



ORIGINAL
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The geography of speciation in narrow-range endemics of the 'Haenydra' lineage (Coleoptera, Hydraenidae, *Hydraena*)

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ABSTRACT

Aim We test whether species of western Mediterranean aquatic Coleoptera of the 'Haenydra' lineage (Hydraenidae, *Hydraena*) originated through: (1) successive periods of dispersal and speciation, (2) range fragmentation by random vicariance, or (3) range fragmentation by geographic isolation owing to a general reduction of population density.

Location Europe.

Methods To discriminate between scenarios we use contrasting predictions of the relationship between phylogenetic and geographic distance. The phylogeny was based on 3 kb of four mitochondrial and two nuclear gene fragments of about half of the known species of 'Haenydra', including most western Mediterranean taxa. Divergences were estimated using a molecular clock. The relationship between phylogenetic and geographic distance was tested using bivariate plots, Mantel tests and comparison of the observed phylogeny with the one minimizing geographic distances between species, as measured using Euclidean minimum spanning trees (EMSTs).

Results The monophyly of 'Haenydra' was strongly supported, although its phylogenetic placement was not resolved. 'Haenydra' was estimated to be of late Miocene age, with most species originating during the Pleistocene. In two clades (*Hydraena tatii* and *Hydraena emarginata* clades) there was a significant association between geographic and phylogenetic distance, and the reconstructed phylogeny was identical to that obtained through the EMST, demonstrating a strong non-randomness of the geographic distribution of the species. In two other clades (*Hydraena iberica* and *Hydraena bitruncata* clades) there was no association between geographic and phylogenetic distance, and the observed phylogeny was not the one minimizing geographic distances. In one of the clades this seems to be due to a secondary, recent range expansion of one species (*H. iberica*), which erased the geographic signal of their distributions.

Main conclusions We show that it is possible to obtain strong evidence of stasis of the geographic ranges of narrow-range endemic species through the study of their phylogenetic relationships and current distributions. In at least two of the studied clades, current species seem to have originated through the fragmentation of a more widely distributed species, without further range movements. A process of range expansion and fragmentation may have occurred repeatedly within the 'Haenydra' lineage, contributing to the accumulation of narrow-range endemics in Mediterranean Pleistocene refugia.

Keywords

Aquatic Coleoptera, Hydraenidae, Iberian Peninsula, narrow-range endemics, Pleistocene refugia, range expansion, speciation.

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INTRODUCTION

Since the early definitions of speciation modes as sympatric, allopatric or peripatric (Mayr, 1963) the fundamental role of geography has been recognized, and there have been many attempts to reconstruct the history of speciation through the distributions of current species (e.g. Lynch, 1989; Barraclough & Vogler, 2000; Fitzpatrick & Turelli, 2006). There is, however, a recognized weakness common to all these studies: species do change their geographic ranges, and it cannot be assumed that the current geographic range of a species is the same as that at the time of speciation, or that ranges are maintained through the cladogenetic process (Gaston, 2003). This has prompted many authors to conclude that evolutionary inferences concerning the geography of species in the past will often not be reliable (Chesser & Zink, 1994; Gaston, 1998; Losos & Glor, 2003). It would be equally wrong, however, to assume that all species have suffered modifications in their ranges large enough to erase any geographic signal from the past, as in some cases there is strong evidence to support the stasis of geographic ranges, either through the fossil record (e.g. Jablonski, 1987) or with indirect evidence from ecological and phylogenetic data (e.g. Carranza & Wade, 2004; Martínez-Solano *et al.*, 2004). Lineages with an abundance of narrowly distributed, mostly allopatric species are particularly difficult cases. The reduced range (often the result of specialized ecological requirements) and non-overlapping distribution, sometimes through several cladogenetic events (Fitzpatrick & Turelli, 2006), strongly suggest allopatric speciation, but one then has to ask whether the species originated, and have always persisted, where they are currently found.

A possible way to test the persistence of a geographic signal in the current distribution of a clade of species is through the comparison of observed phylogenetic and spatial relationships with a random null model (Barraclough & Nee, 2001). Using this approach, we test here three potential scenarios for the origin of several clades with mostly allopatric, narrowly distributed species in a genus of European water beetles.

In scenario 1, range expansion occurs through successive bouts of dispersal with subsequent speciation. This would be generally equivalent to stepping-stone colonization ('island hopping', MacArthur & Wilson, 1967), or, to some extent, to the progression rule of Hennig (1966). The starting situation is a small range to which new areas are added sequentially, to be eventually removed again owing to speciation. The resulting pattern will be a general positive relationship between phylogenetic and geographic distances, with more distant species having the oldest divergences. This relationship will be asymmetrical (triangular in a bivariate plot, Fig. 1a): while there could not be species that are geographically distant but phylogenetically close (unless there is long-range dispersal), there could be species that are geographically close but phylogenetically distant (e.g. species resulting from the initial, most ancient splits). The age of the species will generally increase towards the geographic origin of the range expansion [as postulated by Hennig's (1966) progression rule]. Typical

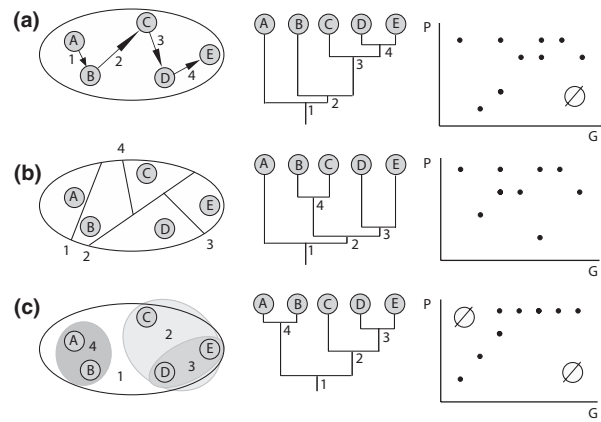


Figure 1 Schematic representation of the various hypothesized scenarios of speciation after a range expansion. The first column shows the geographic distribution of the species (A to E); the second column, the phylogenetic relationships among them; the third column, a bivariate plot of the 10 geographic linear distances (G) versus phylogenetic distances (P) (approximate values; note that some of the species pairs have identical values). (a) Speciation by stepping-stone colonization; (b) speciation by vicariance owing to the formation of random barriers to gene flow (represented by lines); (c) speciation by increased reduction of gene flow between favourable patches. The three scenarios result in different patterns in the relationship between phylogenetic and geographic distances: (a) triangular relationship, with no closely related species at large geographic distances; (b) no relationship; and (c) positive relationship, with neither closely related species at large geographic distances nor distant phylogenetic species at close geographic distances (see text for details). Numbers indicate the temporal sequence of the cladogenetic processes.

examples would be the colonization of archipelagos (Gillespie & Roderick, 2002; Keppel *et al.*, 2009), or of new available areas, for example by progressive deglaciation (Hewitt, 2000).

Alternatively, range expansion may occur with subsequent speciation owing to a fragmentation of the initial range. In this case the starting situation is the maximum range, which becomes fragmented and reduced with time, leading to speciation. Depending on the nature of the barriers fragmenting the initial range, one of two outcomes may occur, providing our second and third scenarios. In scenario 2, if the range is fragmented as a result of vicariance events that have a location independent of the distribution of the species, there should be no correlation between geographic and phylogenetic distances (Fig. 1b). Typical examples could be fragmentation of a range by an increase of sea level, or by tectonic fragmentation of microplates (e.g. Sanmartín, 2003). Under this scenario, species that are close geographically may have large phylogenetic divergences, and vice versa. In scenario 3, the range may be fragmented owing to a reduction of gene flow when there is a progressive and more or less uniform degradation of the general conditions that allowed the initial range expansion. This would be equivalent to the refuge speciation of Moritz *et al.* (2000) or to the vicariance by niche conservatism of Wiens (2004), when the barriers resulting

from suboptimal conditions are not established independently of the spatial distribution of the species (as in the previous scenario) but occur through the lines of minimum gene flow, which would correspond to the largest area with the lowest population density (Fig. 1c). In this case, the general relationship between geographic and phylogenetic distances should increase monotonically. The age of the species will generally increase with respect to the centroid of the distribution of the clade. Under a perfect scenario, the match between geographic and phylogenetic distances should be optimal; that is, the topology of the observed phylogeny should be the one that minimizes the geographic distances between species.

We test these three scenarios using a lineage of aquatic beetles with an abundance of narrowly distributed allopatric species, the 'Haenydra' lineage of the genus *Hydraena*, family Hydraenidae (Hansen, 1998). Our specific aim is to ascertain whether the south-western European species of the group can be said to have persisted in the areas in which they are currently found since their origin; that is, whether they are local endemics or have suffered range shifts (e.g. as consequence of glacial cycles) large enough to have erased the phylogenetic signal of their original distribution. The three possibilities outlined above are model situations that differ in the resulting relationship between geographic and phylogenetic distances, although the power of the conclusions depends on the observed pattern: if a strict positive relationship is found (according to the third scenario), this can be taken as compelling evidence of a non-random geographic arrangement of the species. However, the existence of deviations from this strict proportionality (either partially, as in Fig. 1a, or completely, as in Fig. 1b) could indicate either a compliance with the predictions derived from the initial hypotheses or the breakdown of the expected pattern owing to subsequent changes in the range of some of the species.

MATERIALS AND METHODS

Background on the taxonomy of the group

The genus *Hydraena* Kugelann, with *c.* 850 known species and many more to be described, is the most diverse of the family Hydraenidae, and probably the most diverse among the aquatic Coleoptera (Jäch & Balke, 2008; Perkins, in press). Adults of most species of *Hydraena* are typically aquatic, living in the riparian area of small streams and rivers. Many authors have recognized groups of apparently closely related species based on external morphology, which have in some cases been named as subgenera, or, more recently, as informal species groups (Jäch *et al.*, 2000). One of these traditionally recognized groups is *Haenydra*, described by Rey (1886) as a separate genus, and considered by different authors as a genus (e.g. Ieniştea, 1968; Rocchi, 2009) or a subgenus (e.g. Berthélemy, 1986; Perkins, 1997; Hansen, 1998). In a cladistic analysis of *Hydraena sensu lato*, Jäch *et al.* (2000), even acknowledging their likely monophyly, synonymized *Haenydra* (together with

all previously defined subgenera with the exception of *Hydraenopsis* Janssens), as they would render *Hydraena sensu stricto* paraphyletic, and considered it only as an informal species group (the *Hydraena gracilis* group).

Currently there are 86 recognized species of the 'Haenydra' lineage (Hansen, 1998; Jäch, 2004; Audisio *et al.*, 2009), usually found in clean, fast-flowing waters, often in mountain streams. They share the absence of parameres in the aedeagus and a similar external morphology, typically narrow and elongated (Fig. 2). They are distributed in Europe and the Middle East, from Iberia to Iran (Hansen, 1998; Jäch, 2004). Many species of this lineage have very restricted distributions, often limited to a single valley or mountain system, but there are also some species with very wide geographic ranges, for example *Hydraena gracilis* is present throughout Europe from north Iberia to the Urals (Jäch, 2004).

Taxon sampling

We undertook a comprehensive sampling of the western European species of 'Haenydra', including all species occurring in the Iberian Peninsula, plus a representation of species from other Mediterranean areas (see Appendix S1 in the Supporting Information). Missing species in some of the species groups could be tentatively placed according to the external morphology and the characteristics of the male genitalia. In total, we included examples of 39 named species of 'Haenydra'.

The monophyly of *Hydraena* is generally accepted (Perkins, 1989; Jäch *et al.*, 2000; Beutel *et al.*, 2003), but there is no agreement on the internal phylogeny and, in particular, on the phylogenetic position of the 'Haenydra' lineage. We sampled as outgroups a comprehensive selection of species of *Hydraena*, including most species groups as defined from morphology (Jäch *et al.*, 2000) (Appendix S1). Trees were rooted in *Adelphydraena*, hypothesized to be the sister group of *Hydraena* (Perkins, 1989).

The taxonomy and nomenclature of Jäch (2004) is followed for the species of 'Haenydra', except for *H. saga*. *Hydraena saga* as currently recognized has a disjunct distribution, in the Pyrenean region in the west, and from the eastern Alps to Bulgaria in the east (Jäch, 2004; Fig. 3). Preliminary molecular and morphological data strongly suggest that the populations in these two areas are not closely related (unpublished observations), and we consider here only the Pyrenean populations as the 'H. saga complex'.

DNA extraction and sequencing

Specimens were collected alive in the field and directly killed and preserved in 96% ethanol. DNA was extracted from whole specimens by a standard phenol-chloroform extraction or using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). Vouchers and DNA samples are kept in the collections of the Museo Nacional de Ciencias Naturales (MNCN, Madrid) and the Institute of Evolutionary Biology (IBE, Barcelona) (Appendix S1). DNA extraction was

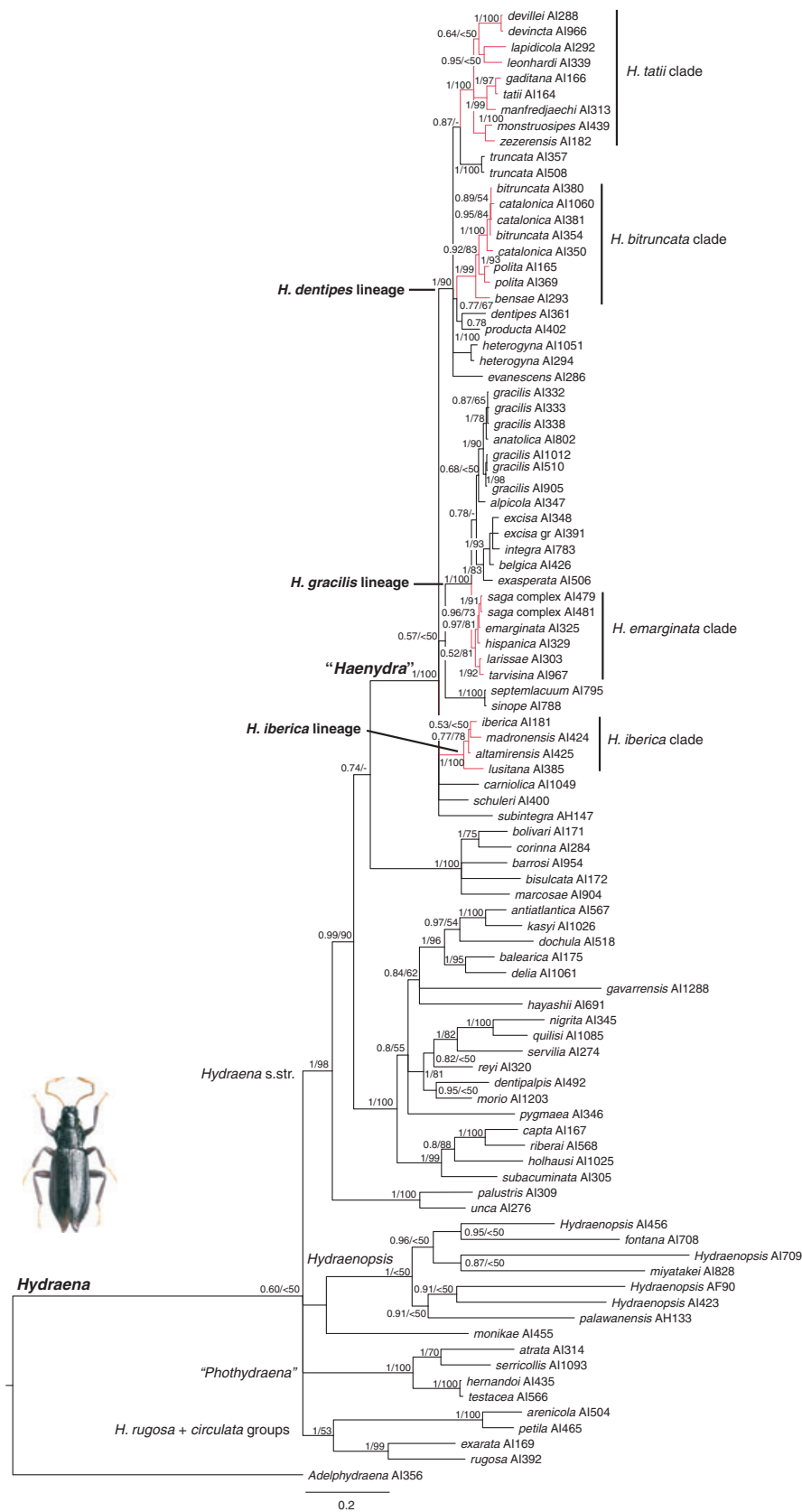


Figure 2 Phylogram of the species of *Hydraena* obtained in MRBAYES. Numbers at nodes denote Bayesian posterior probability/bootstrap support in RAXML; ‘-’ marks nodes not present in the RAXML analyses; vertical bars denote the four clades used in the geographic analyses. Habitus: *H. catalonica*. See Appendix S1 for the codes of the species.

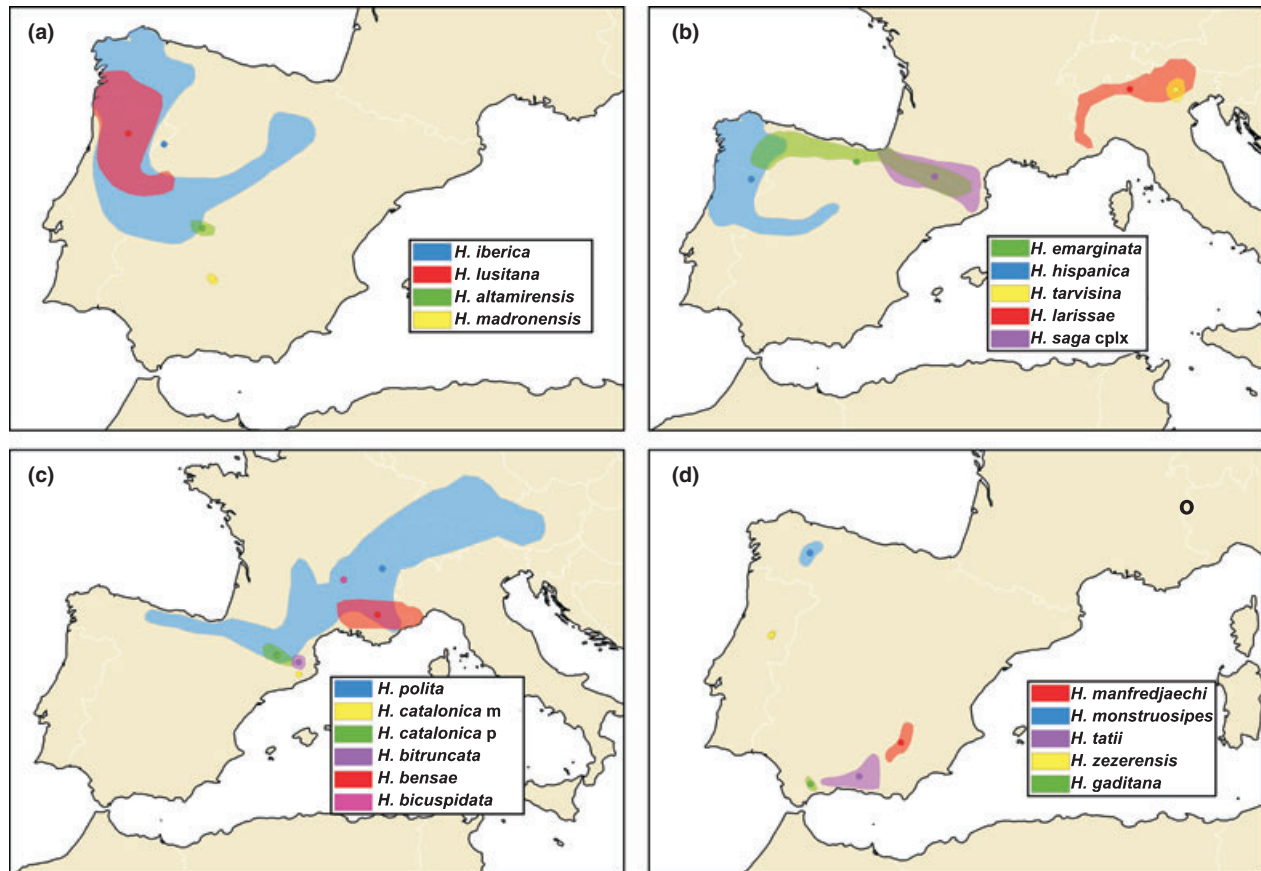


Figure 3 Maps with the distribution of the species of *Hydraena* of the ‘*Haenydra*’ lineage included in the clades used in the geographic analyses, with their centroids (see Table 1 for the coordinates). (a) *Hydraena iberica* clade; (b) *H. emarginata* clade; (c) *H. bitruncata* clade; (d) *H. tatii* clade (in the Alps Maritimes, minimum distance from the species of the Alpine group).

non-destructive, in order to preserve voucher specimens for subsequent morphometric and morphological study. Typically, only males were sequenced, and the male genitalia (used for the identification of the species) were dissected and mounted prior to the extraction to ensure a correct identification.

We sequenced four fragments, namely two mitochondrial fragments including four genes (the 3′ end of cytochrome *c* oxidase subunit 1, COI; and the 3′ end of the large ribosomal unit plus the leucine transfer plus the 5′ end of NADH dehydrogenase subunit 1, *rrnL+trnL+nad1*) and two nuclear fragments (small ribosomal unit, SSU; large ribosomal unit, LSU) (see Appendix S2 for the primers used). For each fragment both forward and reverse sequences were obtained. In some specimens the COI fragment was amplified using internal primers to obtain two fragments of around 400 bp each (Appendix S2). Sequences were assembled and edited with SEQUENCHER 4.7 (Gene Codes, Inc., Ann Arbor, MI). New sequences have been deposited in GenBank with accession numbers HM588308–HM588600 (Appendix S1). Protein-coding genes were not length-variable, and the ribosomal genes were aligned with the online version of MAFFT 6 using the G-INS-i algorithm and default parameters (Kato & Toh, 2008).

Phylogenetic analyses

Bayesian analyses were conducted on a combined data matrix with MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001), using five partitions corresponding to the sequenced genes (the *rrnL+trnL* fragment was considered a single partition) and a GTR+I+G model independently estimated for each partition. MRBAYES was run for 15×10^6 generations using default values, saving trees after every 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 five gene partitions as used in MRBAYES.

Estimation of divergence times

To estimate the relative age of divergence of the lineages we used the Bayesian relaxed phylogenetic approach implemented in BEAST 1.4.7 (Drummond & Rambaut, 2007), which allows

variation in substitution rates among branches. We implemented a GTR+I+G model of DNA substitution with four rate categories, using the mitochondrial data set only and pruning specimens with missing gene fragments. We used an uncorrelated lognormal relaxed molecular clock model to estimate substitution rates and the Yule process of speciation as the tree prior. Well-supported nodes in the analyses of the combined sequence (mitochondrial and nuclear) were constrained to ensure that the BEAST analyses obtained the same topology. We ran two independent analyses for each group, sampling every 1000 generations, and used TRACER 1.4 to determine convergence, measure the effective sample size of each parameter and calculate the mean and 95% highest posterior density interval for divergence times. The results of the two runs were combined with LOGCOMBINER 1.4.7, and the consensus tree was compiled with TREEANNOTATOR 1.4.7 (Drummond & Rambaut, 2007).

The analyses were run for 30×10^6 generations, with the initial 10% discarded as burn-in. Because of the absence of a fossil record with which to calibrate the trees we used as a prior a rate of 2.0% of pairwise divergence per million years (Myr^{-1}), established for subterranean species of a closely related family (Leiodidae) for a combination of mitochondrial markers (including those used here) and using as a calibration point the tectonic separation of the Sardinian microplate (Ribera *et al.*, 2010a). We set as a prior rate a normal distribution with an average rate of 0.01 substitutions $\text{site}^{-1} \text{Myr}^{-1}$, with a standard deviation of 0.001.

Geographic analyses

Contour maps of the distributions of the species of the 'Haenydra' lineage included in the studied clades were compiled from published and unpublished sources (Jäch, 2004; Sánchez-Fernández *et al.*, 2008; Checklist of the species of the Italian fauna, v. 2.0, <http://www.faunaitalia.it>) (Fig. 3). Species' range centroids (centre of mass of the polygon representing the distribution of a species) and distances between centroids were calculated using ARCGIS 9.2 (Environmental Systems Research Institute Inc., Redlands, CA, USA) (Table 1). To check the association between phylogenetic and geographic distances between the centroids we used the following three approaches.

1. Bivariate plots of the linear distance between the centroids of the species ranges and the branch lengths of the ultrametric trees, which is the estimated age of divergence between species (i.e. their phylogenetic distance) (Fig. 1).
2. Mantel tests for the general association between the matrices of geographic and phylogenetic distances. Multiple Mantel tests were performed using zt 1.1 (Bonnet & Van de Peer, 2002), with 10,000 randomizations to generate a null distribution and assess the significance of the results.
3. An optimization procedure to assess the match between the observed geographic distribution and the topology obtained from the phylogeny. We first compute the Euclidean minimum spanning tree (EMST), that is, the minimum spanning tree of a set of n points in the plane (the centroids of the distributions),

Table 1 Centroids of the distributions of the species of *Hydraena* in the clades used for the geographic analyses (in decimal coordinates). See Fig. 3 for the distributions of the species.

Clade	Species	X	Y
<i>H. iberica</i>	<i>H. altamirensis</i>	-4.868	39.504
	<i>H. iberica</i>	-6.474	41.221
	<i>H. lusitana</i>	-7.614	41.277
	<i>H. madronensis</i>	-4.316	38.391
<i>H. emarginata</i>	<i>H. emarginata</i>	-2.746	42.894
	<i>H. hispanica</i>	-7.157	41.588
	<i>H. larissae</i>	9.395	46.041
	<i>H. saga complex</i>	-0.015	42.630
	<i>H. tarvisina</i>	11.588	46.003
<i>H. bitruncata</i>	<i>H. bensae</i>	6.000	44.175
	<i>H. bicuspidata</i>	4.430	45.350
	<i>H. bitruncata</i>	2.314	42.253
	<i>H. catalonica p</i>	1.262	42.415
	<i>H. catalonica m</i>	2.384	41.815
	<i>H. polita</i>	6.071	45.869
<i>H. tatii</i>	<i>H. gaditana</i>	-5.293	36.679
	<i>H. manfredjaechi</i>	-2.541	38.228
	<i>H. monstrosipes</i>	-6.858	42.771
	<i>H. tatii</i>	-3.757	37.132
	<i>H. zezerensis</i>	-7.584	40.385

where the weight of the edge between each pair of points is the linear distance between those two points. The EMST connecting n vertices will have $n(n-1)/2$ edges, which are computed through a standard minimum spanning tree algorithm (see e.g. Graham & Hell, 1985), a trivial task for graphs of fewer than six nodes. The result is a graph connecting all points minimizing the weight of the edges, that is, the distances among centroids. In the scenario outlined in Fig. 1c, the temporal sequence of cladogenetic events will be determined by the length of the edges connecting the centroids: the first split will be between the taxa at either extreme of the longest edge, the second will be between taxa at either end of the second longest, and so on until the two closest species are separated.

To assess the probability that the observed topology could be identical to that obtained with this optimization procedure, we obtained all possible unrooted bifurcating topologies of each of the studied clades in PAUP* 2 (Swofford, 2002), and considered them as a null distribution against which the probability of each individual topology was estimated. Note that the use of the EMST determines not only the final topology but also the relative order of all the cladogenetic events. We did not consider the relative order in the cases in which the cladogenesis occurs in two different branches of the tree, as this does not affect the final topology.

RESULTS

Phylogeny of *Hydraena*

The final matrix included 94 taxa and 2831 aligned characters. Part of the *rrnL+trnL+nad1* fragment was missing for two

species (Appendix S1), and for two of the repeated specimens of *H. gracilis* only COI was sequenced. The nuclear markers (SSU and LSU) were sequenced only for a representation of the species of 'Haenydra' owing to the general low variability within this lineage, with many identical sequences between closely related species (Appendix S1).

The runs of MRBAYES converged to split frequencies lower than 0.01 at 11×10^6 generations, leaving a total of $4 \times 2 \times 10^6$ generations for the sampling of the tree space (= 16,000 trees). The monophyly of *Hydraena* and the basal relationships among its major clades were not well supported (Fig. 2). There are five well-supported lineages within the genus *Hydraena*: (1) the subgenus *Hydraenopsis* (as defined in Jäch *et al.*, 2000); (2) the South African *H. monikae*; (3) the 'Phothydraena' lineage (*H. testacea* species group in Jäch *et al.*, 2000); (4) the *H. rugosa* and *H. circulata* species groups, sisters with good support in the analysis with Bayesian probabilities although not in the maximum likelihood analysis; and (5) the main lineage within *Hydraena sensu stricto* (including the 'Haenydra' lineage), which was well supported in both analyses (Fig. 2; see Appendix S1 for the composition of the species groups).

Within the main lineage of *Hydraena sensu stricto*, the *H. palustris* group was sister to the rest (in agreement with Jäch *et al.*, 2000), which were in turn divided into three well-supported main clades: (1) *H. bisulcata* and its allies; (2) a clade broadly including the *H. riparia*, *H. minutissima*, *H. rufipes* and *H. holdhausi* groups; and (3) the 'Haenydra' lineage (Fig. 2). The relationship between these three main clades was not well resolved, with MRBAYES favouring a sister relationship between 'Haenydra' and the *H. bisulcata* group, and RAxML favouring a sister relationship with the *H. riparia* group (*sensu lato*), albeit in both cases with low support. In all cases the monophyly of the 'Haenydra' lineage was strongly supported (Bayesian posterior probability, BPP = 1.0; maximum likelihood bootstrap, MLb = 100%; Fig. 2).

Phylogeny of the 'Haenydra' lineage

There were three well-supported main lineages within 'Haenydra', namely the *Hydraena iberica*, *H. gracilis* and *H. dentipes* lineages (Fig. 2). There were also three species with an isolated position, namely *Hydraena carniolica*, *H. schuleri* and *H. subintegra*.

The *H. iberica* lineage included four Iberian endemics (Fig. 3a). The *H. gracilis* lineage included the *H. emarginata* clade, with the Iberian endemics *H. saga* complex, *H. emarginata* and *H. hispanica* as sister to two species from the Alps (Figs 2 & 3).

The third main group within 'Haenydra', the *H. dentipes* lineage, included two clades with narrow-range western Mediterranean endemics. The first, the *Hydraena bitruncata* clade, included *H. catalonica*, *H. bitruncata*, *H. polita* and *H. bensae* (Figs 2 & 3c). The first two species have narrow distributions in the north-east of the Iberian Peninsula and southern France, *H. polita* has a widespread distribution from north Iberia to the eastern Alps, and *H. bensae* is endemic to the Alpes Maritimes

(Fig. 3c). The sister of this clade was not well established. All of these species lack a small flagellum in the apical part of the median lobe of the aedeagus, which is present in the rest of the species of the *H. polita* group (*H. dentipes*, *H. producta* and *H. heterogyna* among those included in the study). *Hydraena bicuspidata*, from the Massif Central in south-east France (close to Lyon, Ganglbauer, 1901), should also be included in this clade, as it lacks the flagellum and has a very similar body shape.

The second clade within the *H. dentipes* lineage was the *H. tatii* clade, including five Iberian narrow-range endemics, *H. tatii*, *H. manfredjaechi*, *H. gaditana*, *H. zezerensis* and *H. monstrosipes*, as sister to some species in the Alps and Italy, namely *H. devincta*, *H. devillei*, *H. leonhardi* and *H. lapidicola* (Figs 2 & 3d). The sister relationship between the species *H. tatii*, *H. manfredjaechi* and *H. gaditana* (i.e. the 'H. tatii group' *sensu* Fresneda *et al.*, 1994) with *H. zezerensis* plus *H. monstrosipes* was not well supported, although the node was present in all analyses (maximum likelihood and Bayesian, both with the full combined sequence and with the mitochondrial data only). The sister group of the *H. tatii* clade was *H. truncata* (although with low support, Fig. 2), which has a widespread European distribution that includes the north-west of the Iberian Peninsula. There are three likely missing species in this clade: *H. sanfilippo*, close to *H. lapidicola* (Audisio & De Biase, 1995); *H. bosnica*, close to *H. leonhardi* (Audisio *et al.*, 1996); and *H. hungarica*, also related to *H. bosnica* and *H. leonhardi* (the three share with other species of the group the female gonocoxite with two small symmetric depressions). *Hydraena sappho* Janssens, from the small island of Levkás (Greece), has been associated with the *H. tatii* clade (Audisio *et al.*, 1996). A closer examination of the only known specimen (the holotype, Janssens, 1965) showed that it is most likely to be related to species from the eastern Mediterranean, not to the Iberian species (M.A. Jäch, unpublished observation).

Two of the species of 'Haenydra' were found to be paraphyletic: in the *H. gracilis* complex (*sensu* Jäch, 1995), the north Iberian populations were sister to specimens sampled from sites from Britain to Turkey, including *H. anatolica* (Jäch, 1995); and specimens of *H. catalonica* from the Montseny Massif (central Catalonia) were sister to the Pyrenean *H. catalonica* plus *H. bitruncata*. For the geographic analyses, *H. catalonica* was split into its two geographic lineages, the populations from the Pyrenees ('*H. catalonica* p') and the populations from the Montseny Massif ('*H. catalonica* m').

Estimation of divergence times

From the results of the BEAST runs, and using a calibration of 0.01 substitutions site⁻¹ Myr⁻¹, the origin of the 'Haenydra' lineage was estimated to be c. 8.5 Ma (late Miocene), with a wide confidence interval (Fig. 4). The three main lineages (*H. iberica*, *H. gracilis* and *H. dentipes* lineages) originated c. 6 Ma, and most species, including all Iberian endemics, were estimated to be less than 2.6 Myr old, that is, of Pleistocene origin (Fig. 4). There were relatively deep divergences within some of the species with wider distributions, such as *H. polita* (0.8 Myr between

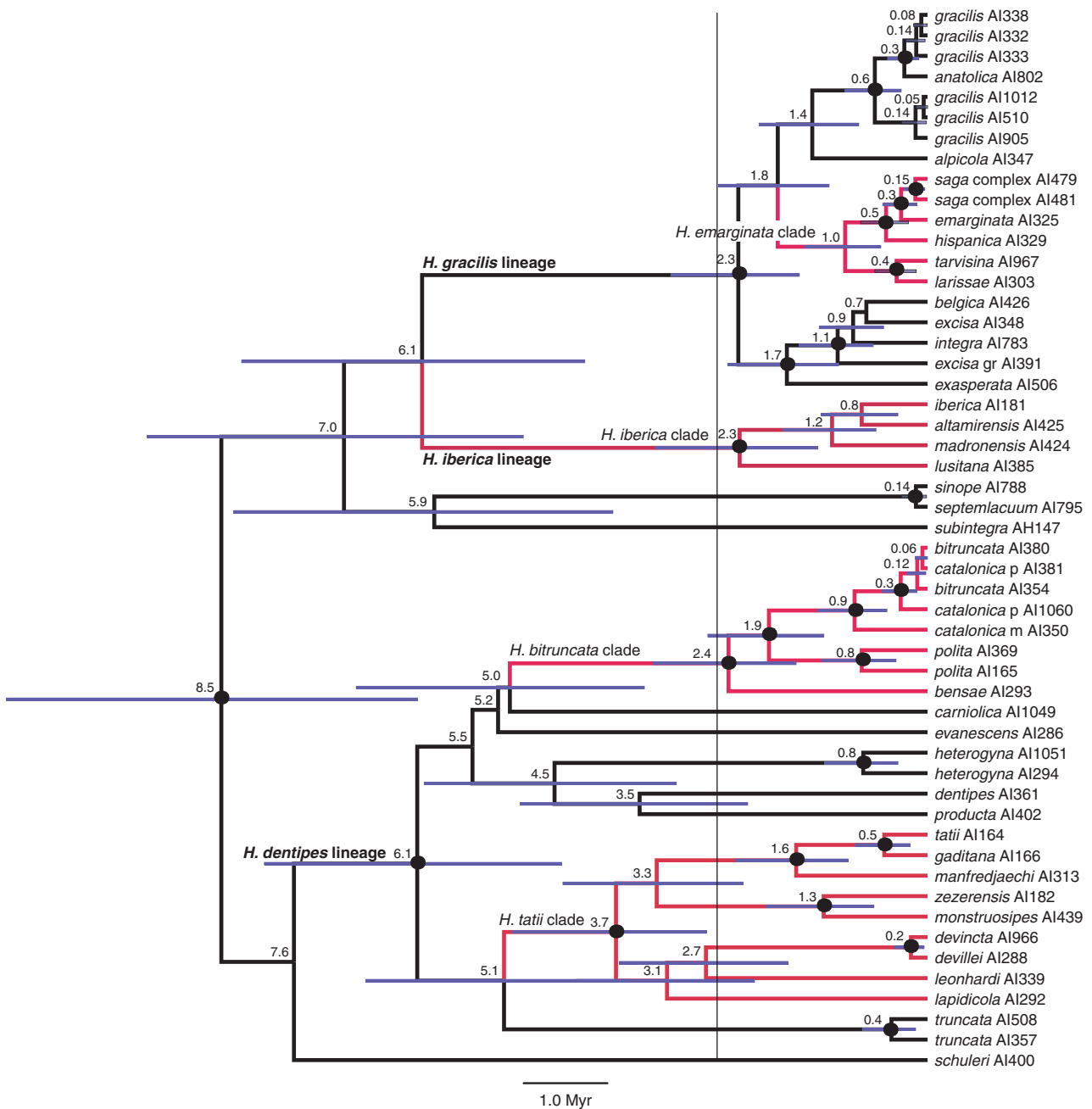


Figure 4 Ultrametric tree obtained using BEAST, using the mitochondrial data from a selection of the *Hydraena* specimens in the ‘*Haenydra*’ lineage and constraining the well-supported nodes of the topology represented in Fig. 2 (black circles). The four clades studied in the geographic analyses are marked in red. Numbers at nodes denote the age estimate (Ma); blue bars, 95% confidence intervals; vertical bar, the Pliocene/Pleistocene transition (2.6 Ma). See Appendix S1 for the codes of the species.

specimens from the Pyrenees and south Germany) and *H. heterogyna* (0.8 Myr between specimens from central Italy and the French Alpes Maritimes; Fig. 4; Appendix S1), suggesting the possible existence of unrecognized cryptic diversity.

Geographic analyses

For the geographic analyses, four well-supported clades of ‘*Haenydra*’ were selected, including most of the south-western

narrowly distributed species and for which the sampling (according to morphology) could be considered complete or with at most one or two missing species. These were the *H. iberica* (1), *H. emarginata* (2), *H. bitruncata* (3) and *H. tatii* (4) clades (see above and Fig. 2 for their composition, and Fig. 3 for the distribution of the species).

The *H. iberica* lineage had only four species, which is below the minimum number necessary for the implementation of Mantel tests in ZT (Bonnet & Van de Peer, 2002). For the other

Table 2 Matrices of the geographic linear distances between the centroids of the species of *Hydraena* (in km) and the phylogenetic distances (i.e. age estimates, in Ma). See text and Fig. 2 for the sister of the *H. tatii* clade. In the *H. bitruncata* clade, ‘*H. catalonica* p’ refers to populations from the Pyrenees, and ‘*H. catalonica* m’ to populations from the Montseny Massif (central Catalonia). In the *H. bitruncata* clade, the age estimate of *H. bicuspidata* (not included in the study) is the middle point of the branch between the two nodes in which it is hypothesized to be placed based on morphological evidence (see text).

<i>H. iberica</i> clade	<i>H. altamirensis</i>	<i>H. iberica</i>	<i>H. lusitana</i>		
<i>H. iberica</i>	236 km/1.6 Ma				
<i>H. lusitana</i>	308/4.5	96/4.5			
<i>H. madronensis</i>	133/2.3	367/2.3	431/4.5		
<i>H. emarginata</i> clade	<i>H. emarginata</i>	<i>H. hispanica</i>	<i>H. larissae</i>	<i>H. saga</i> complex	
<i>H. hispanica</i>	390/1.0				
<i>H. larissae</i>	1025/2.0	1415/2.0			
<i>H. saga</i> complex	226/0.6	601/1.0	840/2.0		
<i>H. tarvisina</i>	1190/2.0	1580/2.0	170/0.7	998/2.0	
<i>H. bitruncata</i> clade	<i>H. bensae</i>	<i>H. bicuspidata</i>	<i>H. bitruncata</i>	<i>H. catalonica</i> p	<i>H. catalonica</i> m
<i>H. bicuspidata</i>	175/4.8				
<i>H. bitruncata</i>	367/4.8	380/1.9			
<i>H. catalonica</i> p	431/4.8	415/1.9	89/0.1		
<i>H. catalonica</i> m	394/4.8	450/1.9	49/1.8	115/1.8	
<i>H. polita</i>	188/4.8	100/3.8	501/3.8	542/3.8	538/3.8
<i>H. tatii</i> clade	<i>H. gaditana</i>	<i>H. manfredjaechi</i>	<i>H. monstrosipes</i>	<i>H. tatii</i>	<i>H. zezerensis</i>
<i>H. manfredjaechi</i>	296/3.2				
<i>H. monstrosipes</i>	691/6.5	628/6.5			
<i>H. tatii</i>	146/1.0	161/3.2	683/6.5		
<i>H. zezerensis</i>	460/6.5	501/6.5	271/2.5	496/6.5	
[sister outgroup]	1300/7.5	1000/7.5	1100/7.5	1100/7.5	1300/7.5

clades, geographic and phylogenetic distances, as measured with a Mantel test with 10,000 permutations, were significantly correlated in the *H. emarginata* ($r = 0.9$, $P < 0.05$) and the *H. tatii* clades, both when only the five Iberian species were included ($r = 0.90$, $P < 0.05$) and when the pooled Alpine species were included as a non-overlapping outgroup to the Iberian species ($r = 0.83$, $P < 0.01$) (Table 2). In the *H. bitruncata* clade, the Mantel test was not significant at the standard level (Table 2; $r = 0.68$, $P = 0.08$). When the missing species *H. bicuspidata* was added to the phylogeny as sister to *H. bitruncata* plus *H. catalonica* (the most likely position according to morphology, see above), it became significant, despite the reduction in the correlation, owing to the increase in power ($r = 0.32$; $P < 0.05$).

The bivariate plot between geographic and phylogenetic distance for the *H. iberica* clade had three data points clearly outside a hypothetical linear relationship, corresponding to the pairwise distances including *H. iberica* (Fig. 5a). The centroid of the distribution of this species was too close to *H. lusitana* and too distant from *H. altamirensis* and *H. madronensis* to obtain a linear relationship between geographic and phylogenetic distance, suggesting a possible secondary range expansion of *H. iberica*. To explore this possibility, we sequenced the COI fragment of five additional specimens of *H. iberica* through their range (Appendix S1). They had identical sequences, with

the exception of a difference of one base pair for the specimen from south Portugal (voucher MNCN-AI386, Appendix S1), at the south-western limit of the species distribution (Fig. 3a), supporting the hypothesis of a recent expansion.

For the *H. emarginata* and *H. tatii* clades, the bivariate plot (Fig. 5b, d) showed a monotonic increase of phylogenetic distance as the distance between centroids increased, without apparent outliers and in particular without phylogenetically distant species pairs in close geographic proximity. In the *H. bitruncata* clade, the pairwise distances corresponding to *H. polita* (the more widespread species of the group) were outliers from a linear relationship in the bivariate plot (Fig. 5c).

For the *H. iberica* and *H. bitruncata* clades, the observed topology was not in agreement with that obtained from the EMST (Fig. 6a, c). The topology optimizing the geographic distances (EMST) for the *H. iberica* clade placed *H. altamirensis* as sister to *H. madronensis*, in contrast with the observed relationship (*H. iberica* sister to *H. madronensis*, Fig. 2). In any case, owing to the low number (three) of different unrooted trees for four taxa, the observed tree could not be said to be different from a random geographic arrangement. For the *H. bitruncata* clade, two topologies had a better match with the EMST: [(*H. bitruncata*, *H. catalonica* m) *H. catalonica* p] and [(*H. catalonica* m, *H. catalonica* p) *H. bitruncata*] (Fig. 6c,

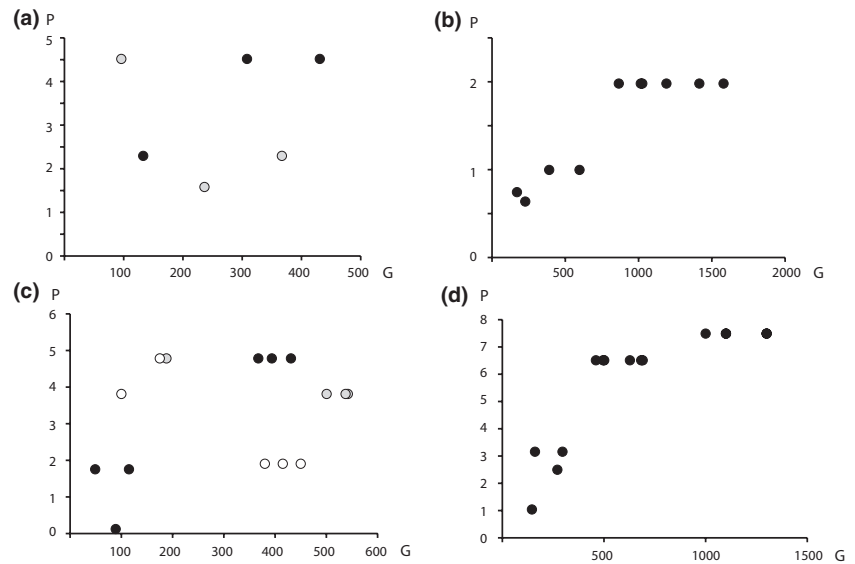


Figure 5 Bivariate plots of the geographic (linear) distance between the centroids of the ‘*Haenydra*’ species (G, km) versus the phylogenetic distance (P, age estimate in Ma). (a) *Hydraena iberica* clade; grey circles, distances to *H. iberica*; (b) *H. emarginata* clade; (c) *H. bitruncata* clade; grey circles, distances to *H. polita*; white circles, distances to *H. bicuspidata*; (d) *H. tatii* clade.

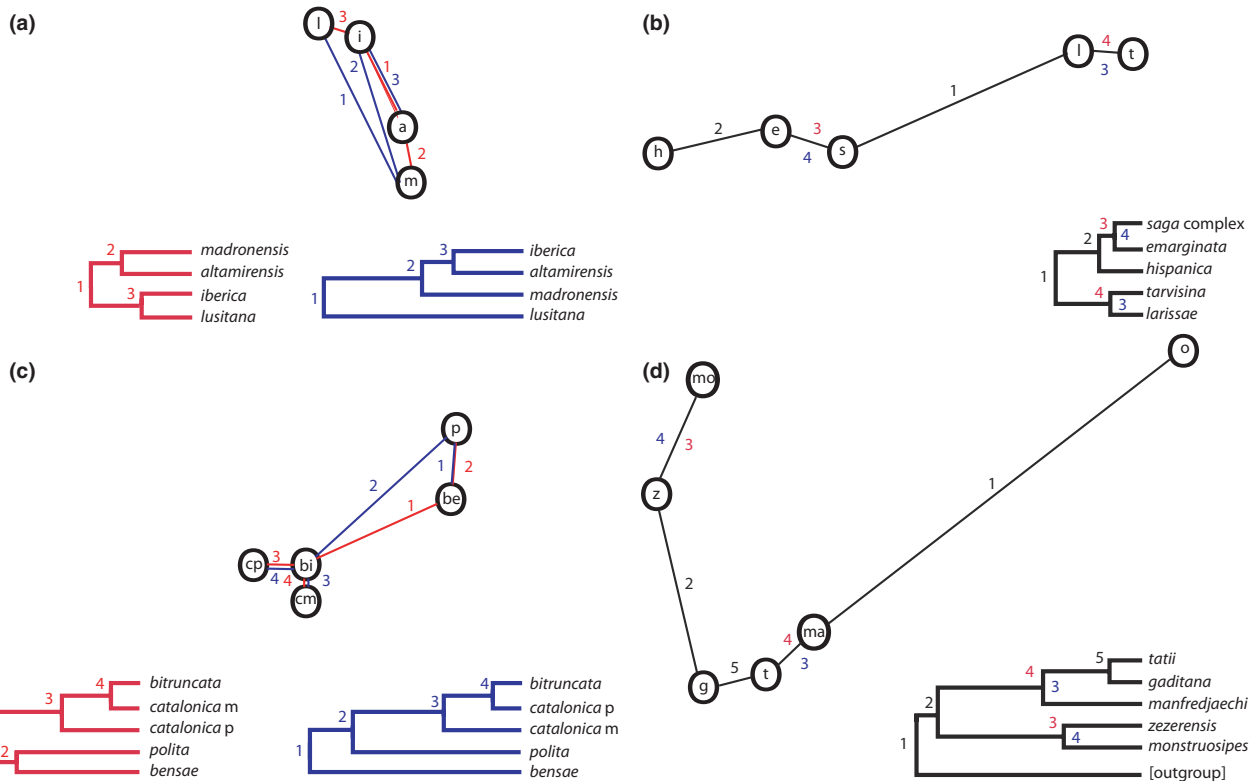


Figure 6 Schematic representation of the Euclidean minimum spanning trees (EMSTs) among the centroids of the species in the four clades of the ‘*Haenydra*’ lineage used for the geographic analyses: (a) *Hydraena iberica* clade; (b) *H. emarginata* clade; (c) *H. bitruncata* clade; (d) *H. tatii* clade. Circles represent the geographic positions of the centroids, as in Fig. 3. Lines between centroids represent cladogenetic events. Numbers represent the temporal order of the cladogenetic events. In (b) and (c), the reconstructed phylogeny (taken from Fig. 4) agrees with the EMST, except for the temporal order of some nodes in various branches, which does not affect the topology (numbers in blue, observed sequence; in red, temporal sequence according to the EMST). In (a) and (c) the observed phylogeny (in blue) does not agree with the phylogeny reconstructed from the EMST (in red).

$P = 3/15 = 0.2$). With the inclusion of *H. bicuspidata* in its hypothesized phylogenetic position there were five topologies that matched the EMST better than the observed one, with a marginal significance ($P = 6/105 = 0.057$).

For the *H. emarginata* and *H. tatii* clades, of all possible unrooted topologies with five taxa (15), the observed one was identical to that determined by the EMST. The observed relative order of two of the nodes in different branches in each

of the trees was reversed with respect to the order expected with the EMST (Fig. 6b, d), although they were estimated to have occurred in close temporal proximity, and the 95% confidence intervals fully overlap each other (Fig. 4). Owing to the low number of possible topologies (15), the geographic distribution of the species could only be considered to be marginally different from random ($P < 1/15 = 0.067$). For the *H. tatii* clade, when the fully allopatric Alpine sister group was included with a pooled geographic distance considered to be the shortest from the Iberian Peninsula (i.e. the Alpes Maritimes, Table 2), the relationship became highly significant owing to the increase to six taxa ($P = 1/105 < 0.01$). Similarly, alternative topologies to the sister relationship between the *H. tatii* group and *H. monstrosipes* plus *H. zezerensis* (placing each of them as sister to a species pair from the Alps) resulted in equally significant non-random geographic distributions when the two groups were considered separately.

DISCUSSION

Origin and phylogeny of the 'Haenydra' lineage

There was strong support for the monophyly of the species of the 'Haenydra' lineage, including *H. schuleri*, which has a deviating morphology. Although the phylogenetic position of the lineage was not resolved, it was nested within the main clade of *Hydraena sensu stricto*, in agreement with the conclusions of Jäch *et al.* (2000), and not as sister to the rest of the genus, as hypothesized by Berthélemy (1986) and Perkins (1997). Jäch *et al.* (2000) suggested that the *H. armipalpis* group (China) could be the sister to *Haenydra*, owing to the base of the parameres being fused with the median lobe of the aedeagus and a similar general structure of the pronotum and elytra, but no species of this group could be obtained for the molecular study.

Within the wider *Hydraena*, the main trends of our phylogeny also agree with the results of Jäch *et al.* (2000), with several well-defined lineages including *Hydraenopsis* and other species groups considered to be plesiomorphic (*H. monikae*, *H. rugosa* group, 'Phothydraena' and *H. circulata* group). Our results confirm the inclusion of the species of the *H. minutissima* group (the former 'Hadrenya') within the main lineage of *Hydraena sensu stricto*, as hypothesized by Jäch *et al.* (2000), but not close to 'Haenydra', as assumed by previous authors (d'Orchymont, 1925; Perkins, 1997).

According to our calibration, the 'Haenydra' lineage dates from the late Miocene, but the main diversification of the group, and the origin of most of the species, took place during the Pleistocene. Post-Pliocene diversification would explain the absence of any species of the group in North Africa, despite intensive search efforts in the area by numerous entomologists and the obvious dispersal abilities of some species through continuous landmasses. There is a record of *Hydraena exasperata* from Morocco in d'Orchymont (1935) (a male, deposited in the Institut royal des Sciences naturelles de Belgique, Brussels), but, as suggested by the same author, it may be a

labelling mistake or a specimen carried over from the previous collecting sites in south Spain. The only species present in western European islands are *H. gracilis* in Britain and Ireland, which are likely to have been connected to mainland Europe during the early Holocene after the Last Glacial Maximum (Lambeck & Chappell, 2001), and three species in Corsica and Sardinia (Audisio *et al.*, 2009). The latter species are often hypothesized to be the result of vicariance owing to the tectonic separation of the Corsican microplate during the Oligocene (see Audisio *et al.*, 2009 for an overview of possible scenarios). According to our estimations based on the same vicariant split in a related family (Leiodidae, Ribera *et al.*, 2010a), the Corsican *Hydraena evanescens* has an estimated age of 5.2 Ma, that is, the end of the Messinian. This opens the possibility of a colonization of Corsica and Sardinia through land connections during the late Miocene, with vicariance of the island populations after the opening of the Straits of Gibraltar with the re-filling of the Mediterranean (García-Castellanos *et al.*, 2009). Some other Sardinian endemics have been estimated to be of a similar age, in particular some cave salamanders of the genus *Hydromantes* (Carranza *et al.*, 2008), and the painted frog *Discoglossus sardus* (Zangari *et al.*, 2006). The dispersal of these taxa would have required a land corridor that was probably also suitable for *Haenydra*.

All the Iberian endemic species were estimated to be of Pleistocene age, in agreement with some groups of aquatic beetles (e.g. family Dytiscidae, Ribera & Vogler, 2004), but in sharp contrast with others, such as the two Iberian endemic species of *Enicocerus* Stephens (Hydraenidae, Ribera *et al.*, 2010b) and the western Mediterranean species of *Hydrochus* (A. Hidalgo-Galiana & I. Ribera, in preparation), all of them of late Miocene origin. The most widespread and common species of the lineage, *H. gracilis*, has a recent origin nested within a clade with predominantly narrow-range endemics. Differences between specimens through the range (Latvia, Britain, Bulgaria) were minimal, strongly suggesting a recent, late Pleistocene range expansion. By contrast, other widespread species of the group (*H. polita*, *H. truncata*) have deep divergences between specimens from different parts of their species' range (estimated to be *c.* 1 Myr), suggesting the existence of frequent cryptic diversity within the lineage. This is also the case for the species found to be paraphyletic (including *H. gracilis sensu lato*), or known to be a composite of polyphyletic lineages (as for the current concept of *H. saga*).

Geographic analyses

The difficulty in determining past geographic ranges in the absence of a fossil record is a major impediment in studies of speciation and diversification (Gaston, 2003; Losos & Glor, 2003). In some cases the accumulation of indirect evidence (genetic, ecological, geographic) adds to the credibility of a given scenario, but it is often not possible to contrast its likelihood against alternative possibilities. The use of null models as a reference for the comparison of the observed pattern allows a more rigorous assessment (Barraclough &

Nee, 2001). We did not test the geography of speciation in the classic sense (Mayr, 1963), because this is not possible using only data of current distributions. In our scenario, speciation ultimately may occur as a result of isolation through rarefaction of the range, but whether this can be considered allopatric or peripatric depends on the precise distribution of the species at the time of speciation, which is not possible to establish without detailed genetic data (Butlin *et al.*, 2008).

The finding that for a given clade there is a strong correlation between geographic and phylogenetic distance is evidence of the non-randomness of the spatial distribution of the species. This correlation may be the result of a process in which the probability of speciation is inversely proportional to the distance to the nearest neighbours, as could happen in the progressive rarefaction of a species' range owing to changes in general climatic or ecological conditions (Fig. 1c). In the traditional models of isolation by distance (Wright, 1943; Kimura, 1953), geographic distance is also proportional to genetic distance, but this proportionality is maintained through the continuous presence of gene flow, more likely to occur between neighbouring individuals (or populations). Given a widespread species showing isolation by distance through its range, if the general conditions were to deteriorate so that gene flow diminished progressively, it could be expected that the first interruption would occur among the groups of populations separated by the longest distance, followed by the next longest, and so on successively, resulting in a topology matching an EMST among the final species.

We have not assessed the degree of overlap between species (as in Lynch, 1989 or Barraclough & Vogler, 2000) because our results depend only on the relative position of the centroid of the distribution of each species in relation to the others, not on possible range expansions or contractions around this centroid. The relative position of the centroids seems to be less evolutionarily labile than the extension of the range, which will usually change faster than the rate of speciation (Coyne & Orr, 2004). Our scenarios also do not assume that the range of the ancestral species is the sum of that of the descendants, only that the relative position of the centroid of their distribution is intermediate between the two. Of the two statistical tests we applied to the geographic data, the Mantel test is the least stringent, and may still be significant when the position of the centroids of two close species swap, or when (as a result of, for example, a geographically biased range expansion) the current centroid changes its relative position with respect to the nearest neighbours. By contrast, the optimization test through the comparison of the topologies is more restrictive, in that any change of the relative position of even nearest-neighbour species would result in a suboptimal topology. This is clearly exemplified in the *H. bitruncata* clade, which has a significant correlation between geographic and phylogenetic distances as measured with a Mantel test when *H. bicuspidata* is included in its most likely position, but is not significantly different from a random arrangement as there are several topologies with a better match to the geographic optimum.

A limitation of our approach is that, while a positive result is a clear indication that species have maintained their relative geographic positions, when there is no significant correlation, or the topology does not optimize geographic distances, it is not possible to affirm that there has been range movement. As seen in Fig. 1, other modes of speciation (e.g. vicariance by random breaks) will result in this lack of correlation even if the species remain in place. Even assuming that the main diversification mechanism of the '*Haenydra*' lineage is the succession of cycles of expansion of some species with subsequent fragmentation (Fig. 1c), the geographic signal, as measured here, will persist only until the next expansion of a species of the clade. This could be the case in the *H. iberica* clade, in which both the genetic uniformity and the deviation from the linear correlation of *H. iberica* strongly suggest a recent expansion from its original range, which may be in central Iberia based on the interpolation of the geographic distances and assuming a linear relationship with phylogenetic distances in Fig. 5a. The most widespread species of the '*Haenydra*' lineage, *H. gracilis*, was found to be of recent origin (c. 0.5 Ma), and the few data available show that the central and northern European form is very homogeneous through its range, as expected after a recent expansion. Although we did not analyse this clade in detail owing to the possibly high number of closely related missing species, the recognition of distinct taxa in the periphery of its current distribution (Jäch, 1995) suggests that this could be an example of a species complex in the early stages of range fragmentation. Some of these peripheral taxa are, however, island endemics (e.g. *Hydraena elisabethae* on the island of Thassos, and *H. nike* in Samothrace; Jäch, 1995). In these cases, the isolation would be produced by vicariant barriers and there does not need to be a correlation between geographic and phylogenetic distances.

The *H. tatii* clade shows the strongest evidence for a non-random distribution among the four tested. The five Iberian endemics have a common origin by the late Pliocene. This is coincident in time with an acute cooling period that may have facilitated the expansion of the ancestral species, prior to the origin of the Mediterranean climate at c. 3.1–3.2 Ma, with its strong seasonality and increase in aridity (Suc, 1984; Mijarra *et al.*, 2009). Subsequent cladogenetic events within the *H. tatii* clade would have taken place during the Pleistocene glacial cycles within the Iberian Peninsula, and without changes in the geographic location of the resulting species – or at least with changes not large enough to erase the geographic signal in their current distributions.

Although with lower support, the *H. bitruncata* clade also showed some evidence of geographic structure when *H. bicuspidata* was included in its most likely phylogenetic position, with a significant overall correlation between geographic and phylogenetic distances. The origin of this clade was estimated to be at the Pliocene–Pleistocene transition, again a cold period (Lisiecki & Raymo, 2007) that could have made possible the expansion of species typical of cold mountain streams. The distribution of this clade, between north-east Iberia (north of the Ebro valley) and the Alps falls outside the traditionally recognized Pleistocene refugia (the

southern peninsulas), but still shows signs of conservation of the geographic ranges.

Glacial cycles may induce regular expansions during favourable times (either glacials or interglacials, depending on the autecology of the species), followed by range contraction to refugia when conditions turn adverse (Dynesius & Jansson, 2000; Bennett & Provan, 2008; Stewart *et al.*, 2010). These repeated cycles produce different degrees and patterns of phylogeographic structure (Hewitt, 2000). However, it seems that for some lineages, among them *Haenydra*, the range expansions are infrequent and affect only some taxa. Periods of range contraction result in the generation of multiple, isolated residual species. The process would thus not be cyclical, in the sense that conditions do not return to the same original state, but accumulative: each expansion produces a set of new species that do not contribute to the next cycle, and overlap with the species resulting from the previous ones. The concept of 'refugia' (as defined by Stewart *et al.*, 2010) will apply not to species, as they would not suffer cyclical periods of contraction–expansion, but to the lineage: successive glaciations would eradicate populations (or species) in the glaciated areas, allowing the survival (and accumulation) of the species only in the refugia. If the species are able to expand their ranges only occasionally, as seems to be the case for '*Haenydra*', either they remain in the refugia as narrow-range endemics, or, when they expand, they do not mix with the populations that are left, as they would already be different species.

Concluding remarks

With our approach we have shown that under some circumstances it is possible to obtain strong evidence of stasis of the geographic ranges of narrow-range endemic species through the study of their phylogenetic relationships and their current distributions. At least some of the studied clades seem likely to be formed by true endemics, originating in the areas in which they are currently found through the fragmentation of a more widely distributed species during the late Pliocene and Pleistocene. This speciation within refugia supports increasing evidence of the complexity of the evolutionary processes that took place in the Mediterranean peninsulas during Pleistocene glacial cycles, with refugia being far more than simple repositories of accumulated genetic diversity (Gómez & Lunt, 2007).

ACKNOWLEDGEMENTS

We thank all colleagues mentioned in Appendix S1 for supplying material for study, A. Faille and A. Hidalgo for help in the laboratory, W. Zelenka for the habitus of Fig. 2, P. Abellán for geographic data, and A. Cieslak, J. Castresana, P. Abellán and two anonymous referees for comments. This work was funded through the EU program Synthesys (projects AT-TAF-217, 1613, 2201 and 2391 to I.R., J.A.D., L.F.V. and J.G., respectively) and projects CGL2004-00028 and CGL2007-61665 to I.R.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Specimens used in the study, with locality, collector, voucher reference numbers and accession numbers for the sequences.

Appendix S2 List of primers used for sequencing.

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BIOSKETCH

I. Ribera is interested in the evolution, systematics and biogeography of Coleoptera. This work is part of an ongoing collaboration among the authors to study the Iberian species of *Hydraena*.

Author contributions: I.R. conceived the study; I.R., A.C., J.G., L.F.V. and M.A.J. provided material and data; A.I. did most of the laboratory work; I.R. carried out the phylogenetic analyses and outlined a first draft; A.C., J.G., J.A.D., L.F.V. and M.A.J. discussed the results and contributed to the final version of the manuscript.

Editor: Pauline Ladiges

Appendix S1

Specimens used in the study, with locality, collector, voucher reference numbers and accession numbers for the sequences.
Specimens "a" to "e" of *H. iberica* were sequenced to check for intraspecific variability and not included in the main analyses (see text).
Nomenclature follows Hansen (1998) and Jäch (2004).

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No	species	species group	voucher	ref MNCN	country	locality	date	collector	cox1	rrnL+trnL+na d1	SSU	LSU
1	<i>Hydraena alpicola</i>	" <i>Haenydra</i> "	MNCN-AI347	23693	Austria	11 Au, Niederösterreich, Schwarzenbach, lat. rd. 18 W Sankt Veit an der Gölsen, 448m N48°04'42.4" E15°40'42.9"	6.8.2005	I. Ribera & A. Cieslak	HM588354	HM588498		
2	<i>Hydraena altamirensis</i>	" <i>Haenydra</i> "	MNCN-AI425	23771	Spain	Ciudad Real, Navas de Estena, río Estena	18.8.2005	A. Castro	HM588371	HM588515	HM588578	HM588435
3	<i>Hydraena anatolica</i>	" <i>Haenydra</i> "	MNCN-AI802	24148	Turkey	TR09 KASTAMONU, Rd. 759 to Senpazar, Çocukören Çoylu, fast stream in mixed forest, 591m N41°49'38" E33°25'03"	26.4.2006	I. Ribera	HM588395	HM588539		
4	<i>Hydraena belgica</i>	" <i>Haenydra</i> "	MNCN-AI426	23772	Austria	Niederösterreich, Schaubach ca. 270 m a.s.l., Schauboden, 3.5 km N. Purgstall, 15°08'20"E, 48°05'23"N	25.9.2005	M.A. Jäch	HM588372	HM588516		
5	<i>Hydraena bensae</i>	" <i>Haenydra</i> "	MNCN-AI293	23639	France	6, river Carai, 2 km N Monti	29.7.2000	I. Ribera & A. Cieslak	HM588338	HM588482	HM588564	HM588421
6	<i>Hydraena bitruncata</i>	" <i>Haenydra</i> "	MNCN-AI354	23700	Spain	1 ES Barcelona, Guardiola del Berguedà, Te. Gavarrós, rd. to La Pobla de L., 850m N 42°15'05.9" E1°55'20.1"	25.7.2005	I. Ribera	HM588357	HM588501		
7	<i>Hydraena bitruncata</i>	" <i>Haenydra</i> "	MNCN-AI380	23726	Spain	2 ES Barcelona, La Pobla de Lillet. Te. de Solís, Rd. to S. Jaume. de Frontanyà, 1055m N 42°12'26.2" E2°00'23.5"	25.7.2005	I. Ribera	HM588362	HM588506		
8	<i>Hydraena carniolica</i>	" <i>Haenydra</i> "	MNCN-AI1049	24395	Italy	1 IT Veneto, Treviso, stream by rd. 1 km S Collalto, 100m N45°52'20.3" E12°11'17.3"	14.8.2006	I. Ribera & A. Cieslak	HM588314	HM588458	HM588549	HM588406
9	<i>Hydraena catalonica</i>	" <i>Haenydra</i> "	MNCN-AI350	23696	Spain	2 ES Barcelona, Santa Fe del Montseny, rd. Turo del Home, Sot de Bernadal, 1134m N 41°45'45.2" E2°26'55.0"	23.7.2005	I. Ribera	HM588356	HM588500		
10	<i>Hydraena catalonica</i>	" <i>Haenydra</i> "	MNCN-AI381	23727	Spain	2 ES Barcelona, La Pobla de Lillet. Te. de Solís, Rd. to S. Jaume. de Frontanyà, 1055m N 42°12'26.2" E2°00'23.5"	25.7.2005	I. Ribera	HM588363	HM588507		
11	<i>Hydraena catalonica</i>	" <i>Haenydra</i> "	MNCN-AI1060	24406	Spain	1 ES Huesca, Bonansa, Coll de Bonansa, rd. Borda Ansuilo, 1454m N42°25'21.7" E0°37'54"	1.8.2006	I. Ribera & J. Fresneda	HM588316	HM588460		
12	<i>Hydraena dentipes</i>	" <i>Haenydra</i> "	MNCN-AI361	23707	Slovakia	Nizke Tatry Mts., Maluziny rv.	24.6.2005	F. Ciampor	HM588360	HM588504		
13	<i>Hydraena devillei</i>	" <i>Haenydra</i> "	MNCN-AI288	23634	Italy	4, r. Staffora, San Ponzio, rd. 461	28.7.2000	I. Ribera & A. Cieslak	HM588336	HM588480		
14	<i>Hydraena devincta</i>	" <i>Haenydra</i> "	MNCN-AI966	24312	Italy	Treviso, Falzi di Piave, Ruio della Mina	10.6.2006	G. Ferro	HM588400	HM588543		
15	<i>Hydraena emarginata</i>	" <i>Haenydra</i> "	MNCN-AI325	23671	Spain	5 ES Lugo, Os Ancares, rd. LU723. Sa. Do Agulleiro, Donis: r. da Vara, 1006m N42°49'31.2" W6°52'50.7"	10.7.2005	I. Ribera & A. Cieslak	HM588346	HM588490		
16	<i>Hydraena evanescens</i>	" <i>Haenydra</i> "	MNCN-AI286	23632	Corsica	6, Col de Bavella: Arggiavara	18.9.1999	I. Ribera & A. Cieslak	HM588335	HM588479	HM588563	HM588420
17	<i>Hydraena exasperata</i>	" <i>Haenydra</i> "	MNCN-AI506	23852	Spain	E-1) Puerto de Panderrueda (León)	12.7.2005	L.F. Valladares	HM588382	HM588526	HM588585	HM588442
18	<i>Hydraena excisa</i>	" <i>Haenydra</i> "	MNCN-AI348	23694	Austria	11 Au, Niederösterreich, Schwarzenbach, lat. rd. 18 W Sankt Veit an der Gölsen, 448m N48°04'42.4" E15°40'42.9"	6.8.2005	I. Ribera & A. Cieslak	HM588355	HM588499		
19	<i>Hydraena excisa</i> gr.	" <i>Haenydra</i> "	MNCN-AI391	23737	Bulgaria	Rila mts. Stream above Bacevo. 1,500m	6.7.2005	D.T. Bilton	HM588365	HM588509		
20	<i>Hydraena gaditana</i>	" <i>Haenydra</i> "	MNCN-AI166	23512	Spain	Málaga, río Genal	7.8.2004	A. Castro	HM588324	HM588468		
21	<i>Hydraena gracilis</i>	" <i>Haenydra</i> "	MNCN-AI905	24251	Spain	E-1) Puerto de Panderrueda (León)	12.7.2005	L.F. Valladares	HM588398			
22	<i>Hydraena gracilis</i>	" <i>Haenydra</i> "	MNCN-AI1012	24358	Spain	2 ES Guipuzkoa, Oiartzun, N43°16'10.2" W1°48'20.5" 240m, Aiko Herria N.P., Incensoro erreka	16.7.2006	I. Ribera & A. Cieslak	HM588311			
23	<i>Hydraena gracilis</i>	" <i>Haenydra</i> "	MNCN-AI332	23678	Latvia	Gaujais NP stream in forest.	13.6.2005	D.T. Bilton	HM588348	HM588492		
24	<i>Hydraena gracilis</i>	" <i>Haenydra</i> "	MNCN-AI333	23679	England	Cumbria river Irthing at Spadeadam Waste	26.6.2005	D.T. Bilton	HM588349	HM588493	HM588570	HM588427
25	<i>Hydraena gracilis</i>	" <i>Haenydra</i> "	MNCN-AI510	23856	Spain	E-7) Beges (Cantabria)	13.7.2005	L.F. Valladares	HM588384	HM588528		
26	<i>Hydraena gracilis balcanica</i>	" <i>Haenydra</i> "	MNCN-AI338	23684	Bulgaria	Rhodope mts. Goce Delcev-. Dospat Rd. Stream @1,000m below	10.7.2005	D.T. Bilton	HM588350	HM588494		
27	<i>Hydraena heterogyna</i>	" <i>Haenydra</i> "	MNCN-AI294	23640	France	6, r. Carai, 2 km N Monti	29.7.2000	I. Ribera & A. Cieslak	HM588339	HM588483		
28	<i>Hydraena heterogyna</i>	" <i>Haenydra</i> "	MNCN-AI1051	24397	Italy	Emilia Romagna, La Lama, Bagno di Romagna (Forlì), 710m	13.6.2006	P. Mazzoldi	HM588315	HM588459		
29	<i>Hydraena hispanica</i>	" <i>Haenydra</i> "	MNCN-AI329	23675	Spain	2 ES Ourense, Sa. de Queixa, 2 km S Manzaneda afl. r. San Lázaro, 770m N42°17'04" W7°13'21.2"	9.7.2005	I. Ribera & A. Cieslak	HM588347	HM588491		
30	<i>Hydraena iberica</i>	" <i>Haenydra</i> "	MNCN-AI181	23527	Portugal	Serra de Arga	9.5.2005	D.T. Bilton	HM588330	HM588474		
31	<i>Hydraena integra</i>	" <i>Haenydra</i> "	MNCN-AI783	24129	Turkey	TR05 BOLU, Rd. 750 btw Yeniçaga & Mengen, fast stream in mixed forest, 844m N40°50'49" E32°03'47.5"	24.4.2006	I. Ribera	HM588392	HM588536		

32	<i>Hydraena lapidicola</i>	" <i>Haenydra</i> "	MNCN-AI292	23638	France	5, Tributary river Bevera, 5 km n Moulinet	28.7.2000	I. Ribera & A. Cieslak	HM588337	HM588481		
33	<i>Hydraena larissae</i>	" <i>Haenydra</i> "	MNCN-AI303	23649	Italy	4 Italy, Brescia, val Trompia, ca. 1500 m, stream rd. to Guogo di Bala	17.10.2002	I. Ribera & A. Cieslak	HM588340	HM588484		
34	<i>Hydraena leonhardi</i>	" <i>Haenydra</i> "	MNCN-AI339	23685	Bulgaria	Rila Mts. River in Kiril Meadow. Above Rila Monastery ca. 1,300m	12.7.2005	D.T. Bilton	HM588351	HM588495		
35	<i>Hydraena lusitana</i>	" <i>Haenydra</i> "	MNCN-AI385	23731	Portugal	4 PORTUGAL Serra Estrela, Sabugueiro, r. above village, 1100m N40°24'20" W7°37'43"	12.5.2005	I. Ribera	HM588364	HM588508		
36	<i>Hydraena madronensis</i>	" <i>Haenydra</i> "	MNCN-AI424	23770	Spain	Ciudad Real Fuencaliente, arroyo Pradillo	8.8.2005	A. Castro	HM588370	HM588514		
37	<i>Hydraena Manfredjaechi</i>	" <i>Haenydra</i> "	MNCN-AI313	23659	Spain	SPAIN Albacete, Robledo, Laguna de el Arquillo	2.6.2002	I. Ribera & A. Cieslak	HM588343	HM588487	HM588567	HM588424
38	<i>Hydraena monstruosipes</i>	" <i>Haenydra</i> "	MNCN-AI439	23785	Spain	León, Puerto de Ancares, Arroyo llegada Río Cuiña	11.9.2005	J. Garrido	HM588374	HM588518		
39	<i>Hydraena polita</i>	" <i>Haenydra</i> "	MNCN-AI165	23511	Spain	Huesca, Valle de Hecho, rio Aragón, Subordán.	8.2004	A. Castro	HM588323	HM588467		
40	<i>Hydraena polita</i>	" <i>Haenydra</i> "	MNCN-AI369	23715	Germany	9 D Bayern, Bad Heilbrunn, rd. 472 8Km W Bad Tölz, stream, 678m N47°44'33.5" E11°27'21.9"	4.8.2005	I. Ribera & A. Cieslak	HM588361	HM588505		
41	<i>Hydraena producta</i>	" <i>Haenydra</i> "	MNCN-AI402	23748	France	Corrèze, Ambrugeat, 700m	20.7.2005	P. Queney	HM588368	HM588512		
42	<i>Hydraena saga</i> gr.	" <i>Haenydra</i> "	MNCN-AI479	23825	Spain	2 ES Barcelona, Santa Fe del Montseny, rd. Turo del Home, Sot de Bernadal, 1134m N 41°45'45.2" E2°26'55.0"	23.7.2005	I. Ribera	HM588378	HM588522		
43	<i>Hydraena saga</i> gr.	" <i>Haenydra</i> "	MNCN-AI481	23827	Spain	4, Logroño, Posadas: Río Oja N 42°12'36.0" W 3°4'27.8" 1159 m	23.10.2004	I. Ribera	HM588379	HM588523		
44	<i>Hydraena schuleri</i>	" <i>Haenydra</i> "	MNCN-AI400	23746	Austria	12 Au, Niederösterreich, Pressbauch, trib. river Haberbach, 357m N48°11'42.8" E16°00'50.0"	13.8.2005	I. Ribera & A. Cieslak	HM588367	HM588511	HM588576	HM588433
45	<i>Hydraena septemlacuum</i>	" <i>Haenydra</i> "	MNCN-AI795	24141	Turkey	TR08 BARTIN, Rd. to Yeniköy from Kapisuyu I, calcareous stream in mixed forest, 161m N41°47'08" E32°43'07"	25.4.2006	I. Ribera	HM588394	HM588538		
46	<i>Hydraena sinope</i>	" <i>Haenydra</i> "	MNCN-AI788	24134	Turkey	TR07 BARTIN, Rd. to Topallar from Kakraz, calcareous stream in mixed forest, 25m N41°45'50.5" E32°29'11"	25.4.2006	I. Ribera	HM588393	HM588537		
47	<i>Hydraena subintegra</i>	" <i>Haenydra</i> "	MNCN-AH147		Macedonia	Macedonia, Mavrovo district, Mata Reka, 2km below Gari	13.9.2007	D.T. Bilton	HM588310	HM588455		
48	<i>Hydraena tarvisina</i>	" <i>Haenydra</i> "	MNCN-AI967	24313	Italy	Triviso, Falzi di Piave, Ruio della Mina	10.6.2006	G. Ferro	HM588401	HM588544		
49	<i>Hydraena tati</i>	" <i>Haenydra</i> "	MNCN-AI164	23510	Spain	Granada, Sierra Nevada, Peña del Perro	9.10.2004	A. Castro	HM588322	HM588466		
50	<i>Hydraena truncata</i>	" <i>Haenydra</i> "	MNCN-AI357	23703	France	2 FR Haute Provence, rd. N202 2km N Moriez, stream, 834m N43°57'43.4" E6°26'14.4"	30.7.2005	I. Ribera & A. Cieslak	HM588359	HM588503	HM588574	HM588431
51	<i>Hydraena truncata</i>	" <i>Haenydra</i> "	MNCN-AI508	23854	Spain	E-2) Soto de Valdeón (León)	12.7.2005	L.F. Valladares	HM588383	HM588527		
52	<i>Hydraena zezerensis</i>	" <i>Haenydra</i> "	MNCN-AI182	23528	Portugal	2, Manteigas, river Zezere	12.5.2005	D.T. Bilton	HM588331	HM588475		
53	<i>Hydraena atrata</i>	" <i>Phothydraena</i> "	MNCN-AI314	23660	Spain	SPAIN Albacete, Robledo, Laguna de el Arquillo	2.6.2002	I. Ribera & A. Cieslak	HM588344	HM588488	HM588568	HM588425
54	<i>Hydraena hernandoi</i>	" <i>Phothydraena</i> "	MNCN-AI435	23781	Spain	Córdoba, Priego de Córdoba, Arroyo Genilla	30.7.2005	A. Castro	HM588373	HM588517	HM588579	HM588436
55	<i>Hydraena serricollis</i>	" <i>Phothydraena</i> "	MNCN-AI1093	24439	Tenerife	Chamorga, N28°34'02.98" W16°09'28.97" 550m	20.7.2006	A. Castro	HM588319	HM588463	HM588552	HM588409
56	<i>Hydraena testacea</i>	" <i>Phothydraena</i> "	MNCN-AI566	23912	Morocco	28 MOROCCO, Oued Massa, Assif-Oumarhouz, 29°47'53"N 9°05'59"W, 1500m	25.4.2000	I. Ribera	HM588386	HM588530	HM588587	HM588444
57	<i>Hydraena barrosi</i>	<i>bisulcata</i>	MNCN-AI954	24300	Spain	1 ES Segovia Puerto de los Cotos, small stream in rd. SG615 pk 2.5, 1825m N40°47'49" W3°59'06.5"	1.7.2006	I. Ribera & A. Cieslak	HM588399	HM588542	HM588595	HM588452
58	<i>Hydraena bisulcata</i>	<i>bisulcata</i>	MNCN-AI172	23518	Spain	Córdoba, Sierra de Córdoba, Arroyo Linares	6.11.2004	A. Castro	HM588328	HM588472	HM588558	HM588415
59	<i>Hydraena bolivari</i>	<i>bisulcata</i>	MNCN-AI171	23517	Spain	Córdoba, Sierra de Córdoba, Arroyo Don Lucas	26.11.2004	A. Castro	HM588327	HM588471*	HM588557	HM588414
60	<i>Hydraena corinna</i>	<i>bisulcata</i>	MNCN-AI284	23630	Spain	4, Logroño, Posadas: Río Oja N 42°12'36.0" W 3°4'27.8" 1159 m	23.10.2004	I. Ribera	HM588334	HM588478	HM588562	HM588419
61	<i>Hydraena marcosae</i>	<i>bisulcata</i>	MNCN-AI904	24250	Spain	2 ES Avila, Sa. Gredos, rv. Pelayo in Arenas de S. Pedro/rd. AV924, ca. 2km W Arenas, 500m N40°12'5.6" W5°06'41.5"	28.5.2006	I. Ribera & A. Cieslak	HM588397	HM588541	HM588594	HM588451
62	<i>Hydraena arenicola</i>	<i>circulata</i>	MNCN-AI504	23850	California	14 US California, Trinity co., junc. Rd. 36 & 14, Forest Glen. Rattlesnake Creek.	22.6.2000	I. Ribera & A. Cieslak	HM588381	HM588525	HM588584	HM588441
63	<i>Hydraena petila</i>	<i>circulata</i>	MNCN-AI465	23811	California	15 US California Humbolt co., Avenue of the Giants, Stream nr. Red Crest	22.6.2000	I. Ribera & A. Cieslak	HM588377	HM588521	HM588582	HM588439
64	<i>Hydraena capta</i>	<i>holdhausi</i>	MNCN-AI167	23513	Spain	Jaén, Valepeñas de Jaén. Las Chorreras.	1.11.2004	A. Castro	HM588325	HM588469	HM588555	HM588412
65	<i>Hydraena holhausi</i>	<i>holdhausi</i>	MNCN-AI1025	24371	Turkey	TR04 DÜZCE, Rd. to Kartalkaya from Çayduzt, fast stream in coniferous forest, 1540m N40°39'20" E31°47'8.5"	23.4.2006	A. Castro	HM588312	HM588456	HM588547	HM588404
66	<i>Hydraena iberai</i>	<i>holdhausi</i>	MNCN-AI568	23914	Morocco	28 MOROCCO, Oued Massa, Assif-Oumarhouz, 29°47'53"N 9°05'59"W, 1500m	25.4.2000	I. Ribera	HM588388	HM588532	HM588589	HM588446
67	<i>Hydraena subacuminata</i>	<i>holdhausi</i>	MNCN-AI305	23651	Corsica	Cassamozza: r. l'Abatescu, 7	19.9.1999	I. Ribera & A. Cieslak	HM588341	HM588485	HM588565	HM588422
68	<i>Hydraena</i>	<i>Hydraenopsis</i>	IBE-AF90		New Caledonia	N. Caled. Prov. Sud. Col d'Amien 489m 21°6'1172S 165°80805E	7.3.2008	J. Gómez-Zurita	HM588308	HM588453	HM588545	HM588402
69	<i>Hydraena</i>	<i>Hydraenopsis</i>	MNCN-AI423	23769	Australia	Brisbane, Forest Park	10.2003	M. Balke & C. Monteith	HM588369	HM588513	HM588577	HM588434

70	<i>Hydraena</i>	<i>Hydraenopsis</i>	MNCN-AI709	24055	Venezuela	Venezuela: 100m, Tobogan de la Selva, S Puerto Ayacucho	24-25.1.2004	M. Balke	HM588391	HM588535	HM588592	HM588449
71	<i>Hydraena cooperi</i>	<i>Hydraenopsis</i>	MNCN-AI456	23802	South Africa	19 SA, W Cape 25.3.2001/Franschhoek, rd. R45/river 3 km SE Franschhoek/I.Ribera & A. Cieslak leg.	25.3.2001	I. Ribera & A. Cieslak	HM588376	HM588520	HM588581	HM588438
72	<i>Hydraena fontana</i>	<i>Hydraenopsis</i>	MNCN-AI708	24054	Nepal	NEPAL 1: small pools (unshaded) in swampy meadow, slope fen, near western boundary wall of Kathmandu University Campus (KUC), west of Dhulikel, ca. 20 km ESE of Kathmandu, ca. 1450 m a.s.l. (GPS) / 1500 m a.s.l., 27°37'05.4"N/85°32'07.4"E	18.11.2005	M.A. Jäch	HM588390	HM588534	HM588591	HM588448
73	<i>Hydraena miyatakei</i>	<i>Hydraenopsis</i>	MNCN-AI828	24174	Japan	Honshu, Kamiyaji, Toyota-Cho, Shimonoseki-Shi, Yamaguchi pref.	17.3.2006	K. Kawano	HM588396	HM588540	HM588593	HM588450
74	<i>Hydraena palawanensis</i>	<i>Hydraenopsis</i>	MNCN-AH133		Philippines	PHIL.: N[orth] Palawan, Taytay [Municipality]; [Barangay/District] Poblacion, Manguao Stream, near sitio [village] Danao, rural, c.30m asl [above see level], 10°46'19"N 119°31'13"E, (66)M[annual collection]	2007	H. Freitag	HM588309	HM588454	HM588546	HM588403
75	<i>Hydraena pygmaea</i>	<i>minutissima</i>	MNCN-AI346	23692	Austria	11 Au, Niederösterreich, Schwarzenbach, lat. rd. 18 W Sankt Veit an der Gölsen, 448m N48°04'42.4" E15°40'42.9"	6.8.2005	I. Ribera & A. Cieslak	HM588353	HM588497	HM588572	HM588429
76	<i>Hydraena monikae</i>	<i>monikae</i>	MNCN-AI455	23801	South Africa	19 SA, W Cape, Franschhoek, rd. R45, river 3 km SE Franschhoek	25.3.2001	I. Ribera & A. Cieslak	HM588375	HM588519	HM588580	HM588437
77	<i>Hydraena palustris</i>	<i>palustris</i>	MNCN-AI309	23655	Austria	1 Austria, Voralberg, Pfänderstock, Bodensee. Hörbranz vic., Leiblach-Mündung, 400 m N47°32'01" E 9°43'46"	2.7.2001	I. Ribera	HM588342	HM588486	HM588566	HM588423
78	<i>Hydraena unca</i>	<i>palustris</i>	MNCN-AI276	23622	Spain	Córdoba, Sierra de Córdoba, arroyo don Lucas	26.11.2004	A. Castro	HM588333	HM588477	HM588561	HM588418
79	<i>Hydraena dentipalpis</i>	<i>riparia</i>	MNCN-AI492	23838	Turkey	TR: ARTVIN, 25 km NNW Borcka (small stream).	9.2005	A. Kasapoglu	HM588380	HM588524	HM588583	HM588440
80	<i>Hydraena morio</i>	<i>riparia</i>	MNCN-AI1203	24549	Greece	ÍPEIROS (IOANNINA). Kipi, vieux pont turc sur la rivière Voidomatis. N39°51'42.2", E020°47'10.4", alt. 730 m. (rivière, ripisylve)	20.7.2006	P. & V. Ponel	HM588320	HM588464	HM588553	HM588410
81	<i>Hydraena nigrita</i>	<i>riparia</i>	MNCN-AI345	23691	Austria	11 Au, Niederösterreich, Schwarzenbach, lat. rd. 18 W Sankt Veit an der Gölsen, 448m N48°04'42.4" E15°40'42.9"	6.8.2005	I. Ribera & A. Cieslak	HM588352	HM588496	HM588571	HM588428
82	<i>Hydraena quillisi</i>	<i>riparia</i>	MNCN-AI1085	24431	Spain	Jaén, Sierra de Cazorla, Ayo. Km 43.3 cta. Del Tranco	3.8.2006	A. Castro	HM588318	HM588462	HM588551	HM588408
83	<i>Hydraena reyi</i>	<i>riparia</i>	MNCN-AI320	23666	Poland	3 POL. Zachodniopomorsky, Wlosciborz: Parseta rv., N54°05'32" E15°42'48", -7m	18.8.2004	I. Ribera & A. Cieslak	HM588345	HM588489	HM588569	HM588426
84	<i>Hydraena servilia</i>	<i>riparia</i>	MNCN-AI274	23620	Spain	Málaga, río Genal	7.8.2004	A. Castro	HM588332	HM588476	HM588560	HM588417
85	<i>Hydraena antiatlantica</i>	<i>rufipes</i>	MNCN-AI567	23913	Morocco	28 MOROCCO, Oued Massa, Assif-Oumarhouz, 29°47'53"N 9°05'59"W, 1500m	25.4.2000	I. Ribera	HM588387	HM588531	HM588588	HM588445
86	<i>Hydraena balearica</i>	<i>rufipes</i>	MNCN-AI175	23521	Mallorca	5, Ternelles, Torrent de Ternelles, 72m, N 39°53'37.2" E3°00'14.9"	14.10.2004	I. Ribera & A. Cieslak	HM588329	HM588473	HM588559	HM588416
87	<i>Hydraena delia</i>	<i>rufipes</i>	MNCN-AI1061	24407	Spain	2 ES Lleida, Llesp, Barranc Basculina, 1012m N42°27'24.5" E0°44'57"	1.8.2006	I. Ribera & J. Fresneda	HM588317	HM588461	HM588550	HM588407
88	<i>Hydraena dochula</i>	<i>rufipes</i>	MNCN-AI518	23864	Bhutan	BHUTAN loc. 16: small stream, ca. 1-2 m wide, flowing from forest, crossing street, eastern slope of Dochu La [pass]; ca. 12 km east of Timphu Town; Timphu Prov.; ca. 2800 m a.s.l.; 27°29'15"N/89°45'50"E	24.11.2005	M.A. Jäch	FM946170	FM946171 / FM946172 / FM946173	FM946174	FM946175
89	<i>Hydraena kasyi</i>	<i>rufipes</i>	MNCN-AI1026	24372	Turkey	TR10 KASTAMONU, Akçaçam, rd. btw Agli & Azdavay, calcareous stream in open area, 909m N41°41'17" E33°24'01"	26.4.2006	A. Castro	HM588313	HM588457	HM588548	HM588405
90	<i>Hydraena gavarrensis</i>	<i>rufipes s.l.</i>	MNCN-AI1288	24634	Spain	Girona, Gavarres	16.11.2006	C. Hernando & M. Martinoy	HM588321	HM588465	HM588554	HM588411
91	<i>Hydraena hayashii</i>	<i>rufipes s.l.</i>	MNCN-AI691	24037	Japan	Shimane Pref., Mitarai-taki, Jinji river, Shimane-cho, Matsue City	19.2.2006	M. Hayashi	HM588389	HM588533*	HM588590	HM588447
92	<i>Hydraena exarata</i>	<i>rugosa</i>	MNCN-AI169	23515	Spain	Arroyo de los Arenales, Sierra de Córdoba	7.3.2004	A. Castro	HM588326	HM588470	HM588556	HM588413
93	<i>Hydraena rugosa</i>	<i>rugosa</i>	MNCN-AI392	23738	Spain	Castil de Vela, Palencia	5.2005	L.F. Valladares	HM588366	HM588510	HM588575	HM588432
94	<i>Adelphydraena orchymonti</i>		MNCN-AI356	23702	Venezuela	Venezuela: 100m, Tobogan de la Selva, S Puerto Ayacucho	24-25.1.2004	M. Balke	HM588358	HM588502	HM588573	HM588430
a	<i>Hydraena iberica</i>	" <i>Haenydra</i> "	MNCN-AI279	23625	Spain	Guadalajara, r. Berbedillo, 7km E Cardoso de la S. 1159m, N41°05'34.3" W3°25'32.1"	3.6.2005	I. Ribera & A. Cieslak	HM588596			
b	<i>Hydraena iberica</i>	" <i>Haenydra</i> "	MNCN-AI281	23627	Spain	2, Burgos, Fresneda de la Sierra: r. Tirón N 42°16'46.2" W 3°7'56.0" 1085 m	22.10.2004	I. Ribera	HM588597			
c	<i>Hydraena iberica</i>	" <i>Haenydra</i> "	MNCN-AI326	23672	Spain	5 ES Lugo, Os Ancares, rd. LU723. Sa. Do Agulleiro, Donis: r. da Vara, 1006m N42°49'31.2" W6°52'50.7"	10.7.2005	I. Ribera & A. Cieslak	HM588598			

d	<i>Hydraena iberica</i>	" <i>Haenydra</i> "	MNCN-AI386	23732	Portugal	6 PORTUGAL Serra de Sao Mamede, Portalegre, rd. to Sao Julia, r. Xévorá, 450m N39°17'50" W7°17'34"	13.5.2005	I. Ribera	HM588599			
e	<i>Hydraena iberica</i>	" <i>Haenydra</i> "	MNCN-AI902	24248	Spain	2 ES Avila, Sa. Gredos, rv. Pelayo in Arenas de S. Pedro, rd. AV924, ca. 2km W Arenas, 500m N40°12'5.6" W5°06'41.5"	28.5.2006	I. Ribera & A. Cieslak	HM588600			

Appendix S2

List of primers used for sequencing.

F: forward, R: reverse

Gene	Name		Sense Sequence	Reference
COI	Jerry (M202)	F	CAACATTTATTTTGATTTTTGG	Simon <i>et al.</i> (1994)
	Pat (M70)	R	TCCA(A)TGCACTAATCTGCCATATTA	Simon <i>et al.</i> (1994)
	Chy	F	T(A/T)GTAGCCA(T/C)TTTCATTA(T/C)GT	Ribera <i>et al.</i> (2010)
	Tom	R	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	Ribera <i>et al.</i> (2010)
	Tom-2	R	A(A/G)GGGAATCATTGAATAAA(A/T)CC	Ribera <i>et al.</i> (2010)
rrnL-nad1	16saR (M14)	F	CGCCTGTTTA(A/T)CAAAAACAT	Simon <i>et al.</i> (1994)
	16Sa	R	ATGTTTTTGTAAACAGGCG	Simon <i>et al.</i> (1994)
	16Sb	R	CCGGTCTGAACTCAGATCATGT	Simon <i>et al.</i> (1994)
	16SAlf1	R	GCATCACAAAAAGGCTGAGG	Vogler <i>et al.</i> (1993)
	ND1A (M223)	R	GGTCCCTTACGAATTTGAATATATCCT	Simon <i>et al.</i> (1994)
	16Sbi	F	ACATGATCTGAGTTCAAACCGG	Simon <i>et al.</i> (1994)
	FawND1	R	TAGAATTAGAAGATCAACCAGC	Simon <i>et al.</i> (1994)
SSU	5'	F	GACAACCTGGTTGATCCTGCCAGT	Shull <i>et al.</i> (2001)
	b5.0	R	TAACCGCAACAACCTTTAAT	Shull <i>et al.</i> (2001)
LSU	Ka	F	ACACGGACCAAGGAGTCTAGCATG	Ribera <i>et al.</i> (2010)
	Kb	R	CGTCTGCTGTCTTAAGTTAC	Ribera <i>et al.</i> (2010)

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