within the family Hydraenidae and certainly one of the most speciose genera among aquatic Coleoptera. Within this genus, Jäch *et al.* (2000) recognized some monophyletic derived species groups, and defined them as "lineages". Among them, the "Haenydra" lineage (or *H. gracilis* species group) includes ca. 90 species distributed exclusively in the western Palaearctic from Ireland and Portugal to the Urals and Iran (Jäch 2004; Audisio *et al.* 2009).

The monophyly of the "Haenydra" lineage is supported, among other characters, by the absence of parameres in the aedeagus (Jäch *et al.* 2000) and by a combination of mitochondrial and nuclear genes (Ribera *et al.* in press). Within the lineage, several species clades can be identified on the basis of external morphology and aedeagal characters. One of these is the *Hydraena emarginata*–*saga* clade, (Figs 1–11, 13) which includes at least six described sibling species: *H. emarginata* Rey, from northern Spain and the French Pyrenees, *H. hispanica* Ganglbauer, from the Iberian Peninsula, *H. larissae* Jäch and Díaz and *H. tarvisina* Ferro from northern Italy, *H. alpicola* Pretner from the eastern Alps, and finally *H. saga* Orchymont thought to be widely distributed from Spain to the Ukraine. Other species, such as *H. samnitica* Fiori from central Italy, *H. pangaei* Jäch from north–eastern Greece, *H. pelops* Jäch from the Peloponnese, *H. dalmatina* Ganglbauer from the Balkans, and the widely distributed *H. belgica* Orchymont (Belgium to Romania) were thought to be less closely related to the *H. emarginata*–*saga* clade based solely on morphological data. However, molecular data (see below) suggest closer affinities within this cluster of species, despite their marked morphological diversification.

*Hydraena saga* was described by Orchymont (1930) from Germany, near Bonn. Preliminary molecular analyses confirmed that the Iberian/Pyrenean populations were in fact not directly related to the eastern populations of *H. saga* (Ribera *et al.* in press). Subsequent morphological examination of material of *Hydraena saga* s.l. from Austria, Bosnia, Czech Republic, France, Hungary, Montenegro, Poland, Romania, Serbia, Slovakia, Spain and Ukraine confirmed these differences and further revealed that the populations from Spain and France in fact represent two new species, which are described below (Figs 1–8). We also include a preliminary molecular phylogeny of the *H. emarginata*–*saga* clade to better understand the relationships among the studied species.

## Acronyms

BMNH	The Natural History Museum, London, UK.
CSR	Zoological Museum, Dipartimento di Biologia e Biotechnologie "C. Darwin", Sapienza Università di Roma, Italy.
IBE	Institut de Biologia Evolutiva (UPF-CSIC), DNA&Tissue collection, Barcelona, Spain.
JFL	Javier Fresneda Collection, Llesp, Spain.
MNCN	Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain.
NMW	Naturhistorisches Museum Wien, Austria.

## Material and methods

**Taxon sampling, DNA extraction and sequencing.** Molecular methods follow the procedure outlined in Ribera *et al.* (in press). Specimens were preserved in absolute ethanol in the field (Table 1). Extractions of single specimens were non-destructive, using a standard phenol-chloroform method or commercial column kits. Vouchers and DNA samples are kept in the collections of the MNCN, IBE and CSR. We included all specimens of the "*H. gracilis* lineage" studied in Ribera *et al.* (in press), plus additional specimens of the two new species, two specimens of *H. saga* from Austria (considered to be the true *H. saga*), and examples of *H. pangaei* and *H. samnitica* (Table 1). Trees were rooted in the species pair *H. sinope* and *H. septemlacuum*, according to the results of Ribera *et al.* (in press). We amplified fragments of four mitochondrial genes: 3' end of *cox1*; 3' end of *rrnL*; full *trnL* and 5' end of *nad1* (see Ribera *et al.* in press for primers and other details). Variation in the nuclear genes *SSU* and *LSU* among the species of the *H. gracilis* lineage was minimal and not informative, so these genes were not included. New sequences have been deposited in GenBank (NCBI) with Accession Numbers displayed in Table 1.

**Phylogenetic analyses.** Bayesian analyses were conducted on a combined data matrix with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001), which runs two independent, simultaneous analyses, using two partitions corresponding to the *cox1* and *rrnL+trnL+nad1* fragments with evolutionary models estimated with jModeltest (Posada 2008). MrBayes ran for 10<sup>6</sup> generations using default values, saving trees at each 500 generations. "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 used Maximum Likelihood as implemented in the on-line version of RAxML (which includes an estimation of bootstrap node support, Stamatakis *et al.* 2008), using GTR+G as the evolutionary model and the same gene partitions used in MrBayes.

**Material of *Hydraena saga* examined.** 1 male and 2 females Czech Rep. or Germany, Böhmerwald, Eisenstein (NMW); 1 male Upper Austria, Mühlviertel, Reichenau (NMW); 1 male, Poland, Srebrna Góra (NMW); 2 males Czech Rep., Kaplice (NMW); 2 males and 3 females Czech Rep., "Mährisch Weißkirchen" [Hranice] (NMW); 1 male Czech Rep., "Hof" [Dvorce] (NMW); 2 males Czech Rep., "Aussee" [Úsov] (NMW); 1 male and 5 females Czech Rep., Beskids (NMW); 2 females Lower Austria, Merkersdorf, Kajabach (NMW); 1 male Lower Austria, Stiefernbad (NMW); 3 males and 3 females Lower Austria, Retzbach (NMW); 6 males and 10 females Slovakia, Muran (NMW, CSR); 2 males Slovakia, Nizke Tatry Mountains, Poltica river (IBE, MNCN, DNA voucher numbers MNCN-AI358 and MNCN-AI485); 1 male and two females Hungary, Aggtelek N.P., Szögliget (NMW); 1 male Hungary, Bükk Mountains, Nagyvisnyó (NMW); 1 male Hungary, Gyertyanliget (NMW); 1 male and two females Hungary, Pálháza (NMW); 1 male Hungary, Körmöcbánya (NMW); 1 female Hungary, Szklénófürdő (NMW); 1 male Romania, Csík Mountains, Kászón (NMW); 1 male Bosnia, Patkovina (NMW); 1 female Bosnia, Prevrac (NMW), 2 males and 2 females Bosnia, Višegrad (CSR); 4 females Montenegro, Kolašin Pčinja (NMW); 1 male Romania, Máramaros (NMW); 1 male Romania, Transylvania, Caraş-Severin, Sasca Montana (NMW); 2 males and 2 females Romania, Sinaia, Cumpatu (NMW); 1 female Serbia, Kućište (NMW); 2 males Ukraine, Worochta (NMW).

### *Hydraena diazi* n. sp. Trizzino, Jäch & Ribera

(Figs 1–4)

**Type locality.** Spain, Barcelona province, Montseny, La Costa, Sot de Ritronyes, 620 m, N41°45'23.5" E2°24'49.8".

**Type material. Holotype** male (MNCN): “ES Barcelona, Montseny 10.9.2006 / La Costa de M.: Sot de Ritronyes / 620m N41°45'23.5" E 2°24'49.8" /Ribera, Hernando & Aguilera leg.”. **Paratypes** (58 specimens: CSR, IBE, MNCN, NMW): 6 males and 10 females same locality and data as holotype; one of the males used for DNA extraction and sequencing, voucher number MNCN-AI479. 2 males and 4 females Spain, Barcelona province, Montseny, Santa Fe del Montseny, road Turo del Home to Sot de Bernadal, 1134 m a.s.l., N41°45'45.2" E2°26'55.0", 23.7.2005, Ribera leg. 2 males Spain, Guipuzkoa province, Oiartzun, 240 m a.s.l., N43°16'10.2" W01°48'20.5", 16.7.2006, Ribera & Cieslak leg.; one used for DNA extraction and sequencing, voucher number MNCN-AI1011. 1 male and 1 female Spain, Girona province, Meranges, Fontanera, torrent de la Mata de l'Os, Ribera & Aguilera leg. 10.6.1995. 1 male Spain, Barcelona, Bagà, Riera de Gréixer, N38°12'52.4" E02°59'18.5", 1000 m, 22.6.1994, Fresneda leg. 5 males and 8 females Spain, Barcelona, Mosqueroles, Riera Ciuret, N41°43'50.5" E02°26'04.2", 440 m, 20.3.1989, Fresneda leg. 1 male and 4 females Spain, Barcelona, Mosqueroles, Riera Ciuret, N37°46'20.5" E03°21'34.8", 440 m, 23.3.1989, Fresneda leg. 1 male Spain, Vizcaya, Villaro, Río Arratia, N38°55'45.9 E03°47'35.9", 140 m, 5.6.1985, Rico leg. 1 male and 6 females Spain, Lleida, Salardú-Naut Aran, Montgarri, N38°35'49.2" E02°27'49.7", 1650 m, 15.10.1985, Fresneda leg. 1 female Spain, Lleida, Es Bordes, Artiga de Lin, N42°43'04.3" E0°43'26.3", 1012 m, 11.5.1986, Fresneda leg. 2 males and 2 females Spain, Lleida, Vielha-Mig Aran, Boca nord túnel de Vielha, N38°31'26.2" E02°20'41.7", 1510 m, 2.4.1989, Fresneda leg.

**Additional material.** 1 male France, Eastern Pyrenees, Les Angles. 1 male and 1 female France, Eastern Pyrenees, Larrau. 1 male France, Eastern Pyrenees, Suchon. 1 male France, Eastern Pyrenees, Gabas. 2 males Spain, Catalonia, Lleida.

**Description.** 2.2–2.4 mm long. Head, pronotum and elytra dark brown to black, distal third of elytra in some specimens dark reddish brown to paler reddish brown. Palpi and legs paler reddish brown.

Head: labrum with V-shaped notch anteriorly (but not as deep as in e.g. *Hydraena larissae*). Fronto-clypeal suture slightly arcuate, not strongly impressed. Middle of frons moderately densely punctate, lateral portion of frons very densely punctate, as in related species. Occipital ridge well developed. Eyes moderately large, maxillary palpi very long, about twice as long as distance between eyes.

Pronotum wider than long, moderately cordiform, anterior margin emarginate, anterior angles more or less rounded. Lateral margin slightly denticulate. Disc slightly more convex than in *Hydraena larissae*, rather densely punctate near anterior and posterior margins, along midline and around posterior foveae. Foveae hardly impressed.

Elytra elongate, parallel-sided, rather strongly declivous laterally. Nine rows of punctures between suture and shoulder. Strial punctures moderately large, slightly more deeply impressed than in *H. saga* and *H. larissae*. Explanate margin of elytra moderately wide, as in all members of the *H. emarginata*–*saga* complex.

Mentum and submentum microreticulate. Posterior genal ridge not particularly prominent, mostly glabrous. Prosternum with indistinct median keel. Angles between mesoventral disc and mesoventral process small as in e.g. *H. larissae* and *H. saga*. Metaventral plaques well developed, divergent posteriorly. Glabrous areas behind metacoxal sockets not very pronounced; abdominal ventrites III–VI more or less entirely covered with hydrofuge pubescence, abdominal ventrites VII and VIII largely glabrous, as in related species.

Aedeagus (Fig. 1): Length of main piece 572 µm (holotype), with four setae, three on left side and one very reduced (typical of members of the *H. emarginata*–*saga* group) on right side. Apex distinctly more rounded than in *H. saga* and *H. alpicola* (Figs 9, 10); a peculiar and clearly visible small V-shaped incision on pre-apical dorsal side of main piece. Distal half of main piece in right side view markedly more curved and wider (about 1.3 times) than in *H. saga*. Pre-basal tooth not very prominent, phallobase symmetrical in ventral view. Distal lobe as in *H. saga* and *H. alpicola*, seemingly without variability in size.

Gonocoxite and female tergite X as in Figs 2–3.

Secondary sexual characters: male elytral apices conjointly rounded, with very small sutural notch. Female elytral apices slightly conjointly rounded (Fig. 4), with a more or less large sutural notch. Female ventrites V and VI with fringe of long setae. Male ventrite VI enlarged. Male mesotibia with a row of ca. eight minute denticles along mesial face of posterior half. Male metatibia with fringe of long setae along mesial face of posterior half. Male tergite X as in *H. saga* and related species.

**Differential diagnosis.** *H. diazi* is clearly related to *H. saga*, *H. larissae*, *H. emarginata*, *H. alpicola*, and especially to the second new species described in this paper (see below; see also Fig. 12). The aedeagus of *H. diazi* (Fig. 1) could be easily distinguished from the other related species by the markedly curved and wide apical half of the main piece and by the peculiar distal apex of the main piece. The aedeagus of *H. emarginata* (Fig. 11) can be distinguished also by its distinctly larger size (PL: 610–665 µm), and by the distinctly larger distal lobe.

**Distribution.** *H. diazi* has a typical Pyrenean distribution, occurring in the southern (Spanish) and in the northern (French) side, and extending to some mountain massifs in Catalonia (Fig. 13). All Pyrenean specimens previously attributed to *H. saga* almost certainly belong to *H. diazi*.

**Etymology.** We are pleased to dedicate this species to our dear friend and colleague Juan Angel Díaz (University of Santiago de Compostela, Spain).

### *Hydraena fosterorum* n. sp. Trizzino, Jäch & Ribera

(Figs 5–8)

**Type locality.** Spain, Burgos province, Fresneda de la Sierra, Tirón river, 1085 m a.s.l., N42°16'46.2" W3°7'56.0".

**Type material.** **Holotype** male (MNCN): "ES Burgos 22.10.2004 / Fresneda de la Sierra: r. Tirón / N42°16'46.2" W3°7'56.0" / 1085m I.Ribera leg.". **Paratypes** (129 specimens: BMNH, CSR, IBE, MNCN, NMW): 8 males and 4 females same locality and data as holotype; one male used for DNA extraction and sequencing, voucher number MNCN-AI282. 4 males and 8 females Spain, La Rioja province, Logroño, Posadas, Río Oja, 1159 m, N42°12'36.0" W3°4'27.8" 23.10.04 Ribera leg.; one male used for DNA extraction and sequencing, voucher number MNCN-AI481. 8 males and 5 females Spain, La Rioja province, Logroño, Posadas, Río Oja, artificial pool, 1200 m, N42°12'36.0" W3°4'27.8" 23.10.04 Ribera leg. 33 males and 52 females Spain, Burgos, Fresneda de la Sierra Tirón, Río Tirón, N42°16'46.2" W3°7'56.0", 1100 m, 15.8.1994, Fresneda leg. 1 male Spain, Soria, Vinuesa, 2km Pto. Sta. Inés, 16.7.1991, Fery leg. 1 female Spain, Soria, Vinuesa, Laguna Negra, 13.6.1990, Fresneda leg. 3 males and 1 female Spain, Zaragoza, Moncayo, Fuente del Pedregal, 9.4.1993, Fresneda leg. 1 female Spain, Zaragoza, Moncayo, entre Veruela y Na. Sierra Del Moncayo, 9.4.1993, Fresneda leg.

**Description.** 2.15–2.30 mm long. Male habitus as in *Hydraena diazi*.

Aedeagus (Fig. 5): Length of main piece 555 µm (holotype), with four setae, three on left side and one very reduced (typical of members of the *H. emarginata*–*saga* complex) on right side. Apex distinctly more rounded than in related species (including *H. diazi*); pre-apical dorsal incision absent. Apical half of the main piece in right side view markedly more curved and wider (about 1.3 times) than in *H. saga* and *H. alpicola* (Figs 5, 9, 10). Pre-basal tooth not very prominent, phallobase symmetrical in ventral view. Distal lobe as in *H. saga* and *H. alpicola*, seemingly without variability in size.

Gonocoxite and female tergite X as in Figs 6–7.

Secondary sexual characters: male elytral apices conjointly rounded, with a small sutural notch. Distal third of female elytral apices very widely explanate (Fig. 8), conjointly rounded, with a very large sutural notch (resembling *H. samnitica* and *H. pangaei*). Female ventrites V and VI with fringe of long setae. Male ventrite VI enlarged. Male mesotibia with a row of ca. eight minute denticles along mesial face of posterior half. Male metatibia with fringe of long setae along mesial face of posterior half. Male tergite X as in *H. saga* and related species.

**Differential diagnosis.** *H. fosterorum* is clearly related to *H. diazi*, *H. saga*, *H. larissae*, *H. emarginata*, and *H. alpicola* (see also fig. 12). The aedeagus of *H. fosterorum* (Fig. 5) could be easily distinguished from the other related species by the markedly curved and markedly wide distal half of the main piece, and from *H. diazi* (Fig. 1) by the rounded apex of the main piece and by the absence of the small dorsal pre-distal V-shaped incision. The aedeagus of *H. emarginata* (Fig. 11) can be distinguished also by its distinctly larger size (PL: 610–665 µm), and by the distinctly larger distal lobe. Moreover, *H. fosterorum* could be easily distinguished from any other species of the *H. emarginata*–*saga* complex by the peculiar female elytral shape (Fig. 8).

**Distribution.** *H. fosterorum* is known only from the north of the Iberian System mountains, in Sierra de la Demanda and del Moncayo (Fig. 13). Specimens from that region previously attributed to *H. saga* (e.g. Valladares *et al.* 2000) almost certainly belong to the new species.

**Etymology.** We are pleased to dedicate this species to our friends Sue and Garth Foster, who accompanied IR when collecting some of the paratypes.

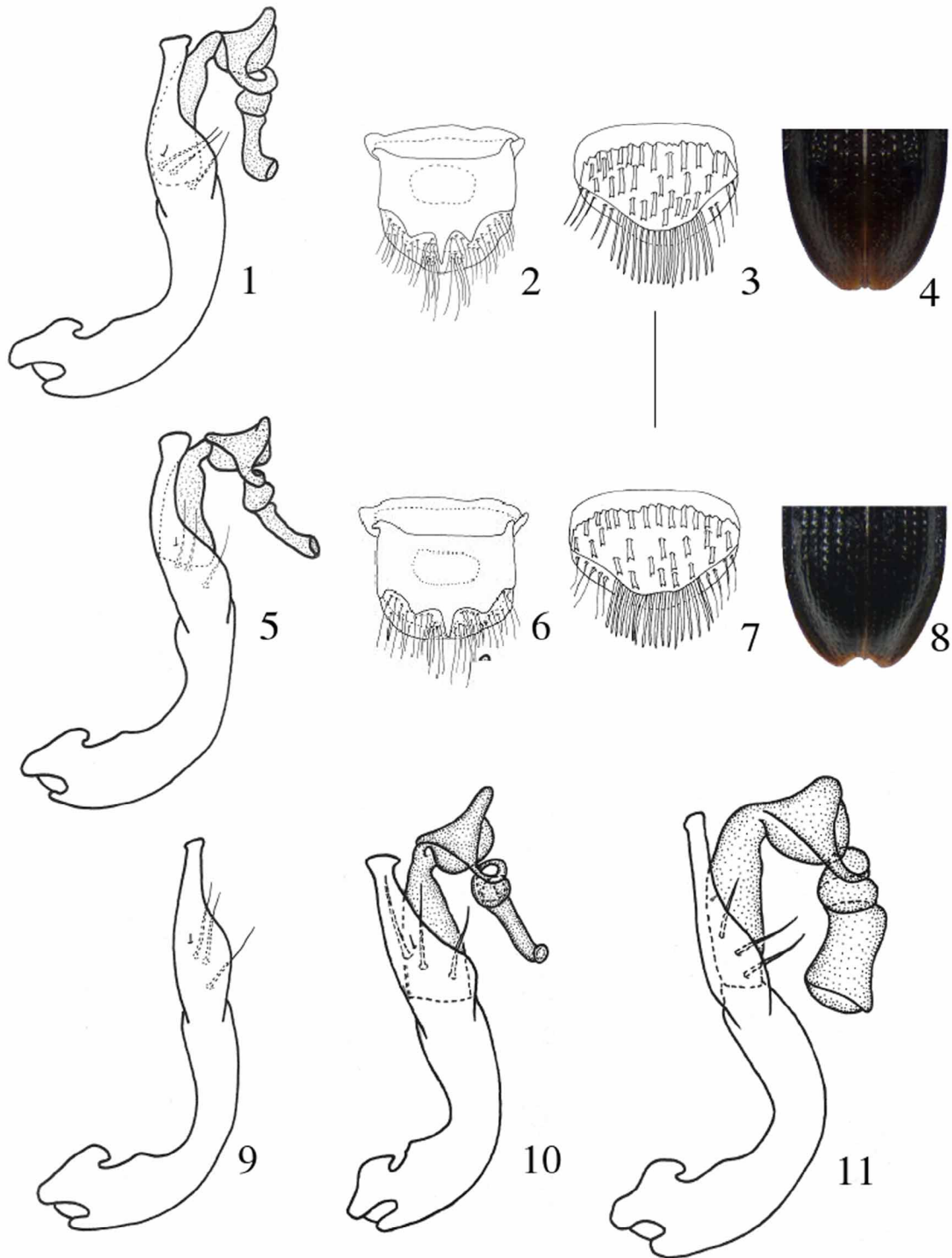
TABLE 1. Specimens used in the molecular study, with locality, collector, voucher reference numbers and accession numbers for the sequences.

Species	Voucher	Ref	Country	Locality	Date	Collector	cox1	rrnL+trnL+nad1
<i>H. alpicola</i>	MNCN-AI483	23829	Austria	Niederösterreich, Schwarzenbach, road 18 W Sankt Veit an der Gölsen, 448 m, N48°04'42.4", E15°40'42.9".	6.8.05	I. Ribera & A. Cieslak	FR717887	
<i>H. alpicola</i>	MNCN-AI347	23693	Austria	Niederösterreich, Schwarzenbach, road 18 W Sankt Veit an der Gölsen, 448 m, N48°04'42.4", E15°40'42.9".	6.8.05	I. Ribera & A. Cieslak	HM588354	HM588498
<i>H. anatolica</i>	MNCN-AI802	24148	Turkey	Kastamonu, Road 759 to Senpazar, Çocukören Çöyl, fast stream in mixed forest, 591 m, N41°49'38", E33°25'03".	26.4.06	I. Ribera	HM588395	HM588539
<i>H. belgica</i>	MNCN-AI426	23772	Austria	Niederösterreich, Schaubach, ca. 270 m, Schauboden, 3.5 km N Purgstall, N48°05'23", E15°08'20".	25.9.05	M.A. Jäch	HM588372	HM588516
<i>H. diazi</i>	MNCN-AI479	23825	Spain	Barcelona, Santa Fe del Montseny, road Turo del Home, Sot de Bernadal, 1134 m, N41°45'45.2", E2°26'55.0".	23.7.05	I. Ribera	HM588378	HM588522
<i>H. diazi</i>	MNCN-AI1011	24357	Spain	Guipuzkoa, Oiartzun, 240 m, Aiako Herria N.P., N43°16'10.2", W1°48'20.5".	16.7.06	I. Ribera & A. Cieslak	FR717888	
<i>H. emarginata</i>	MNCN-AI325	23671	Spain	Lugo, Os Ancares, road LU723, Serra do Agulleiro, Donis: Río da Vara, 1006 m, N42°49'31.2", W6°52'50.7".	10.7.05	I. Ribera & A. Cieslak	HM588346	HM588490
<i>H. exasperata</i>	MNCN-AI506	23852	Spain	León, Puerto de Panderrueda.	12.7.05	L.F. Valladares	HM588382	HM588526
<i>H. excisa</i>	MNCN-AI348	23694	Austria	Niederösterreich, Schwarzenbach, road 18 W Sankt Veit an der Gölsen, 448 m, N48°04'42.4", E15°40'42.9".	6.8.05	I. Ribera & A. Cieslak	HM588355	HM588499
<i>H. cf. excisa</i>	MNCN-AI391	23737	Bulgaria	Rila Mts., stream above Bacevo, 1500 m.	6.7.05	D.T. Bilton	HM588365	HM588509
<i>H. fosterorum</i>	MNCN-AI282	23628	Spain	Burgos, Fresneda de la Sierra, Río Tirón, 1085 m, N42°16'46.2", W3°07'56.0".	22.10.04	I. Ribera	FR717889	FR717818
<i>H. fosterorum</i>	MNCN-AI481	23827	Spain	Logroño, Posadas: Río Oja, 1159 m, N42°12'36.0", W3°4'27.8".	23.10.04	I. Ribera	HM588379	HM588523
<i>H. gracilis</i>	MNCN-AI332	23678	Latvia	Gaujas N.P., stream in forest.	13.6.05	D.T. Bilton	HM588348	HM588492
<i>H. gracilis</i>	MNCN-AI333	23679	England	Cumbria, Irthing river at Spadeadam Waste.	26.6.05	D.T. Bilton	HM588349	HM588493

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TABLE 1. (continued)

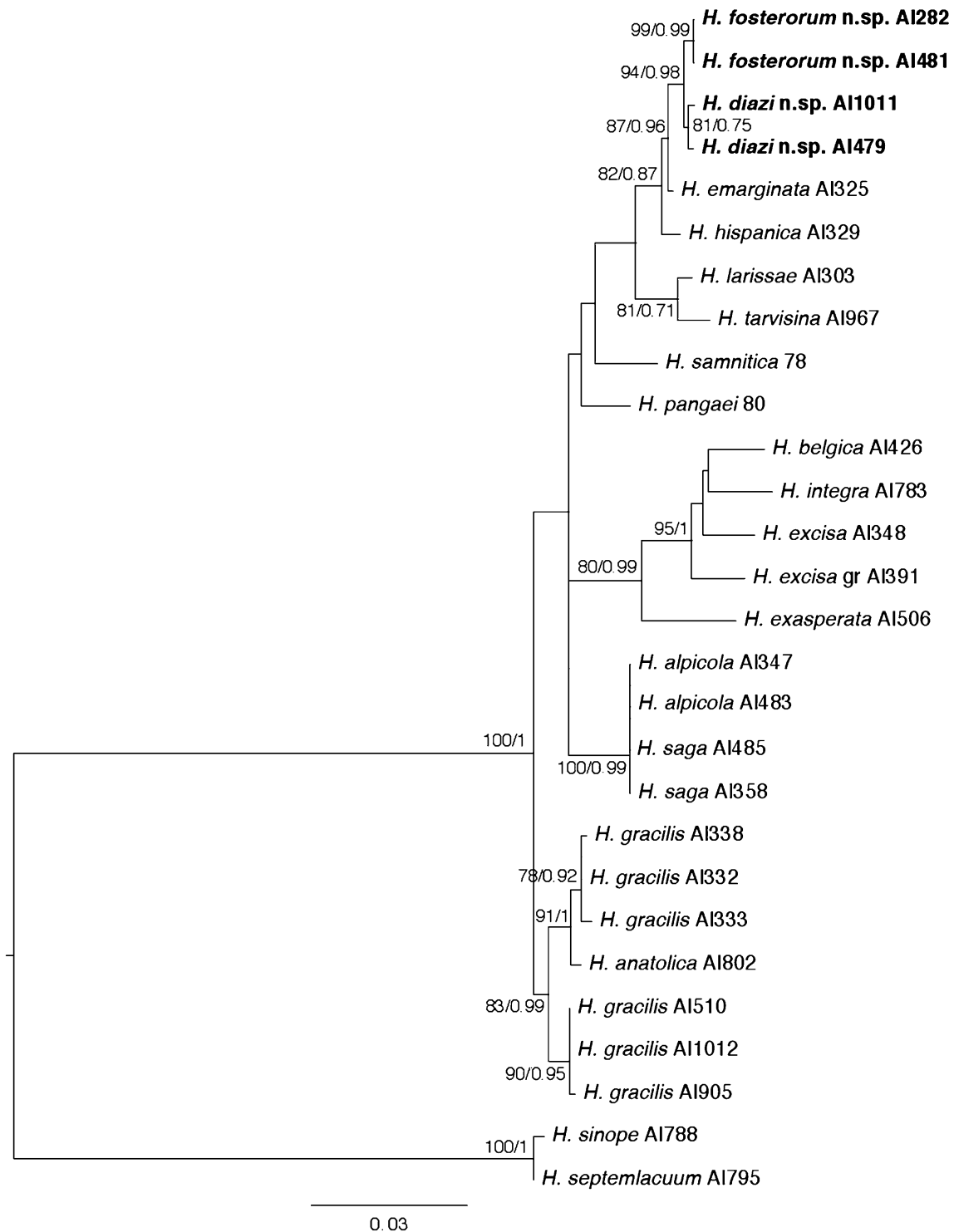
Species	Voucher	Ref	Country	Locality	Date	Collector	cox1	rrnL+trnL+nad1
<i>H. gracilis</i>	MNCN-AI338	23684	Bulgaria	Rhodope Mts., road Goce Delcev to Dospat, stream, 1000 m.	10.7.05	D.T. Bilton	HM588350	HM588494
<i>H. gracilis</i>	MNCN-AI510	23856	Spain	Cantabria, Beges.	13.7.05	L.F. Valladares	HM588384	HM588528
<i>H. gracilis</i>	MNCN-AI1012	24358	Spain	Guipuzkoa, Oiartzun, 240 m, Aiako Herria N.P., N43°16'10.2", W1°48'20.5".	16.7.06	I. Ribera & A. Cieslak	HM588311	
<i>H. gracilis</i>	MNCN-AI905	24251	Spain	León, Puerto de Panderrueda.	12.7.05	L.F. Valladares	HM588398	
<i>H. hispanica</i>	MNCN-AI329	23675	Spain	Ourense, Serra de Queixa, 2 km S Manzaneda, tributary of San Lázaro river, 770 m, N42°17'04", W7°13'21.2".	9.7.05	I. Ribera & A. Cieslak	HM588347	HM588491
<i>H. integra</i>	MNCN-AI783	24129	Turkey	Bolu, road 750 btw. Yeniçaga and Mengen, fast stream in mixed forest, 844 m, N40°50'49", E32°03'47.5".	24.4.06	I. Ribera	HM588392	HM588536
<i>H. larissae</i>	MNCN-AI303	23649	Italy	Lombardia, Brescia, val Trompia, road to Gioio della Bala, stream, ca. 1500 m.	17.10.02	I. Ribera & A. Cieslak	HM588340	HM588484
<i>H. pangaei</i>	CSR-80_1	H080_1	Greece	Kavala, Pangeon, road to Sky Center, deviation after 15 km, small spring, 994 m, N40°53'59.5", E24°08'01.9".	9.9.08	M. Trizzino & P. Audisio	HQ425789	HQ425835
<i>H. saga</i>	MNCN-AI358	23704	Slovakia	Nizke Tatry Mts., Politica river.	25.6.05	F. Ciampor	FR717891	
<i>H. saga</i>	MNCN-AI485	23831	Slovakia	Nizke Tatry Mts., Politica river.	25.6.05	F. Ciampor	FR717890	FR717819
<i>H. samnitica</i>	CSR-78_1	H078_1	Italy	Abruzzo, Teramo, Pietracamela, stream Arno, 1000 m.	24.7.08	M. De Pace	HQ425788	HQ425834
<i>H. septemlacuum</i>	MNCN-AI795	24141	Turkey	Bartın, road to Yeniköy from Kapisuyu, calcareous stream in mixed forest, 161 m, N41°47'08", E32°43'07".	25.4.06	I. Ribera	HM588394	HM588538
<i>H. sinope</i>	MNCN-AI788	24134	Turkey	Bartın, road to Topallar from Kakraz, calcareous stream in mixed forest, 25 m, N41°45'50.5", E32°29'11".	25.4.06	I. Ribera	HM588393	HM588537
<i>H. tarvisina</i>	MNCN-AI967	24313	Italy	Veneto, Treviso, Falzè di Piave, Riuo della Mina, riva Sant'Anna, 207 m, N45°51'01.5", E12°11'37.5".	10.6.06	G. Ferro	HM588401	HM588544



**FIGURE 1.** Aedeagus in right side view of *Hydraena diazi* (Spain, Guipuzkoa prov., Oiartzun). Scale bar = 0.2 mm.  
**FIGURE 2.** Female gonocoxite of *Hydraena diazi* (Spain, Barcelona prov., Santa Fe del Montseny). Scale bar = 0.2 mm.  
**FIGURE 3.** Female tergite X of *Hydraena diazi* (Spain, Barcelona prov., Santa Fe del Montseny). Scale bar = 0.2 mm.  
**FIGURE 4.** Female elytral apices (not scaled) of *Hydraena diazi* (Spain, Barcelona prov., Santa Fe del Montseny).  
**FIGURE 5.** Aedeagus in right side view of *Hydraena fosterorum* (Spain, Burgos prov., Fresneda de la Sierra). Scale bar = 0.2 mm.  
**FIGURE 6.** Female gonocoxite of *Hydraena fosterorum* (Spain, Burgos prov., Fresneda de la Sierra). Scale bar = 0.2 mm.  
**FIGURE 7.** Female tergite X of *Hydraena fosterorum* (Spain, Burgos prov., Fresneda de la Sierra). Scale bar = 0.2 mm.  
**FIGURE 8.** Female elytral apices (not scaled) of *Hydraena fosterorum* (Spain, Burgos prov., Fresneda de la Sierra).  
**FIGURE 9.** Aedeagus in right side view of *Hydraena saga* (Slovakia, Muran), distal lobe omitted. Scale bar = 0.2 mm.  
**FIGURE 10.** Aedeagus in right side view of *Hydraena alpicola* (Slovenia, Valdekom). Scale bar = 0.2 mm.  
**FIGURE 11.** Aedeagus in right side view of *Hydraena emarginata* (Spain, León, Puerto San Isidro). Scale bar = 0.2 mm.

## A molecular phylogeny of the *H. emarginata*–*saga* clade

The final alignment included 826 bp of the gen *cox1* and 724 bp of the ribosomal plus *nad1* fragment. The optimal evolutionary models as selected by jModeltest among those implemented in MrBayes were GTR+I+G for the *cox1* fragment and HKY+I for the *rrnL+trnL+nad1* fragment. There was no length variation in the protein coding genes, and in the ribosomal gene length variation was reduced to a single nucleotide deletion in the outgroups (*H. sinope* and *H. septemlacuum*), and thus the alignment was trivial.



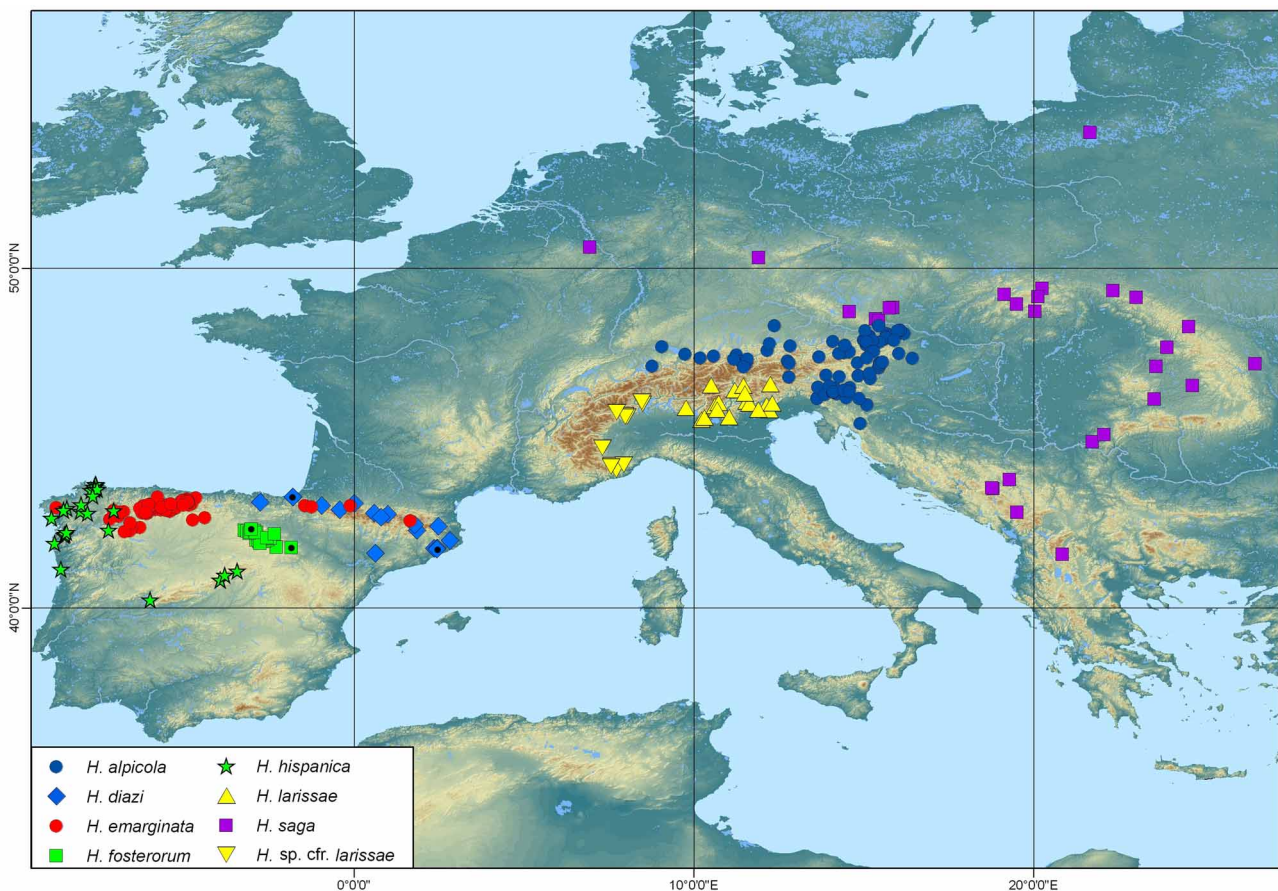
**FIGURE 12.** Phylogram of the species of the *H. gracilis* lineage obtained with RAxML. Numbers at nodes represent Bootstrap support values in RAxML / Bayesian posterior probabilities (only if > 70 % and 0.7 respectively).



The Bayesian runs converged at ca. 600,000 generations, with a standard deviation of the split frequencies around 0.01. The 1600 sampled trees provided reliable estimates of all parameters, as established by the effective sample size (measured in Tracer v1.5, Drummond & Rambaut 2007) and the convergent diagnostics in MrBayes (Huelsenbeck & Ronquist 2001). The topology (Fig. 12) of the well supported nodes (i.e. Bayesian posterior probability > 0.9, ML Bootstrap > 70 %) was identical for both methods used, with differences occurring only in the poorly supported nodes. The mitochondrial haplotypes of the two eastern specimens of *H. saga* were identical to those of *H. alpicola* from Austria, suggesting a very recent divergence between both species. The western *H. diazi* and *H. fosterorum* were included in a clade with two other Iberian endemics (*H. emarginata* and *H. hispanica*) with moderate support, but clearly not directly related to the eastern *H. saga* (Fig. 12). The position of *H. samnitica* and *H. pangaei*, not included in the analyses of Ribera *et al.* (in press), were not recovered with good support. Other results fully agree with those obtained by Ribera *et al.* (in press).

There was no variation between the two sequenced specimens of *H. fosterorum*, which were from nearby localities in Burgos and Logroño. Only two nucleotides (ca. 0.3 %) in the *cox1* differed between the specimens of *H. diazi* from the Montseny and Guipuzkoa, which represent opposite sides of the known distribution. The two species had identical haplotypes for the *rrnL+trnL+nad1* fragment, and differences in the *cox1* gene were ca. 0.5%. The estimated time of divergence between the two species, obtained with a Bayesian relaxed clock and a calibration using a combined (protein coding plus ribosomal) mitochondrial rate of 2% per MY, was 150,000 years, in the late Pleistocene (see Ribera *et al.* in press for details).

The low genetic divergence between the two newly described species, and their allopatric but geographically nearly contiguous distribution fully agree with the diversification model for some of the species of the "*Haenydra*" lineage suggested by Ribera *et al.* (in press). Thus, it seems likely that the current species of the *H. emarginata*–*saga* complex are the result of the fragmentation of a common ancestor that expanded its range through the north of the Iberian Peninsula ca. 0.5 MY ago, with the resulting species remaining in their original ranges through the speciation process.



**FIGURE 13.** Geographic distribution of members of the *H. emarginata*–*saga* clade. Sequenced populations of the two new species are marked with a black dot within the symbol.

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